

# **Soil and Plant Interactions with Salinity**

**Kearney Foundation  
Five-Year Report  
1980–1985**

**Agricultural Experiment Station  
University of California  
Division of Agriculture and Natural Resources  
Special Publication 3315**





# **Soil and Plant Interactions with Salinity**

## **Kearney Foundation Five-Year Report 1980–1985**

Coordinating Editor: John Letey  
Kearney Foundation Director  
1980–1985

Agricultural Experiment Station  
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# Preface:

## The Kearney Foundation of Soil Science

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The establishment of the M. Theo Kearney Foundation of Soil Science by the University of California Regents in 1951 was made possible by a bequest by M. Theo Kearney, a successful farmer in the Central Valley. The bequest exceeded \$2 million, and UC President Robert Gordon Sproul felt that it would be appropriate to use it to study soil science, in view of the fact that Mr. Kearney had expressed concern about soil problems in the great interior valleys of California. He had observed on his ranches hundreds of acres of once-highly productive land deteriorate and become barren under irrigation and the consequent accumulation of alkali salts. President Sproul saw that the solution to these problems lay in the field of soil science.

The Kearney bequest was set up as an endowment, with the annual income from it dedicated to accomplishing these goals: (1) to study the physical, chemical, biological, and hydrological relations of soil and water to plants, particularly in arid and semiarid environments, and (2) to strengthen the University's education of soil scientists and prepare teachers of soil science and related subjects to assist farmers of California in selecting crops that will yield a fair return to producers and maximum satisfaction to consumers.

Initially, the foundation was located at UC Berkeley with a specifically identified director, physical facilities, equipment, and staff. It was moved from Berkeley to UC Davis in 1963. In 1970, the operation and administration of the foundation were changed to permit it to conduct research on definitive missions of public concern in the fields of soils, plant nutrition, and water science. Useful contributions were considered likely to be made in five-year increments. Missions were to be selected each five years with a new director for each. The director is responsible to the Vice President, Agricultural and Natural Resources, through the director of the Agricultural Experiment Station and the associate director of the Agricultural Experiment Station located on the campus where the foundation director is located. Within this administrative framework and directed mission goals, the Foundation has been expanded to encompass the entire Agricultural Experiment Station and Cooperative Extension.

The Kearney Foundation today is a highly coordinated and effective research and extension program in soil science. The knowledge of soil management this program creates is extremely valuable to the state of California, to the scientists of the University of California, and to soil scientists throughout the world. In addition, the Kearney Foundation has become a model for effective research and extension activities on a systemwide basis in UC's Division of Agriculture and Natural Resources.

LOWELL N. LEWIS  
Director  
Agricultural Experiment Station

# Introduction:

## The 1980–1985 Mission

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A very broad mission, "Soil and Crop Plant Response to Water and Fertilization under High Salinity and Limited Water Supply," was originally selected as the 1980–1985 mission for the Kearney Foundation of Soil Science. The foundation's technical and advisory committees narrowed the focus to "Soil and Plant Interactions with Salinity."

This mission has historical significance for California. Professor E. W. Hilgard's 1877 report to the President of the University of California is credited as the first publication dealing with saline soils in the U.S. Mr. Kearney provided land for some of the field experiments. Hilgard and colleagues attributed the development of soil salinity to excess irrigation water which led to a rise in the water table and/or seepage at low-lying areas. Evaporation, which tended to concentrate salts at the surface, was also implicated. Subsurface drainage and subsequent leaching were considered essential to solving the problem. Indeed, Hilgard in 1889 stated, "The time is not far distant when in California the laying of underdrains will be considered an excellent investment on any land as valuable as all irrigated land is likely to be; and when that day comes, alkali will be at an end on irrigated lands in this state." Hilgard properly identified irrigated land in California as valuable and that installation of drainage systems would be necessary on much of this land. What Hilgard failed to comment on in his writing is that drainage systems would require safe disposition of the drainage water, a factor which has prevented the fulfillment of his prophecy.

In providing an endowment to the University of California, Mr. Kearney showed gratitude for the research conducted by Dr. Hilgard and other UC scientists to preserve productivity on agricultural lands he owned. He also recognized that not all problems had been solved. His bequest was to be used to study these and other soils-related problems. The 1980–1985 mission addressed problems close to Mr. Kearney's heart.

### Contributions of the 1980–1985 Mission

Twenty-four research projects directed by scientists at UC Berkeley, Davis, and Riverside and UC Cooperative Extension were funded. Two of these projects were cooperative projects with other agencies. This publication summarizes the research, providing general descriptions of the problem investigated, procedures followed, results, and significance of the research. Detailed descriptions and results have been or will be published in technical journals and these reports are referenced for those who desire more specific information.

## Information Dissemination

The Kearney Foundation has sponsored a number of activities, besides this report, to foster information dissemination pertinent to the mission. One such activity was the support of an International Conference on "Soil Salinity under Irrigation--Processes and Management." Organized by the Israel Society of Soil Science and sponsored by the Subcommittee on Salt-Affected Soils of the International Society of Soil Science, the conference took place in March 1984 in Bet-Dagan, Israel. The reports of the invited lecturers to that conference, who summarized the state of the art, were published in 1984 in a book, Soil Salinity under Irrigation--Processes and Management, edited by I. Shainberg and J. Shalhevet for Ecological Studies 51, and published by Springer-Verlag, Heidelberg (349 pp.).

A review of the interaction between soils and sodic and saline conditions was published in 1984 in the University of California journal, Hilgardia. (That review can be found in: I. Shainberg and J. Letey, "Response of Soils to Sodic and Saline Conditions," Hilgardia 52:1-57).

A book, Salinity and Crops--Principles and Management, written by J. Shalhevet, A. Lauchli, and J. Letey under foundation sponsorship, will be published by Springer-Verlag.

Finally, a report is being prepared which specifically addresses the present salinity-drainage problems on the west side of California's San Joaquin Valley. This report, Agricultural Drainage in California's Central Valley--A History and Analysis of Alternatives, will be published by the University of California in a format similar to that of this publication.

JOHN LETEY  
Kearney Foundation Director  
1980-1985

# I. Physiological Response to Salt Stress

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## 1. Membrane Organization and Function in Seed Germination and Salinity

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**PRINCIPAL INVESTIGATOR:** W. W. Thomson

These studies on membrane organization and function, particularly in regard to the plasmalemma of seed cells and salinity, were based on the following premises: (1) The primary determinant of cell permeability is the bounding membrane of the cell, the plasmalemma. (2) The organization of the constituents of this membrane, primarily the phospholipids, determine the degree of permeability, particularly in regard to the entrance of water and, for example, exogenously applied solutes. (3) Most evidence and current hypotheses indicate that insensitivity to salts is achieved by the isolation of toxic species into compartments, primarily the wall and the vacuole, delimited, respectively, by the plasmalemma and the tonoplast. Thus, the initial objectives of these studies were to assess the organization of the plasmalemma in relation to porosity and the effects of salinity.

We chose to study seed germination because: (1) Several reports indicate that for many plants, seed germination is the most sensitive stage in the life cycle of the plant. (2) Imbibition and seed germination have been well characterized in the literature as to structural and functional changes. (3) Since seeds are basically dormant, the events of germination are initiated at a known time, that is, with imbibition. Thus, seed germination offers an excellent experimental system to investigate membrane changes with time, development, and as affected concomitantly by salt.

One characteristic of many seeds is that they contain low amounts of free water, 5 to 10 percent, and when placed in water to initiate germination, rapid uptake of water into the tissue occurs. Concomitant with water uptake, there is a large and rapid leakage of solutes, ions, amino acids, sugars, peptides, and organic acids into the medium. When we started these studies in 1980, a major hypothesis in membrane research was that when the water content of cells falls below 20 to 30 percent, the membranes are disordered from their normal, bilayered structure into a micellar or hexagonal organization. Thus, when dry seeds are placed in water, leakage of solutes can be expected until the bilayer organization of the membrane is established. that is, when the water content rises above approximately 30 percent during imbibition. A logical extension of the above is that, if leakage occurs because the membranes are disorganized, the plasmalemma will not be an effective permeability barrier to salts during imbibition.

## EXPERIMENTAL PROCEDURES AND RESULTS

To examine the question of whether the plasmalemma in dry seeds is bilayered in organization and thus presumably semipermeable, we used freeze-fracture electron microscopy. The material of choice was seeds of cowpea (*Vigna unguiculata* L.), since they appear to be quite sensitive to salt. We examined the radicle, embryo, and primary leaves. In these studies, and with both dry and hydrated seeds, we observed large, planar, internal fracture faces of the membrane. Such fracture faces are indicative of a normal lipid bilayer organization. Other forms of lipid organization, such as micellar or hexagonal, have different patterns of organization and are readily recognized in freezer-fracture replicas.

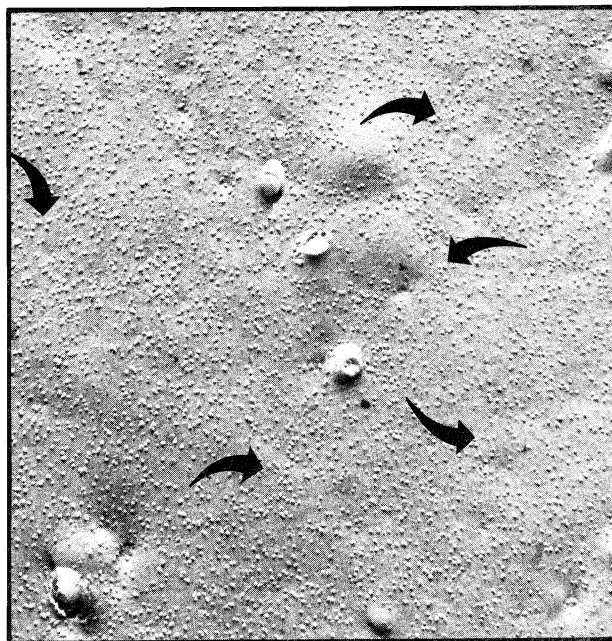
Further, lanthanum, as an ion, is not able to permeate across the plasmalemma in hydrated tissue, presumably because of the bilayer organization of the membranes. Thus, if the membrane is disorganized, lanthanum should be free to enter the cells. When we imbibed seeds in the presence of lanthanum and then examined the tissue with the transmission electron microscope, we found that the lanthanum was totally restricted to the walls and did not cross the plasmalemma. Ionic lanthanum has a hydrated radius of  $9.04 \times 10^{-10}$  m. This suggests again that the plasmalemma is a bilayer and, if it is porous, the pore openings must be less than this diameter. However, the hydrated radius of both  $\text{Na}^+$  and  $\text{Cl}^-$ , the major ions of interest in these salinity studies, is smaller than that of lanthanum and could possibly penetrate the membrane through smaller pores. Indeed, we found that  $\text{Cl}^-$  could penetrate the plasmalemma. After seeds were imbibed for 4 hours in a NaCl solution, we observed accumulations of chloride within the cells using silver to precipitate the chloride and examined the tissue with the electron microscope for the deposition of silver chloride.

In a subsequent study on the radicles of cowpeas, we examined the changes in plasmalemma organization with imbibition in water and in a NaCl solution. In these studies, we concentrated on freeze-fracture electron microscopy. Because we observed no basic difference or perturbations in the smooth areas of the membrane faces and since these areas presumably represent the lipid-enriched region, we concentrated our analysis on the integral membrane proteins (IMPs). These are represented in the replicas of internal membrane faces by "bumps" (fig. 1). In both salt- and water-imbibed tissue, the number of IMPs per unit area declined considerably as compared to that of the dry radicle, but less so in the salt-imbibed material. Calculations for membrane expansion that occurs with hydration indicated that such expansion could account for the decrease in the density of IMPs for the inner, but not the outer, half of the membrane. In contrast, membrane expansion could not account for the change in particle density in the salt-imbibed tissue, since there was no significant difference in membrane expansion in salt- or water-imbibed tissue. Thus, salt appears to affect the organization of the plasmalemma during imbibition, particularly in regard to protein density within the membrane. This may be because salt affects the hydrophobic-hydrophilic properties of the membrane proteins and/or the chemical activity of water at the



surfaces of the membrane. Such changes could alter the depth of insertion of proteins in the membrane, bringing more into the center where they would be visualized in freeze-fracture replicas. Also, the altered density of proteins in the membranes of salt-imbibed, as opposed to water-imbibed tissues, suggest the possibility of altered membrane function, but these are unlikely to be related to permeability per se, since this characteristic is probably more directly related to the organization of the membrane lipids.

Fig. 1. A freeze-fracture replica of a portion of the internal face of the outer half of the plasmalemma of a cell from the radicle of cowpea after imbibition in 0.2 M NaCl for 20 hr. The intramembranous proteins, IMPs, are identified by arrows (X 100,000).



Although there is some evidence that suggests that salt inhibition of germination is a toxic effect, most evidence indicates that the inhibition is due to osmotic effects. An osmotic effect would implicate the semipermeability properties and, therefore, the organization of the lipid bilayer of the plasmalemma. More recently, we have investigated the role of membranes in regard to osmotic sensitivity and salt tolerance with salt-sensitive and salt-tolerant cultivars of barley. The examination of different strains of the same species that differ in sensitivity to salt could provide primary clues as to the mechanism of salt tolerance.

