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Aspects of salt tolerance of Salicornia Pacifica standl. var. Utahensis (Tidestrom) Munz

Dennis J. Hansen
Brigham Young University - Provo

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ASPECTS OF SALT TOLERANCE OF SALICORNIA PACIFICA STANDL. VAR. UTAHENSIS (TIDESTROM) MUNZ.

A Thesis
Presented to the L2
Department of Botany and Range Science
Brigham Young University

In Partial Fulfillment
of the Requirements for the Degree of
Master of Science

by
Dennis Jay Hansen
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Additional thanks are also expressed to Dr. Jack D. Brotherson for his influence in gaining an appreciation for the esthetics of botany, and to Dr. W. M. Hess for his encouragement and help with the light microscopy and electron micrographs.

Special appreciation is expressed to my wife, Evelyn, and our children, Michael and Tamara, for their patience, love, and encouragement.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Approximately one-third of the land surface of the world falls into the category of semiarid or arid land. In these areas where evaporation rates are high, rainfall does not reduce the salinity of the soil and in many cases salt which is leached from the soil collects in dead bodies of water resulting in the formation of saline playas.

At least four-fifths of the surface of the earth is covered with a salt solution containing, among many other constituents, approximately 0.5 M NaCl. Very few species of higher plant can withstand these conditions. In fact, most terrestrial species are unable to tolerate even one-tenth of the salt concentration of ocean water without a serious setback in water and nutrient balance or metabolism (Waisel 1972). A group of higher plants, know as halophytes, can, however, grow under saline conditions. Species of the genus *Salicornia* are some of the more salt tolerant forms of halophytic plants. Approximately fifty species of *Salicornia* can be found growing in moist saline habitats of temperate and subtropical regions of the world, several of which are found in inland areas where saline playas provide a suitable habitat for growth (Chapman 1960).

Species of the genus *Salicornia* are known to concentrate salts in succulent tissues, presumably to maintain a favorable free energy gradient from soil substrate to extremities of the above ground organs (Yabe *et al*. 1965, Hansen *et al*. 1972). These plants tolerate salt concentrations in their habitats and often have osmotic potentials well above 100 atmospheres if the soil moisture is high. This investigation was undertaken to obtain information about physiological and morphological aspects of the salt tolerant nature of *Salicornia pacifica* Standl. var. *utahensis* (Tidestrom) Munz.

LITERATURE REVIEW

Taxonomy

There is a considerable amount of confusion about the identification and naming of species in the genus Salicornia. In some cases one plant may have as many as three different names (Langlois 1961). The species of Salicornia studied during this investigation was named first as Salicornia utahensis in 1913 by Tidestrom. In 1935 Munz changed the name to Salicornia pacifica var. utahensis (Tidestrom) Munz.

Anatomy and Morphology

Several investigators have described aerial shoots of plants in the genus Salicornia as aphyllous stems (Babington 1904, De Bary 1915), or stems with highly reduced leaves (Ganong 1911). Therefore the chlorenchyma tissue of the succulent internodes has been regarded as the cortex of the stem. Others have concluded that the succulent internode is foliar in origin and is formed by the fusion of the decurrent bases of the two small opposite leaves (de Fraine 1912). Fahn and Arzee (1959) studied several articulated chenopods and concluded that the cortex was not foliar in origin and that the fleshy tissue external to the central vascular cylinder was true cortex tissue.

Waisel (1972) compared the effects of NaCl, $CaCl_2$, $MgCl_2$, $NaNO_3$, and Na_2SO_4 or various combinations of these salts on the succulence of S. herbacea. He concluded that the effects of various ions upon succulence appeared to parallel the lyotropic series and that NaCl was the most efficient salt in promoting succulence. His investigations indicated that chloride rather than sodium was responsible for the induction of succulence. Similar conclusions were reached by Williams (1960).

Germination

Seeds of *Salicornia* are sensitive to prolonged innundation, and according to Waisel (1972), the seeds have to be free from excess water at least 2 to 3 days in order to germinate satisfactorily. Chapman (1960) showed that seedling development of *S. perennis* occurred in salt marshes of the eastern coast of England during the neap tides and that seedlings required 2 to 3 days free from tidal immersion to become established.

According to Waisel (1972), conflicting data exist regarding germination of *S. herbacea* in salt solutions. He stated that while some investigators reported highest germination percentages in salt-free media, others reported optimal germination in 50% sea water. Levitt (1972) showed that optimal germination of *S. herbacea* occurred in nonsaline media, and that growth was optimal only in the presence of NaCl. Loetschert (1970) also showed that *S. stricta* germinates best in pure water. In studies sodium chloride decreased germination of *Salicornia* but increased the survival rate of seedlings (Waisel 1972). Rivers and Weber (1971) reported a reduction in germination rates with increasing salt in the germination media. They also reported that maximum germination of *S. bigleovii* occurred at a sea salt concentration of 4.04% which is very close to the salinity of the sea. Ungar (1962) found that germination of *S. europaea* seeds was impeded at 5% NaCl but that seedling were uninjured at this salt concentration. The degree of inhibition (Shive 1916) and delay of germination (Ayers and Hayward 1948, Ayer 1952) was proportional to an increase of the external osmotic potential.

For germination chloride salts were more toxic than sulfate salts, which were less toxic than carbonate salts (Stewart 1898, Harris and Pittman 1918). However, these investigators pointed out that toxicity of NaCl and Na_2SO_4 appeared to be dependent upon concentration but toxicity of Na_2CO_3 was primarily dependent upon the presence of organic matter in the soil. Harris (1915) found that the relative toxicity of soluble salts was in the following descending order: NaCl, $CaCl_2$, KCl, $MgCl_2$, KNO_3, $Mg(NO_3)_2$, and Na_2SO_4. He also concluded that salt mixtures were not as toxic in soils as in culture solutions.

An interaction between the effects of temperature and salinity on seed germination was reported by Ungar (1967). In his investigation S. europaea, the most salt tolerant species studied, was stimulated by high temperatures, but other plants were inhibited by the highest temperature, 32°C.

Salt Tolerance

Bernstein and Hayward (1958) were the first to provide a comprehensive review of the physiology of salt tolerance. Later, Levitt (1972) provided one of the better treatises on salt and ion stresses. An excellent review of the biochemical aspects of extreme halophilism in bacteria was also written by Larsen (1967). His review included various aspects of enzyme modification and activation by NaCl and other salts, metabolic pathways, protein synthesis under saline conditions, and the requirement for salt to maintain the cell envelope. In his review the evidence are overwhelmingly strong that the enzyme systems have been modified rather than protected by partitioning or compartmentation of salt. This however, does not necessarily mean that extrapolation to higher plants is valid.

Salt Injury

Bernstein (1961) classifies the effect of salinity on plants as osmotic, nutritional, and toxic. According to Bowen and Rovira (1966), ions may cause toxicity in various ways: 1) acting as antimetabolites; 2) binding or precipitating various metabolites; 3) catalyzing rapid decomposition of essential elements; 4) combining with cell membranes and effecting their permeability; and 5) replacing essential elements but failing to fulfill their functions. They suggested that salt injury is not due to a direct effect of the salts, but to the indirect effects of one or more of the above mentioned metabolic disturbances. Waisel (1972) singled out nitrogen metabolism as the area affected by high salts. The salt-induced growth retardation leads to an accumulation of unused substances which may be toxic (Gauch and Eaton 1942).

Changes in root cell ultrastructure were observed by Udovenko et al. (1971) in plants of different salt resistance when grown under saline conditions. They observed that changes in relation to stress were about the same for either plants grown in NaCl or Polyethylene glycol (an osmoticum of extremely low toxicity). After a period of stress, the restoration of normal organelle structure proceeded more rapidly in plants with a higher salt resistance. It is also interesting that restoration of organelle ultrastructure occurred with the passage of time in nearly all cases after initial breakdowns. The rate of normalization was different in different organelles of the plant. Leucoplasts of plants grown in a NaCl-salinized medium were significantly altered until completion of the experiment.

The degree of salt injury produced may be affected by a number of environmental factors. Waterlogged soils may effect the tolerance of plants to salt. Some plants have adapted to tolerate waterlogged conditions. According to Adams (1963), S. foliosa not only tolerates waterlogged habitats but appears to derive benefit from them, because of increased capacity to obtain iron under these conditions which avoids chlorosis. In habitats with marked fluctuations in salt concentration of the root medium, only the species with high shock resistance (Salicornia sp.) can survive (Levitt 1972). Even most halophytes are sensitive to direct salt effects produced by a sudden salt shock (Levitt 1972). Some of the facultative halophytes such as S. rubra are found at the highest salinities, yet are capable of growing normally in low to nonsaline environments (Ungar et al. 1969).

Ion Uptake and Distribution

Waisel (1972) reported that sodium and chloride ions were taken up by roots of halophytes in nonequivalent quantities. In his investigation, higher contents of sodium were reported for S. herbacea plants and a higher uptake of sodium over chloride was reported for other halophytes even under conditions where the chloride content of the growth media was much higher than the sodium content. Under these conditions he reported that glycophytes (nonhalophytes) usually absorb much more chloride than sodium.

The content of sodium and chloride in various organs of Salicornia plants increased from the root upward and in young seedlings the highest salt content was found in the upper parts of the hypocotyl and in the cotyledons (Hill 1908). This excess accumulation of chloride reduces uptake of phosphate and nitrate resulting in a reddish color in these plants (Waisel 1972).

Very little is known regarding the localization of chloride within cells or tissues. According to Levitt (1972), the cytoplasm of cells of halophytic species is rich in unbound free chloride ions. Waisel and Eshel (1971) were able to scan leaf mesophyll cells of Suaeda monoica and follow the distribution of elements such as sodium, chloride, potassium, and phosphorus within those cells. Sodium was located mainly in the cytoplasm of mesophyll cells. A similar distribution was found for potassium. The content of phosphorus inside the vacuoles of the same leaf cells was negligible, and most of it was concentrated in the cytoplasm. Sodium was concentrated primarily in the cytoplasm, while chloride was distributed throughout the cell sections examined.

Growth and Nutrition

Waisel (1972) showed that S. herbacea required a 2% NaCl solution for optimal growth. Webb (1966) reported that the optimum growth of S. bigelovii occurred at 1% NaCl whereas growth of S. olivieri and S. ramosissima was greatest when 2 to 3% NaCl was present in the medium (Halket 1915). Waisel (1972) reported a higher optimum growth for Salicornia in the field. Growth increased proportionally with soil salinity up to a salt content of 6% of the soil solution.

Very little has been published on the effect of essential elements on the growth of Salicornia. There appears to be some evidence that Na^+ and Cl^- can be considered as essential elements for Salicornia. According to Waisel (1972), plants such as S. stricta exhibit restricted growth, abnormal leaf development, and high sensitivity to various infections in sodium-deficient media. In his investigation addition of sodium ions stimulated the growth of plants, but the concentrations required were of magnitudes of 50 to 150 mM NaCl, far above those of micronutrients. Webb (1966) reported that S. bigelovii plants did not survive without addition of NaCl. Very little is known about factors controlling flowering of halophytes. According to Halket (1915), S. olivieri plants did not flower unless salt was present in the growth medium (3.4 to 5% NaCl).

Osmotic Potentials

When a plant is subjected to external osmotic stress it passes through the following sequential events: 1) osmotic stress; 2) reduction in growth; 3) increase in internal solute concentration; 4) partial recovery of growth; and 5) stabilization of the internal level of osmotically active substances (Greenway and Thomas 1965).

Osmotic adaptation in plants occurs mostly because of an increase in the chloride content which is especially high in halosucculents (Adriani 1958). Waisel (1972) has shown that chloride ions account for 67% to 88% of the increase of osmotic potential of different species of salt marsh plants, whereas other osmotically active substances had only a negligible effect. Levitt (1972) reported that chloride accounted for 80% of the total osmotic potential in *S. ambigua*, 91% in *S. stricta*, and 93% in *S. mucronata*. Harward and McNulty (1965) on the other hand, found that chloride did not account for more than 49% of the osmotic potential in *S. rubra*. In their investigation sodium accounted for almost as much osmotic potential as chloride and in some cases even exceeded it.

Yabe *et al.* (1965) reported osmotic values for leaf saps of a number of herbaceous halophytes, including *S. herbacea*, which ranged from 25 to 75 atm when grown in salt marshes. Mullan (1932, 1933) reported that the osmotic potentials of *S. herbacea* were 16.80 atm in tap water, 26.88 atm in 6.04 atm of salt solution, 30.24 atm in 11.02 atm of salt solution, 33.60 atm in 16.04 atm of salt solution, and 36.96 atm in 22.13 atm of salt solution. Seasonal changes in osmotic potentials in *S. rubra* were reported to range from 40 atm to over 100 atm over a two month period (Harward and McNulty 1965). Higher osmotic potential values have been recorded for a number of halophytes, for example, Waisel (1972) reported that osmotic potentials of *Rhizophora* and *Avicennia* leaves reached values of 148 and 163 atm respectively.

Chemical Analysis and Enzyme Sensitivity

Greenway and Osmond (1972) studied enzymes from *S. australis* and suggested that malic dehydrogenase, glucose-6-phosphate dehydrogenase, and aspartic transaminase were as sensitive to salt as enzymes from non-halophytes. The *in vitro* effect of sodium chloride on the enzyme activity of *S. ramosissima* was investigated by Flowers (1972) and it was found that activity of malic dehydrogenase, glucose-6-phosphate dehydrogenase, and peroxidase were, in general, affected by sodium chloride in a similar manner to that reported for salt sensitive species. He found that sodium chloride, however, stimulated ATPase in *Salicornia*. He suggested that salt tolerance in higher plants involves a very different mechanism from salt tolerance in bacteria and concluded that presumably salt and enzymes are not subjected to high concentration *in vivo*. Andersen *et al*. (In preparation) also concluded that the enzyme ribulosediphosphate carboxylase (RuDPCase) from *S. pacifica* was as sensitive to salt *in vitro* as RuDPCase from salt sensitive species. However, he showed that the specific activity of the enzyme from *S. pacifica* was higher than that of the salt sensitive species, tomato.

Parekh and Rao (1965) reported the chemical composition of *S. brachiata*, a species in India, and suggested its use as a feed for cattle since the essential micronutrients that were present were higher than in most grasses. Analysis showed 69.81% crude protein.

Avoidance Mechanisms

Diurnal fluctuations in sodium and in chloride content of *S. europaea* were described for plants growing in two saline habitats, lowflooded and elevated (Waisel 1972). In both habitats, the salt content of plants was at its peak twice a day, in the late morning and in the late afternoon. In the early afternoon and night hours, the salt content dropped significantly. Since no external leaching of the plants was observed which could account for this phenomenon it was suggested that Na^+ and Cl^- were transported from the shoot to the roots, and were periodically excreted to the medium around the roots. Substances accumulated by aerial tissues of plants may eventually be transported back to the roots through the phloem and may be lost to the surrounding medium. This was well established for the transport of sodium in squash (Cooil et al. 1965), and it is probably also true for many halophytes as well. In plants with low chloride content, retranslocation of chloride from older leaves was appreciable. Thus, it seems that retranslocation of ions is not a special characteristic of halophytes (Greenway et al. 1966, Waisel 1972).

According to Chapman (1960), succulents such as Salicornia discard portions of their fleshy cortex together with their leaves, which results in the release of large quantities of salt from plants and enables survival.

Ecology

There appears to be two groups of thought explaining why Salicornia is restricted to a saline environment. Newwohner (1938) stated that halophytes fail to succeed in fresh-water habitats due to competition with other species. This idea was supported by investigations of Stalter and Batson (1969) who stated that survival and growth rate data of transplanted salt marsh vegetation suggest that several species of halophytes (S. virginica included) can tolerate conditions not found in their usual zones. Waisel (1972), on the other hand, claimed that this explanation of plant distribution must be displaced by the concept of "direct ecological salt action." He pointed out that S. herbacea has an optimum growth between 1.5 and 3% salt. Webb (1966) also pointed out that that Salicornia plants grown without salt soon die. Waisel (1972) stated that the proportion of chlorides to sulfates and the total salt content were important in determining distribution. For example, S. herbacea was more sensitive to a high proportion of sulfates than was *Aster tripolium*. Levitt (1972) reported that *Allenrolfea occidentalis* and *S. subterminalis* were highly resistant to salt but sensitive to alkalinity. He suggested that a change from saline to sodic soils (higher pH) may kill these plants. Both of these plants are distributed in soils with a highly saline top layer, but with only moderately saline lower horizons (Waisel 1972).

METHODS AND MATERIALS

Description of the Study Area

This investigation was begun in April of 1972 and continued through August of the same year. This represented a typical growing season for Salicornia pacifica. The study sites chosen for the investigation were salt desert playas. Sites 1 and 2 were located six miles north of the town of Goshen, Utah. Site 3 was located about one half of a mile east of Goshen, Utah. These sites were chosen because of the homogeneous nature with regards to Salicornia plants. Site 1 was especially selected because it was an ecotone between a stand of S. pacifica and a stand of Distichlis stricta. It was hoped that the data from this site could be used to explain some of the environmental factors responsible for separating the Salicornia communities from the Distichlis communities.

The ground at Site 1 was covered with a thin layer of dried algae of the genus Oscillatoria. This covering formed a surface mulch which increased the moisture of the soil surface. The other two sites lacked this surface layer of algae and had dryer soil surface layers throughout the growing season except directly following rain storms. Site 2 was located about 100 yards west of Site 1 on the opposite side of a large drainage basin. Site 3 (Fig. 1) was located 6 miles from Sites 1 and 2 and was adjacent to a natural drainage system. It was selected because of reduced fluctuations in soil moisture throughout

Fig. 1.--Site 3, located one mile east of Goshen, Utah, showing a a typical stand of Salicornia pacifica. (Photograph courtesy of W. M. Hess)

the growing season. Soil and plant samples were taken every two weeks in all three sites.

A weekly hygrothermograph unit was maintained which measured temperature and relative humidity continuously. The housing unit for the hygrothermograph was located a short distance from Sites 1 and 2. Measurements were recorded for the months of May through September 20, 1972. Trouble was experienced in maintaining the unit in an operative condition as the housing unit provided an excellent scratching post for nearby cattle. Troubles were also experienced in earlier experiments when the cattle removed quadrat marking stakes, apparently for the salts that had been absorbed and evaporated on the stakes. The cattle were sold during early April and this eliminated the problem.

Analysis of Soil Samples

Moisture Content

Soil samples were taken with a core borer. Each core measured one inch in diameter and was extended to a depth of ten inches. The cores were separated to provide surface and subsurface soil samples. The surface samples consisted of the upper two inches of the core. The subsurface samples consisted of soil taken from six to ten inches in depth. Five to eight core samples were taken at each site and pooled to obtain the soil sample. The samples were placed in plastic bags, sealed, and immediately taken to the laboratory for analysis. Samples were weighed to the nearest one hundredth of a gram and dried in an oven at 110° C for 48 hours. The samples were weighed again and the percentage of moisture was calculated.

Soil pH

Measurements of the soil pH were determined from saturated soil paste samples using the Sargent-Welch pH/Activity Meter, Model PAX with a combination electrode (0-14 pH, 0-80° C).

Conductivity-resistance Measurements

Resistance measurements were determined with a standard Wheatstone bridge by methods described by Richards (1954).

Osmotic Potential of Soil Samples

Osmotic potentials of soil samples were determined from saturated soil pastes by freezing point depression methods according to the procedure outlined by Hansen and Weber (In press).

Ion Determination

Soil samples were leached of exchangeable cations and anions by placing 5 grams of a dry powdered soil sample in a filter paper which was flushed with four 25 ml volumes of normal-neutral ammonium acetate. Each volume was allowed to drain before the next was applied. The normal-neutral ammonium acetate was prepared by adding 57.5 ml of glacial acetic acid to 800 ml of distilled water and then adding 65 ml of concentrated NH_4OH (C.P.). The pH was adjusted to 7.0 with NH_4OH using the methods described under the "Soil pH" heading above. A total of about 70 ml of NH_4OH was required if the NH_4OH was full strength. The solution was then diluted to one liter with distilled water. The cations, sodium, potassium, magnesium, and calcium were detected in the filtrate according to procedures outlined by Perkin-Elmer (1971) on the Model 290B Atomic Absorption Spectrophotometer using an air and acetylene fuel mixture. The anion, chlorine, was detected in the filtrate according to the procedure outlined by Marius/Fiske (1972) with a Marius/Fiske Chlor-o-counter.

Analysis of Plant Samples

Osmotic Potential

Osmotic potential measurements for Salicornia were determined by freezing point depression techniques as described by Cary and Fisher (1969, 1971) and Fisher (1972). The circuitry was modified by replacing the two 1.35 vdc Hg battery cells with an alkaline 9 vdc battery. A 10K 1-turn potentiometer was installed to correct for voltage drop that occurred with time and usage. Measurements were made at each internode for several plants. Measurements were occasionally made using a vapor pressure osmometer, Model 301 Mechrolab Inc., to verify freezing point measurement values.

Ion Determination

Cation concentrations were determined for sodium, potassium, magnesium, and calcium according to the procedures described by Perkin-Elmer (1971) using the microkjeldahl method of digestion.

The chloride concentration was determined by the use of a newly developed procedure. The plant tissue was prepared by freeze-drying the aerial shoots in the New Brunswick Freeze Dryer B64 and ground into a fine powder by the use of a Wiley mill. Five grams of the powdered plant tissue was soaked in 10 ml of distilled water overnight and filtered. The remaining plant material was further washed with three separate 5 ml volumes of distilled water. Distilled water was then added to the combined filtrates to obtain a final volume of 25 ml. The chloride concentration of the filtrate was then determined using the Marius/Fiske Chlor-o-counter.

Crude Protein Analysis

The percentage of crude protein in each plant sample was determined according to the methods described by Horwitz (1970).

Germination

Seeds of *S. pacifica* were collected from Site 3 in the fall of 1971. Seeds of *S. bigelovii* were collected September 22, 1968 from the Texas City Dike extending into Galveston Bay, Texas by W. G. Rivers. Solutions of sodium chloride, sodium sulfate, and sodium carbonate were prepared with complete, full-strength Hoaglands solution (Epstein 1972) to produce treatments of osmotic values equal to 10, 20, 30, and 40 atm of pressure. Each series of the salt treatments was tested using a vapor pressure osmometer, Model 301 Mechrolab Inc., to insure iso-osmotic concentrations.

Compartments in a plastic germination tray measuring 3 cm by 3 cm by 2.5 cm were filled with a 1.5 cm thick piece of gauze cut into squares and placed into the bottom of each compartment. Twenty seeds were placed in each compartment. Fifteen ml of each salt solution were added to each compartment. This raised the level of solution to near the top of the gauze squares so that the seeds were not completely immersed. By this procedure the concentration of salt could be held osmotically constant by the addition of distilled water as evaporation reduced the level of the solution. A glass plate was placed on top of the plastic germination tray to reduce evaporation and allow for gaseous diffusion into each compartment.

The growth chamber controls were set to provide a 30° C day of 14 hours in duration and a 16° C night of 10 hours in duration. Light and dark germination requirements were determined using temperatures of 16° C and 30° C under continuous light and continuous dark. Twenty seeds were scattered on filter paper in petri dishes and moistened with distilled water. Dark conditions were obtained by wrapping the petri dishes in black paper and placing them in a light tight box to exclude any light and still allow for gaseous exchange. The relative humidity was near 100% for both the light and dark treatments. Seeds were inspected daily to detect germination.

Initial inhibitor studies were carried out under both light and dark conditions. Two treatments were used in this investigation. In one treatment twenty seeds were evenly spaced on moistened filter paper. In the other treatment twenty seeds were grouped together to insure that each seed had ample access to the moisture and oxygen. The experiment was replicated three times.

Subsequent inhibitor studies were carried out by first washing the seeds one hour to reduce freely leachable salts from the seeds and then the seeds were soaked for 24 hours in distilled water. The water soluble extract was concentrated by use of a flash evaporator with a water bath temperature of 50° C. Various dilutions of this concentrated extract were applied to inhibitor leached seeds and unwashed seeds. The chloride concentration of the extract was determined by the Marius/Fiske Chlor-o-counter.

Partial isolation of the inhibitor from the water soluble extract was accomplished by paper chromatography using large sheets of

Whatman No. 1 filter paper and a two phase solvent system of 4 parts n-butanol: 1 part acetic acid: 5 parts water. The upper phase (water saturated n-butanol) was placed in the upper trough of a descending chromatography tank and the lower phase was placed in the bottom of the tank. The separation was allowed to proceed for 32 hours.

Determination of R_f values was accomplished by calculating the distance to the solvent front from the origin and dividing by the distance to the band from the origin.

After 48 hours of drying, bands of the chromatograph were cut into pieces and the water soluble materials were eluted off. The elutant from each band was concentrated using a flash evaporator. Two pieces of filter paper in the bottom of small petri dishes, 2.5 cm in diameter, were used to support 8 dry unwashed seeds. Five-tenths of a ml of each conconcentrate was added to separate petri dishes. The experiment was replicated three times.

Further characterization of the inhibitor was attempted by scanning with the DB Spectrophotometer in the visible and UV range and classification of general compounds by various sprays.

Electron and Light Microscopy

Electron micrographs and light microscopy photographs were provided by W. M. Hess. Specific staining with silver acetate was used to precipitate and fix the chloride ions in situ (Philpott 1965).

Uptake and Distribution of $^{36}Cl^-$

Information regarding the the uptake and distribution of $^{36}Cl^-$ was obtained using 18 excised aerial shoots of uniform size from mature plants collected on June 7, 1973. The shoots were placed in a 100 ml beaker containing a solution of radioactive chloride in 10 ml of full-strength Hoaglands solution (Epstein 1972). Three randomly selected shoots were then removed from the solution after intervals of 15 min, 30 min, 60 min, 3 hours, 12 hours, and 24 hours. The plants were placed immediately into distilled water to remove excess radioactive solution and were then allowed to stand for 5 min. The three plants were then cut into 5 segments. When possible the segments were divided at nodes. The 5 segments for each shoot were numbered from 1 to 5 from the base upward. Generally each segment consisted of at least 2 internodes. The weight of each segment was then determined. The samples were frozen to rupture the cell membranes. Upon thawing, the samples were crushed with a glass rod and 1 ml of distilled water was added to the tissue mixture. This mixture was then allowed to stand overnight to insure diffusion of the radioactive chloride from the tissue. One half ml of aqueous solution was then taken from each sample and added to a 10 ml mixture of scintillation solution. The scintillation solution was prepared by adding 7.3 grams of Pre Mix "P" (Packard Instruments Company, Inc.) and 100 grams of naphthalene to 1 liter of 1, 4 dioxane. Counts were then determined by using the Packard Tri-carb Scintillation Spectrometer Model 3002. The counts were corrected for quench and efficiency. The radioactivity of each segment was expressed as disintegrations per minute (dpm) per gram of fresh weight.

RESULTS

Climatic Factors

Continuous hygrothermograph monitoring of climatic factors indicated that temperatures fluctuated consistantly throughout the growing season. The temperatures gradually increased through the months of May and June. A peak was reached during the month of July followed by a gradual decrease from August to September (Fig. 2). The average of the daily highs during the month of July was 34° C. The hottest temperature recorded in July was 38° C on July 12. The average of the daily lows during the month of July was 11° C. The coolest temperature recorded in July was 6° C on July 25.

Lowest daily means (calculated on an hourly basis) for relative humidity occurred from July 15 through August 20 (Fig. 3). A series of rain storms increased the relative humidity during late August and early September. Rain was also common during late May and early June. No quantatative data concerning the amount of rain was taken. Relative humidity reached 100% every night except for about six days during the growing season.

Soil Moisture

The subsurface and surface soil moisture readings for Site 1 were highest during the month of June and gradually decreased throughout the growing season (Fig. 4). The subsurface soil moisture for this site was less than the surface soil moisture from April 1 through

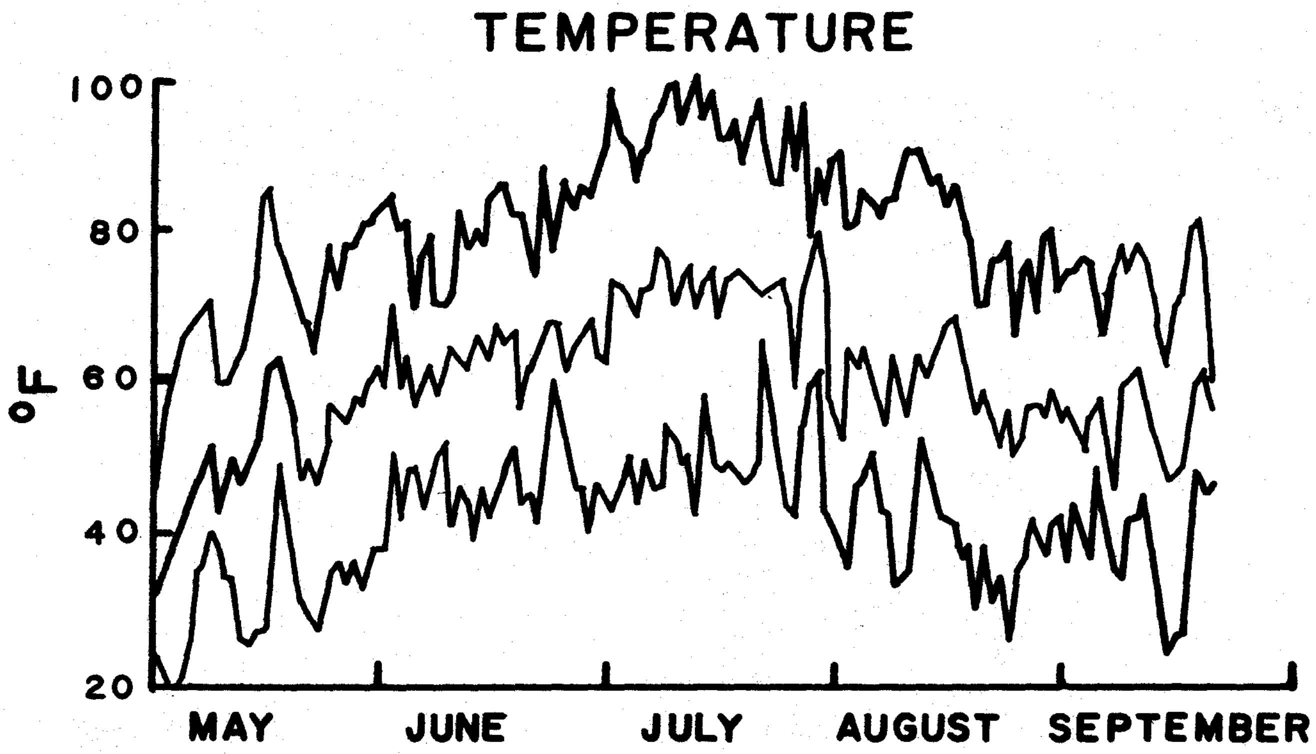

Fig. 2.--Temperatures recorded throughout the growing season (May 1 through September 20, 1972). The upper graph indicates the plot of the daily highs. The middle graph indicates the plot of the daily means calculated on an hourly basis. The lower graph indicates the plot of the daily lows.

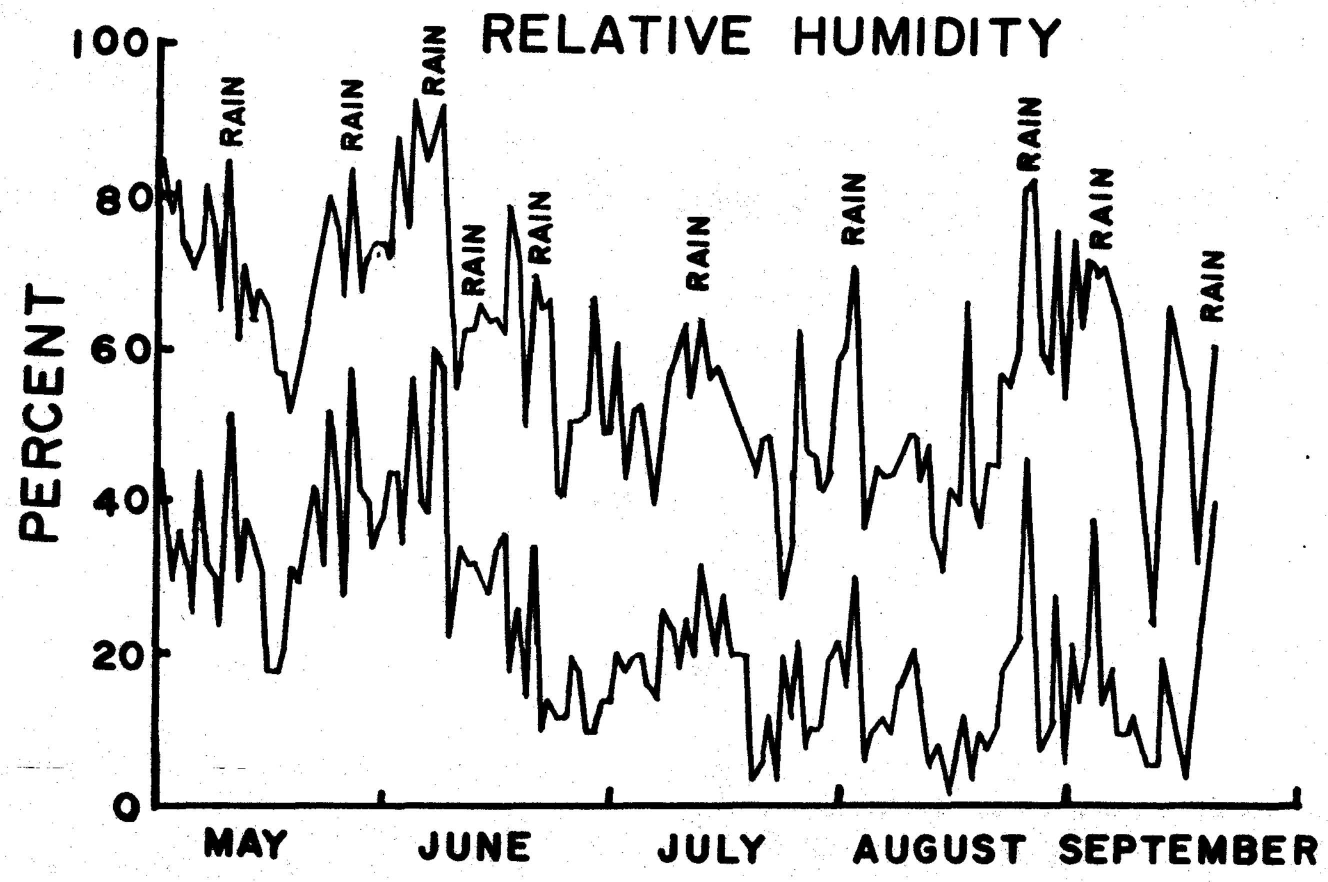

Fig. 3.--Relative humidity recorded throughout the growing season (May 1 through September 20, 1972). The upper graph indicates the plot of the daily means. The lower graph indicates the plot of the daily lows. The daily highs were 100% every day except for six days in July.

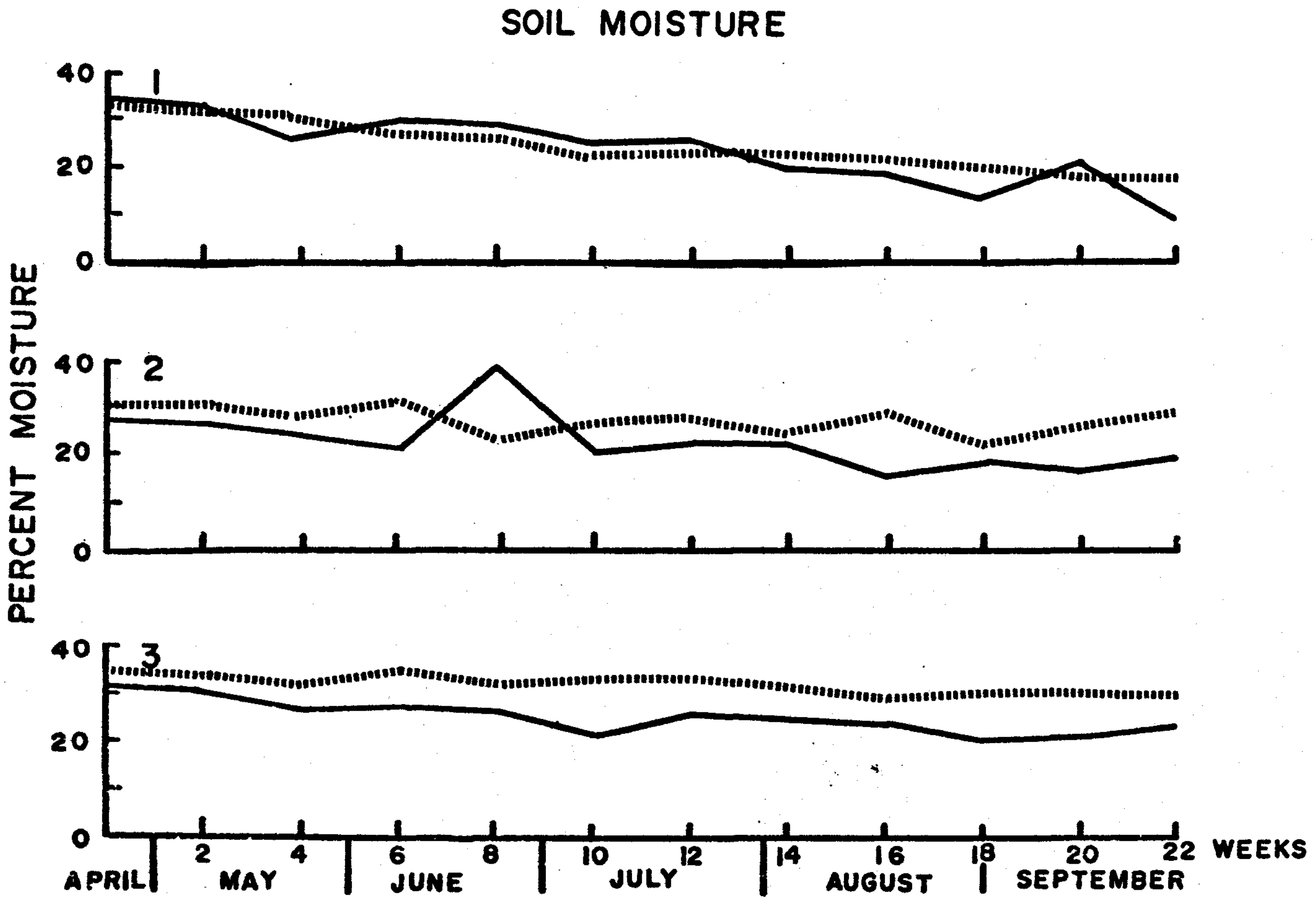

Fig. 4.--Soil moisture for Sites 1, 2, and 3. Solid lines indicate the plot of percentage values for surface soil samples. Dashed lines indicate the plot of percentage values for subsurface soil samples.

July 15 which was probably due to the mulching effect of deposits of dead algae. However, from mid-July through the rest of the season this trend was reversed. In Sites 2 and 3 the subsurface soil moisture was consistently higher than the surface soil moisture throughout the growing season except for one week in June when Site 2 had an increase in the surface soil moisture due to rainfall. Surface and subsurface soil moisture percentages at Site 3 were well above moisture percentages of Sites 1 and 2. From June through July soil moisture in Site 3 was above 30% whereas soil moisture of Sites 1 and 2 was well below 30%. This was probably due to underground seepage from the nearby drainage system adjacent to Site 3.

Soil pH

The surface soil pH on Site 1 increased during the growing season and reached a peak of 8.8 by August 5 (Fig. 5). The subsurface soil pH on this site decreased during the growing season until September 2 when it reached a pH of 8.1. From September 2 to September 20 the soil pH increased rapidly to a pH of 8.6.

The surface soil pH was generally higher and fluctuated more than the corresponding subsurface pH on Sites 2 and 3 (Fig. 5). The subsurface pH of these sites changed very little during the growing season. The subsurface soil pH was more constant in Site 3 than in Sites 1 and 2. In Site 3 the soil pH averaged 7.7. Soil samples from Site 1 had the highest pH values for all sites with a seasonal average of about 8.3. Soil samples from Site 2 had an average value of about 8.1. The decrease in the pH of the soil surface appeared to be strongly correlated with the amount of rainfall. This effect was probably

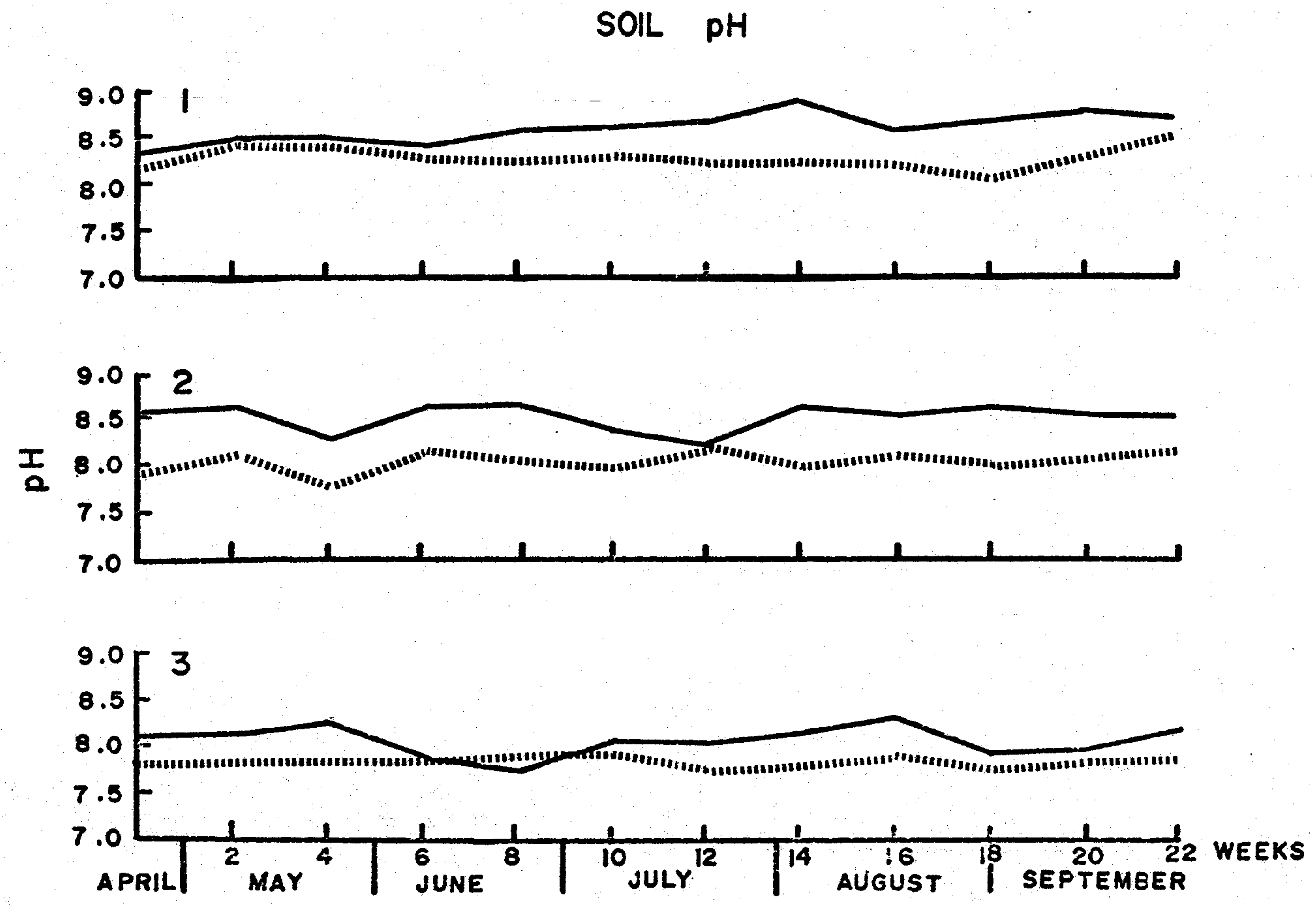

Fig. 5.--Soil pH as recorded for Sites 1, 2, and 3. The solid lines indicate the plot of the pH for surface samples. The dashed lines indicate the plot of the pH for subsurface samples.

due to a transport of soluble salts into the subsurface layers with the percolating rain. A decrease in the pH of the surface was inversely proportional to the increase in the subsurface pH.