The results of these studies indicate the effects of salt on the earliest stage of seed germination, that is, during imbibition, is osmotic rather than toxic. For example, we found no significant differences in the inhibitory effects on germination between solutions of the nontoxic compounds, betaine and mannitol, and that of NaCl when applied at equal osmotic potential. Also, the difference in salt sensitivity between the salt-tolerant and salt-sensitive varieties was reflected in a corresponding difference in sensitivity to betaine and mannitol, indicating that they differ in osmotic sensitivity rather than having a different toxic response to salt. This latter point is further emphasized by the observations that there was no difference between the two varieties in the absorption of water from a salt solution.

The osmotic effect of a solute, such as salt, would have the effect of reducing the hydration level of the seed, and when seeds are placed in a subinhibitory solute concentration, germination is delayed as compared to water-imbibed seeds. Our observations are consistent with the suggestion that a threshold level of hydration is required for the seed to commence germination.

However, reports that salt has a toxic effect on germination cannot be totally ignored. In a series of experiments, we preimbibed barley seeds, presumably past the hydration threshold, and then exposed them to salt; as expected, salt inhibited further germination. However, germination was better when seeds were preimbibed and then challenged with betaine solutions at concentrations that were iso-osmotic to that of the salt solutions. This suggests that following the attainment of the hydration threshold, salt has a toxic effect on germination, considering the fact that betaine is a relatively nontoxic solute.

Of particular interest was the observation that high concentrations of salts do not damage seeds if the salt challenge is applied before the hydration threshold is reached. When seeds were imbibed in high concentrations of salt or betaine for several hours, then placed in water, germination was virtually identical to the controls in water only. However, salt rapidly killed seeds if applied after the hydration threshold had been reached. Further, a number of reports have indicated that calcium ameliorated the inhibitory effects of salt on seed germination, but we found no such effect of calcium with seeds imbibed in the presence of salt or betaine. However, calcium alleviated the toxic effects of salt on germination on preimbibed, hydrated seeds. Therefore, the effects of calcium appear to be limited to the posthydration threshold stage of development.

As pointed out earlier, there are good reasons to suspect that the site of the toxic effect of NaCl is the cell membranes and particularly the plasmalemma. Similarly, the protective effect of calcium could well be at the membrane also. It is known that proteins that bind calcium have dissociation constants in the micromolar range, while membranes have calcium dissociation constants in the millimolar range. We found the effects of calcium in alleviating the toxic properties of NaCl to occur at a concentration of 5 mM. No calcium effect was observed with concentrations at the micromolar level.

### **SIGNIFICANCE**

During the past 5 years and with support of the Kearney Foundation, we have made the following advances in understanding the nature of membranes in seeds and the effects of salinity on membranes during seed germination:

- (1) Structurally, membranes in dry seeds have a bilayer organization similar to that of membranes in hydrated seeds.

- (2) Osmotic experiments show that membranes in imbibing seeds show properties of semipermeability. Thus, functionally, the plasmalemma must have bilayer organization.
- (3) The plasmalemma is permeate to chloride with imbibition of several hours; however, the larger hydrated ion, lanthanum, is impermeate, indicating that if the plasmalemma is porous, pore size is restricted.
- (4) With imbibition, salt affects the protein components of the plasmalemma by increasing their density per unit area as compared to the control in water.
- (5) The effect of salt on decreasing germination, when applied at the onset of imbibition, is an osmotic response.
- (6) Seeds must reach a critical level of hydration before they become committed to germination.
- (7) Salt has a toxic effect on germination if applied after the critical level of hydration has been reached.
- (8) Calcium ameliorates this toxic effect of salt, probably through an interaction with the plasmalemma.

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#### **SUPPORTING PERSONNEL**

R. D. Bliss (postgraduate research botanist), K. Platt-Aloia (SRA)

## 2. Compartmentation of Osmotic Solutes in Plants Exposed to Saline Stress

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**PRINCIPAL INVESTIGATORS:** Lester Packer, Rolf J. Mehlhorn, and Ian V. Fry

Current irrigation techniques are leading to a progressive increase in topsoil and groundwater salinity. If such trends continue, we are faced with the prospect of diminishing conventional crop yields, unless crop selection can be modified. One means of coping with increased soil salinity is the utilization of salt-resistant crop plants. However, the choice of available plants is severely limited, and often, growth of the plant in highly saline environments renders the plant unpalatable for human consumption. These disadvantages of halotolerant plants may, perhaps, be overcome if new varieties can be identified. Alternatively, basic research on salt-tolerance mechanisms may help to devise strategies for plant selection, growth regimes, or genetic manipulations that may be successful in obtaining useful crop plants that can be grown at high salinities.

We have used two species of halotolerant higher plants and one species of halotolerant cyanobacterium to investigate how increases in salinity affect the biochemical pathways involved in the salt-tolerance phenomenon.

Beta vulgaris and Avicennia germinans, both halotolerant higher plants which also grow at low salinity, were used as source material for the isolation of the subcellular compartment responsible for photosynthesis, the thylakoid. The isolated thylakoids were subjected to various hyperosmotic regimes using NaCl, KCl, and nonpermanent solutes, to determine the permeability properties of the thylakoid membrane.

Cyanobacteria (blue-green algae), microorganisms which share many features of photosynthesis with higher plants, which exhibit a broad salinity tolerance in their natural environments, and which are an important component of the soil ecosystem, were cultured under various saline regimes, and their biochemical processes were monitored to elucidate mechanisms that enable them to tolerate salinity.

### EXPERIMENTAL PROCEDURES

Beta vulgaris, Avicennia germinans, and Synechococcus 6311 were cultivated under high and low salinities (NaCl). Beta vulgaris and Avicennia germinans were used as source material for the photosynthetic subcellular organelle, the thylakoid. Synechococcus 6311, a unicellular prokaryote, was used without further treatment.

In these studies, we have found the application of new electron spin resonance (ESR) techniques for rapidly and accurately monitoring intracellular and intraorganelle aqueous volumes to be particularly useful. This method has enormously facilitated the characterization of the osmoregulatory changes that occur during the adaptation to salt and in the subsequent dynamic changes in membrane permeability. In conjunction with this technique, we have characterized electron paramagnetic resonance (EPR) signals arising from the terminal oxidase in the cyanobacteria, an enzyme that plays a major role during the adaptation to salt.

Using standard biochemical analysis in conjunction with the novel techniques mentioned above, we have been able to elucidate the temporal sequence of events leading to salt adaptation in the cyanobacterium Synechococcus 6311.

## RESULTS

Isolated thylakoids acted as perfect osmometers when subjected to hypersaline shock. From the rates of recovery of the internal volume, and assumptions made of the surface area, a net flux of NaCl across the thylakoid membrane of  $2.3 \times 10^{-13}$  mole  $\text{cm}^{-2}$   $\text{s}^{-1}$  was estimated, indicating a finite, but low, passive permeability to NaCl, irrespective of the salinity of the growth conditions.

Using the cyanobacteria it was found that the initial entry of salt into the cells is in the time range of seconds, resulting in a marked inhibition of photosynthesis, which may be due to detachment of the light harvesting phycobilisomes, and growth. During the initial phase of salt stress, endogenous respiration increases tenfold (fig. 1) which is a result of the de novo synthesis of the cytochrome oxidase as determined by an increase in membrane  $\text{Cu}^{2+}$ . It is the marked increase in respiratory activity which is proposed to be the force, in the form of the trans-cytoplasmic membrane pH gradient, which drives the extrusion of salt via a  $\text{H}^+/\text{Na}^+$  antiporter. Depletion of intracellular salt concentrations relieves the inhibition on cellular processes, and photosynthesis returns to control values. At this time, the osmotic imbalance due to the extrusion of salt is overcome by the accumulation of inorganic and organic solutes and there is a marked increase in the storage carbohydrate glycogen. The cell is now competent to exclude salt, and to maintain a suitably high internal osmoticum, it is adapted to the saline environment which is reflected in the return to near-normal growth rates (see flow diagram, fig. 2).

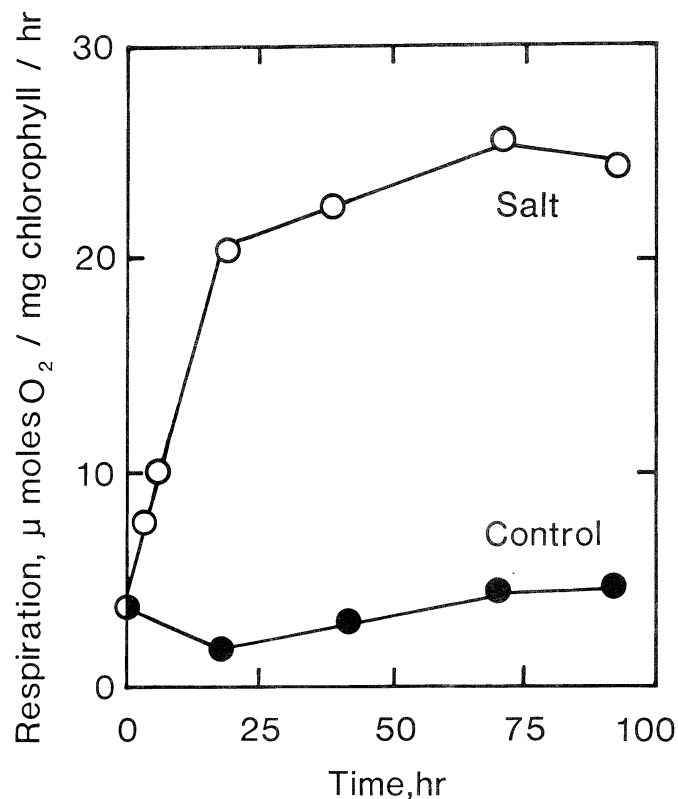


Fig. 1. Effect of salt (0.5 M NaCl) on the endogenous rate of respiration in Synechococcus 6311.

Temporal sequence of events leading to salt adaptation of the freshwater cyanobacterium Synechococcus 6311

- I. sec. Rapid entry of NaCl into the cell  
Inhibition of photosynthesis  
(Salt stress)  
↓
- II. 4-10 hrs. De novo synthesis of cytochrome c oxidase  
Decrease in intracellular Na<sup>+</sup>  
Increase in intracellular K<sup>+</sup>  
Accumulation of glycogen  
(Water stress)  
↓
- III. 10-20 hrs. Recovery of photosynthesis  
Increase in organic osmoticum, sucrose, glucose  
↓
- IV. 25-50 hrs. Return to control growth rates  
(Adaptation)

Fig. 2. Temporal sequence of events leading to salt adaptation of the freshwater cyanobacterium Synechococcus 6311.

## SIGNIFICANCE

The observation of parallel responses of salt-tolerant cyanobacteria and higher plant thylakoids to salt, that is, a significant membrane permeability to sodium chloride, suggests that a capacity of membranes to conduct ions may be fundamental to the salt-tolerance phenomenon. In the cyanobacteria, a high membrane permeability to NaCl could readily provide a means for the cell to maintain turgor when presented with sudden hypersaline shock, without undue demands upon the cell's energy reserves. It is conceivable that higher plants also may experience sudden changes in the ionic composition of root, and perhaps leaf, cells; a similar mechanism of ion conduction could provide a means of volume maintenance under such circumstances.

The adaptive bioenergetic events which lead to the adaptation of an organism to a change (increase) in salinity are complex. Of major concern is the energy requirement for the removal of the salt. Ultimately, in photosynthetic organisms, this energy derives from photosynthesis. However, in the cyanobacterium under investigation, it was found that respiration was the major mechanism for salt removal. Indeed, under conditions when greater energy expenditure is known to occur (intracellular salt removal) there is in fact a 15-fold increase in storage carbohydrate (glycogen). Perturbation of energy metabolism per se is apparently not the cause of the initial transient inhibition of growth. The mechanism and the reason of how and why the energy management of the cell is directed to storage products in cyanobacteria (and, perhaps, higher plants) exposed to salinity has not yet been elucidated, but is obviously of paramount importance to the understanding and utilization of this phenomenon in basic and applied research.

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### 3. Salinity Responses of Crop Plants in Terms of Leaf Expansion and Photosynthesis

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**PRINCIPAL INVESTIGATORS:** Norman Terry and Lawrence J. Waldron

Although manipulating the soil environment to minimize salt levels will continue to be the principal strategy for coping with salinity, development of new, more salt-tolerant crops will undoubtedly become important in salinity management. The first steps in breeding plants for salinity resistance are to learn how plant growth is restricted by salt and to identify the mechanisms by which salt-tolerant plants adapt to salinity. These were our objectives in the project reported here.

#### **EXPERIMENTAL APPROACH**

Plant biomass production is determined largely by the accumulation of carbon products in photosynthesis. This, in turn, depends on two components, the rate of photosynthesis per leaf area and the total area of photosynthetic surface (total leaf area). In this project, we analyzed the effects of salinity on plant dry-matter yield in terms of the two physiological components, photosynthesis/area and the expansion of the leaf surface. This was carried out initially using classical plant-growth analysis techniques in combination with leaf gas exchange to measure changes in photosynthetic rate. Later, we used more sophisticated techniques to provide more specialized information at cellular and subcellular levels of organization.