Ion Content of the Soil

Site 1

Soil paste resistance measurements for surface soil samples indicated that the greatest concentration of soluble salts occurred during the month of July (Fig. 6). The three high peaks of subsurface concentrations of soluble salts occurred on May 10, July 6, and September 20. There was an increase of soluble salts throughout the growing season.

Osmotic potential measurements of the surface soil were high during July and September. Osmotic potential values reached as high as 135 atm (Fig. 7). Osmotic potential measurements of subsurface soil samples were considerably lower than osmotic potential measurements of the surface. Osmotic potential values for the subsurface soil samples gradually increased. The highest value reached was 48 atm. This value was recorded on September 20 when the study terminated. Values during the hottest month did not exceed 23 atm.

Individual ion analysis of soil samples from the surface (Fig. 8) and the subsurface layers (Fig. 9) showed that sodium and chloride were the two ions responsible for most of the osmotic potential. The increasing ion accumulation of the soil closely paralleled the increase in osmotic potential previously described. The concentrations of sodium and chloride were highest during the months of July and September. The concentrations levels of calcium and potassium ions were

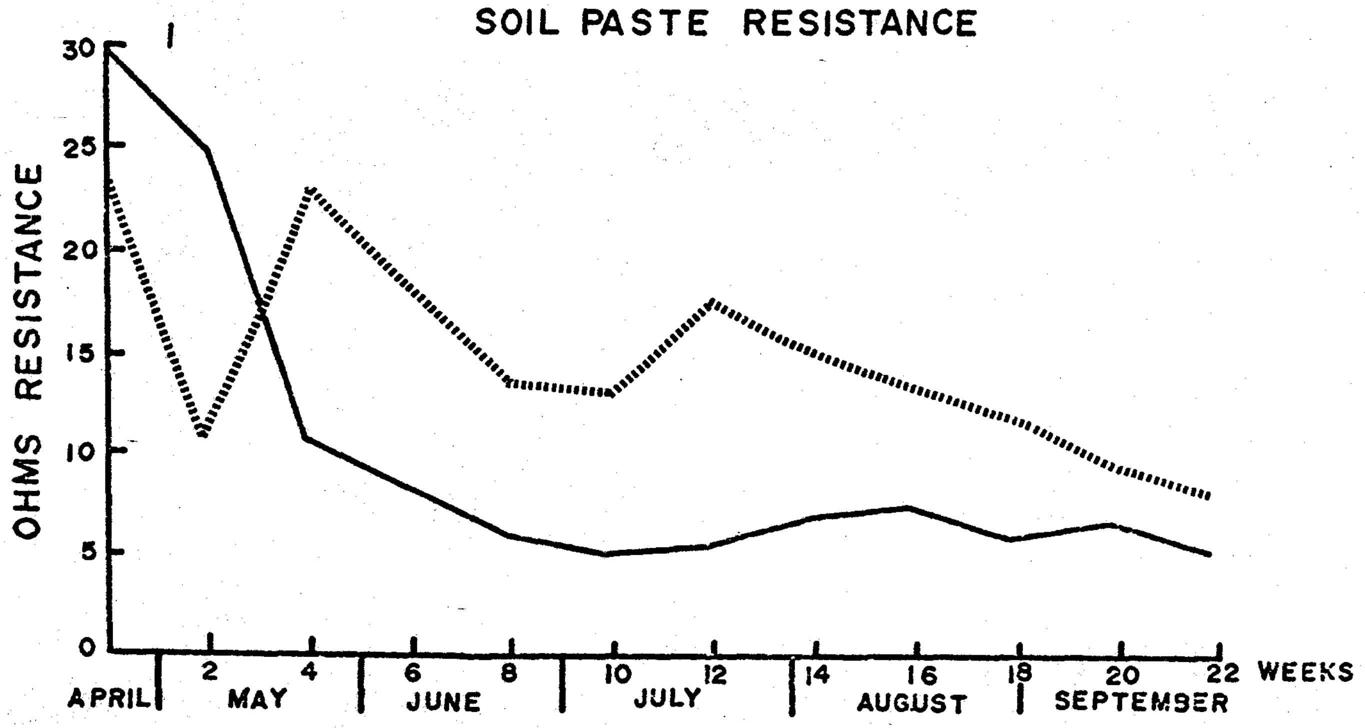

Fig. 6.--Soil paste resistance for Site 1 expressed as ohms resistance. The solid line indicates the plot of resistance values for the surface samples. The dashed line indicates the plot of resistance values for the subsurface samples. Low ohms resistance indicates high soluble salts.

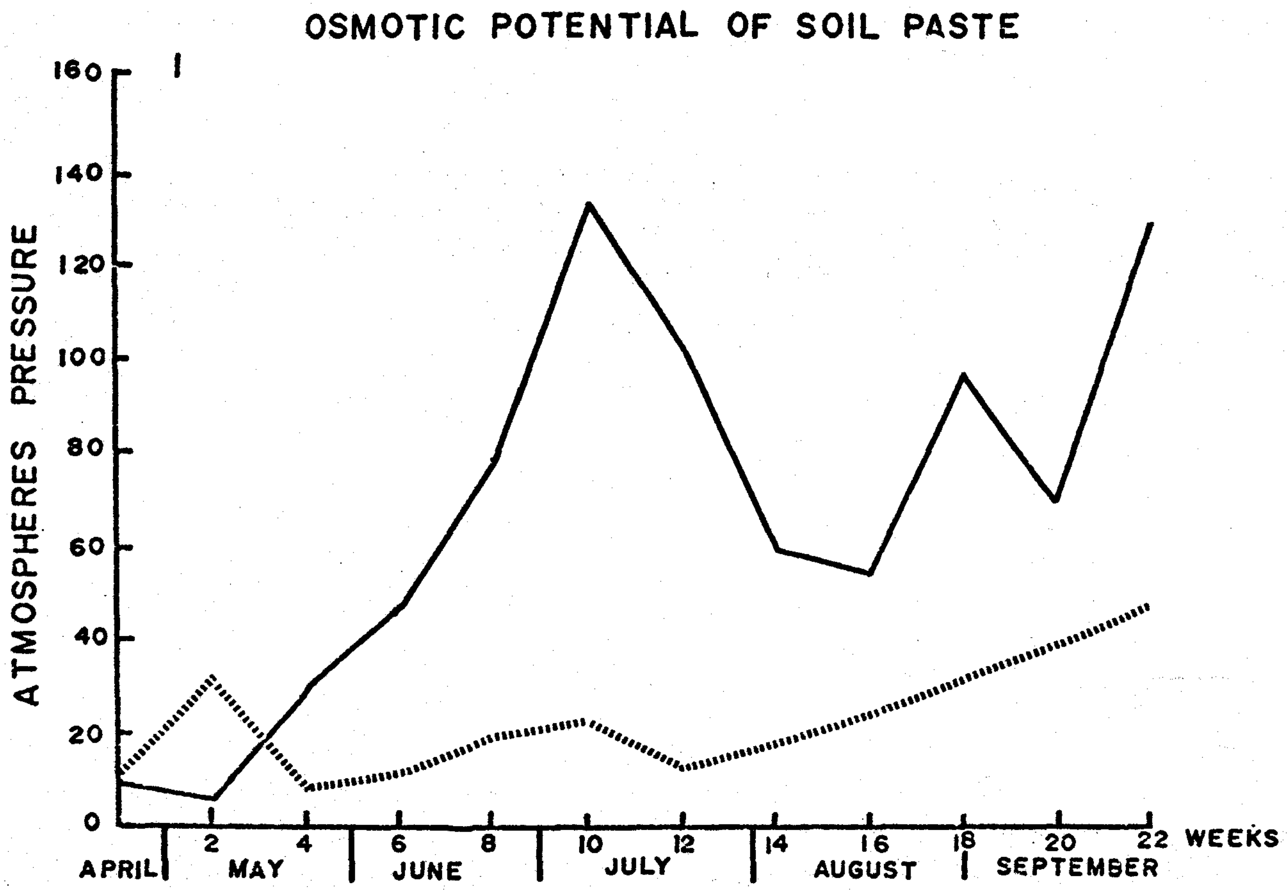

Fig. 7.--Osmotic potential of saturated soil paste for Site 1 expressed as atmospheres pressure. The solid line indicates the plot of osmotic potential values for the surface soil samples. The dashed line indicates the plot of osmotic potential values for the subsurface samples.

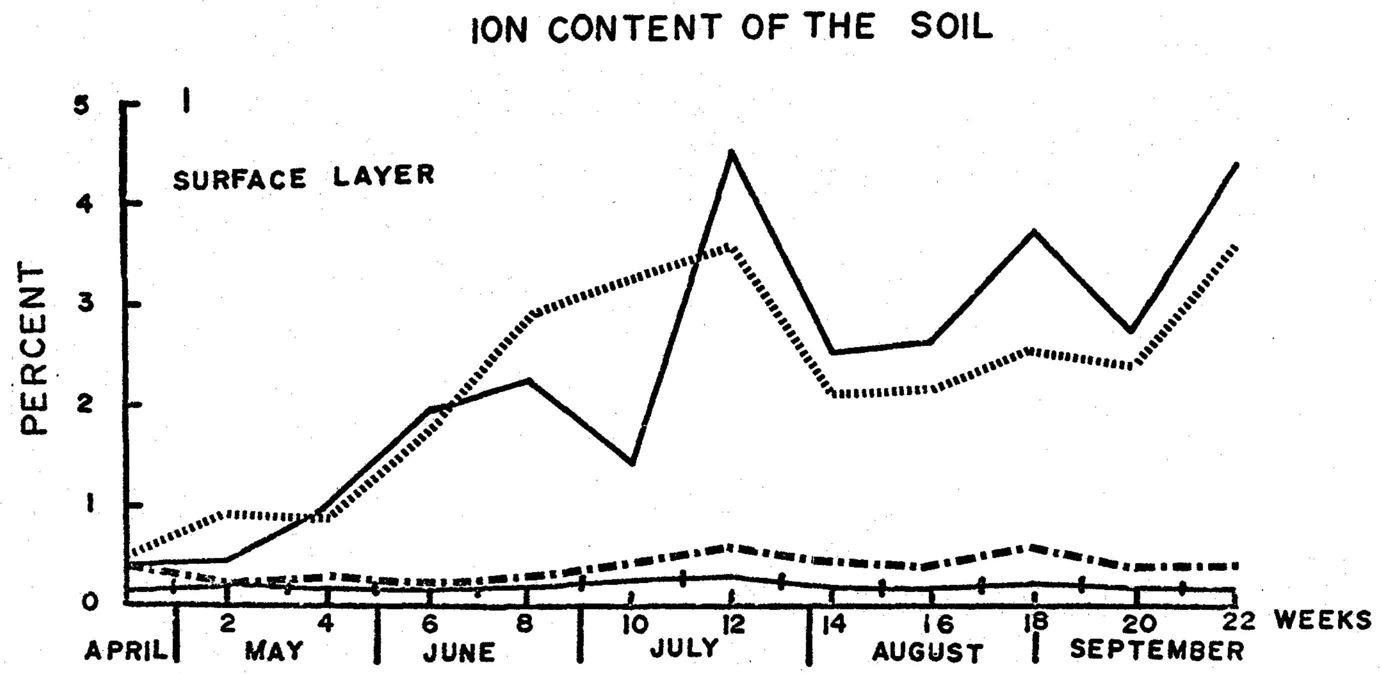

Fig. 8.--Ion content of the soil for the surface layer of Site 1 expressed as a percentage of the dry soil. The solid line indicates the percentage of chloride. The dashed line indicates the percentage of sodium. The dash-dotted line indicates the percentage of calcium. The solid vertical-barred line indicates the percentage of potassium.

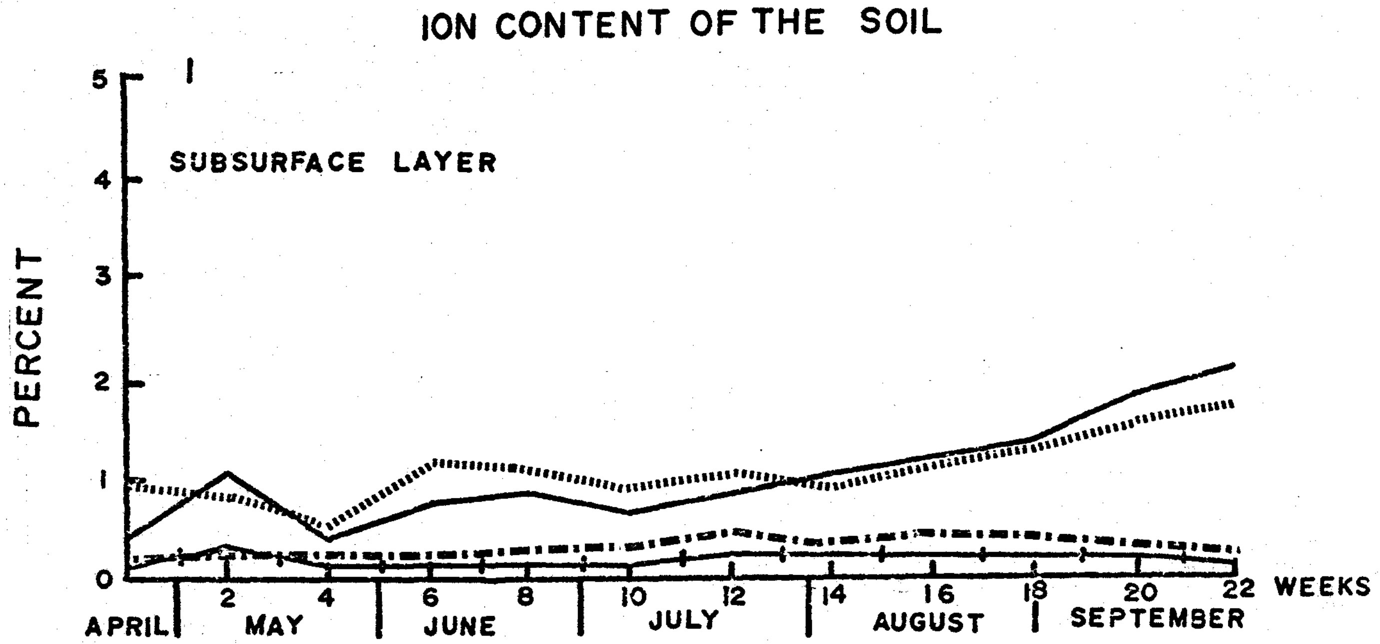

Fig. 9.--Ion content of the soil for the subsurface layer of Site 1 expressed as a percentage of the dry soil. The solid line indicates the percentage of chloride. The dashed line indicates the percentage of sodium. The dash-dotted line indicates the percentage of calcium. The solid vertical-barred line indicates the percentage of potassium.

fairly constant throughout the season. Concentration values seldom exceeded 0.5% of the dry soil weight in either the surface or the subsurface layers.

Site 2

Measurements of soil paste resistance for the surface soil samples indicated that the concentration of soluble salts remained relatively constant throughout the growing season (Fig. 10). However, there was a slight decrease in the amount of soluble salts during the months of July and Augsut. Resistance measurements of the subsurface soil samples suggested a gradual increase in the concentration of soluble salts.

Osmotic potential values for the surface layer samples were uniform and high (over 130 atm) throughout the season with the exception of a substantial decrease on August 29 when values dropped to 80 atm (Fig. 11). The subsurface values steadily increased throughout the growing season to a high of about 96 atm on September 20. In most cases osmotic potential values of the subsurface soil samples were 50 to 100 atm lower than osmotic potential values of the surface soil samples. Therefore, deep rooted plants experienced significantly lower moisture stresses than plants with roots near the surface.

Ion analysis of the surface layer soil samples showed that sodium and chloride ions were responsible for most of the osmotic potential of the soil samples (Fig. 12). The calcium ion concentration of the surface layer for this site was considerably higher than for Site 1. The subsurface calcium and potassium ion concentrations were comparable with other sites and rarely exceeded 0.5% of the soil dry weight (Fig. 13).

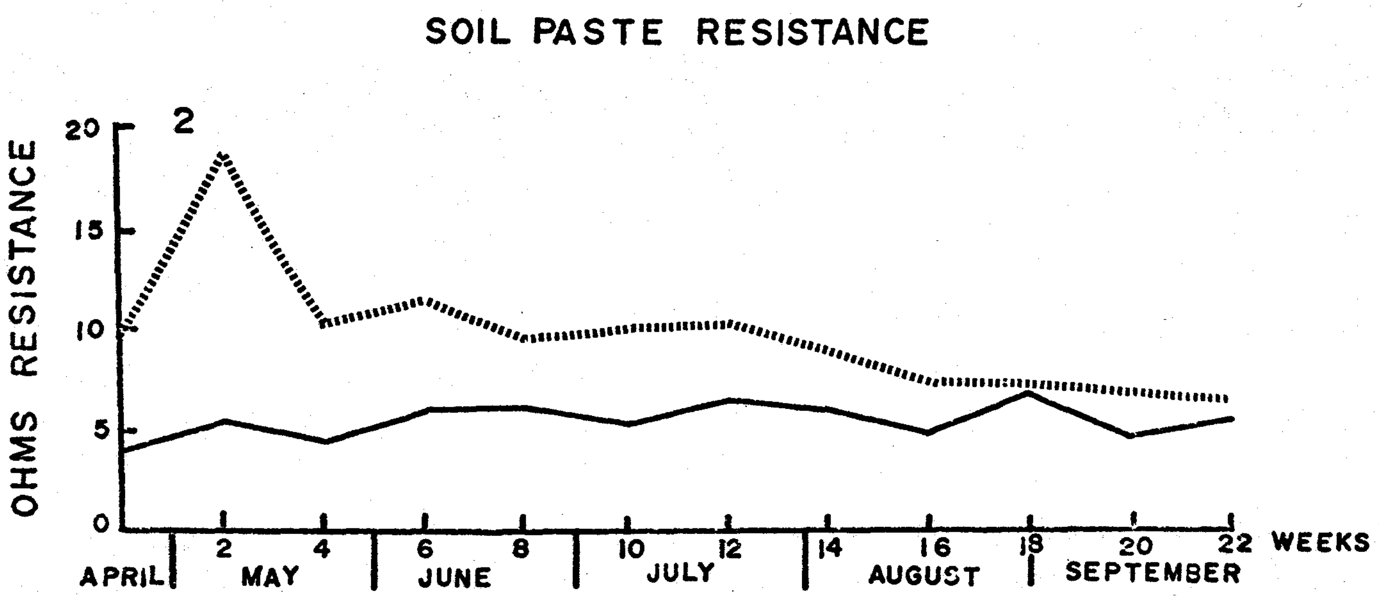

Fig. 10.--Soil paste resistance for Site 2 expressed as ohms resistance. The solid line indicates the plot of resistance values for the surface samples. The dashed line indicates the plot of resistance values for the subsurface samples. Low ohms resistance indicates high soluble salts.

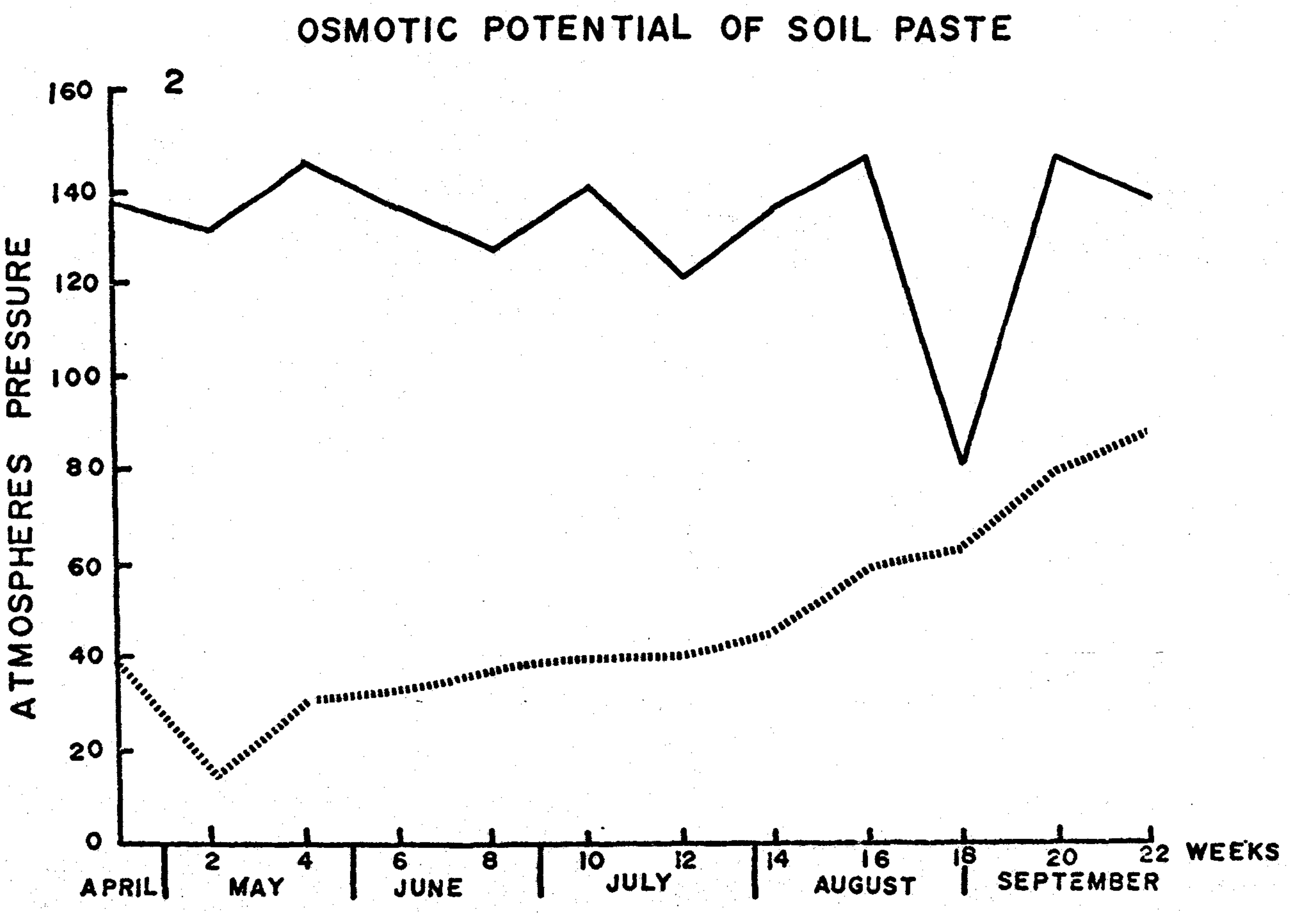

Fig. 11.--Osmotic potential of saturated soil paste for Site 2 expressed as atmospheres pressure. The solid line indicates the plot of osmotic potential values for the surface soil samples. The dashed line indicates the plot of osmotic potential values for the subsurface soil samples.

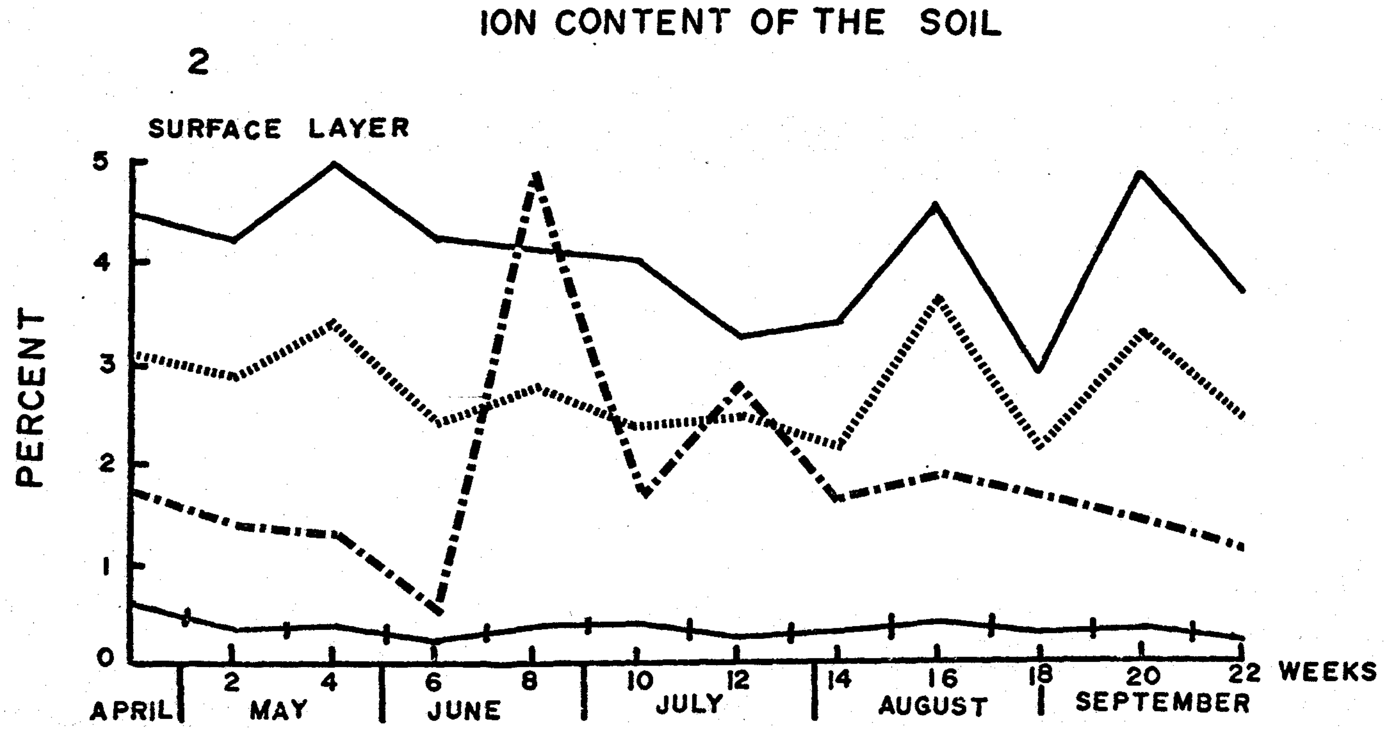

Fig. 12.--Ion content of the soil for the surface layer of Site 2 expressed as a percentage of the dry soil. The solid line indicates the percentage of chloride. The dashed line indicates the percentage of sodium. The dash-dotted line indicates the percentage of calcium. The solid vertical-barred line indicates the percentage of potassium.

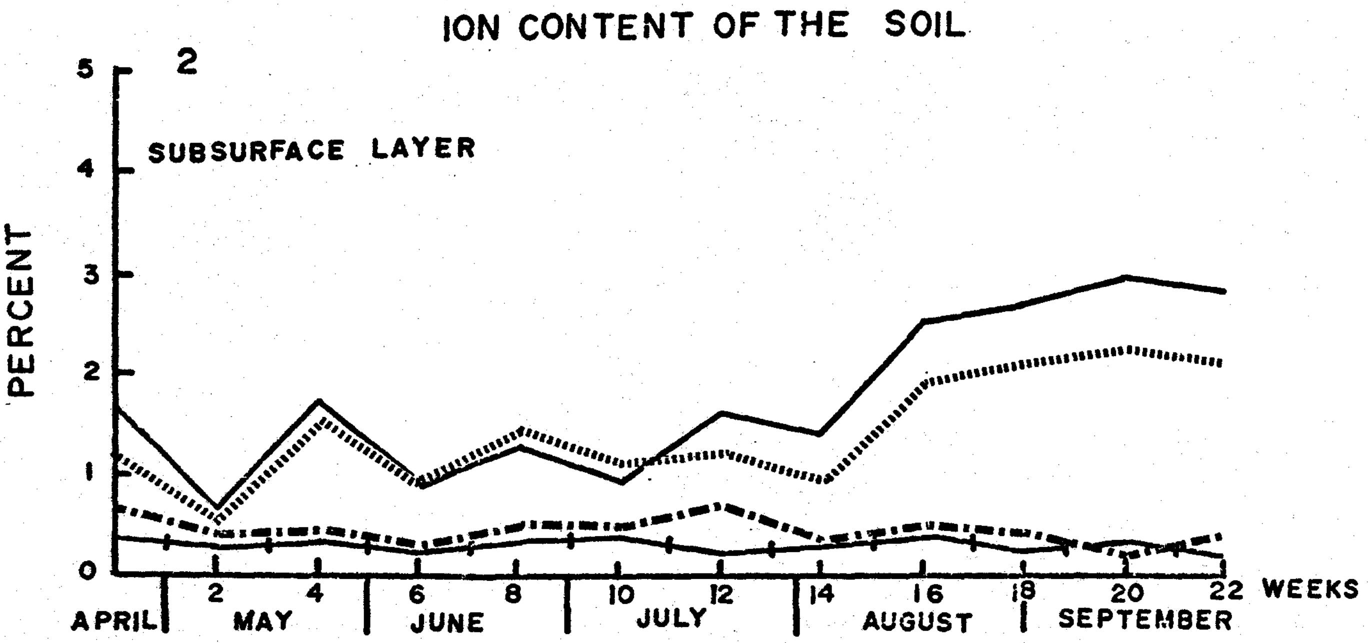

Fig. 13.-- Ion content of the soil for the subsurface layer of Site 2 expressed as a percentage of the dry soil. The solid line indicates the percentage of chloride. The dashed line indicates the percentage of sodium. The dash-dotted line indicates the percentage of calcium. The solid vertical-barred line indicates the percentage of potassium.

The combined ion concentration in the subsurface layer increased gradually over the growing season and reached a peak of about 6% soluble salts on September 20.

Site 3

Soil paste resistance measurements of the soil samples taken from the surface layer showed that soluble salts increased gradually until July 20 and then gradually decreased (Fig. 14). Concentrations of soluble salts in the subsurface layer followed a similar pattern but increased on September 20.

Osmotic potential measurements of soil samples on this site gradually increased in both the surface and subsurface layers. Measurments were highest on July 20 and September (Fig. 15) and were comparable with measurements from Site 2, both of which were considerably higher than Site 1.

Chloride ion content from the surface layer fluctuated considerably but the sodium content was more stable (Fig. 16). The major increase in osmotic potential of the subsurface layer from July 20 through September 20 was chiefly due to the chloride ion content. The calcium ion concentration level of this site was considerably higher than for Site 1 and not as high as for Site 2. The potassium ion concentration level was less than 0.5% and did not fluctuate significantly. The concentration gradually increased to a peak of 0.5% on July 20 and then gradually decreased to 0.3% on September 20. Concentrations of sodium and chloride ions in the subsurface soil samples gradually increased (Fig. 17). Calcium and potassium ion concentrations in this layer remained low and relatively constant through the growing season

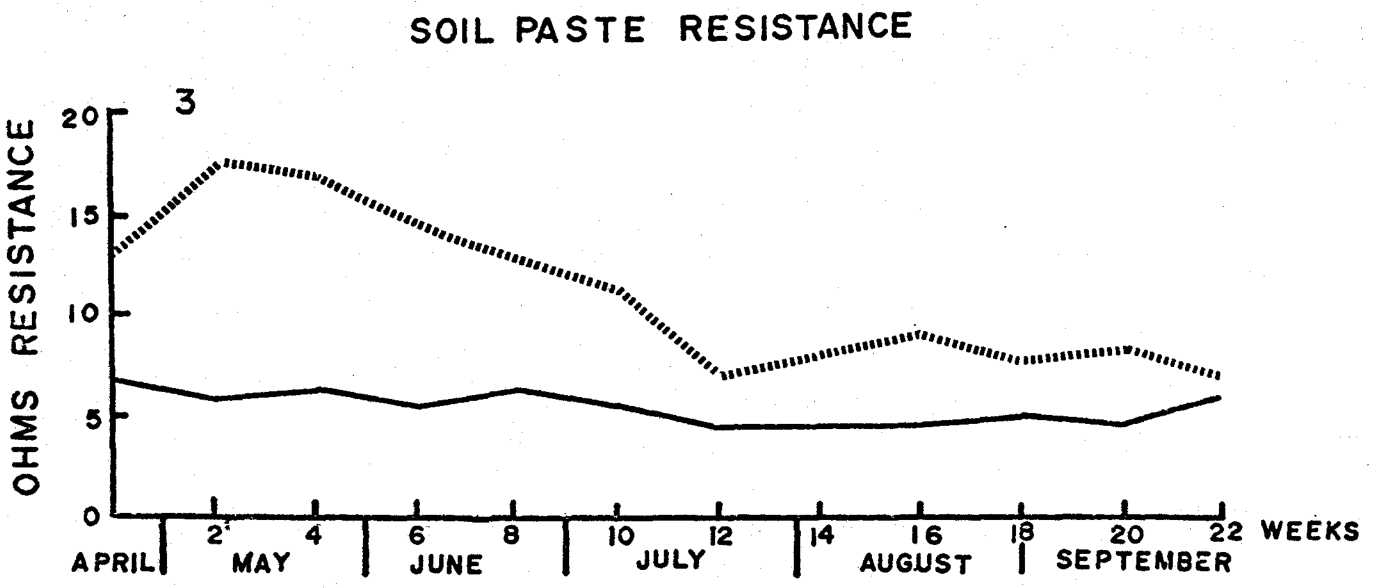

Fig. 14.--Soil paste resistance for Site 3 expressed as ohms resistance. The solid line indicates the plot of resistance values for the surface samples. The dashed line indicates the plot of resistance values for the subsurface samples. Low ohms resistance indicates high soluble salts.

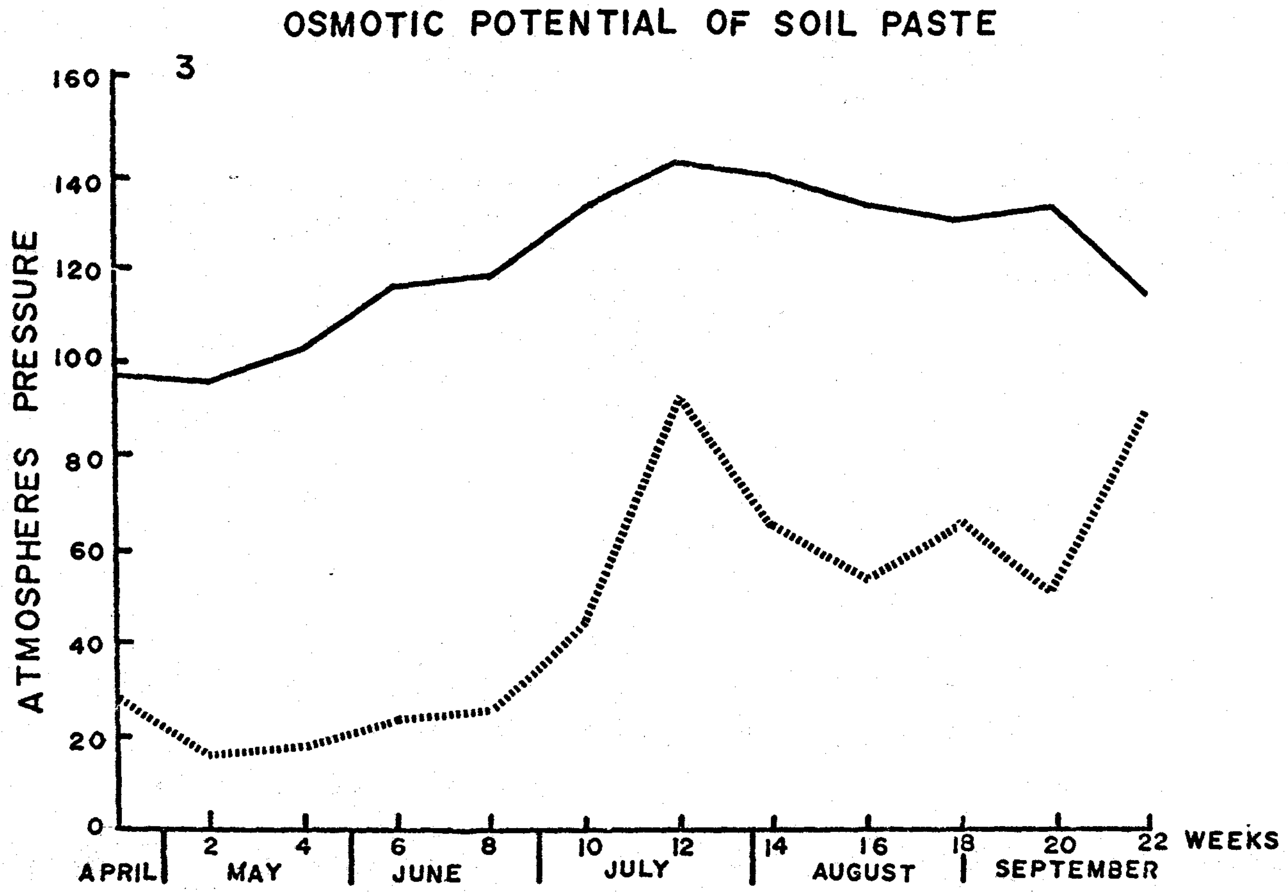

Fig. 15.--Osmotic potential of saturated soil paste for Site 3 expressed as atmospheres pressure. The solid line indicates the plot of osmotic potential values for the surface soil samples. The dashed line indicates the plot of osmotic potential values for the subsurface samples.

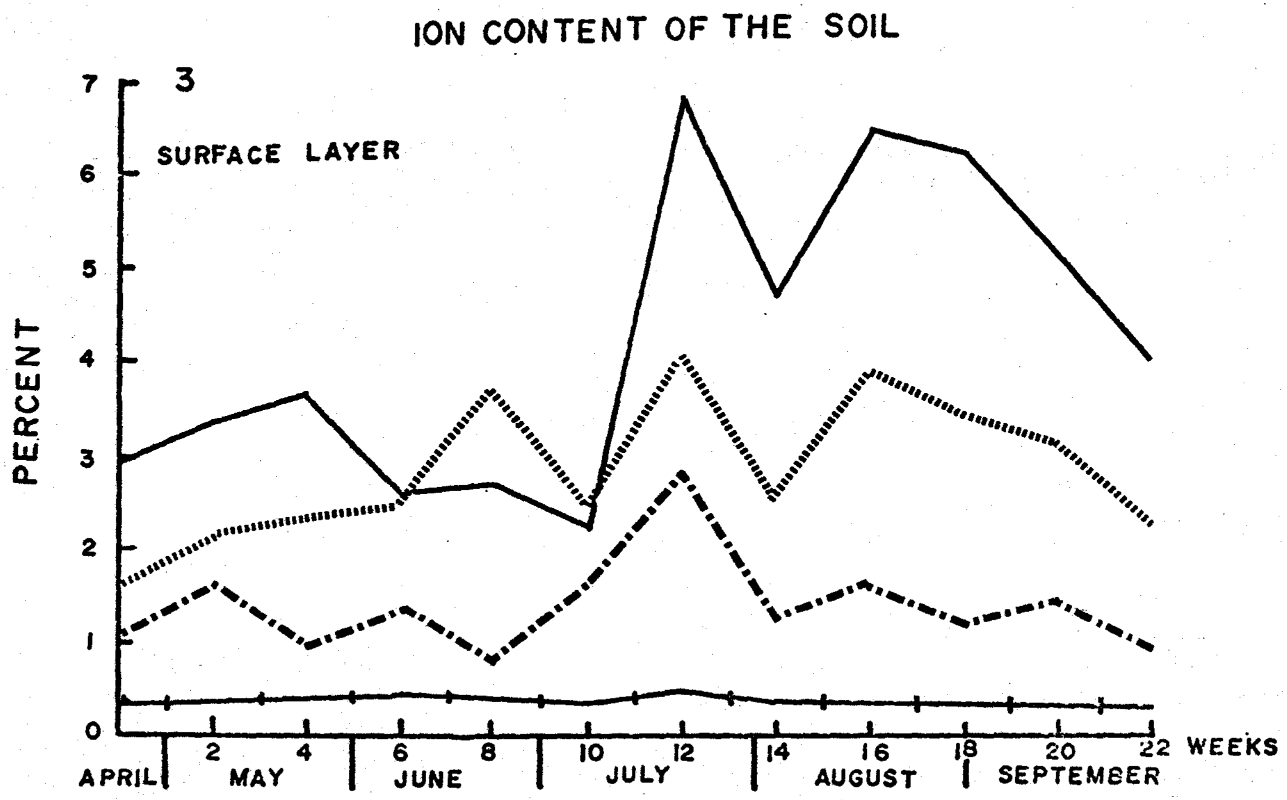

Fig. 16.--Ion content of the soil for the surface layer of Site 3 expressed as a percentage of the dry soil. The solid line indicates the percentage of chloride. The dashed line indicates the percentage of sodium. The dash-dotted line indicates the percentage of calcium. The solid vertical-barred line indicates the percentage of potassium.