Many different aspects of photosynthesis were studied in addition to the gas exchange ( $\text{CO}_2$  uptake and transpirational water vapor loss) of attached leaves of intact plants. Rates of photosynthetic  $\text{O}_2$  evolution of leaf slices and of intact chloroplasts were measured, as well as the activities of photosynthetic enzymes (ribulose biphosphate carboxylase, fructose biphosphatase). Several aspects of the photosynthetic electron-transport process were investigated, including thylakoid membrane composition (membrane lipids and proteins, pigments, reaction centers, and electron carriers).

A major conclusion to emerge from this project: Salinity exerted its effects primarily via an effect on leaf expansion. Accordingly, we developed equipment (linear variable differential transducers, LVDTs) which could monitor with precision changes over time in leaf length, width, and thickness. By this means, we investigated leaf-growth responses to changes in environmental factors, for example, variation in rootzone salinity and atmospheric humidity.

Most of our work centered on salt-tolerant plants, especially sugarbeet, the major test plant of this laboratory. Other species examined included two species of mangrove, Avicennia maritima and A. germinans, and some membrane characteristics of the salt-sensitive cucumber.

## MAJOR FINDINGS

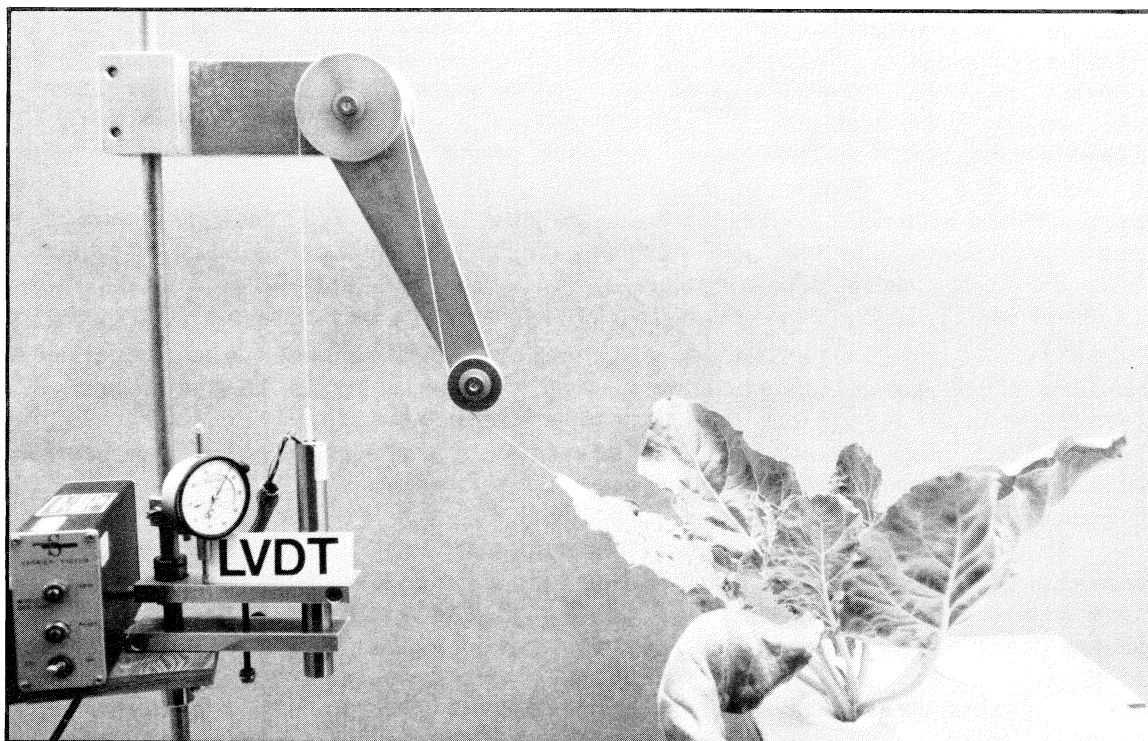
### Overall Effects on Plant Growth

Growth-analysis studies revealed that a salinity level of as low as 25 mM NaCl diminished total plant dry matter significantly in sugarbeets. Further increases up to 500 mM NaCl diminished plant growth progressively.

When these growth effects were analyzed in terms of the effects of salinity on the two components of dry-matter yield, photosynthesis/area and area of photosynthetic surface, it was evident that in sugarbeet the primary effect of salinity was on the expansion of photosynthetic surface (that is, total leaf area) and not on the rate of photosynthesis per area. Papp et al. (1983) showed that leaf growth was reduced progressively with increase in salinity from 0.5 to 500 mM NaCl, whereas photosynthesis (as measured by the rate of O<sub>2</sub> evolution of leaf slices) was not diminished until salinities of 350 to 500 mM had been reached. Similar results were obtained using gas exchange of leaves of intact plants: rates of photosynthetic CO<sub>2</sub> uptake (per area) were not significantly affected by salt at salinities lower than 200 mM NaCl.

The effect of salinity on the expansion of the leaf surface was manifested primarily via the effect of salinity on the expansion of individual leaves, and not on the rate of unfolding of new leaves by the terminal bud. Papp et al. (1983) showed that both the rate of leaf growth and final leaf size were linearly and negatively related to increase in salinity. In terms of cell growth, salinity diminished the rate of leaf growth mostly by reducing the rate of cell enlargement, although there was some evidence of a minor negative effect of salinity on the initial rate of cell division.

There were some interactive effects between mineral nutrition and salinity. Salinity effects on growth were more marked at high levels of nitrogen supply; or, stated in a different way, when nitrogen supply was limiting growth, salinity impaired plant growth less. This effect was particularly pronounced for growth in average leaf size. There was some evidence that salinity effects on growth were diminished when phosphorus supply limited growth, the effect--as with nitrogen supply--being most pronounced with respect to growth in leaf size. No such interactive effects were found between salinity and potassium nutrition, probably because the low K treatments were insufficiently low to diminish growth significantly in the 2-week period of these experiments.



Special instrument used for continuous measurement of leaf growth.

An important observation of these experiments is that in all instances (N, P, or K experiments), total leaf-water potentials decreased markedly with increase in salinity while turgor pressures were not significantly affected. The lack of effect of different levels of NaCl-salinity on turgor pressure contrasted with the data for low nitrogen supply, which significantly increased turgor pressure, and for low potassium supply, which decreased turgor pressure. The low N effect was probably due to the continued accumulation of solutes in plants which were growing more slowly than they were accumulating salts. The low K effect suggests that potassium has an important role in maintaining cell turgor.

### **Effects on Photosynthesis**

When salinity was increased by increments to moderate levels (for example, 150 mM NaCl), per-area rates of photosynthesis in sugarbeets exhibited no decline (measured at normal ambient CO<sub>2</sub> levels of 300  $\mu$ l l<sup>-1</sup> CO<sub>2</sub>). This was presumably because the plants had a chance to osmoregulate before serious water stress developed. Rates of photosynthesis per chlorophyll, however, did exhibit a small linear decrease with time over the 4-week experimental period. Stomatal conductance was decreased significantly, lowering the intercellular CO<sub>2</sub> concentrations, but, as is discussed below, did not affect photosynthesis/area.

As plants acclimated to moderate salinity, their leaves underwent distinct changes. Chlorophyll contents increased with time as did the content of other components of the photosynthetic apparatus (for example, P700, cytochrome f, thylakoid membrane lipids and proteins, ribulose biphosphate carboxylase, soluble protein, etc.). The reason that salinity did not decrease photosynthesis/area, even though stomatal conductance was decreased, is because the lowered internal CO<sub>2</sub> values were compensated by the accompanying increase in photosynthetic capacity (that is, more photosynthetic apparatus per leaf area in the saline-treated leaf). The reduction in photosynthesis per chlorophyll with salinity was due to the increase in chlorophyll content which resulted in less light being transmitted to the mesophyll cells in the lower layers of the leaf. This was experimentally verified by illuminating leaves from the underside during measurement, thereby increasing light saturated photosynthetic rates to control levels.

In general, the data show that salinity has little effect on photosynthetic rate in sugarbeet plants until relatively high salinities have been reached, at least 200 to 350 mM NaCl. Measurement of photosynthesis/area as a function of intercellular CO<sub>2</sub> showed that, at high salinities, leaf photosynthesis was reduced via an effect on stomatal conductance and on chloroplast photosynthesis. The decrease in stomatal conductance led to a reduced rate of transpiration and an elevated leaf temperature.

### **Thylakoid Membranes as Site of Adaptation to High Salinity**

One theory to explain how salt-tolerant plants cope with high salt concentrations is that they develop membranes which differ structurally and functionally from salt-sensitive plants. Photosynthetic membranes (thylakoids) have been proposed as a site of such an adaptation to high salinity. Chloroplasts of salt-tolerant plants may contain as much as 3 to 5 times higher salt concentrations than normal plants, suggesting that some structural modification of membranes could have occurred to handle the excessive salt levels. Also, it has been proposed that certain structural/functional aspects of photosystem II may have changed in salt-tolerant plants in some kind of adaptive response to high salinity, that is, that the chloride requirement of photosystem II may be 10 to 100 times greater than in salt-sensitive plants.

In studies with two salt-tolerant plants, sugarbeet and mangrove, we have found no evidence of an adaptive response in thylakoid membranes (Ball et al., 1984). The data suggest that the amount of membrane per leaf area increases with increase in the level of salinity from 50 to 500 mM NaCl, leading to increases in chlorophyll content. However, the ratio of various membrane constituents to chlorophyll content did not change, for example, chlorophyll/P700, chlorophyll/cytochrome f. There was no apparent change in membrane composition with respect to thylakoid lipids or proteins. Various assays of thylakoid membrane function also showed no change in response to increase in growth salinity from 50 to 500 mM NaCl, nor did they differ from similar assays conducted with thylakoids isolated from the salt-sensitive cucumber

plant. Such assays included uncoupled rates of electron transport through photosystem I, photosystem II, and photosystem I + II measured with different strengths of sorbitol (to vary water potential) and NaCl (to vary ionic strength), that is, there was no evidence that membranes of salt-tolerant plants functioned better at low-water potentials or at high NaCl concentrations. There was also no evidence for a specific structural/functional change in salt-tolerant plants with respect to photosystem II: we found that photosystem II required normal amounts of chloride ion to function properly, provided the thylakoids were isolated isotonicity.

The lack of an effect of salt on thylakoids and photosynthetic electron transport suggests that the reduction in photosynthesis at high levels of salinity is probably due to an effect on the enzymes of the Calvin cycle rather than on photosynthetic electron transport. However, we have little direct evidence on this point.

Measurements of intrathylakoid aqueous volumes by electron spin resonance spectroscopy was used to study ionic permeability properties of thylakoid membranes isolated from two salt-tolerant species, sugarbeet and mangrove (*Avicennia germinans* L.), grown in low- and high-salinity nutrient solutions (Ball et al., 1985). Thylakoids behaved as perfect osmometers in the presence of sorbitol or betaine and the passive permeability to NaCl and KCl was low. No consistent permeability differences were observed between thylakoids from plants grown at low and high salinities, further supporting the view that salinity does not affect the structural/functional properties of thylakoid membranes.

### **Photosynthesis and Ion Transport**

The results suggest that when plants are first exposed to salinity (for example, 150 mM NaCl), they appear to osmoregulate rapidly so that turgor pressures are maintained to prevent wilting. In sugarbeet, this osmoregulation appears to be due to the transport of sodium (and probably chloride, although chloride was not measured) into the leaf. This transport occurs most rapidly during the first 48 hours and continues at slower rates throughout the life of the leaf. Studies with nonaqueously isolated chloroplasts showed that under saline conditions, the concentrations of salt may reach 2 to 3 times those normally found in chloroplasts. Since photosynthetic rates in vivo were unchanged by salinity up to 250 mM NaCl, it is evident that photosynthetic enzymes, for example, of the Calvin cycle, were unaffected by the apparently high concentrations of salt in the chloroplast.

### **Dynamic Studies of Leaf Expansion**

Because the effect of salinity on plant growth is manifested primarily via an effect on the rate of expansion of individual leaves, we used LVDTs to study the changes in leaf length, leaf width, and leaf thickness with time (Waldron et al., 1985; Waldron and Terry, 1986).

The data show that changes in leaf area correlated with changes in leaf width, but not necessarily with leaf thickness. The expansive growth of leaves (that is, leaf length x leaf width) occurs mainly at night, almost certainly because of the improved leaf-water status associated with the reduced evaporational load (no heat from illumination). When salinity (for example, 100 mM NaCl) is imposed on the plant, the expansive growth by day ceases almost completely. At night, the rates of expansive growth are much reduced by salinity.

Humidity greatly affects the growth response of leaves to salinity treatment. At 35 percent relative humidity, a level not uncommon in irrigated areas of California, an increase in salinity of 100 mM NaCl caused a 65 percent decrease in the average rate of leaf extension. This reduction in leaf growth could be almost completely offset when the humidity was increased to 85 percent.

### **Overall Conclusions**

The main conclusion emanating from this work is that salinity affects growth primarily through an effect on the expansion of the leaf surface and not via an effect on photosynthesis/area. Leaf (and whole plant) growth may be impaired at salinity levels as low as 25 mM NaCl: photosynthesis is not impaired until salinity has reached at least 10 times this value.

The results are consistent with the view that salinity influences sugarbeet growth via an effect on plant-water relations. All growth reductions with salinity were associated with a decrease in total (and solute) leaf-water potentials but not with changes in bulk leaf turgor. An imposition of a brief period of salinity (for example, 48 hours) resulted in an immediate reduction in leaf growth as measured by LVDTs. Restoration of high-water potentials at the root caused an immediate increase in leaf-growth rates to presaline treatment levels. These data suggest that salinity effects are mediated via changes in leaf-water status. Further support for this view comes from the data showing that increases in relative humidity could almost offset the effect of salinity.

There was no support for the view that salinity reduced growth in sugarbeets via specific ion effects on metabolic processes. Rates of photosynthesis and respiration were not affected at moderate levels of salinity, despite very high concentrations of salts in chloroplasts and leaves.

### **SIGNIFICANCE**

From a fundamental science viewpoint, we have demonstrated unequivocally the importance of increasing research efforts to understand the mediation of salinity effects on leaf expansion growth, especially in relation to the control of leaf growth via changes in leaf water sta-

tus. This area requires use of LVDT technology, together with better equipment for ascertaining leaf-water status, particularly the turgor pressures of specific cellular regions of the leaf. Perhaps the Zimmermann pressure probe would be valuable in this respect.

From a practical standpoint, we see the possibility that salinity may be managed more effectively by using some kind of mist-irrigation device to increase humidity levels in the immediate environment of the leaf, particularly during periods of high evaporative demand. This would diminish losses in plant growth due to salinity and also the amount of water loss from the soil by evapotranspiration.

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### SUPPORTING PERSONNEL

M. C. Ball (postdoctoral researcher), S. E. Taylor (postdoctoral researcher), J. Nemson (SRA), C. Carlson (SRA), J. Krall (SRA), J. C. Papp (lab helper)

#### 4. Additive and Interactive Effects of Soil Salinity and Water Regimes on Crop-Growth Responses and Osmoregulation

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**PRINCIPAL INVESTIGATOR:** Theodore C. Hsiao

Salinity and water shortage, problems common to agriculture in semiarid zones, are closely connected. As the soil dries or as salt levels build up, both water potential and osmotic potential in the soil decline, thus affecting plant growth and processes. On the other hand, salinity stress involves excesses of ions, whereas water stress is due primarily to a deficit of water without a direct role of ions. Nonetheless, from a practical viewpoint of crop yields, early approaches have assumed that the effect of salt in the soil (osmotic-potential effect) and the effect of soil drying (matric-potential effect) are additive. In addition, analysis of some field- and growth-chamber studies revealed that reducing soil-water potential, either through reduced osmotic potential or reduced matric potential, resulted in the same reduction in water uptake and dry-matter yield.

Under either salinity or water stress, plants exhibit lower-leaf water potential. Despite this obvious similarity, some clear distinctions exist between plant responses to salt and water stress. One common observation is the lack of wilting under salt stress at water potentials that cause wilting under water stress. In addition, the osmotic adjustment processes under these two stresses differ. Osmoregulation in response to salinity may utilize ions from the soil, particularly those ions in excess, whereas under drought in the absence of salinity, the necessary solutes have to be produced mostly within the plant. Analysis shows that adjustment through ion uptake is energetically more efficient than adjustment through the production of organic solutes, but may be associated with ion toxicity.

Experiments designed to compare quantitatively the effects of salinity with those of water stress are few. Those available often simulated water stress with the application of polyethylene glycol and showed that the simulated water stress was more detrimental to growth and other physiological processes than salinity of equal water potential. However, it is not clear whether polyethylene glycol per se may have its own detrimental effect.

This project was conducted to evaluate the similarities and differences in response of crop growth, osmotic adjustment, transpiration, and photosynthesis to equivalent reductions in soil-water potential brought about by either soil drying or salinity.




## EXPERIMENTAL APPROACHES

The study was carried out in controlled-environment chambers and in the laboratory on cotton, pepper, and bean plants growing in pots. These plant species were chosen for their contrasting tolerance for salinity. The basic approach used was to compare selected functions of the plant at a given degree of soil-water stress with those of the same plant at the equivalent salt stress in the soil. The plants were allowed to transpire to deplete water in the pot to a desired level of soil-water potential. At that time, leaf-water potential and its components were measured. Leaf growth, transpiration, and sometimes photosynthesis, were monitored concurrently. The soil was then salinized by adding salt solutions ( $\text{CaCl}_2\text{-NaCl}$ , 1:1 ratio on the solute potential basis) of the same water potential as the droughted soil. After 2 or more days of adjustment, leaf-water potential and components and plant functions were measured again. This experimental approach permits an evaluation of how plants acclimated to water stress adjust themselves to salinity stress. In addition, the kinetics of changes in photosynthesis and underlying parameters as a plant was shifted from water stress to salinity stress were examined, to gain insights on differences in plant responses elicited by these two stresses.

## RESULTS

### Osmotic Adjustment

Osmotic adjustment enables the plant to maintain turgor and tissue-water content at lower tissue water potentials. In this study, under water stress leaf-turgor pressure of cotton was found to be lower than under salinity stress of similar levels. When cotton plants were shifted from soil-water stress to equivalent soil-salinity stress, leaf-turgor pressure recovered to levels expected of unstressed plants. For a range of soil-salinity levels (-3.3 to -10.6 bars), turgor was maintained at a constant level of about 0.4 MPa in the leaves two days after the shift. The better turgor maintenance under salinity is attributable to the accumulation of solutes in leaves and possibly also to a lower hydraulic resistance to water flow from the soil to leaves. Solute potential was lower by several bars in salinized leaves, reflecting higher solute concentration. Under water stress a higher proportion of the solutes was made up by organic compounds (indicated by refractive index measurements) than under salinity stress. When water stress was changed over to salinity stress, more electrolytes were taken into cotton leaves, making them the major tissue solutes 4 days after salinization. The most marked increase was in chloride (to approximately 100 mmole per g fresh weight), whereas the increase in sodium content was less dramatic.



Pepper plants also responded differently to the 2 stresses, with turgor maintenance through osmotic adjustment only under salinity stress (-1.6 to -6.5 bars). Very little osmotic adjustment occurred under water stress of similar magnitudes. Another difference between cotton and pepper was that the major portion of solutes in pepper leaves consisted of electrolytes under both stresses. Leaves of beans also exhibited substantial osmotic adjustment in response to a shift to salt stress. However, leaves began showing signs of permanent damage 2 days after the shift and did not recover their physiological functions when salt was leached out from the soil.

### **Growth and Transpiration**

Expansive growth of cotton leaves was markedly inhibited by soil-water potential of -2.5 bars or lower. Two days after being shifted to a soil-salinity level of -3.0 to -6.5 bars, however, leaf growth resumed at a rate equal to or approaching that of the control plants. In spite of this, fresh weight of the plants after a total of 6 days of stresses was highly correlated with growth ratio during the water-stress period, although salt stress constituted 4 of the 6 days. Transpiration was also higher under salinity than under water stress of equivalent magnitudes, but remained depressed when compared to the control. With pepper plants, a similar difference in the effect on transpiration between salt and water stress was observed, but expansive growth appeared to be similarly reduced under either stress.

### **Photosynthesis**

In pepper, leaf CO<sub>2</sub> assimilation was higher 1 to 2 days after the shift to salinity than before, when the plants were under equivalent water stress. Similar deductions regarding assimilating differences may be made from the transpiration data for cotton. To gain more insights on the parameters responsible for the changes in photosynthesis, gas-exchange analyses were carried out and photosynthesis response curves to CO<sub>2</sub> were determined for cotton at various times before and after the shift from water stress to salinity stress. The results showed that CO<sub>2</sub> assimilation was reduced under both water and salt stress by a reduction in biochemical capacity for photosynthesis. In addition, stomatal closure under stress was sometimes a contributing factor. Limited data indicated that leaching to remove salt from the soil several days after the initiation of salt stress permitted the nearly complete recovery in leaf photosynthetic capacity, despite the fact that most of the accumulated chloride and sodium ions remained still in the leaf. These results, if confirmed, would suggest that the reduced photosynthesis of cotton under salt stress is not the consequence of ion toxicity. In contrast, beans, subjected to the same soil salinity (-6.5 bars), did not recover and their leaves exhibited signs of permanent damage after salt was removed from the soil by leaching.

Preliminary results obtained on transient changes in photosynthesis as cotton plants were shifted from water stress to salinity stress are also interesting.  $\text{CO}_2$  assimilation increased within minutes after salt solution was added to the soil, replacing the effect of soil drying. This elevated rate of assimilation was maintained, however, for only several hours, after which the rate declined, often to a level lower than that at the time of the shift. The changes in rate were associated with corresponding changes both in stomatal conductance and "mesophyll" (or biochemical) conductance of the leaf. Possibly the decline in assimilation after the initial elevation was associated with the arrival of salt ions at the leaf. In any event, then transient changes appear to contradict some of the results of the long-term experiments, which showed that salt stress was generally less detrimental to plant functions than water stress. These discrepancies will have to be resolved by future experimentation.

### **SIGNIFICANCE**

When subjected to soil stress of the same or similar water potential, plants (cotton and pepper) were able to adjust osmotically to maintain nearly full turgor only under salt stress, in contrast to very limited or incomplete adjustment under water stress. Rates of leaf expansion, photosynthesis, and transpiration were higher under salinity stress than under equivalent water stress. However, the rates were still depressed compared to control plants in spite of the full osmotic adjustment under salinity. These results point to the need to reexamine the concept of equal effect of salinity and water stress on crop yields.

The depression in photosynthesis was attributable largely to a loss in biochemical capacity to assimilate  $\text{CO}_2$ . Stomatal closure contributed to this only in a minor way. The depressive effect appeared to be readily reversible, once excessive salt was removed from the soil, while most of the absorbed salt ions remained in the leaf. These results are suggestive regarding mechanisms of salt damage and resistance, and point to future lines of research.

### **PUBLICATIONS**

Shalhevet, J., and T. C. Hsiao. 1986. Salinity and drought: A comparison of their effects on osmotic adjustment, assimilation, transpiration, and growth. *Physiologia Plantarum* (submitted).

### **SUPPORTING PERSONNEL**

J. Shalhevet (senior scientist, The Volcani Center, Bet-Dagan, Israel)

## 5. Genetic Engineering of Osmoregulation and Salt Tolerance in Rhizobium and Symbiotic Legumes

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**PRINCIPAL INVESTIGATORS:** D. Munns and R. C. Valentine

The principal investigators did not provide a summary report.  
They should be contacted directly for information.

## II. Plant Nutrient-Salinity Interactions

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### 6. Cereal-Crop Response to Chloride and Sulfate Salinity and Interaction with Root Aeration

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**PRINCIPAL INVESTIGATORS:** André Läuchli and Emanuel Epstein

This project's general objective was to unravel the physiological changes of cereal crops responding to salinity. The project can be broadly categorized into 4 areas of investigation: (1) ion and nutrient partitioning and transport in barley, wheat and sorghum; (2) chloride partitioning in 4 cereal crops with emphasis on sorghum; (3) physiological responses of sorghum to chloride and sulfate salinity; and (4) interaction of root aeration with NaCl-stress in corn.

#### **ION AND NUTRIENT PARTITIONING AND TRANSPORT IN BARLEY, WHEAT, AND SORGHUM**

As a prelude to more detailed physiological studies in the laboratory, 3 crop species were evaluated under salt-stress conditions in the San Joaquin Valley. Barley (Hordeum vulgare L.) was evaluated in 1981 on a segment of a commercial farm near Hanford suffering from a serious salinity problem. Two cultivars were compared, the salt-sensitive "Arivat" and the more salt-tolerant "Briggs." Plots with differing salinity levels were identified, and at several times during crop growth plants were harvested from these plots for growth and ion-content analysis. From 1982-84 we participated in the field experiment at West Side Field Station. Wheat (Triticum aestivum L.) was studied in spring 1982 and sorghum (Sorghum bicolor L.) was investigated in the summers of 1982-84 at this location. As in the barley study, plants were periodically harvested from the site for growth and ion-content analysis.

Three salient features emerged from the field studies: (1) Na/K relationships were affected by salt stress in barley, (2) salt stress disturbed the Ca nutrition of barley, and (3) in all 3 crops Cl was preferentially accumulated in the leaf sheaths under salt stress, as opposed to the leaf blades. The Na/K relationships are particularly important in terms of maintaining K supply to meristematic regions of barley shoots, despite high levels of the competing and potentially toxic Na. This was confirmed in a second study of barley in solution culture under controlled-environment conditions. The solution culture study also demonstrated that the salt tolerance of a given cultivar may

change with ontogeny, perhaps as a function of the relative proportion of growing and expanded tissue. Salt stress reduced Ca levels in the shoot of the salt-sensitive barley cultivar Arivat more than in Briggs, which is interesting in view of the importance of Ca in salt tolerance.