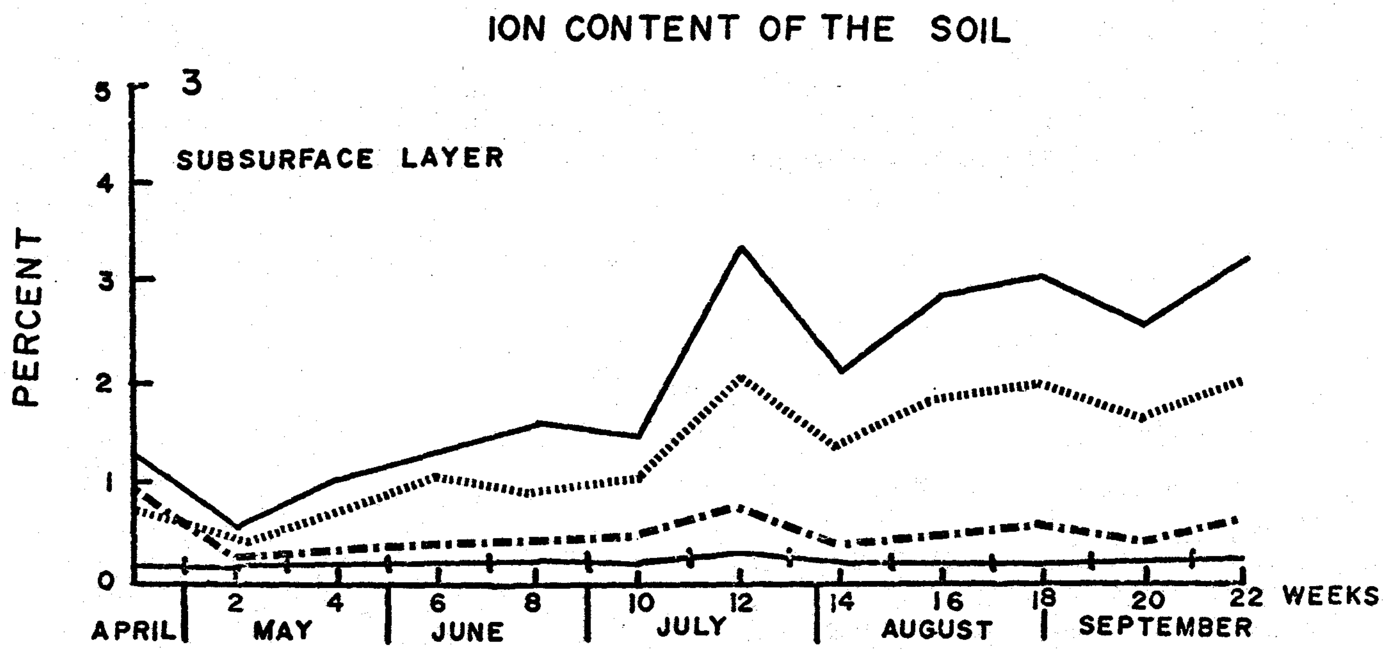

Fig. 17.--Ion content of the soil for the subsurface layer of Site 3 expressed as a percentage of the dry soil. The solid line indicates the percentage of chloride. The dashed line indicates the percentage of sodium. The dash-dotted line indicates the percentage of calcium. The solid vertical-barred line indicates the percentage potassium.

and these ion concentrations were comparable to ion concentrations of the other sites.

Ion Content in Salicornia

Site 1

The chloride ion content in Salicornia tissues remained constant throughout most of the growing season but increased slightly in the month of April (Fig. 18). This increase was followed by a stabilization of the concentration at about 12% of the dry weight. Sodium ion concentration gradually increased from about 4.2 to 9% while potassium ion concentration decreased throughout the season, from 2.2 to 1%.

Site 2

The chloride content gradually increased from 14.2% to 16.1% at the end of the growing season (Fig. 19). Increase in the sodium content was proportional to the increase in the chloride content and was 10.2% at the end of the growing season. The potassium ion concentration increased from 4.5 to 7.5% and was closely correlated to decreases in the sodium ion concentration.

Site 3

The ion content in Salicornia tissues in this site showed a decrease in chloride, sodium, and potassium throughout the season (Fig. 20). The chloride content ranged from 16% on May 1 to 12.4% on September 20. The sodium content ranged from 11% on May 1 to 7.6% on September 20. The potassium content ranged from 4.8% on May 1 to 2.1% on September 20.

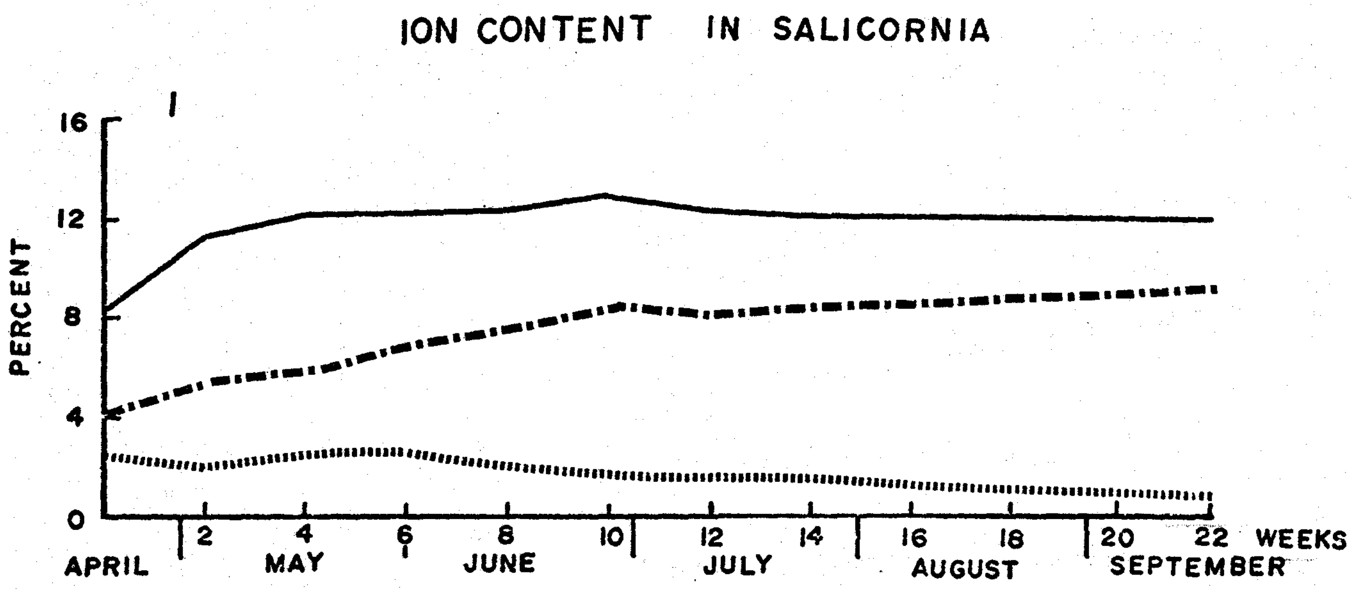

Fig. 18.--Ion content in *Salicornia pacifica* for Site 1 expressed as a percentage of the dry weight. The solid line indicates the plot of the percentage values for chloride ions. The dash-dotted line indicates the plot of the percentage values for sodium ions. The dashed line indicates the plot of the percentage values for potassium ions.

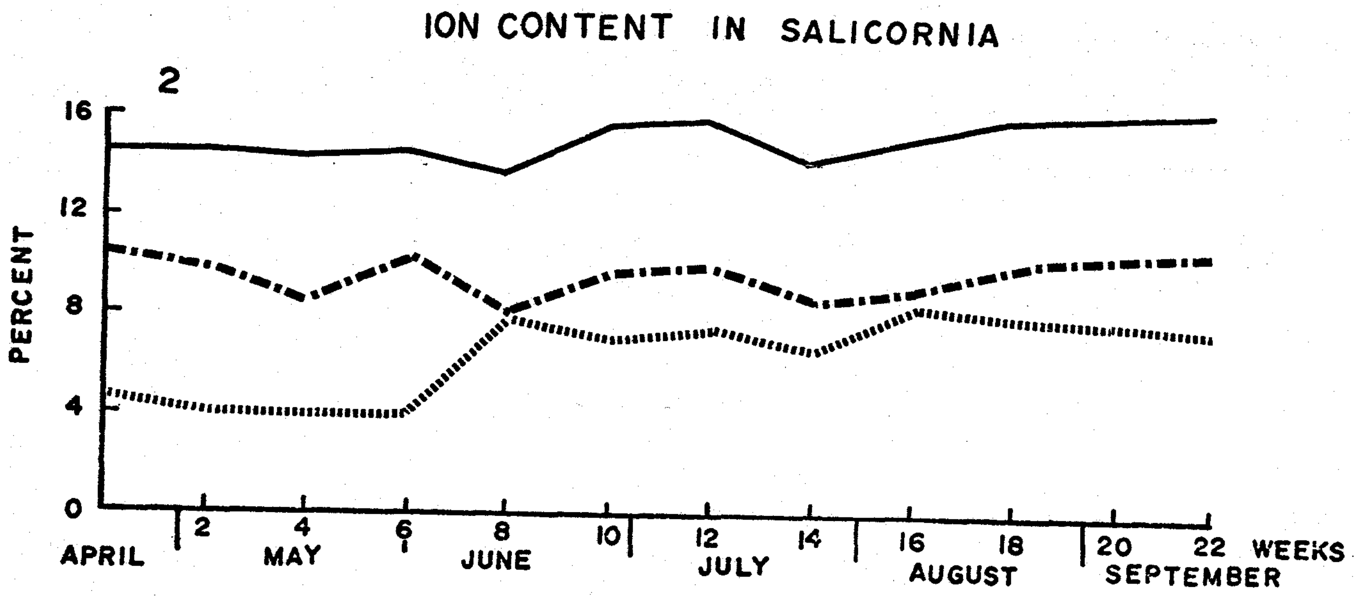

Fig. 19.--Ion content in Salicornia pacifica for Site 2 expressed as a percentage of the dry weight. The solid line indicates the plot of the percentage values for chloride ions. The dash-dotted line indicates the plot of the percentage values for sodium ions. The dashed line indicates the plot of the percentage values for potassium ions.

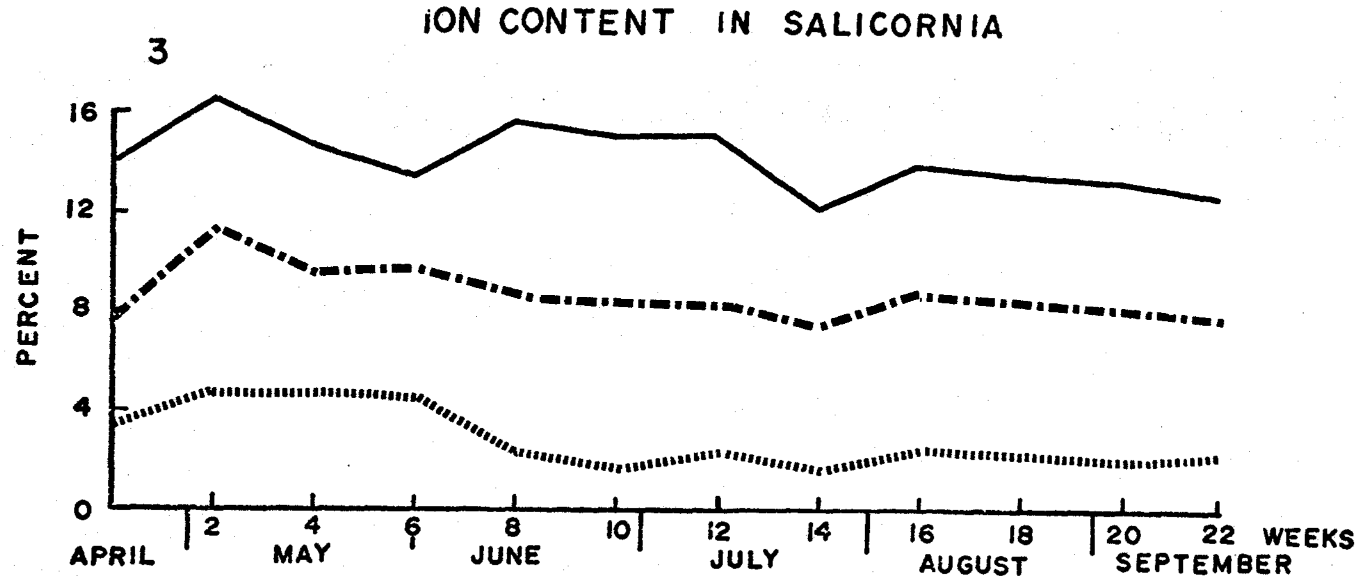

Fig. 20.--Ion content in <u>Salicornia pacifica</u> for Site 3 expressed as a percentage of the dry weight. The solid line indicates the plot of the percentage values for chloride ions. The dash-dotted line indicates the plot of the percentage values for sodium ions. The dashed line indicates the plot of the percentage values for potassium ions.

Osmotic Potentials in Salicornia

Early in the investigation it was discovered that each internode of the plant was osmotically independent from other internodes of the same plant. Freezing point depression measurements showed that osmotic potential values increased from the base of the aerial shoot upward to the top of the plant (Table 1). The lowest internodes near the base generally had a lower osmotic potential than internodes near the middle of the plant. In some cases there was as much as 15 atm difference between the two regions of the cortex, although usually the difference was only about 5 atm. Due to the complexity of such differences in osmotic potential readings only periodic measurements were made on the plants. The measurements ranged from a low of about 80 atm in May to about 130 atm to 150 atm in mid-July and August. However, the average

Table 1.--Osmotic potentials of four different plants showing the values (atm) of the outer and inner "cortex" tissues from the bottom of the shoot (Node No. 1) to the top of the shoot (Node No. 5).

	Plant A		Plant B		Plant C		Plant D	
Node	Inner	Outer	Inner	Outer	Inner	Outer	Inner	Outer
1	76	76	81	81	73	73	84	89
2	69	89	57	57	61	80	64	74
3	71	86	93	86	83	96	71	74
4	67	67	96	106	68	83	73	79
5	106	130	96	106	103	108	84	88
6	89	92	--	--	93	108	--	--
7	106	112	--	--	--	--	--	--

was about 90 to 100 atm.

Uptake of $^{36}Cl^-$ and its Distribution

Analysis of freshly cut aerial shoots exposed to a $^{36}Cl^-$ solution for 15 and 30 min showed a gradual uptake of the radioactive chloride into the first two internodes (Fig. 21). After 60 min the radioactive chloride was present in the third and fourth internodes. After 3 hours of uptake there was an accumulation of chloride in the base of the shoot and in the tip. Chloride concentrations decreased from the tip of the shoot (700 dpm/gm) to the middle of the shoot (300 dpm/gm). Chloride concentrations increased from the middle of the shoot to the base of the shoot (2000 dpm/gm). A similar pattern was observed after 12 hours of uptake. A higher concentration of radioactive chloride accumulated just below the tip rather than in the tip itself. After 24 hours of uptake there was an almost complete reversal in the accumulation pattern. The highest concentration of chloride was found just above the base of the shoot and decreased toward the tip. Investigations on redistribution of chloride after initial uptake revealed complex patterns of redistribution. Further investigations will be required to make valid conclusions.

Crude Protein Analysis

Crude protein analysis showed a gradual decrease in the total crude protein content in the plants from all three sites from April 1 through July 30. Crude protein decreased from 20 to 10% on a dry weight basis (Fig. 22). From July 30 through September 20 the content remained about 10%.

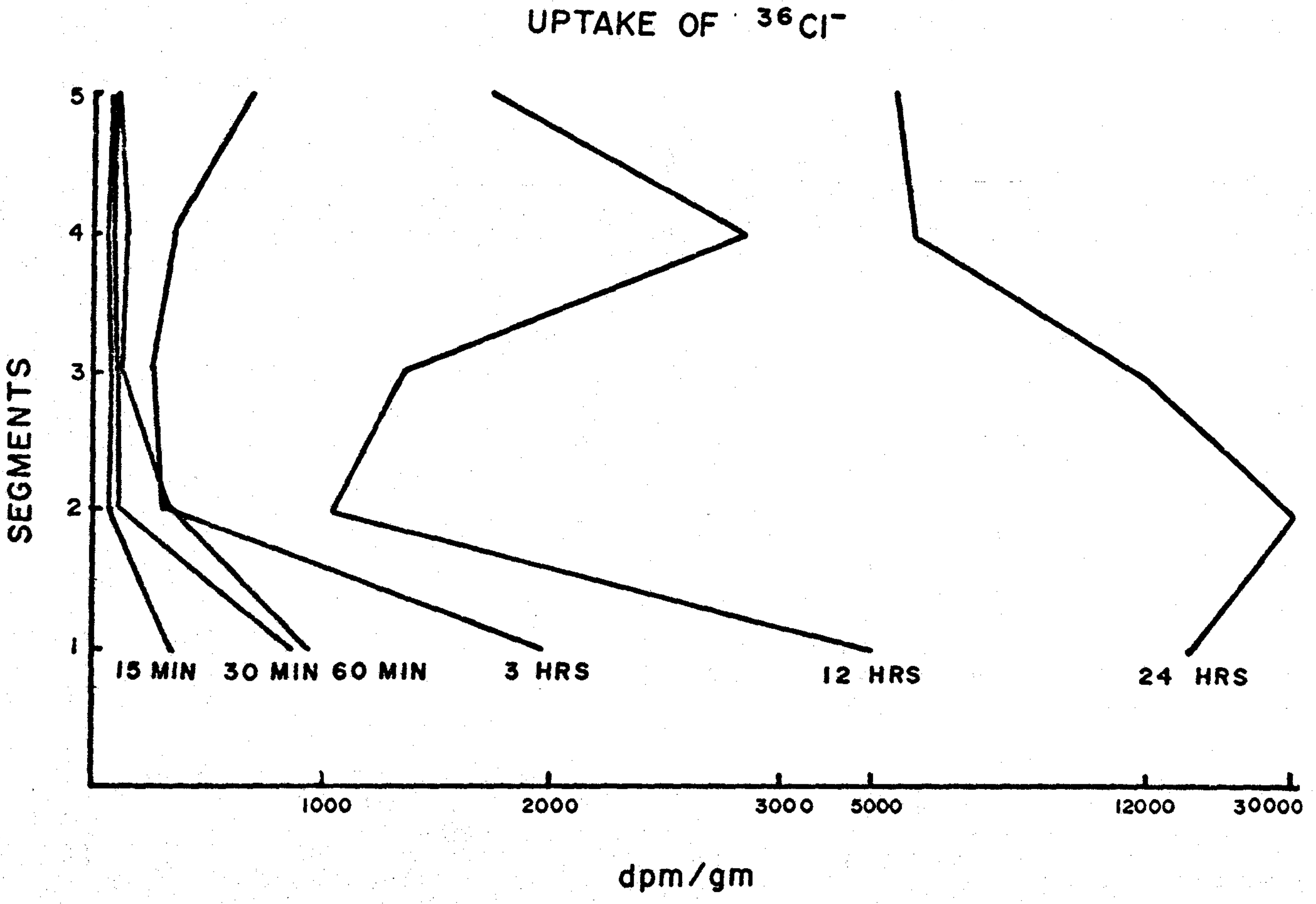

Fig. 21.--Uptake of $^{36}Cl^-$ by Salicornia pacifica aerial shoots expressed as the average disintegrations per minute (dpm) per gram of fresh weight. Each segment consisted of approximately two internodes of the plant and progress from the bottom of the plant shoot (Segment No. 1) to the top of the plant shoot (Segment No. 5).

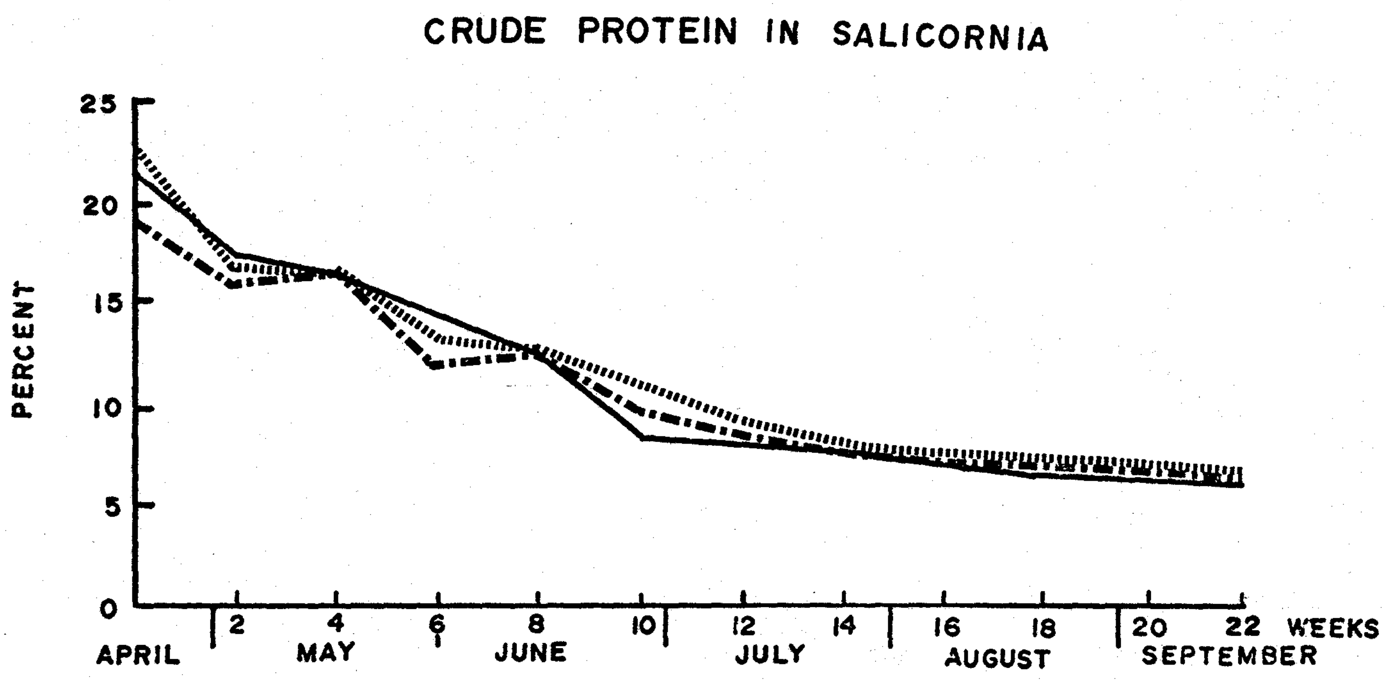

Fig. 22.--Crude protein content in *Salicornia pacifica* shoots through the growing season for Sites 1, 2, and 3. Values are expressed as a percentage of the dry weight. The solid line indicates values from Site 1. The dot-dashed line indicates values from Site 2. The dashed line indicates values from Site 3.