These field observations prompted laboratory studies on the effect of salt stress on K and Ca transport mechanisms in barley. These studies were conducted in solution culture on seedlings at the 3 to 4 leaf stage. The transport of K, Na, and Ca was evaluated with the radionuclides  $^{86}\text{Rb}$  or  $^{42}\text{K}$ ,  $^{22}\text{Na}$ , and  $^{45}\text{Ca}$ , respectively. The release of labeled elements into the xylem was analyzed by collecting exudate from the cut end of excised roots. The uptake of labeled elements into the root was evaluated after 10 minutes of exposure of excised or intact roots to the treatment solution.

Relatively low levels of NaCl (15 to 45 mM) inhibited both K uptake into barley roots and K release into the xylem. In plants that had been previously exposed to 1 mM NaCl, media salinization directly inhibited K release into the xylem. Although we did not detect cultivar differences in our short-term studies, it may be expected that in the field, the tolerance of this process to media salinization may contribute to the plant's overall salt tolerance.

Media salinization (30 mM NaCl) also inhibited Ca transport from root to shoot in barley. This effect is due to a specific inhibition of Ca release into the xylem and is not mediated by salt effects on transpiration. In collaboration with M. Drew we found that NaCl (10 and 50 mM) also retarded Ca transport to the shoot of corn, although the effect was not specific to NaCl, since KCl at equimolar concentrations caused a similar inhibition. Calcium has several functions that are particularly important to salt-stressed plants, so the maintenance of adequate Ca transport to the shoot may be another facet of overall salt tolerance.

#### CHLORIDE PARTITIONING IN FOUR CEREAL CROPS WITH EMPHASIS ON SORGHUM

Extensive tissue ion analysis performed on salinized sorghum plants (Sorghum bicolor L.) grown at West Side Field Station revealed a preferential accumulation of chloride in the sheath tissue of the leaves relative to the blade tissue. A detailed growth study and accompanying ion analysis were conducted in the greenhouse using solution culture on sorghum, corn (Zea mays, 2 cultivars), and barley (Hordeum vulgare, c.v. Briggs) at several salinity levels (1, 50, 100, 150 mM NaCl). This study was conducted primarily to investigate more accurately chloride partitioning under more controlled conditions and to assess its potential role in the salt tolerance of salt-stressed cereal crops.

All species examined showed significant chloride partitioning when results were expressed on a dry-weight basis. However, when chloride ion concentrations were expressed on a tissue-water basis, taking into

account differences in the fresh-weight to dry-weight ratios of the 2 tissues, sorghum had the greatest ability to partition Cl into sheath tissue away from the potentially salt-sensitive photosynthetic regions in the blade tissue. Corn, representing the most salt-sensitive species examined, showed no Cl partitioning whatsoever when chloride concentrations were expressed on a tissue-water basis although differences between the 2 cultivars of corn examined were obtained. Barley was somewhat intermediate in its ability to partition Cl. Chloride partitioning into sheaths may be a protection mechanism against Cl toxicity in leaves.

Additional ion interactions revealed by tissue analysis of the field-grown sorghum included an apparent K-Mg interaction specific to the blade tissue. Higher levels of Mg in the high-salt irrigation water and subsequent uptake of Mg significantly suppressed the K concentrations in the blade of salt-stressed sorghum. This interaction was not found in any of the other tissues examined including the sheath, culm, and inflorescence.

As a result of these field and greenhouse studies, physiological investigations were undertaken with sorghum to understand more fully the physiological basis of sheath chloride partitioning. Ion transport studies were performed utilizing radioactively labeled chloride to estimate chloride influx and efflux rates in sheath and blade tissue of salt-stressed sorghum. Although no significant differences could be detected in the efflux of chloride from the 2 tissue types, significantly greater influx rates were found for sheath than blade tissue, and it is this behavior that is presumed responsible for the observed chloride partition patterns.

Presently electron probe X-ray microanalysis is being utilized to characterize cellular and subcellular distribution patterns of chloride (and other ions) in sheath and blade tissue. This may give us further insight into the mechanism of chloride partitioning in sheath tissue.

#### **PHYSIOLOGICAL RESPONSES OF SORGHUM TO CHLORIDE AND SULFATE SALINITY**

Irrigation water used on the sorghum field plot at West Side Field Station contained considerable quantities of sulfate as well as chloride salts. Thus, a comparative study was undertaken to assess accurately the relative toxicities of these 2 types of salinizing anions. Detailed growth studies and tissue ion analysis were performed on sorghum in the greenhouse utilizing iso-osmotic concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub>.

These investigations revealed no consistent, statistically significant differences between the 2 salts as they affect total vegetative shoot biomass, total root biomass, shoot/root ratios, or plant-leaf area when compared at iso-osmotic concentrations. However, substantial differences were found in the ionic composition of tissue taken from plants salinized with the 2 different salts. The sodium exclusion

mechanism of sorghum, which effectively maintains shoot sodium concentrations at a very low level by sequestering sodium in the roots, was found to function efficiently only under NaCl salinization. Moderate (0.4 MPa) levels of Na<sub>2</sub>SO<sub>4</sub> salinization completely disrupted the sodium exclusion mechanisms, resulting in significant increases of sodium in the shoot tissue.

### INTERACTION OF ROOT AERATION WITH NaCl STRESS IN CORN

The breakdown of the Na exclusion mechanism in oxygen-deficient roots of corn (*Zea mays* L. cv. Pioneer 3906) and a consequent abnormal ratio of Na/K entering the shoot are described. To characterize the location of the exclusion process, we examined the extent to which the mesocotyl acts as a sink for Na, as a possible mechanism for protecting the shoot. We also measured Na efflux and the effects of metabolic inhibitors on Na exclusion from the shoot.

The Na exclusion from the shoot by roots is highly O<sub>2</sub>-sensitive. The process is increasingly inhibited when O<sub>2</sub> concentrations are lowered from ambient (21 percent v/v) to 15 percent or less. Because of a simultaneous inhibition of K transport, the ratio Na/K entering shoots was increased 90 to 200 times when roots became anoxic. This abnormal movement of Na to the shoot is likely to interfere severely in cell metabolism. We conclude that soil O<sub>2</sub>-deficiency, which often accompanies high water tables in irrigation agriculture, can cause failure of the Na-exclusion process and thereby contribute to salinity damage in salt-sensitive crops.

Work elsewhere (Johanson and Cheeseman, Plant Physiol. 73:153-58, 1983) indicates that the mesocotyl, located between the root and shoot, can strongly accumulate Na from the ascending transpiration stream, thereby potentially acting as an alternative sink to protect the shoot from excess Na. To determine the quantitative importance of the mesocotyl as a Na sink, we grew plants with either short (9.0 mm) or long (21 mm) mesocotyls. At 13 days, when measurements of Na uptake were made, there were no perceptible differences between treatments in plant growth. After being raised on Na-free nutrient solution, plants were transferred to <sup>22</sup>Na labeled solution in which the concentration of NaCl was 1.0, 10, and 50 mM. The concentration of Na accumulated in the mesocotyl (per g fresh wt of mesocotyl) exceeded that in the roots that were directly exposed to the nutrient solution in 24 hours. The amount of <sup>22</sup>Na retained in the long mesocotyls was about double that in the short ones and increased with time of exposure and NaCl concentration. At 1 and 10 mM NaCl, the amounts of Na retained in the mesocotyl were 10 to 18 percent of that reaching the shoot in 24 hours, but with 50 mM NaCl, a damaging concentration for corn, this declined to 2 to 4 percent. The mesocotyl even in its fully elongated structure is therefore unlikely to provide an appreciable alternative sink for Na when NaCl reaches injurious concentrations.

A major aim was to determine whether the mechanism of Na exclusion from the shoot was located principally at the plasma membrane of the



epidermal and cortical cells (active efflux pumping) or involved absorption of Na from the transpiration stream by xylem parenchyma cells in the older (basal) part of the root. One approach to this question was to compare the effect of permeant and nonpermeant inhibitors on Na and K transport to the xylem. The roots of intact plants were exposed to a range of different inhibitors for 30 minutes before addition of labeled K (1 mM) and Na (100 mM). The impermeant sulfhydryl reagent p-chloromercuribenzenesulfonic acid appreciably reduced K uptake by roots and transport to shoots, but the exclusion of Na from the shoots was not affected. By contrast, carbonyl cyanide m-chlorophenylhydrazone, an uncoupler of oxidative phosphorylation, and vanadate (a plasma membrane ATP-ase inhibitor), both of which are able to penetrate root tissues, retarded K uptake and transport and reduced the effectiveness of the exclusion mechanism, resembling the effects of anoxia. These results suggest that the site of K entry into the symplast may be at the epidermis and outer cortical cells. Sodium exclusion appears to take place deeper within the root cortex, perhaps at the inner cortical cells or at the xylem parenchyma.

Earlier studies (Shone et al., *Planta* 86:302-14, 1969; Yeo et al., *J. Exp. Bot.* 28:17-29, 1977) indicate that Na moving upwards in the transpiration stream is strongly accumulated by xylem parenchyma cells bordering the xylem. However, the capacity of such cells to act as a sink for Na must be limited, and the question arises as to whether there is an outwardly directed transport mechanism for Na. We studied this by sealing a seminal root of an intact corn seedling into a Plexiglas chamber with 6 compartments. Nutrient solution, with addition of NaCl and labeled with  $^{22}\text{Na}$ , was supplied to the subapical compartment, 10 mm from the root tip. Solution in the other compartments, which contained unlabeled nutrients with or without 200 mM NaCl, was counted for Na and replaced at regular intervals. The results indicate that efflux of Na to the root environment, originating from the xylem, may make an appreciable contribution to removing Na from the transpiration stream moving towards the shoot. Equally important over the duration of this experiment was retention of Na in the older part of the root.

### SIGNIFICANCE

A major conclusion of these studies is that interactions of salinity with uptake and transport of mineral nutrients are an important component of the overall plant response to salinity. In addition, not only the total ionic strength of a saline medium determines the degree of plant response, but also the kinds of salts contributing to salinity, such as chloride and sulfate salts. Sulfate salinity and poor root aeration dramatically interfere with the exclusion of sodium from the shoot. Root aeration should be considered an important factor in crop responses to salinity in the field. Interference of salinity with nutrient acquisition in crops may have practical implications in salt-affected soils that show low availability of the respective nutrient.

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## SUPPORTING PERSONNEL

Dr. Malcolm Drew (research scientist, Long Ashton Research Station, Bristol, England), Pat Boursier (graduate student), Jonathan Lynch (graduate student)

## 7. Plant Response to $\text{Na}^+$ , $\text{K}^+$ , and $\text{K}^+/\text{Na}^+$ Ratios under Saline Conditions

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PRINCIPAL INVESTIGATORS: D. A. Devitt and L. H. Stolzy

Increasing demands for water have resulted from expanded uses by agriculture, municipalities, and industry. Thus, a greater emphasis has been placed on improving water-use efficiency and on using alternate sources of water in agricultural production. One such alternate water source is electrical-generating power-plant blowdown water. In the production of electricity from fossil-fuel plants, large quantities of good-quality water are required in the closed cooling system. A portion of this water, perhaps as high as 20 percent, is removed from the cooling cycle and replaced with fresh water to prevent the accumulation of salt in the cooling system. It is this drawn-off water, called blowdown, which represents a potential water resource. Of course, the presence of large quantities of soluble salts in this water places constraints on its use for agricultural production.

We investigated the effect of using such waters on plant growth and development, with special emphasis on the impact of imbalanced  $\text{K}^+/\text{Na}^+$  ratios under saline conditions on plant response. Two separate experiments dealing with the  $\text{Na}^+$ -salinity interaction with plant response were conducted.

The first study's objective was to establish the relationship for wheat and sorghum, between  $\text{K}^+/\text{Na}^+$  ratios at different osmotic levels in solution, on root growth and development and stomatal response. Under saline-sodic conditions the concentration of  $\text{Na}^+$  in the soil solution can reach disproportionately high levels relative to  $\text{K}^+$ . It is the root system that must first confront the high  $\text{Na}^+$  and osmotic potentials. The extent to which the root system is able to adjust and adapt to these stresses will help determine the plant's overall growth and development. The impact of both the  $\text{Na}^+$  ion and the osmotic potentials on root elongation and growth needed further elucidation. After the uptake of  $\text{Na}^+$  by the root takes place, its mobility within the plant will dictate further plant response. The ability of  $\text{Na}^+$  to substitute or hinder  $\text{K}^+$  involvement in critical physiological roles, such as stomatal response, also needed further investigation.

The second study's objective was to investigate the response of wheat to zonal saline-sodic conditions. When blowdown water is used as an irrigation source, the accumulation of salts in the profile and the distribution of roots therein will influence the plant's overall productivity. A plant's ability to extract selectively soil water of more favorable osmotic potential implies that average soil salinities may not represent the true integrated response of a root system. Distribution of such salts as  $\text{Na}^+$  will be controlled by soil type, both through exchange reactions and water-flow rates. Thus, the zonal distribution of salts in

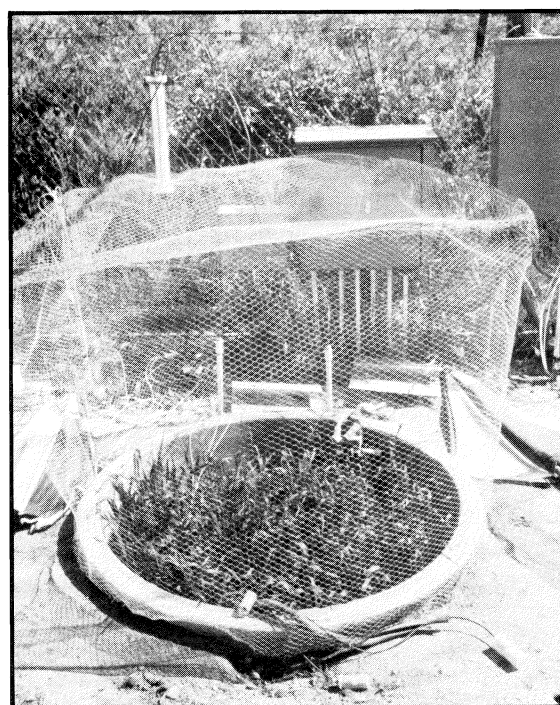
the soil profile needed to be studied in both sand and clay soils. The extent to which water uptake and yield are decreased by the plant's response to their zonal salt distributions (and in particular to the  $\text{Na}^+$  ion) also needed to be assessed more closely.

### EXPERIMENTAL PROCEDURES

To ascertain the interaction of  $\text{K}^+/\text{Na}^+$  ratios at different osmotic potentials on plant response, wheat (Triticum aestivum L. var. Cajeme) and sorghum (Sorghum bicolor L. Moench. cv. Dekalb C-42Y) were grown in outdoor solution tanks containing polyethylene glycol and/or NaCl as osmoticum with one-half-strength Hoagland as the base nutrient solution.

The plants were grown in the experimental solutions for 6 weeks. During the test period, root length, plant height, leaf-water potential, and porometry measurements were taken. Upon harvest, fresh weights were obtained and the plants were washed, subdivided into 2 uppermost leaves, lower leaves, stalks, and roots. Plant tissue was then oven-dried, ground, digested, and analyzed for  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ , and  $\text{Mg}^{++}$ .

To determine the response of wheat to sodium uptake under zonal saline-sodic conditions, wheat was grown in lysimeters with electrical-generating plant blowdown water (E.C. = 6.91 dS/m) as the irrigation water source. The lysimeters were packed with four different soil types (2 clay loams and 2 sandy loams), with 4 replicates per soil type (3 experimental and 1 control). Only the third and final crop (wheat) in a wheat-sorghum-wheat rotation is reported. Irrigation water was applied at a rate to maintain a 10 percent leaching fraction on all of the lysimeters. A hydrologic balance was maintained on all of the lysimeters during the experimental period.



Lysimeter used to investigate the effects of  $\text{Na}^+$  and  $\text{K}^+$  on plant growth under saline conditions.

A pulse of  $^{22}\text{Na}^+$  was applied once to each of the lysimeters at different times to establish different placement of the  $^{22}\text{Na}^+$  relative to the  $^{23}\text{Na}^+$ ,  $\text{K}^+$  and salinity levels.

At the end of each crop, soil cores were taken to a depth of 140 cm. The cores were subdivided into depth increments. All cores were then divided vertically into 2 equal parts. One subsample was used to obtain a saturation extract and a  $^{22}\text{Na}$  count; the other sub-sample was used to obtain a root sample. The extracts were analyzed for all of the major cations along with the EC and  $\text{Cl}^-$ . At harvest, the plant material was separated into forage and grain. Plant tissue was analyzed for  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Na}^+$ ,  $\text{K}^+$  and  $^{22}\text{Na}$ .

## RESULTS

At different Na concentrations with varying osmotic potentials, root-growth patterns of the two species differed. The mass of the root system for both wheat and sorghum was controlled primarily by the osmotic potential; however, root elongation was controlled primarily by the Na concentration. Sorghum-root elongation rates generally decreased in the presence of Na while those for wheat generally increased. The coefficient of root elongation and average whole plant Na concentration in sorghum were inversely related. Sodium was not translocated out of the sorghum-root system until a critical  $\text{Na}^+$  root saturation level of 0.6 moles/kg was obtained. A definite  $\text{Na}^+$ - $\text{Ca}^{++}$  interaction also occurred, resulting in a reduction in the  $\text{Ca}^{++}$  concentrations in the upper leaves at high  $\text{Na}^+$  concentrations. The combination of high  $\text{Na}^+$  and low  $\text{Ca}^{++}$  concentrations in the leaves of sorghum, even when  $\text{K}^+$  concentrations appeared to be adequate (that is, greater than control), was associated with increased diffusive resistance measurements.

The experimental findings would suggest that under saline conditions both wheat and sorghum would be well adapted to exploit regions of more favorable osmotic potential. However, under saline-sodic conditions, the wheat-root system would be superior to the sorghum-root system at this adaptation. Under saline-sodic conditions, sorghum may sequester the Na in the root until a tissue-saturation capacity of Na is exceeded, this directly contributing to reduced rates of root elongation.

The response of wheat to irrigation water, high in both  $\text{Na}^+$  and soluble salts, was highly dependent on soil type. Plants growing in soils with higher CEC and water-holding capacities fared better than those growing in the lower CEC and water-holding capacity soils, apparently in direct response to the salt distribution. An excellent correlation was found between the depth-weighted  $\text{EC}_{\text{SE}}/\theta$  and the cumulative evapotranspiration. Plants that had higher dry-matter production and ET rates also had a higher  $\text{Na}^+$  accumulation and tissue concentration. This, we believe, was manifested by a positive response to more favorable  $\text{EC}_{\text{SE}}/\theta$  regions where  $\text{Na}^+$  was effectively used in osmoregulation. Inclusion of multiple soil parameters did not improve the already high correlation between the  $\text{Na}^+$  concentration in the plant tissue and evapotranspiration rate.

Under zonal salinity, water uptake is usually dictated by plant response to osmotic stress that varies within the active root zone. In this experiment, an excellent correlation was obtained between the evapotranspiration and an  $(EC_{SE}/\theta) \times (W_J)$  parameter that was summed over the entire profile, where  $W_J$  was the fractional water uptake calculated from steady-state  $Cl^-$  distribution data. Sodium-22 results confirmed the  $W_J$  and root-distribution data, which indicated that the roots closest to the surface were by far the most predominant for water and  $Na^+$  uptake. The  $^{22}Na$  results also indicated that  $^{22}Na$  was fairly mobile within the plant, showing a great deal of recirculation out of the tops as the plants reached maturity.

Finally, as the ET rate decreased, a 39 percent reduction in yield was observed between the high and low measured evapotranspiration rates, with separation of yields being linked to soil type.

### SIGNIFICANCE

Yields as high as  $7.5 \text{ Mg ha}^{-1}$  were obtained on a clay-loam soil, suggesting that saline wastewater (electrical-generating plant blowdown water) could be used as a viable alternative under water-shortage conditions, if care were used in the choice of crops, soil types, and management practices.

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### SUPPORTING PERSONNEL

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## 8. N Use Efficiency as Influenced by S Assimilation in Barley Exposed to Salinity

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**PRINCIPAL INVESTIGATORS:** R. C. Huffaker and D. W. Rains

The uptake and assimilation of inorganic macronutrients are critical if plants are to adapt, grow, and reproduce in a saline environment. The focus of the studies was on nitrogen metabolism because of its central role in plant growth and development, and because nitrogenous metabolites, that is, proline and glycine betaine, accumulate during stress and are generally thought to be important for osmoregulation, which allows plant adaptation to salinity. Whether these compounds accumulate simply as symptoms of stress or act as osmoregulants as hypothesized, they place an additional drain on the N economy of the plant and emphasize the importance of characterizing N assimilation during salt stress. The objective of this study was to determine the relationship of the assimilation of inorganic N and phosphate by barley plants to their initial response and adaptation to salinity. The responses were determined for CM 67 and Arivat, cultivars which represent salt-tolerant and salt-sensitive varieties, respectively. Effects were also correlated with seedling growth.

### EXPERIMENTAL PROCEDURES

Barley (*Hordeum vulgare*) seedlings from the cultivars CM 67 and Arivat were placed in specific nutrient solutions containing increasing levels of salt presented as NaCl and Na<sub>2</sub>SO<sub>4</sub>. All studies were done in environmentally controlled growth chambers. Uptake rates of NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, P<sub>i</sub>, K<sup>+</sup>, Ca<sup>+</sup>, Cl<sup>-</sup>, Na<sup>+</sup>, and SO<sub>4</sub><sup>=</sup> were followed over time by determining their disappearance from the solutions. Further assimilation of nutrients is assayed by determining how much of that taken up is converted to other products. The concentrations of proline and glycine betaine were determined according to previously established procedures.

### GENERAL RESULTS

#### Nutrient Transport

The uptake of NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, and P<sub>i</sub> was identified as a key limiting process for the growth of barley seedlings in a saline environment. Uptake of each was little affected up to 100 mM salt; uptake of each then decreased sharply between 100 and 200 mM salt. At 200 mM salt, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, and P<sub>i</sub> uptake was, respectively, 17, 38, and 20 percent of the control (0 salt).

The  $\text{NO}_3^-$  transporter appears to be synthesized only in the presence of  $\text{NO}_3^-$ . The presence of salt had no effect on the appearance of the  $\text{NO}_3^-$  transporter; hence, salt did not affect the synthesis of transporter but generally affected its activity.

Long-term studies showed that the uptake of nutrients and growth were inhibited to the same degree. Uptake and growth simultaneously slowly recovered as the plants adapted to the presence of NaCl. This shows the strong relationship of nutrient assimilation to growth as plants adapt to salt stress. The inhibitory effect of NaCl on the transport of the 3 nutrients was not readily reversed. After a pre-treatment with NaCl, uptake of the nutrients remained inhibited even after the NaCl were removed from the nutrient solution.

At equal osmolality of uptake solutions,  $\text{NO}_3^-$  uptake was inhibited more by  $\text{Cl}^-$  than  $\text{SO}_4^{=}$  salts. At equal concentrations,  $\text{SO}_4^{=}$  salts inhibited  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake more than  $\text{Cl}^-$  salts. Both  $\text{K}^+$  and  $\text{Na}^+$  salts inhibited  $\text{NO}_3^-$  and  $\text{NH}_4^+$  similarly, indicating that the transporters are more sensitive to anionic than cationic salinity.

Because the  $\text{Cl}^-$  and  $\text{SO}_4^{=}$  salts had little effect on transport of nutrients until the salt was greater than 100 mM, it seems that the main effect of the salts may be on membrane stability or conformation rather than on specifically inhibiting the active sites of the transporters.

### **Effect of $\text{Ca}^{++}$**

The presence of  $\text{Ca}^{++}$  greatly protected the  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and  $\text{P}_i$  transporters against NaCl injury. In the absence of NaCl,  $\text{Ca}^{++}$  had no effect on the apparent rate constants of uptake. In the presence of NaCl,  $\text{Ca}^{++}$  did not affect the apparent  $K_m$  for uptake but greatly increased the  $V_{\max}$ . The enhancement of uptake by  $\text{Ca}^{++}$  required the constant presence of  $\text{Ca}^{++}$  in the nutrient solution, since  $\text{Ca}^{++}$  had no effect when supplied before or after salinity stress. Although Mn and Mg enhanced uptake under saline conditions, neither was as effective as  $\text{Ca}^{++}$ . In longer-term studies, increasing the  $\text{Ca}^{++}$  concentration in the saline nutrient solutions resulted in increases in  $\text{NO}_3^-$  assimilation and seedling growth. The protection of the nutrient transporters by  $\text{Ca}^{++}$  may also result from stabilization of the cell membranes.