Germination of *Salicornia pacifica* Seeds

Table 2 shows germination results of *S. pacifica* seeds in various concentrations of different sodium salts. The most toxic salt was Na_2CO_3. There were some seeds that germinated in the 10 and 20 atm Na_2CO_3 treatments. For these treatments maximum germination was 20% for the 10 atm treatment and 13% for the 20 atm treatment. In the Na_2CO_3 solutions the cotyledons of germinating seeds seldom emerged and became green. The more common occurrence was an emergence of the radicle and a

Table 2.--Germination of *Salicornia pacifica* in three kinds of sodium salts at various concentrations (atm pressure). Values indicate the average number of seeds germinating on each day and the percentage germination of the total thirty seeds.

		Cl^-				$SO_4^=$				$CO_3^=$			
Day	0	10	20	30	40	10	20	30	40	10	20	30	40
4	2	1	0	0	0	1	0	0	0	2	0	0	0
5	3	4	1	0	0	0	0	0	0	0	0	0	0
6	6	8	1	0	0	3	3	0	0	0	0	0	0
7	2	0	1	0	0	2	0	0	0	0	0	0	0
8	0	3	2	0	0	2	2	1	0	3	3	0	0
10	1	0	3	1	0	2	2	0	0	0	0	0	0
12	0	1	3	1	0	1	1	0	0	1	1	0	0
14	0	1	1	0	1	1	1	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	14	18	11	2	1	12	10	1	0	6	4	0	0
%	47	60	37	7	3	41	33	3	0	20	13	0	0

subsequent death.

Sodium sulfate was the next most toxic salt. At the 20 atm treatment level seed germination in the NaCl solution was 37% as compared to 33% germination in the Na_2SO_4 solution. The highest germination at 10 atm for S. pacifica seeds was 60% in the NaCl solution and only 41% germination in the Na_2SO_4 solution at 10 atm. Forty seven percent germination occurred in full-strength Hoagland solution.

The results of light and dark studies (Table 3) indicated that

Table 3.--Germination of Salicornia pacifica seeds in the light and dark at 16° C and 30° C. Values indicate the average number of seeds germinating each day and the total percentage of germination out of 20 seeds.

	16° C		30° C	
Day	Light	Dark	Light	Dark
4	0	0	1	0
5	0	0	10	0
6	0	0	6	0
7	1	0	0	1
8	3	0	1	6
9	2	0	1	3
10	3	0	0	0
12	2	1	0	2
14	1	5	0	0
Total	12	6	18	12
%	60	30	90	60

seeds of S. pacifica germinate best in the light at both 16° C and 30° C. However, investigations with inhibitor leached seeds indicated that germination rates for these seeds were equal whether seeds were exposed to the light or dark. When the inhibitor was washed from the seed coats the light treatment was no longer required for germination. Cooler temperatures further intensified the lag in germination between the light and dark treated unleached seeds.

Salicornia pacifica possesses an effective seed coat inhibitor. The results in Table 4 indicate that when seeds are grouped close

Table 4.--Inhibition of germination of Salicornia pacifica seeds as a result of grouping 20 seeds close together in one treatment and spacing the seeds separately in the other treatment. The experiment was carried out at a 30° C day of 14 hours and a 16° C night of 10 hours. Values indicate the average number of seeds germinating and the percentage of germination of the total 20 seeds.

Day	Grouped	Separated
4	0	2
5	0	7
6	2	8
7	1	1
8	2	0
9	4	0
10	3	0
12	1	0
Total	13	18
%	65	90

together germination is inhibited. Widely spaced seeds are not inhibited. In this case twenty seeds were more than sufficient to indicate the effectiveness of the inhibitor. Accumulation of twenty seeds or more in a group in the field would be a very common occurance. Therefore inhibition could be common in nature.

Water extracts of a number of seeds were concentrated and applied to leached and unleached seeds. Table 5 shows the effect of the extracts on washed or inhibitor leached seeds and dry unleached

Table 5.--Germination affected by increasing amounts of inhibitor extract (X) on inhibitor leached and unleached seeds of *Salicornia pacifica*. Figures indicate the average number of seeds germinating on each day and the percentage of germination of the total 20 and 120 seeds. The experiment was carried out with a 30° C day of 14 hours and a 16° C night of 10 hours.

	Leached				Unleached		
Day	H_2O	X	XX	XXX	H_2O	X	XX
4	1	2	1	0	10	2	1
5	7	4	3	0	30	12	8
6	8	9	6	0	23	41	29
7	3	1	4	6	41	30	22
8	0	0	2	4	8	8	15
9	0	0	0	2	0	6	5
10	0	0	0	1	0	0	0
Total	19	16	15	13	112	99	80
%	95	80	75	65	93	82	66

seeds. The results suggest that the inhibition effect is the same for leached and unleached seeds. The unleached seeds showed a slightly lower percentage in germination. This was probably due to the additional amount of inhibitor present in the seed coat.

To determine whether the inhibition was due to an organic compound or the presence of concentrated salts from the seed coat, the concentrated extract was spotted on large sheets of Whatman No. 1 filter paper to obtain chromatographic separation of the compounds in the extract. The chromatographs were dried and sectioned into five bands: No. 1 was the "control" which consisted of the paper exposed to the solvent in the trough, No. 2 was the band of R_f values ranging from 0 (the origin) to 0.25, No. 3 was the band of R_f values ranging from 0.26 to 0.50, No. 4 was the band of R_f values ranging from 0.51 to 0.75, and No. 5 was the band of R_f values ranging from 0.76 to 1.0 (the solvent front). After elution with distilled water and concentration by flash evaporation the elutant was applied to the seeds. The germination results are shown in Table 6. The salts and a yellow-brown pigment were present in Band No. 2. Germination was retarded by about two days in the elutant from this band. Total germination of seeds treated with this band was only 75% as compared to 100% germination for the seeds of the control and seeds treated by elutants from the other bands. The elutant from Band No. 2 was examined in the visible and UV wave lengths with the Beckman DB-G Grating Spectrophotometer. Strong absorption occurred in the blue and violet wave lengths (430 nm to 200 nm). The least amount of absorption (greatest transmission, 41%) occurred at about 760 nm. Further attempts to characterize the inhibitor revealed a contaminate of unknown composition that was apparently used in the

Table 6.--Germination of *Salicornia pacifica* seeds in elutants from five bands of a chromatographic separation of the inhibitor concentrate. Figures indicate the average number of seeds germinating each day and a total percentage of germination for the eight seed replicas. The Experiment was carried out with a 30° C day of 14 hours and a 16° C night of 10 hours.

	Bands				
Day	#1 (Control)	#2	#3	#4	#5
4	3	0	1	2	1
5	5	0	4	6	6
6	0	3	3	0	1
7	0	1	0	0	0
8	0	2	0	0	0
Total	8	6	8	8	8
%	100	75	100	100	100

manufacture of the filter paper. This compound was responsible for the strong absorption in the spectroscopic scan. However, germination was not affected by the presence of the compound. The use of general indicator sprays showed that reducing sugars, alkaloids, amino acids or peptide chains, or phenols were not found in this inhibitor band. The results indicate that the inhibitor is an inorganic compound, probably sodium chloride. The compound with the pigment was present in such a low concentration that it could not be detected by the procedures used. The inhibitor is also effective in restricting or inhibiting the germination of *Lepidium perfoliatum*, a mustard, and *Bromus tectorum*, a grass. Both of these species of plants are present adjacent to the study sites.

Light and Electron Microscopy

The basic anatomy of the aerial shoots was studied by use of light microscopy of sections of Salicornia embedded in Spurr's plastic. The central stele consisting of from 6 to 8 vascular bundles or stelar strands was surrounded by several layers of large parenchyma cells with large vacuoles (Fig. 23). No chloroplasts or vascular tissue were seen in this tissue region. An anastomosing vascular system that branches from two sets of leaf traces coming out of the central stele near the top of each node and on opposite sides of the stem separates the large parenchyma tissue region and the outer chlorenchyma tissue region. Long radially oriented sclereid- or tracheid-like cells extend from the anastomosing vascular system to near the epidermis (Fig. 24). These cells seldom join with the xylem clements, but usually a small intermediate cell connects the two. Numerous chlorenchyma cells can be found between these unusual cells.

Stomata are slightly sunken. The epidermis is covered with a thick cuticle consisting of a waxy layer and numerous wax crystals (Fig. 25). A series of phellogen cells that produced suberized, cork-like cells and numerous fibers were found in the stele of older internodes that were just beginning to shed the outer "cortex". These cells appeared to seal off the central stele and guard against water and nutrient loss. The phellogen was also prevalent in the rhizome segments.

An unusual arrangement of the chloroplasts in the chlorenchyma cells was evident in electron micrographs (Fig. 26). The chloroplasts are located on the perifery of the cell and often come into close contact or even join with adjacent chloroplasts to give the appearance of one continuous chloroplast. There does not appear to be an indication

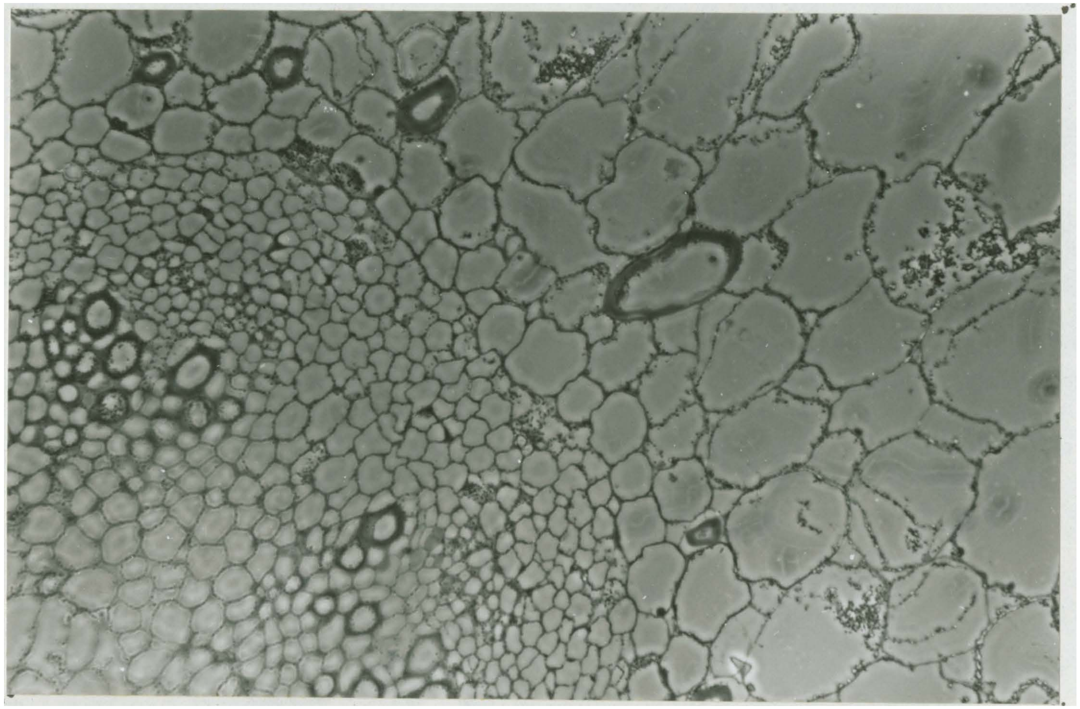

Fig. 23.--Light microscopy showing a cross section through the stem of *Salicornia pacifica* illustrating the central stele and the surrounding inner "cortex" (X 350). Sections have been treated with silver acetate to precipitate and stain the chloride ions which appear as granular particles. (Photograph courtesy of W. M. Hess)

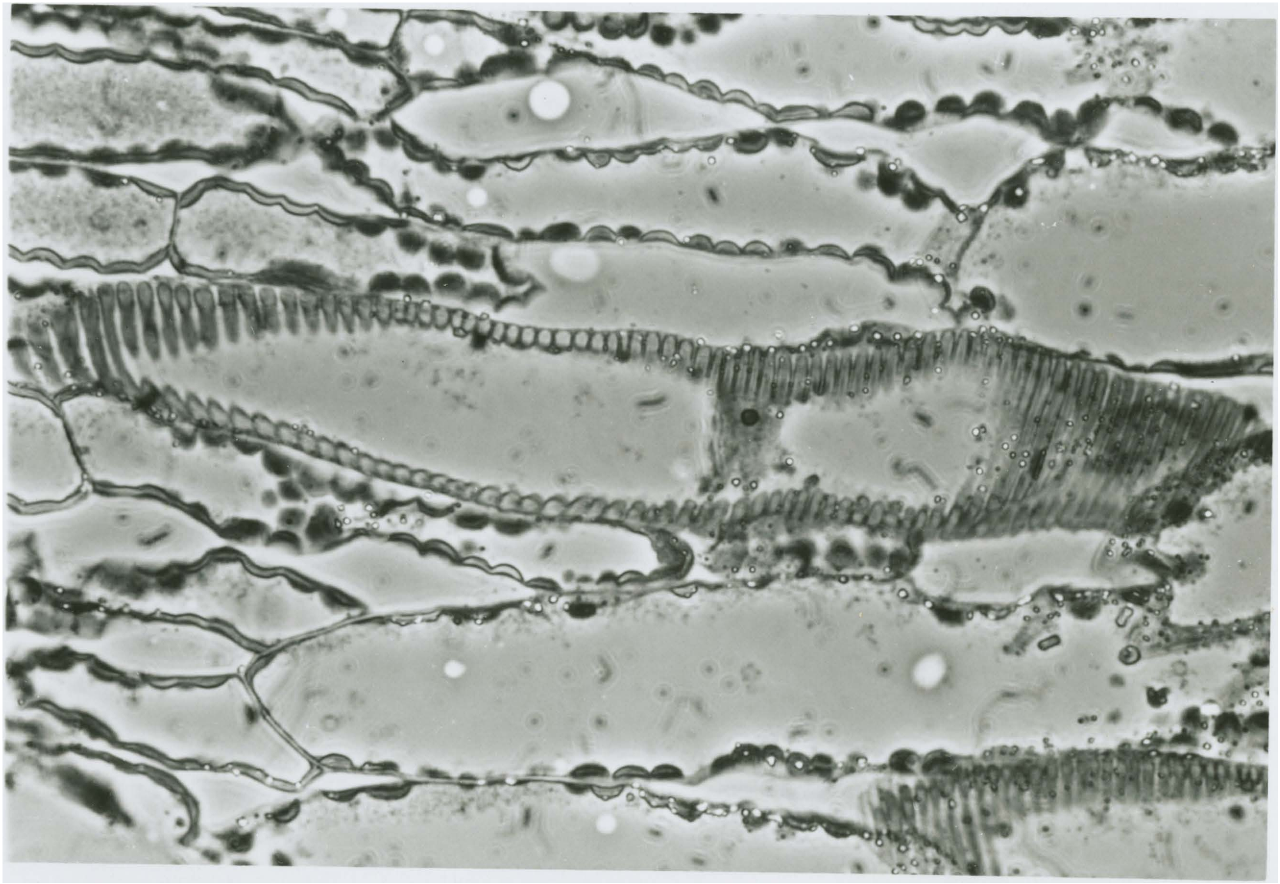

Fig. 24.--Light microscopy showing a longitudinal view of the striated sclereid-like cell treated with silver acetate to precipitate the chloride ions, which appear as granular particles (X 840). (Photograph courtesy of W. M. Hess)

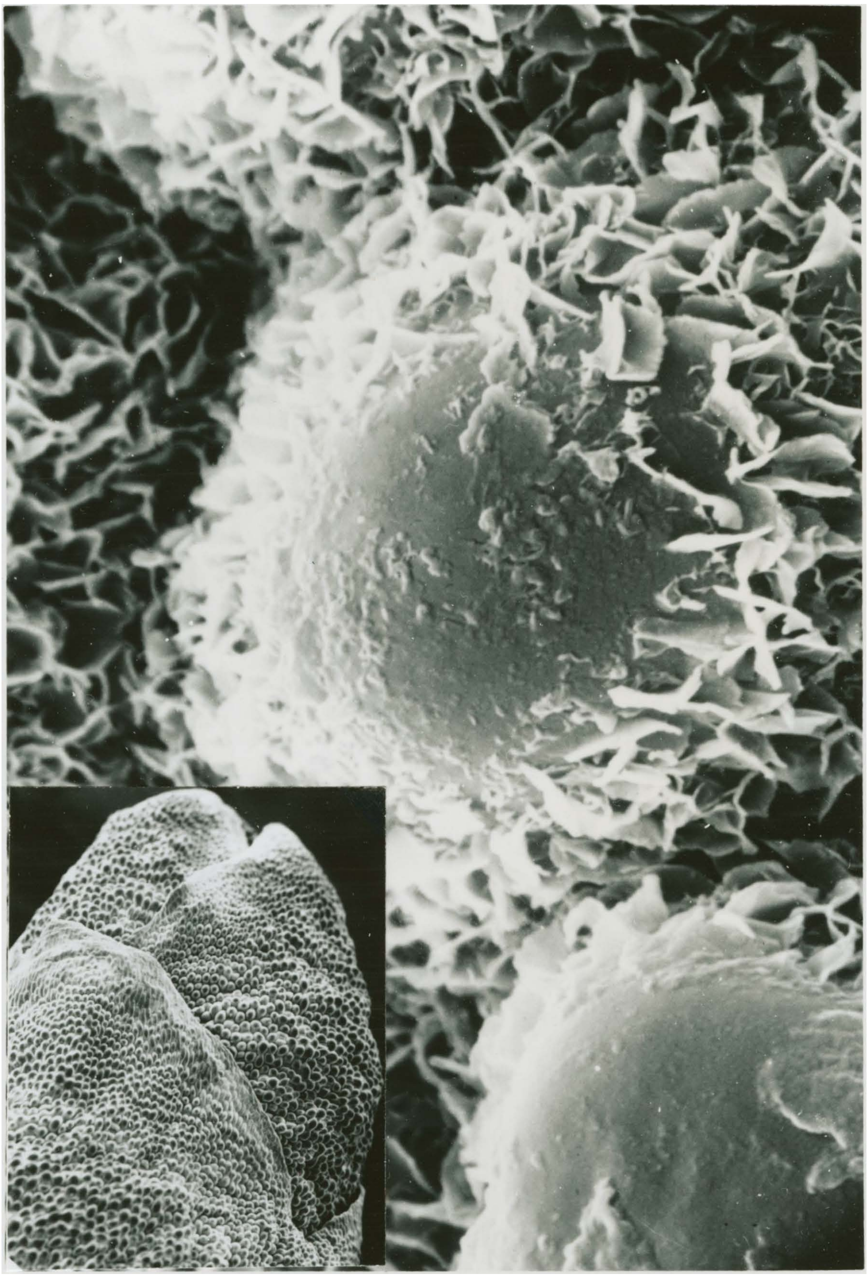

Fig. 25.--The surface of epidermal cells of Salicornia pacifica showing wax crystals (X 5,000). The insert shows a growing tip. Note the protruding epidermal cells (X 50). (Scanning electron micrograph courtesy of Jim Allen)