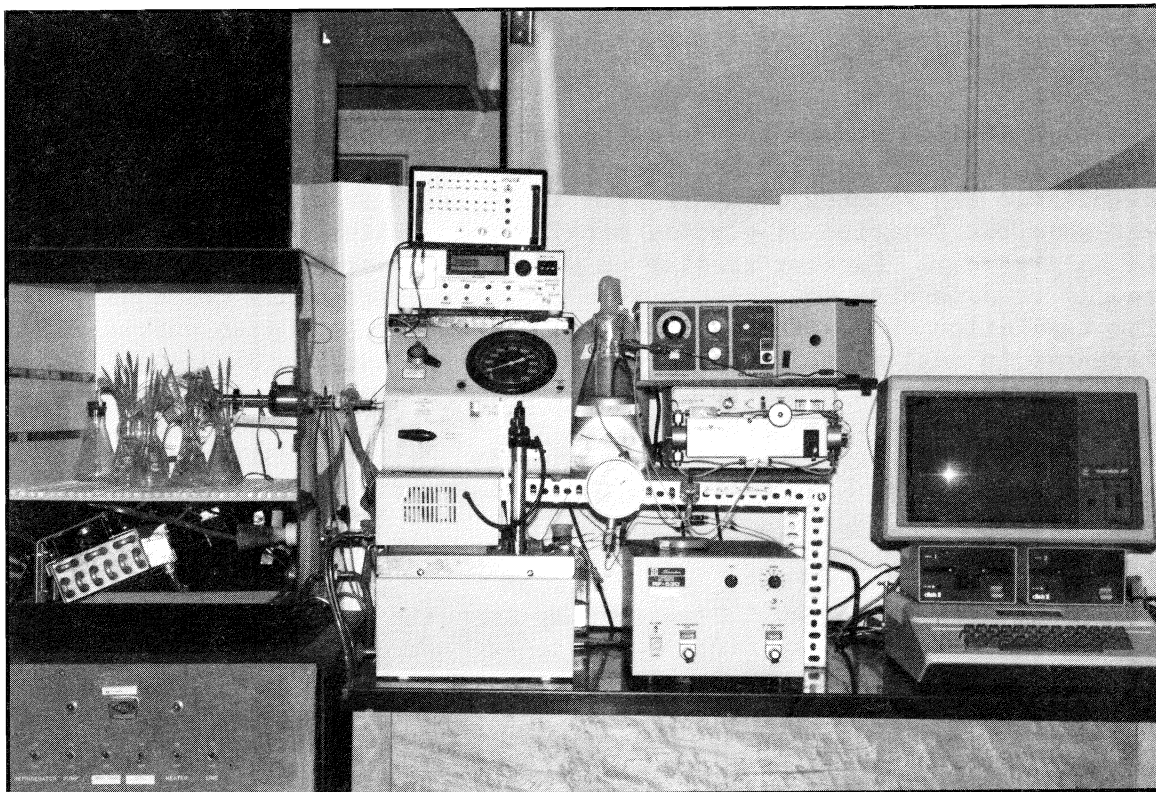
### **Comparative Effects of Salinity on CM 67 and Arivat**

The  $\text{NO}_3^-$  transporter in the roots of barley seedlings slowly adapted to NaCl as a function of salt concentration, and the recovery coincided with the onset of growth. Recovery of  $\text{NO}_3^-$  uptake was tightly correlated to the lowering of plant solute potential. The adaptation was evaluated in 2 barley cultivars differing in response to salinity. CM 67, the more salt-tolerant cultivar, showed a more rapid recovery of  $\text{NO}_3^-$  transport activity after exposure to salt than did Arivat, a less tolerant cultivar.



The accumulation of proline and glycine betaine was also compared in the 2 seedlings to determine whether a correlation existed between the concentration of these 2 compounds and the degree of adaptation to salinity. Betaine accumulation began almost immediately after the plants' exposure to NaCl. At the end of day 36, betaine concentrations were still increasing. This contrasted to proline accumulation by the barley cultivars; after an initial rapid rise of proline, a steady-state concentration was maintained. The steady-state proline concentration was reached within 6 to 8 days.

The relationship between growth and betaine accumulation was ambiguous. Although plants grown at low NaCl concentrations accumulated more fresh weights than those at high NaCl concentrations, the concentration of betaine in both Arivat and CM 67 was independent of the concentration of NaCl in the root media. The solute potential of the plants decreased with increasing NaCl in the root media at NaCl concentrations where growth occurred. There was no obvious correlation between betaine accumulated and solute potential values reached. However, in both CM 67 and Arivat at NaCl concentrations where there was no growth, That is, 200 mM and 175 mM NaCl, respectively, betaine accumulation was much less.



A 16-channel, fully automated, microcomputer-based system for studying ammonium, nitrate, nitrite, and urea transport kinetics in wheat seedlings under saline and nonsaline conditions.

A comparison of betaine concentrations showed no differences between the 2 cultivars at NaCl concentrations where there was growth, although CM 67 accumulated more fresh weight than Arivat at any given NaCl concentration. It is possible that, at the different NaCl concentrations, the partitioning of betaine between the vacuole and the cytoplasm is different. For example, perhaps in plants grown in high NaCl concentrations, more betaine is kept in the cytoplasm than in plants grown at low NaCl concentrations, thus allowing adequate protection of enzyme proteins.

Sodium and chloride uptake was directly related to the NaCl concentrations experienced by Arivat. However, at low NaCl concentrations, chloride was excluded by Arivat plants. A comparison of the cultivars showed there were no differences between chloride and sodium uptake at different NaCl concentrations. Potassium uptake was inhibited by NaCl in the nutrient media in Arivat. When the 2 cultivars were compared, there were no differences between the pattern of potassium uptake of the 2 cultivars. However, a higher K:Na ratio was found in the meristematic portion of the tissues than in older tissues of Arivat at the NaCl concentrations in which the plants survived. High K:Na ratios are thought to be important for normal cell metabolism; therefore, a high ratio in the meristematic parts of plants would ensure conditions necessary for cell division and growth. It may be noteworthy that the NaCl-tolerant cultivar, that is, CM 67, had a higher K:Na ratio in the meristematic portion of the plant than Arivat, and these ratios were maintained at NaCl concentrations where Arivat did not survive.

Our studies showed that proline and glycine betaine accumulated in barley leaves but not roots before the adaptation to salt stress was detected. The results obtained from analysis of intact tissue do not yet show the function of glycine betaine and proline in plants exposed to salt stress. Further studies on cellular compartmentation are needed to determine the osmotic role of these nitrogenous compounds. The regulation of solute potential and growth may be due to subtle differences in cell size, vacuole/cytoplasm ratio, and/or the amount of osmotic partitioned into the vacuole and the cytoplasm rather than to major differences in shifts in metabolism between the 2 cultivars. A difference which may turn out to be important was the ability of CM 67 to maintain higher K:Na ratios in the meristematic portion of the plant than Arivat.

### **Effect of Salinity on $\text{NO}_3^-$ Transport by Bacteria**

Similar to plants,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  transport by Klebsiella was severely inhibited by the presence of NaCl in the growth medium.

### **Effect of Salinity on Metabolism of $\text{NO}_3^-$ , $\text{NH}_4^+$ , and $\text{P}_1$**

Although the uptake of the above compounds was severely inhibited by NaCl, more than 90 percent of the absorbed compounds was further metabolized. The  $\text{NO}_3^-$  reduction was less affected than  $\text{NH}_4^+$  metabolism, probably because most of the  $\text{NO}_3^-$  is reduced in leaves

while  $\text{NH}_4^+$  is metabolized in roots.  $\text{NH}_4^+$  accumulates to much higher concentration in roots of barley during salt stress; however, 90 percent of the absorbed  $\text{NH}_4^+$  is still metabolized. At the same time, glutamine synthetase (GS) in the roots decreases, while glutamate dehydrogenase (GDH) is not affected. It appears that  $\text{NH}_4^+$  is metabolized via GDH rather than GS as salt stress ensues in the roots. The activity of these 2 enzymes in leaves was not affected by salt stress. Although nitrate reductase (NR) was somewhat decreased in the salt-stressed plants, it was still in excess and not rate limiting to  $\text{NO}_3^-$  reduction.

The internal biochemistry of the barley plants is largely protected to allow N metabolism, although small-to-large effects were found for enzymes in N metabolism pathways. Thus, the importance of protecting the transporters is paramount during salt stress.

### SIGNIFICANCE

In summary, we have identified the transport of nutrients as critical to adaptation of barley seedlings to salinity stress. Because the adaptation of the transporters to salinity can be followed, a novel approach is identified to supply basic information concerning changes in the membrane environment or the transporters themselves. Genotypic differences were detected in the ability of barley plants to adapt their root transport systems to salinity. The presence of divalent cations, especially  $\text{Ca}^{++}$ , protected the root transporters during salinity stress. Thus, the symptoms of salinity stress may be partially alleviated by ameliorating the ionic composition of soil solutions and the continued genetic manipulation of agronomically important crop plants.

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#### **SUPPORTING PERSONNEL**

M. Aslam (SRA), A. Sen-Gupta (graduate student), R. Tischner (visiting professor from University of Gottingen), M. R. Ward (graduate student)

## 9. Plant Physiological Responses to Interactions between Salt and Water Stress and N Utilization

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**PRINCIPAL INVESTIGATORS:** V. V. Rendig and F. E. Broadbent

The objectives of these studies were to relate N uptake and utilization by plants to their growth and development under saline conditions.

### **SPLIT-ROOT STUDIES WITH TOMATOES**

One aspect investigated is the N nutrition/growth interrelationship with spatial and temporal differences in the salinity of soil in which plants are growing. For this study, tomato plants were grown in units permitting the root systems to be divided into four sections, each growing in a different compartment (split-root system). Thus, the level of salinity, as well as of individual plant nutrients, could be supplied by differential irrigation of each compartment. With tracers, effects on plant uptake of nutrients and plant composition in each compartment could be determined. Comparisons were made between plants grown in regular containers in some experiments and the 4-compartment units, each containing the same amount of soil and irrigated similarly. Nutrient solutions, with salinity levels ranging from 1 to 10 dS/m, were recycled through the compartments and periodically replenished; composition of the soil solutions was monitored by vacuum extraction with porous cups.

As expected, solution recycling caused salinity levels in the soil solution to increase in all units, but the pattern of the change varied. By the end of the 66-day growing period, extracted solutions from the units with all compartments irrigated with solutions having an initial salinity of 1 dS/m ranged from 9.5 to 10 dS/m; solutions from those irrigated with solutions of 1, 4, 5, and 10 dS/m had 20.1 to 21.6 dS/m. Changes with time in the salinity of the soil solutions, from cultures with undivided root systems and irrigated with solutions with salinity levels comparable to those given the 4-compartment units, were generally similar to changes in solutions taken from the latter units. However, at the end of the 66 days, salinity levels in the extracted soil solution with the undivided root-culture system were near to or slightly above 30 dS/m when the initial level of the applied nutrient solution was greater than 3 dS/m.

Salinity had a much greater effect on decreasing tomato-fruit yields than on shoot or root growth. One or both of 2 possible effects of salinity could account for this response: (1) Interference with production and/or translocation of metabolic products from the shoot and root, or (2) interference with uptake and/or translocation of

needed mineral nutrients. There was a consistent decline in fruit production with increasing salinity, and yields from the plants with undivided roots were consistently lower than those grown in the 4-compartment units. With the undivided root-system culture method, fruit fresh weights and total shoot dry weights were very significantly correlated with both the salinity of the nutrient solution initially applied and the time-averaged salinity of the extracted soil solutions. Neither of the growth parameters was correlated with the salinity of the soil solutions extracted at the end of 66 days. With the split-root method, fruit fresh weights and shoot dry weights correlated significantly and very significantly, respectively, with the initial salinity of the nutrient solutions but not with the salinity levels of the extracted soil solutions. Significant impairment of root growth occurred only on plants grown with their entire root systems in the most highly salinized soil.

To determine effects of salinity on N uptake, the 4 compartments of each culture unit were irrigated with nutrient solutions equally salinized or salinized to different levels and with some solutions containing  $^{15}\text{N}$ . With plants growing in units in which the 4 compartments were irrigated with solutions having initial salinity levels ranging from 1 to 10 dS/m, most (60 percent) of the N was absorbed from the compartment irrigated with the least saline solution. The relative contribution from this compartment decreased with time; the proportion of N provided by the more saline compartments increased. The concentration of  $^{15}\text{N}$  in the recently matured leaves of the plants provided the tagged N in the compartment receiving the least saline solution decreased, possibly because a higher proportion of that absorbed at an early stage of growth was redistributed. Thus, the tagged N moving into the new leaves during the later stages of growth would be diluted by the N entering prior to the time  $^{15}\text{N}$  was included in the nutrient solution. Interpretation of the response would have to take into account the effects of salinity on growth. More adverse effects of salinity on N uptake than on growth or water uptake was indicated when the entire root system was in salinized soil (5.5 dS/m treatment) rather than being split between compartments containing soils receiving the 1, 4, 7 and 10 dS/m salinity treatments.

To evaluate influences of salinity on N nutrition, account should be taken of physiological changes associated with the presence of higher salt levels in the plants. As others have also noted, the nature and level of salinity was found in these studies to affect cation-anion balance. The excess of cations over anions increased with increasing salinity. An estimate of the concentrations of organic anions, calculated from the amounts of soluble and insoluble Ca and Mg (Kirkby and Mengel method), indicated that the "bound" organic anions (uronates and oxalates) increased, while the soluble anion fraction (malate and citrate) decreased with increasing salinity. Through their presence as solute anions and their metabolism, the latter forms have been implicated in N nutrition. Organic anions, in particular malate and citrate, are associated with the alkaline earth cations and thus function in their translocation. They also provide substrate for cellular energy needs and by being metabolized to bicarbonate ions can exchange for the entry of nitrate into root cells.

Apparatus for imposing  
split-root treatments.