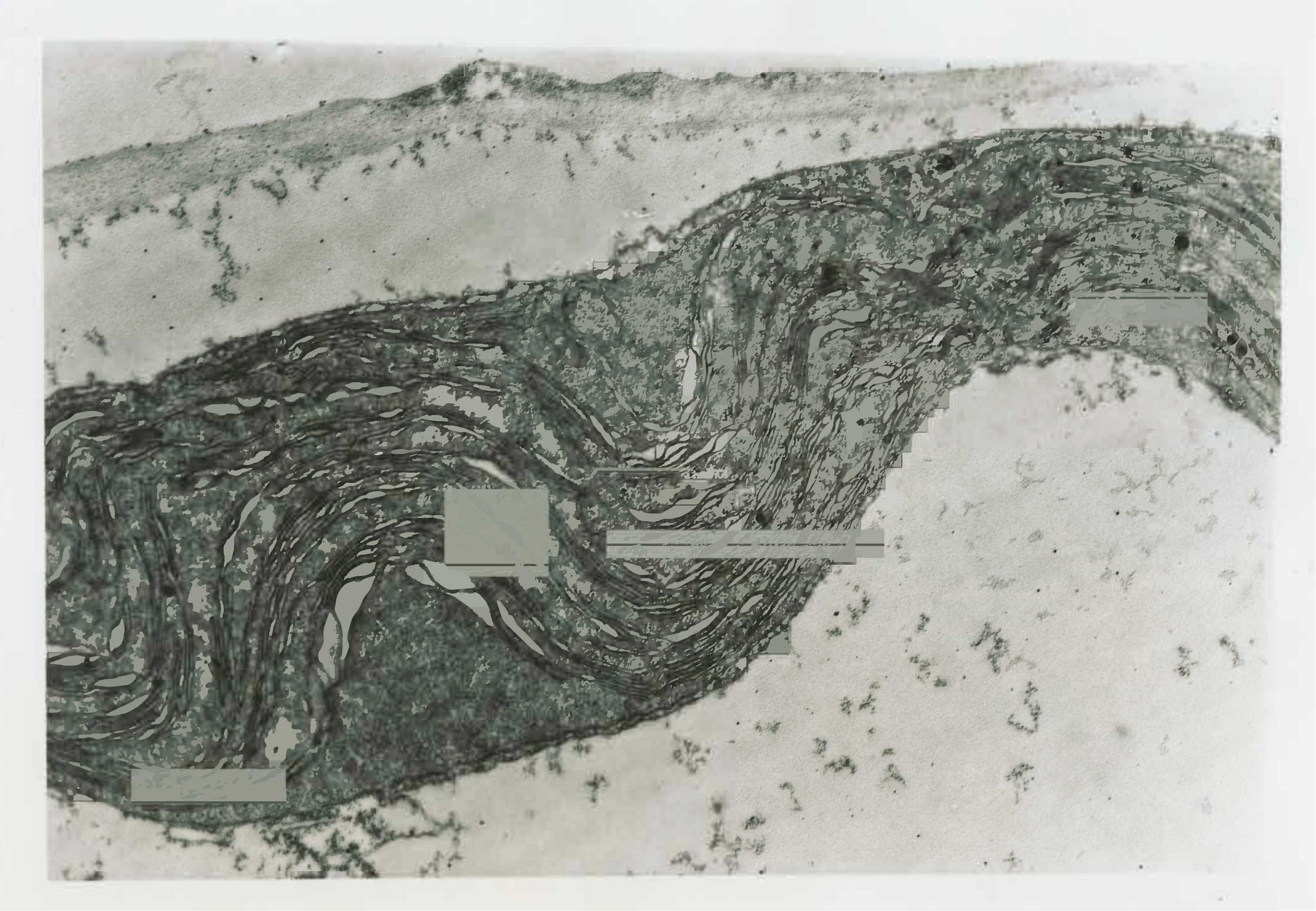

Fig. 26.--Electron micrograph showing an unusual arrangement of the chloroplasts in the chlorenchyma cells. Sections were treated with silver acetate to precipitate the chloride ions which appear as tiny black dots in the chloroplasts and adjacent areas (X 12,000). (Electron micrograph courtesy of W. M. Hess)

of small vesicles. Other cell organelles appear to be normal. Large vacuoles are often associated with the cells. Specific staining with silver acetate failed to reveal discrete localization of chloride and indicated the presence of a general dispersion of chloride throughout the entire cell (Fig. 27). Occasionally it was possible to detect some cells that apparently had more chloride than others but occurance of these cells appeared to be random. The sclereid-like cells contain large vacuoles and other functional organelles (Fig. 28). They appear to be living when mature and have a continuous plasmalemma. Fixation with silver acetate showed that chloride was distributed in these cells (Fig. 29).

Phenology and Morphology

Growth of S. pacifica usually begins with development of the subterranean or near-subterranean axillary buds of older shoots that start to grow in March and emerge in late April or early May. Flowering occurs in mid-June and generally completed by July.

By late August lower internodes begin to wither and die in a sequential manner from the base of the shoot to the tip (Fig. 30). Occasionally internodes may become injured or for other reasons may also wither and die. The central stele continues to function in a normal manner. Seed-producing internodes are the last to die (Fig. 31). It is not known whether the internodes die strictly by senescence or whether accumulation of additional salts into these areas causes death or whether it may be related to some other phenomenon. The independence of one internode from another provides a great protection to the plant from grazing insects or other preditors and appears to be an evolutionary advantage to the plant and the maturing seeds.

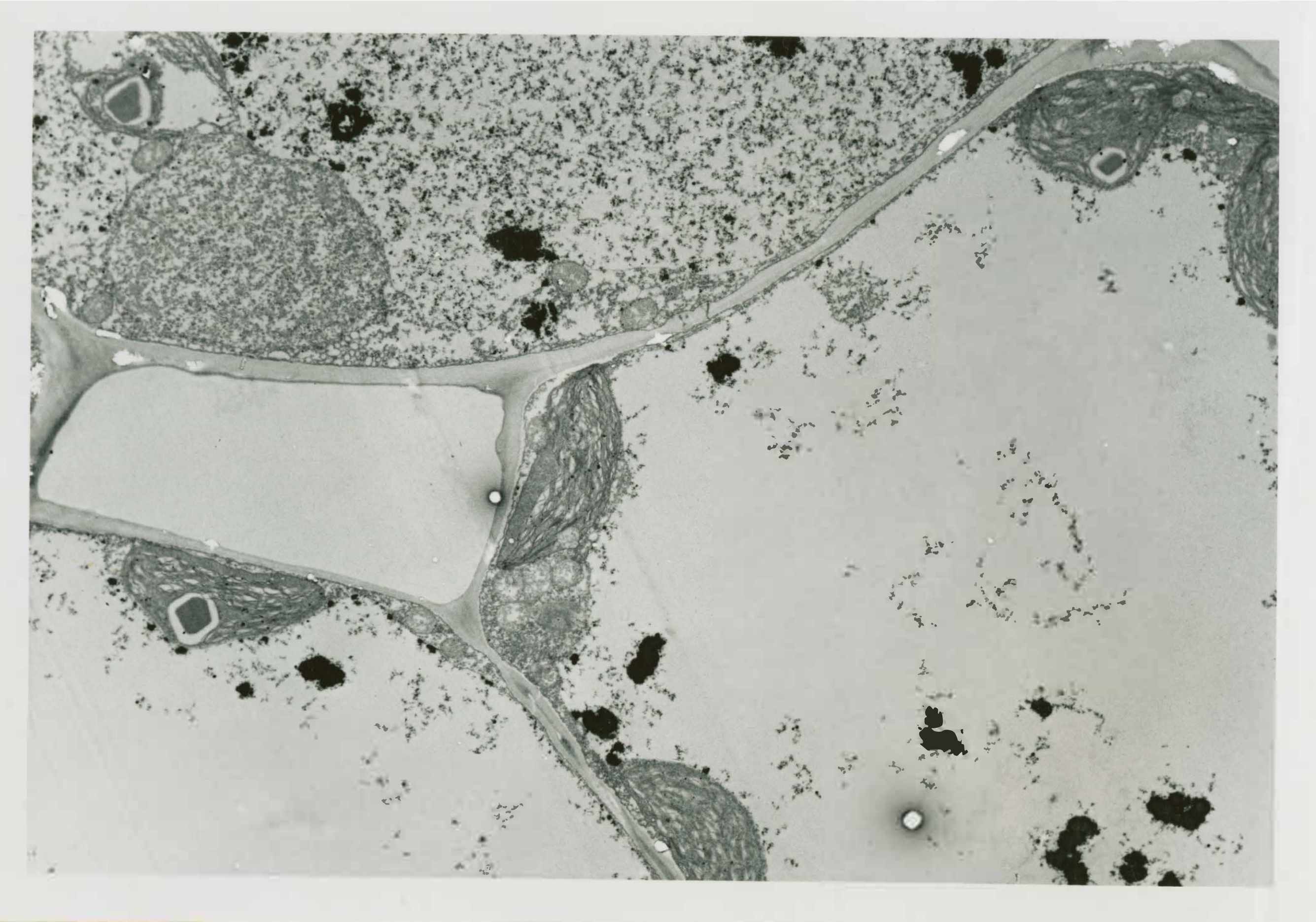

Fig. 27.--Electron micrograph of chlorenchyma cells. Sections were treated with silver acetate to precipitate the chloride ions which appear as tiny black dots or large aggregates throughout the entire cell (X 2,820). (Electron micrograph courtesy of W. M. Hess)

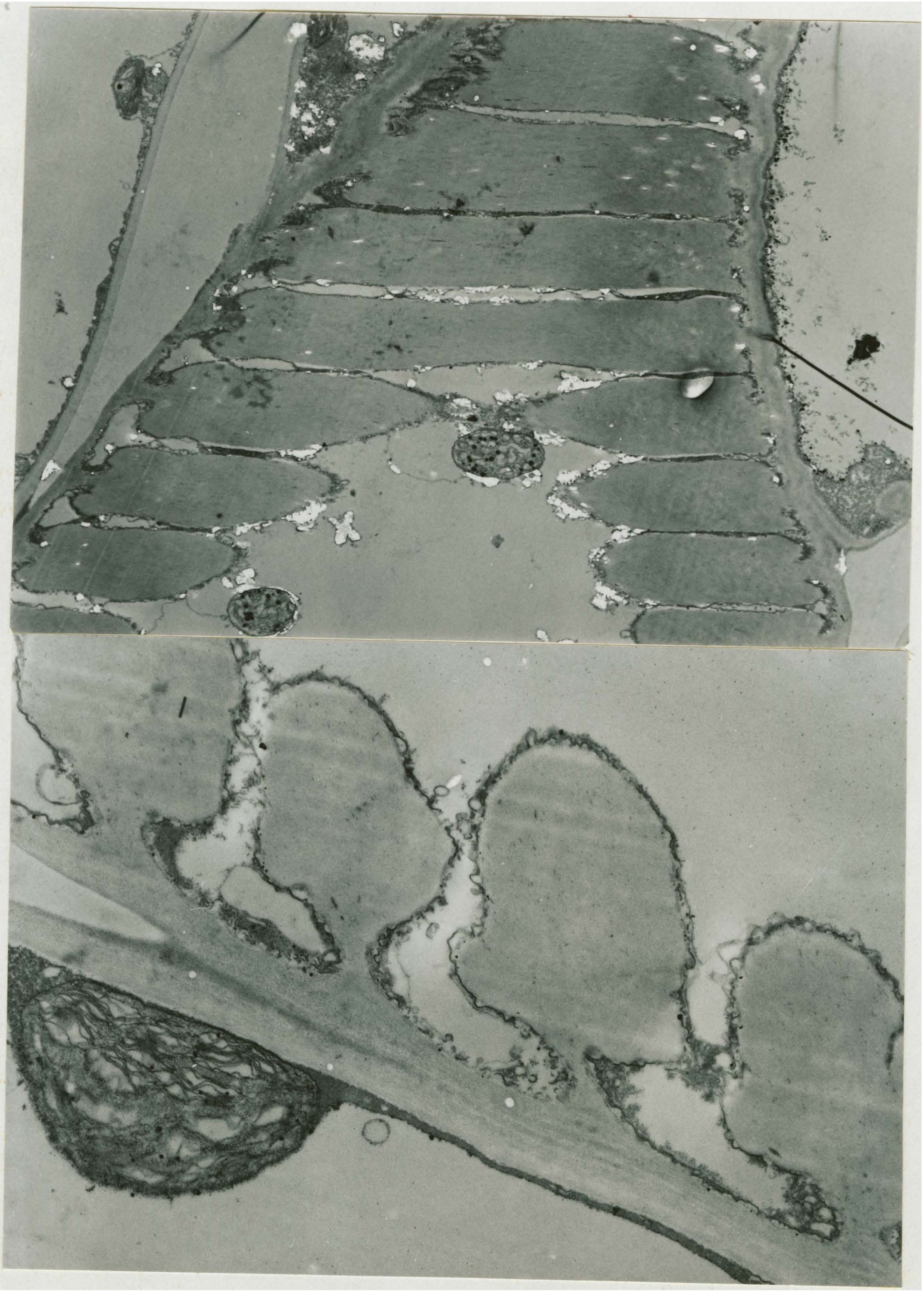

Fig. 28.--Electron micrograph (top) showing the sclereid-like cells with their secondary thickenings and cell organelles (X 2,820). The bottom electron micrograph shows the secondary thickenings of the sclereid-like cells and their attached plasmalemma (X 7,880). (Electron micrographs courtesy of W. M. Hess)

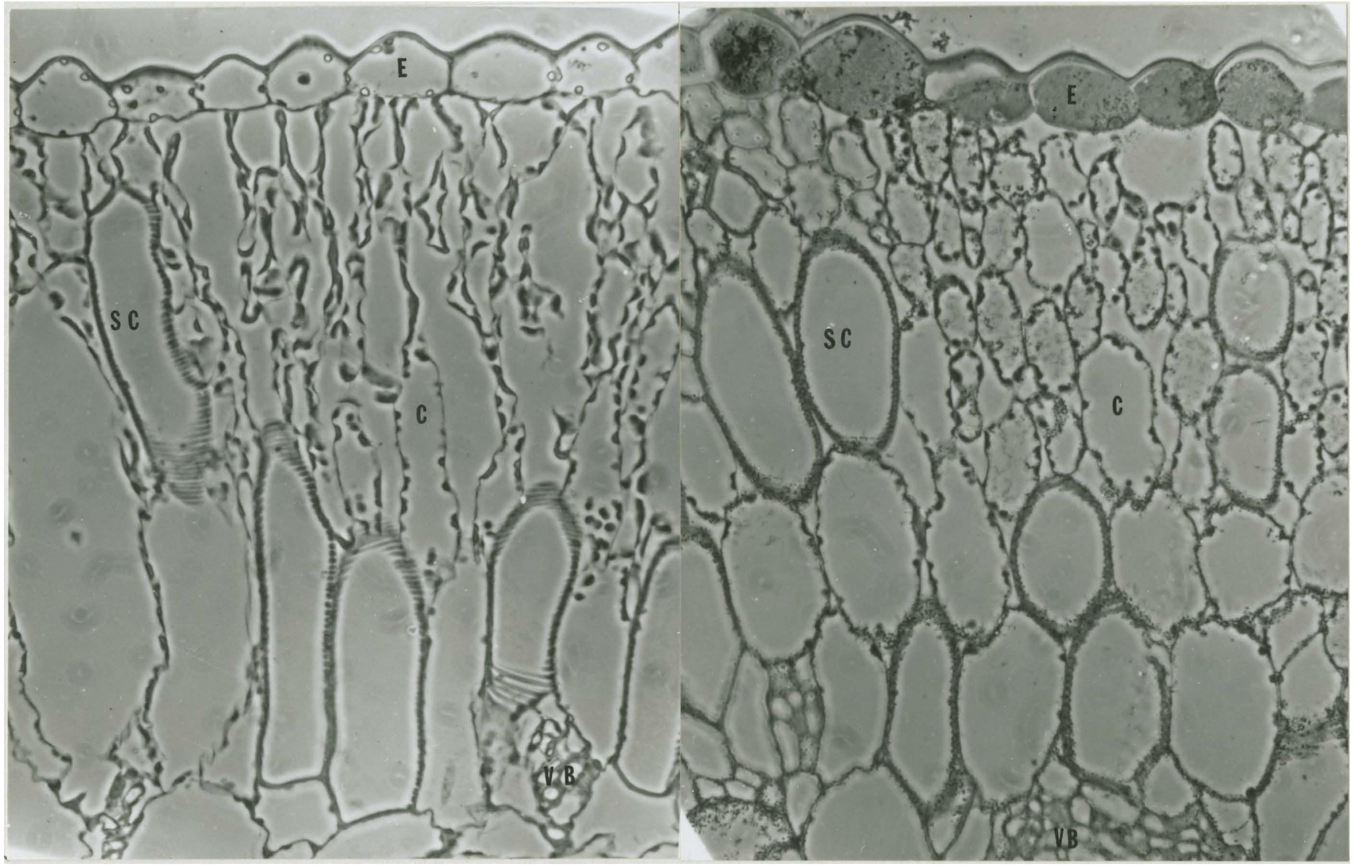

Fig. 29.--Light microscopy contrasting sections treated with (right) and without (left) silver acetate showing the outer "cortex" region with its vascular bundles (VB), sclereid-like cells (SC), chlorenchyma (C), and epidermis (E) (X 350). (Photographs courtesy of W. M. Hess)

Fig. 30.--*Salicornia pacifica* shoots showing a withered internode near the base of the previous years growth. Also note the seeds on the tips of the upper branches. (Photograph courtesy of W. M. Hess)

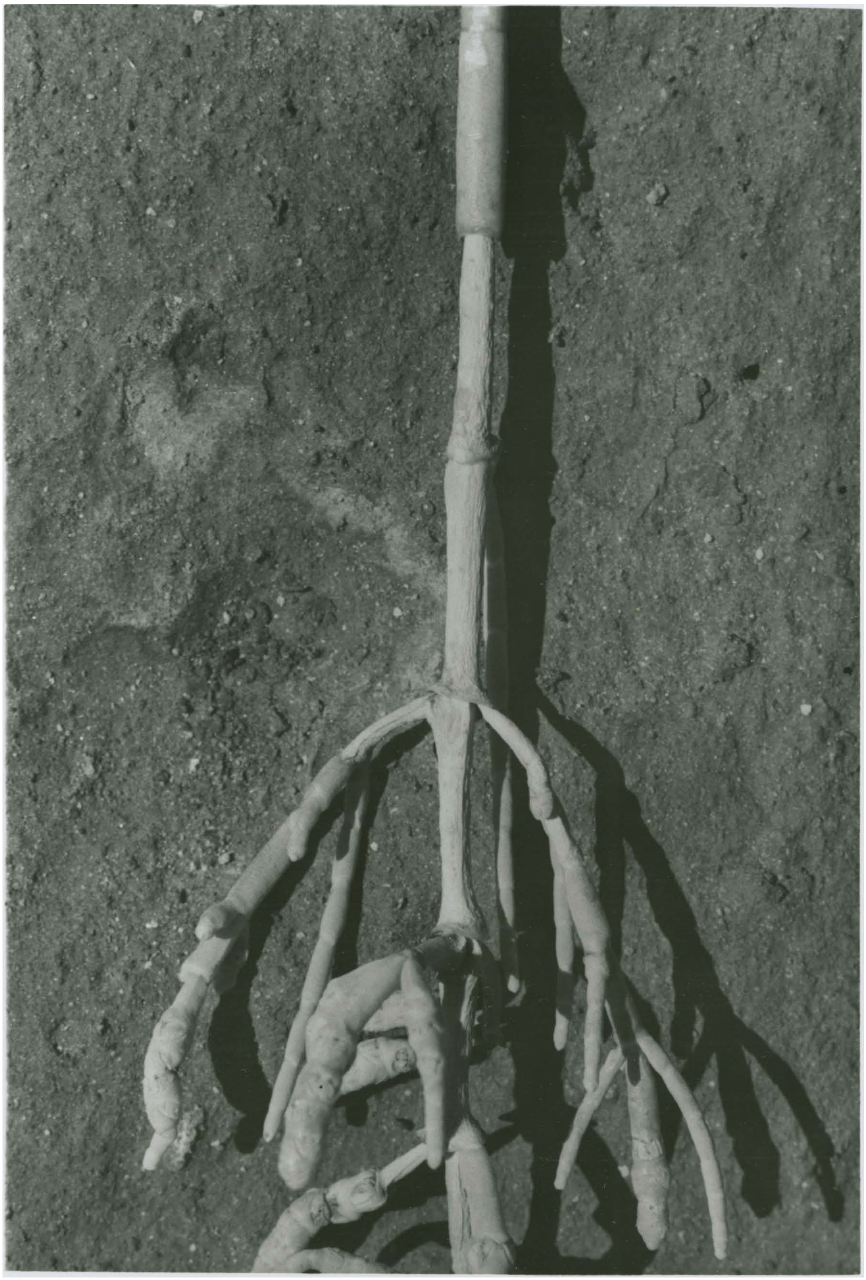

Fig. 31.--Salicornia pacifica shoot showing withered internodes to near the seed bearing branches. (Photograph courtesy of W. M. Hess)

The seeds are shed in October and November. A few seeds adhering to the mother plant until rain or heavy snows separate them. Each seed is protected by a bulky lightweight seed coat that aids in dispersal and adsorption of moisture.

DISCUSSION

The purpose of this investigation was to obtain information that will be useful in explaining the salt tolerant nature of S. pacifica. There appears to be an infinite number of interactions that are of importance in determining the successful adaptation of Salicornia to saline conditions. In this investigation it was only possible to study some factors concerning the salt tolerant nature of this plant.

Germination

Germination was optimal for S. pacifica seeds in a 10 atm solution of sodium chloride salt. The decrease in germination was proportional to the increase of the external osmotic potential. A similar inhibition of germination was also reported by Ayers and Hayward (1948), Ayers (1952), and Waisel (1972). Adaptation by natural selection appears to have produced a genotype that allows for germination in 10 atm of salt. Germination of other species of Salicornia in low salt or non-saline media was reported by Loetschert (1970) and Levitt (1972). Spring rains and precipitation rarely permit lengthly extensions of low salt conditions favorable for germination. Most seeds of S. pacifica begin germination when the concentrations of salts are low in the late winter or early spring. In the spring and summer of 1972 surface water was rarely more dilute than about 30 atm except around drainage ditches and springs. Due to the slow growing habit of S. pacifica one rarely finds a great preponderance of juvenile plants as compared to the annual species *S. rubra* which grows rapidly and sets seed rather quickly. *Salicornia rubra* is found in microniches on all three sites.

Evidently germination of *S. pacifica* seeds is light dependent. Waisel (1972) pointed out that germination of *S. herbacea* seeds was also positively correlated with illumination. The large bulky, lightweight seed coat may aid the seed in maintaining a surface or high light position for germination. The seeds are bouyant in the water. They are carried on the surface of water which is accumulated by winter precipitation and the seeds are deposited by wave action in the areas that are lower in salt than the immediate playa bottom. Dalby (1963) suggested a similar dissemination of seeds of *S. pusilla*. He pointed out that dispersal of *S. pusilla* occurs while the seeds are immersed in the fleshy cortex of stem segments that have been shed. These segments are able to float in seawater for periods of up to 3 months, at the end of which the seeds germinate inside the disseminule. Sea currents serve as a disseminating agent of this species, and the distribution of plants of this species is limited to, or near, the high tidal mark. Wind action may also be responsible for collecting seeds of *S. pacifica* near the surface.

Comparative germination investigations indicate that *S. pacifica* is less capable of germinating at high concentrations of salt than *S. bigleovii*, a costal species. The germination rate of *S. bigleovii* at 40 atm of sodium chloride solution was 32% as compared to 3% germination for *S. pacifica*. Rivers and Weber (1971) reported a reduction in the germination rate of *S. bigleovii* with increasing salt in the germination media but they reported that the maximum germination occurred at a sea salt concentration of 4.04% which is very close to the salinity of the sea. Salicornia bigleovii seeds have a very small amount of seed coat around them. The seeds often sink in the tide or are deposited in areas of high salt concentrations. Due to their higher salt tolerance in germinating under saline conditions, bouyancy is not as important for seed germination and seedling establishment as is the case with S. pacifica. This adds further evidence to support the idea that the bulky seed coat may have evolved to sustain the opportunist nature of S. pacifica seeds and provide the seed with a high light environment necessary for germination. The bulky seed coat would also aid the seed by absorbing moisture.

The leaching of the seed coat inhibitor, which appears to be sodium chloride, also removes the light requirement necessary for germination. The interaction between the seed coat inhibitor and the light requirement will require further investigation. The inhibitor may have evolved to enhance the survival of seedlings by insuring the presence of an adequate supply of moisture for germination which allows a very limited number of seeds to germinate once an aggregate grouping of seeds has occurred. This would also reduce competition between seedlings.

Most of the seeds are shed when the temperatures are too cold for adequate germination. By the time temperatures become favorable for germination, high concentrations of salts have been diluted by winter precipitation thus allowing germination to take place.

Seasonal Variations of the Environment

Temperatures were highest during the month of July while relative humidity was lowest during the months of July and August. These high temperatues would cause increased moisture stress. Levitt (1972) showed that the toxicity of salts increases with the temperature (0 to 33° C). This observation was also corroborated by Waisel (1972). Except for about six days during the growing season, relative humidity reached 100% every night which would tend to reduce transpiration of the plants. The high moisture content of the soil and the subsequent evaporation may have had a cooling effect upon the temperature of the soil, thereby reducing, in part, the toxicity of the salts. This evaporation would also increase the relative humidity. Both low temperatures and high relative humidity have favorable effects upon plant survival.

In all three sites there was an increase in moisture stress through the season primarily due to increases in salt from underground sources and slight decreases in soil moisture. However, soil moisture was generally high and remained constant except in Site 1. This high soil moisture of 25 to 35% is perhaps one of the most important factors responsible for maintaining *S. pacifica* in the environment. Sodium and chloride were the two principle ions responsible for increases in moisture stress of samples. These same two ions were responsible for 85 to 95% of the osmotic potential of *S. pacifica*. The plant appears to have evolved a specific need for these ions and may die if exposed to high concentrations of other ions (Levitt 1972, Waisel 1972). The salt concentration of the surface layers was considerably higher than the salt concentration in the rooting layers. Surface layers were often encrusted with deposits of white salt which gave the impression that the plants were surrounded by extremely high concentrations of salt when, in fact, the rooting zones or layers were only moderately saline. This observation was also reported by Waisel (1972). The wicking action caused by evaporation of water resulted in salt crystalization at the surface which removed salts from the rooting layers and provided a unique environmental niche for Salicornia to occupy. The thick cuticle and waxy layer of the plants and the active phellogen of the central stele appear to protect the upper shoots from the more severe environmental stresses near the surface.

The soil pH was relatively stable throughout the growing season in all three sites. The surface soil pH of each site was about one half of a pH unit higher than the subsurface pH. Decreases in the pH of the surface layer were proportional to increases in pH of the subsurface layer. Conversely, increases in the pH of the surface layer were proportional to decreases in the pH of the subsurface layer. This effect appears to have resulted from a transport of salts up or down in the soil profile with evaporation or leaching which subsequently affect the pH. Calcium ion concentrations in the surface layers were considerably higher in Sites 2 and 3 than in Site 1. Soil pH in Sites 2 and 3 was significantly lower than in Site 1. The pH may have been indirectly affected by the calcium concentration or may have been related to the algal covering of the soil surface in Site 1.

Ecological Interactions and Plant Distribution

The fact that Site 1 is an ecotone between S. pacifica and Distichlis stricta suggests that differences in this site and the other two sites would provide information regarding some parameters of S. pacifica. Distichlis stricta appears to tolerate a higher pH than S. pacifica and may survive in pH values from 8.0 to 8.5. The soil pH would affect the enzymatic reactions taking place at the surface of roots as well as the availability of nutrients. At pH values over 7 the availability of manganese, iron, copper, molybdenum and zinc declines

(Buckman and Brady 1969). At high pH values phosphorus forms complex insoluble calcium phosphates that are also unavailable. Levitt (1972) also pointed out that *Allenrolfea occidentalis* and *S. subterminalis* are highly resistant to salt but sensitive to alkalinity. A change from saline to sodic soils may kill these plants. Site 3, where *S. pacifica* was considered to produce the healthest plants, had an average pH of 7.7 and a moisture content of 32%. In Site 1 reduction in the soil moisture and the increase in the total concentration of salts of the subsurface soil layers during the season apparently also favored growth of *D. stricta* over *S. pacifica*. *Distichlis stricta* appears to be a better competitor for low soil moisture than *S. pacifica*. It seems probable that during the initial adaptation of *Salicornia* to the saline environment competition played a major role in determining plant distribution. As natural selection exerted its selective forces upon the plant through time, adaptations were reinforced that allowed the plant to maintain itself in an optimal salt concentration. Plasticity could have been reduced and that portion of the gene pool lost that allowed the plant to move back into non-saline areas. As such selective forces would be localized, it would be improper to generalize and say that all species of *Salicornia* have optimum growth at high salt concentrations. The variations in salt concentrations that yield optimum growth would bear this out (Halket 1915, Webb 1966, Waisel 1972).

Seasonal growth ring analysis of woody stems of *A. occidentalis* found scattered in Site 3 suggested that the average age of these plants was about 38.5 years old. The annual growth increment in the crown of this plant was estimated to be one growth ring per year. It is possible that the ages may be somewhat in error due to additional growth rings in a given year. *Allenrolfea occidentalis* is a halophyte which will not tolerate innundation or extremely high moisture content of the soil as well as _S. pacifica_. Since no stems of plants younger than about 35 years old could be found near this site it was concluded that the area around Site 3 had become more moist in the last 35 years. In Site 2 there was a gradual decrease in the average age of A. occidentalis stems from the middle of the drainage basin toward the edges (84 years old in the center to 61 years old on the edge). Few juvenile plants were present except at the edge of the basin. Again, this suggests that the soil was dryer in the past. The increase in soil moisture in both areas probably resulted from increases in surface and subsurface water due to irrigation water diversion and several new wells and springs as the area was more fully utilized by early settlers in the Goshen area (Cordova 1969).

The Nature of the Fleshy Stem

Anatomical investigations with light microscopy indicate that as internodes mature or become injured a phellogen which produces fibers and suberized cells is formed in the central stelar region. These cells seal off the conductive tissues from water and nutritive loss as the internodes wither and die. This development would protect the plant when the salt and moisture stresses are greatest near the surface in July and August. Osmotic potential measurements and measurements of uptake and distribution of $^{36}Cl^-$ indicate that the different internodes function independantly. This would allow the plant to survive in the environment if a few of the internodes were attacked and destroyed by insects.

Physiology of Salt Tolerance

Several possibilities exist that might explain the nature of salt tolerance in S. pacifica. Due to the existance of high salt in the succulent tissues of this plant the following possibilities were considered: 1) The salt is excluded from the salt sensitive sites by one or several of the following mechanism; a) by compartmentation within the cell by vacuoles or small vesicles, b) by limitation of uptake and transport of ions into the shoot by a mechanism located in the roots, c) by export of ions from shoots, leaves and roots, d) by increased succulence and dilution of high concentrations of salts. 2) The enzyme systems are tolerant to high salt solutions. This may be the results of a modification in the enzyme proper or by protection of enzymes by proteins such as albumins in the cytoplasm. 3) A compromise exists between the first two possibilities in which there which there is some degree of compartmentation or partial exclusion of salt from sensitive sites and modification of the enzyme systems to tolerate higher levels of electrolytes.

Several procedures were used in attempts to locate areas of high salt concentration in unique compartments within the cell or among tissues. Specific staining for chloride ions revealed a general distribution of chloride throughout the cell and showed no unique vesicles for compartmentation. Electron micrographs also showed that the chloride was distributed in each organelle and in the vacuole where the accumulation of salts is generally accepted. According to Waisel (1972), the cytoplasm of cells of halophytic species is rich in unbound free chloride ions. Waisel and Eshel (1971), using an X-ray microanalyzer, showed that the distribution of chloride and sodium did not coincide; while relatively more sodium was concentrated in the cytoplasm, chloride was more or less evenly distributed throughout the cell sections examined. In

S. pacifica little difference between chloride concentration among tissues could be recognized by specific staining techniques. Occasionally a large build-up of chlorides could be detected in some cells of the vascular tissues but these appeared to be randomly distributed and did not differ significantly in form or structure from other cells.

Osmotic potential measurements of the plants indicated that each internode is osmotically independent from other internodes. Hill (1908) showed that osmotic adaptation differs not only between species but also between organs and even between cells. In his investigation root hairs varied greatly in this respect, even in the same individual plant. Measurements of freezing point depression of S. pacifica internodes indicated that at least two areas of osmotically different tissues were present within one internode, corresponding to the inner "cortex" and outer chlorenchyma tissues. Osmometer measurements on dissected portions of these tissues indicated that the chlorenchyma tissue had a higher osmotic potential. Scholander et al. (1966) suggested that xylem sap of S. pacifica may contain very little salt as freezing point depression of S. pacifica sap was found to approach freezing point depression of pure water. Since chloride ions were equally distributed in these two tissues, the difference was probably due to sodium ions or sugars and other organic molecules that were produced by chloroplasts in the immediate vicinity. Waisel (1972) has shown that chloride ions account for 67 to 88% of the increase of osmotic potential of different species of salt marsh plants, whereas other osmotically active substances had only negligible effects. This increase in chloride was shown to account for increases in the osmotic potential of the entire plant. This does not rule out the possibility that differences in osmotic potentials of individual tissues might be due to other osmotically active substances. Increase in the chloride content of the succulent tissues appears to be the primary means of osmotic adaptation whereby the plant can survive under increased salt and moisture stress. Sodium and chloride ions account for 85 to 95% of the osmotic potential of *S. pacifica*. These results agree with the findings of Harward and McNulty (1965) and Scholander *et al*. (1966). Ion analyses of *S. pacifica* tissues in Site 1 indicated that increases in osmotic potential were due to increases in Na$^+$ but not Cl$^-$. Increases in osmotic potential in Site 2 were due to both Na$^+$ and Cl$^-$. However, in Site 3 there was a decrease in both Na$^+$ and Cl$^-$. As the concentration of soil salts increased, which caused an increase in moisture stress, osmotic potential of the plant also increased. This increase was due chiefly to sugars and other organic molecules.

Ion analysis of *S. pacifica* tissues showed that the chloride ion was present in higher concentrations than sodium or potassium ions. Azizbekova and Babaeva (1970) found that the amount of absorbed Na$^+$, Cl$^-$, and Mg^{++} in *Salicornia* increased with increasing salt concentrations in solution whereas the concentration of K$^+$ and Ca^{++} decreased. In their investigations, salinization with NaCl lowered the K$^+$ concentration in all parts of the plant, regardless of nutritional level. They found that sodium ions weakened absorption of K$^+$ and Ca^{++} and they showed that chloride ions in the plant were not accumulated in the roots, but moved quickly into the stem and leaves. They also demonstrated that K$^+$ distribution was similar in plant parts above and below the ground while sodium ions accumulated in the underground plant parts. It appears then that the differential absorption or uptake of ions and subsequent accumulation in various organs may be of importance for surviving in a saline environment.

The function of the sclereid-like cells with large vacuoles that are found in the chlorenchyma tissues appears to be primarily support in the tissue area rather than a means of removing salt from the tissues. If the turgor should temporarily be reduced by increased environmental stress these cells might also serve as a source of water as well as support. The living nature of the cells rules out the ideas held by some researchers who concluded that the cells were tracheids (Fahn 1967) or sclereids (Waisel 1972).

There was a definite increase in succulence due to the addition of NaCl in seedlings and plants grown in a salt-free medium. Light microscopy showed that the anatomy of plants grown without NaCl salt was similar to the anatomy of plants grown with NaCl. The only apparent difference was a decrease in both the number and size of cells found. The sclereid-like cells were present in both types of plants. The increase in succulence appears to increase the size of the vacuole. This would provide a greater storage volume for injurious electrolytes. At the same time it would reduce the volume where metabolic reactions would occur. This reduction in relative volume would allow the cell to more effectively utilize sugars and other organic molecules to maintain osmotic potential favorable to survival. The linking of the chloroplasts would also support this reduced volume concept.

There is a partial degree of compartmentation in Salicornia, because each internode of the plant is osmotically independent and because differences between the two tissues of a single internode exist. However, it appears unlikely that highly specific compartmentation is present within the cell.

According to Waisel (1972) no changes in respiration and photosynthesis were caused in Salicornia or Suaeda plants by addition of NaCl to the growth media in spite of induced growth stimulation. However, investigations indicated that isolated enzymes of halophytic species studied in vitro to date, are as sensitive to salt as enzymes of non-halophytic species (Flowers 1972, Greenway and Osmond 1972, Anderson et al. In preparation). Therefore, the nature of salt tolerance in Salicornia allowing enzymes to function normally in the presence of large quantities of injurious electrolytes appears to be centered around a protection of the enzymes by other proteins as was suggested by Waisel (1972). These protecting proteins could be proteins that bind to the salt ions. This idea appears to be reasonable since some halophiles (halophytic bacteria) require Na^+, Cl^-, and K^+ to keep enzymes in an activated state, presumably by binding to the enzyme proper to maintain an active conformation (Larsen 1967). The cell envelope of halophiles also requires salts to maintain the proper shape, to prevent leakage of material from the cells, and to prevent envelope disintegration. Proteins from halophiles characteristically have DNA with an extremely high guanine-cytosine content and the bulk of the protein of the cell envelopes is acidic. While crude protein analyses of S. pacifica tissues showed a gradual decline in the percentage of crude protein during the growing season no attempt was made to classify the types of proteins present nor their relative proportions. The decline in the percentage of crude protein was probably due to increases in the size of the vacuoles and elongation of the cell walls resulting in a lower percentage of crude protein for the overall tissue, but this does not necessarily mean that the percentage of crude protein in the cytoplasm declined. The percentage of crude protein in the cytoplasm may have remained constant or actually increased as moisture stresses increased due to salt accumulation in the environment. Whatever the case might be the percentage of crude protein remained stable during the periods of greatest moisture stress in July and August.

The quantity of protein required to bind specific salt cations and anions would be extremely large. From an evolutionary standpoint it would appear that this would not be the most logical means of evolving a protective mechanism nor the most efficient means of conserving energy. The osmotic potential, under these circumstances, would have to be maintained by other osmotically active substances other than salt ions. Freezing point depression measurements indicate that this is not the case. Fixation with silver acetate indicates a general dispersion of chloride ions throughout the cell. Due to the fact that silver acetate reacts only with chloride ions it appears that if such a binding protein exists the bonds must be extremely weak so as to be broken by a proximal silver ion. Interpretation of the present information and data suggests that the protection of sensitive enzymes is not accomplished by specific binding proteins.

Another possibility explaining how sensitive enzymes might be protected is that the protecting proteins could be nonspecific structural proteins found in the various membranes or free in the cytoplasm that function by protecting the active sites of sensitive enzymes from injurious electrolytes. By such a physical or chemical screening of cations and anions it would be possible to allow certain cations and anions to function as coenzymes or enzyme activators while at the same time protecting the sites from Na^+ and Cl^-.

Future investigations should be undertaken to determine the changes in the types of proteins present during the growing season and salt binding or protective capacities of these proteins for various salt ions.

CONCLUSIONS

The following conclusions have been reached regarding the salt tolerant nature of Salicornia pacifica and environmental fluctuations and parameters that are responsible for distribution of this plant in the Goshen, Utah area:

1. Highest temperatures and lowest relative humidities were recorded during the month of July.

2. A constant subsurface soil moisture level of 25 to 35% is necessary to sustain S. pacifica in the environment. Thirty to 35% soil moisture content, which is near field capacity, provided optimum growth for S. pacifica in the sites studied.

3. Seasonal growth ring analysis of woody stems of Allenrolfea occidentalis suggest that the study sites were considerably dryer a number of years ago and have become more favorable for establishment of S. pacifica as soil moisture increased.

4. Moisture stress for S. pacifica increased gradually from May through September due to accumulation of sodium and chloride ions in the subsurface layer.

5. Concentrations of salts in the surface layers were significantly higher than concentrations of salts in the subsurface layers around the roots in all three sites during the time periods studied.

6. A soil pH of about 8.0 ± 0.2 appears to be the upper limit for distribution of S. pacifica. Higher values favor establishment of

Distichlis stricta.

7. Optimum germination for S. pacifica seeds occurred in salt solutions of 10 atm. Therefore S. pacifica appears to be an ecological opportunist.

8. Of the salts studied, chloride salts were the least toxic to germinating seeds of S. pacifica while carbonates were the most toxic. Sulfate salts were intermediate.

9. Seeds of S. pacifica require a light stimulus for germination and appear to have evolved a bulky lightweight seed coat to help maintain a high light environment for germination.

10. Seeds of S. pacifica contain a seed coat inhibitor that must be leached out before germination can result. This inhibitor appears to be sodium chloride.

11. Ion content in S. pacifica from all three sites remained constant for concentrations of sodium, potassium, and chloride ions.

12. Eighty-five to 95% of the osmotic potential of S. pacifica results from concentrations of sodium and chloride ions.

13. Each internode appears to be osmotically independent from other internodes.

14. There are two areas or tissues of different osmotic potentials within each internode. These areas are the inner and the outer "cortex". The outer "cortex" generally had the highest osmotic potential.

15. The living nature of the sclereid-like cells in the outer "cortex" suggests that these cells are not tracheids or sclereids.

16. As the internode matures a phellogen is formed in the central stelar region which produces fibers and suberized cells that

seal off the conductive tissues from water and nutritive loss as the internode withers and dies.

17. Specific staining with silver acetate showed that chloride ions were distributed throughout the entire cell.

18. At the ultrastructural level apparent vesicles or areas of high salt concentrations were not present within the cells or tissues.

19. While there is a partial degree of compartmentation of salts within the plant it appears unlikely that highly specific compartmentation occurs within the cell.

20. The most likely inference about salt tolerance in *S. pacifica* from the results of this investigation appears to be that sensitive enzyme systems are protected from the harmful effects of salt by other proteins.

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ASPECTS OF SALT TOLERANCE OF SALICORNIA PACIFICA STANDL. VAR. UTAHENSIS (TIDESTROM) MUNZ.

Dennis Jay Hansen

Department of Botany and Range Science

M.S. Degree, April 1974

ABSTRACT

Aspects of salt tolerance of *Salicornia pacifica* were investigated. Survival of this plant in a saline environment is due to many interacting factors. A soil pH of 7.5 to 8.0 ± 0.2 and a constant subsurface soil moisture level of 25 to 35% are necessary for plant survival. A fluctuating lower soil moisture level and higher pH favor establishment of *Distichlis stricta*. The ion content in *S. pacifica* remained constant despite increased moisture stress throughout the growing season due to accumulation of salts which were significantly higher in the surface soil layers than in the subsurface layers around the roots. High salt concentrations in the soil are diluted by winter precipitation which allows for germination of seeds of *S. pacifica*. The seeds contain a seed coat inhibitor which appears to be sodium chloride. The seeds require a light stimuli for germination. Each internode of the plant was found to be osmotically independent. Chloride ions were distributed in each tissue, cell, and organelle. The most likely inference about salt tolerance in *S. pacifica* is that sensitive enzyme systems are protected from the harmful effects of salt by other proteins of the cell.