#### HYDROPONIC STUDIES WITH TOMATOES

Plant growth/N nutrition interrelationships were also investigated using hydroponic techniques. Two levels of N and 3 levels of salinity (unsalinized nutrient solution, 5 dS/m (SAR=6.35), 10 dS/m (SAR=9.45)) were provided to tomato plants grown in a greenhouse, with solutions changed frequently to maintain salinity levels,  $\pm$  about 10 percent. Plants were harvested 31 and 36 days after transplanting, separating the leaves from the stems. During the final 3 days of growth,  $^{15}\text{N}$  was included in the nutrient solution. The dried tissue was analyzed for total N, nitrate-N and Cl.

Concentrations of nitrate-N in different parts of the plants was affected differently by the nutrient solution's salinity, and the relationship also depended upon the N level. In the stems of plants grown at the 5 dS/m salinity level, nitrate concentrations were lower than with the unsalinized nutrient solution, but at the higher salinity level (10 dS/m) stem-nitrate concentrations were between those found in plants grown with the unsalinized nutrient solution and the

lower (5 dS/m) salinity level. This relationship between salinity and nitrate concentrations was reversed in the lower leaves. The pattern of the response in these leaves was similar to that shown by the occurrence of  $^{15}\text{N}$  that had been supplied in the nutrient solution during the 3 days before harvest. Salinity had the least effect on the nitrate concentrations in the upper leaves. In contrast to the relationship between salinity and nitrate in the stems, greatest effect of saline conditions on the concentrations of organic N was found in the upper leaves, with no significant response shown in either the lower leaves or the stems. Total uptake of N into the shoots was not significantly affected by salinity.

### **SALINITY/K INTERACTION EFFECTS ON N NUTRITION OF CORN**

Another series of experiments involved growth of corn in containers with a sandy loam soil irrigated daily or more frequently with a complete nutrient solution, or with the nutrient solution salinized to 2 levels (2.8 and 4.3 dS/m) with a mixture of  $\text{CaCl}_2$  and  $\text{NaCl}$  (constant SAR 2.35). Two levels (1.5 and 5.0 mM) of K were provided, and  $^{15}\text{N}$  was included in the nutrient solution during growth subsequent to anthesis. Neither salinity nor K levels had significant effects on corn growth. With increasing salinity the proportion of N absorbed in the postanthesis growth period increased. Effects of K on the utilization and/or translocation of N between plant parts were also indicated. At the lower K level a higher proportion of the N in the grain was provided by absorption in the postanthesis growth period. Interactions between N and K also had been indicated in earlier growth chamber studies of effects of salinity stress on corn plants during the vegetative stage of growth.

### **SIGNIFICANCE**

For plants growing in soils in which salts are not uniformly distributed, for example, with a drip system for water application, accessibility of N depends on root activity in the less salinized soil zones. The extent to which salinity stress will influence nitrogen nutrition depends upon the interplay of salt effects on growth and development, and on metabolic reactions associated with the amounts and kinds of cations and anions present.

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#### **SUPPORTING PERSONNEL**

Ioannis Papadopoulos (graduate student), Curt Grossman (graduate student)



# III. Microorganisms and Soil Salinity

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## 10. Influence of Salinity and Sodicity on Transformations of N, S, and P in Soils

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**PRINCIPAL INVESTIGATOR:** F. E. Broadbent

In spite of substantial research on the influences of saline and sodic soils on crop plant growth, little has been done to investigate their effects on soil microorganisms. Of particular interest are the influences of salinity and sodicity on the microbial transformations of the important nutrient elements, nitrogen, phosphorus, and sulfur. Our research objectives were: (1) To measure rates of mineralization of organic forms of N, P, and S at various levels of salinity, and as influenced by exchangeable sodium percentage at given salinity levels; (2) to determine the effects of salinity and sodicity on enzyme kinetics of urease, phosphatase, and sulfatase in soils; (3) to estimate threshold concentrations of salts for inhibition of microbially induced mineralization processes in soils.

### EXPERIMENTAL PROCEDURES

To differentiate between biological activity of living microorganisms and that of cell-free enzymes, which are thought to be stabilized by adsorption to the soil matrix, it is desirable to extract soil enzymes and measure their activities in the absence of intact cells. A part of the work involved developing a method for extracting soil phosphatase and freeing it from the humic substances to which it is strongly bound. This was successfully accomplished by using electron-donating reagents, such as tetramethyl-p-phenylenediamine dihydrochloride (TMePDA), which substitute for the humic substances in the enzyme-humic complex and release the phosphatase.

For investigation of salinity effects on microbial processes in soil systems, soils were salinized with NaCl or CaCl<sub>2</sub> in different ways. One method involved leaching soil columns with 0.1 M NaCl for varying periods. Sectioning the column into 10-cm segments provided soil samples covering a range of values of electrical conductivity and exchangeable sodium percentage. A second method involved adding known amounts of salt to a given quantity of soil, followed by incubation and measurement of the type of activity in question. A third method involved collecting soil samples along a transect of the salinity field trial at the West Side Field Station, where salinity gradients have been imposed through application of saline water through a line-source sprinkler system.

## GENERAL RESULTS

### N-Mineralization

Measurement of nitrogen mineralized from ground alfalfa in column-salinized Diablo clay for 10 days showed that nitrogen release was 35 percent greater in a sample at 48.3 percent sodium than in one at 6.4 percent sodium.

### Nitrification

Rates of nitrification were not significantly different in soil samples collected along a transect at the West Side Field Station. These represented electrical conductivity values ranging from 2.1 to 6.6 dS/m and from 4.5 to 16.6 percent sodium.

### Urease Activity

Measurement of urease activity in the same series of West Side samples, as were used for the nitrification experiment previously discussed, showed a slight downward trend in the EC range between 3 and 7. On the other hand, urease activity in column-salinized Diablo clay was considerably enhanced by salinization, with an approximately linear relationship between level of urease activity and a percentage of Na in the soil.

In experiments involving addition of NaCl solutions to soil, determination of the urease parameters  $K_m$  and  $V_{max}$  indicated increasing affinity of urease for its substrate at high salt concentrations.

### Sulfatase Activity

Measurement of sulfatase activity in column-salinized soil samples showed an approximately 30 percent increase of activity from the lowest to the highest percentage of Na. Addition of NaCl solutions to Diablo clay to provide a range of salt concentrations from 0 to 1800 mM produced only insignificant effects on sulfatase activity at lower salt concentrations, but up to 55 percent inhibition was observed at 1800 mM. A comparable experiment with  $CaCl_2$  instead of NaCl had a similar effect, but a lesser degree of inhibition at the higher salt concentrations. At an osmotic potential comparable to 1800 mM NaCl, inhibition of sulfatase activity by  $CaCl_2$  was only 24 percent.

### Phosphatase Activity

In column-salinized soil treated for varying periods of leaching with NaCl ranging from 2 to 34 days, 2 days of salinization produced about a 10 percent increase in phosphatase activity, but with longer periods of salt treatment phosphatase activity decreased progressively.

The maximum inhibition observed, after 34 days of salinization, was about 45 percent. Attempts to relate phosphorus in microbial biomass to phosphatase activity in these soil samples showed a positive correlation only in samples salinized 34 days.

No significant effects of salinization in the West Side transect series (percent of Na from 4.5 to 16.6; EC from 2.1 to 6.6) on phosphatase activity were found. Addition of NaCl solutions to Diablo clay in concentrations up to 1800 mM produced progressive inhibition of phosphatase activity, reaching 65 percent at the 1800 mM level. Similar results were obtained with  $\text{CaCl}_2$  solutions, which produced 52 percent inhibition of phosphatase activity at 1047 mM. Measurement of the influence of added salt on phosphatase activity of humic substances extracted from soil and of purified supernatant from humic extracts showed only small decreases in phosphatase activity over the range from 0 to 1800 mM NaCl.

### **Nature of Salt Effects**

If salts act directly on the enzyme by some mode of action inducing changes in molecular configuration, for example, through coiling or uncoiling of proteins, consistent effects of salinity on enzyme activity would be expected. On the other hand, if the influence of salts is secondary in character, salinity might either enhance or inhibit enzyme action, as was observed in the soil system. It is hypothesized that a pH shift in the diffuse double layer resulting from addition of salts may account for a substantial part of the observed effects of NaCl and  $\text{CaCl}_2$  on activity of phosphatase and sulfatase. Whether enhancement or decrease in enzyme activity accompanies salt addition depends on whether the pH is shifted toward or away from the optimum pH.

### **SIGNIFICANCE**

In practical terms, the most important finding of this work is that in field soils the microbiological processes measured were not significantly altered at EC and percent of Na values which nevertheless produced substantial depression in crop plant growth. In other words, soil microorganisms appear to be more salt tolerant than are higher plants. Another contribution of the research is the development of a procedure for obtaining phosphatase free of clay minerals and released from complexes with humic substances. This procedure, with some modification, may be applicable to other soil enzymes as well. A rapid method for estimating soil urease activity by conductimetric analysis was also developed.

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#### **SUPPORTING PERSONNEL**

U. Gosewinkel (graduate student)

## 11. Microbial Nutrient Transformations in Saline Soils and Adaptation of Microorganisms to Soil Salinity

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**PRINCIPAL INVESTIGATOR:** M. K. Firestone

The availability of soil nutrients to plants largely depends on numerous microbial nutrient transformations. Effects of soil salinity on microbial processes in soil will thus influence soil fertility. Some aquatic microorganisms are highly adapted to saline (even hypersaline) conditions. Thus, it is reasonable to expect that microbes indigenous to saline soils may be adapted to saline conditions. Understanding the physiological basis of microbial adaptation to soil salinity may provide knowledge useful in developing agricultural systems with greater tolerance to salinity. Determination of the degree to which soil salinity affects microbial nutrient transformations will provide better understanding of how nutrient supply must be managed in saline soils.

This project's 2 main objectives were: (1) to determine whether microorganisms indigenous to saline soils are specifically adapted for growth at salt concentrations (or fluctuations in concentration) characteristic of these soils, and (2) to learn whether rates of microbial nutrient transformations are affected by salinity or by fluctuations in salinity which occur during normal wetting/drying cycles in saline soils, thus influencing nutrient availability to plants.

### **Experimental Approach**

Two general types of research protocol were employed. The more basic research involved isolation and physiological characterization of bacteria from saline soils. The second general approach utilized assays of nutrient transformations in soils exposed to varying salinities and water potential fluctuations.

Surface soils of varying salinities (0.9 to 59 dS/m) were sampled and salt-tolerant bacteria were isolated on selective media. Major emphasis was placed on isolation and characterization of actinomycetes from saline soils, since these organisms are reportedly important in soils of high alkalinity and prolonged drought. The physiological strategies utilized by these filamentous bacteria to tolerate high salinities were determined by identifying solutes accumulating inside of cells of 2 species of *Streptomyces* growing in the presence of varying concentrations of salt. Growth efficiencies of salt-stressed *Streptomyces griseus*, growing in the presence and absence of the compatible solute proline, were used to quantify the metabolic cost of salt stress in these organisms.

To assess the adaptation of nitrifying organisms to soil salinity, nitrification activity was assayed in 4 soils sampled during winter, spring, summer, and fall. Two saline and two nonsaline soils were tested for nitrification activity under conditions of varying salinity. The tolerance of the nitrifying populations in each soil was indicated by the relative inhibition of nitrification occurring with increasing salinity of the assay solution. Specific ion effects were determined by comparing the effects of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Mg}^{++}$ .

The effects of water-potential fluctuation on nutrient availability were investigated by determining the effect of water-potential upshocks (wetting events) on the soil microbial biomass. This was done by comparing the carbon released when a soil was wet with water, when a soil was wet with a KCl solution (maintaining a constant total soil-water potential), and when the total biomass carbon was released by chloroform fumigation.

## RESULTS

### Adaptation of Organisms Indigenous to Saline Soils

Two species of Streptomyces (S. griseus and S. californicus), isolated from saline and saline-sodic soils, were selected to assess physiological response to salinity. NaCl was more inhibitory to growth rates and specific growth yields than were equivalent concentrations of KCl. Both species effectively excluded  $\text{Na}^+$  and concentrated  $\text{K}^+$ . Streptomyces griseus was less sensitive to salt stress than was S. californicus, which is consistent with the soil distribution of the 2 Streptomycetes. Intracellular concentrations of the free amino acid pool increased similarly in response to NaCl- and KCl-salt stress. While the neutral free amino acids proline, glutamine, and alanine accumulated as salt concentration increased, concentrations of the acidic-free amino acids glutamate and aspartate were reduced. Above a salinity threshold of about 0.75 M (-3.8 MPa), there was little further intracellular accumulation of free amino acids, while accumulation of  $\text{K}^+$  salts increased sharply.

Uptake of exogenous  $^{14}\text{C}$ -proline and internal synthesis of proline were quantified in cells growing at salt concentrations from 0 to 1 M NaCl. Externally supplied proline accounted for an increased proportion of the intracellular pool of free proline as salt concentration was increased, but neither the concentration nor the composition of the internal amino acid pool was substantially altered by supply of exogenous proline. Uptake of exogenous proline significantly increased the specific growth yield of S. griseus growing under salt stress; the increased yield was proportional to reductions in proline synthesis.

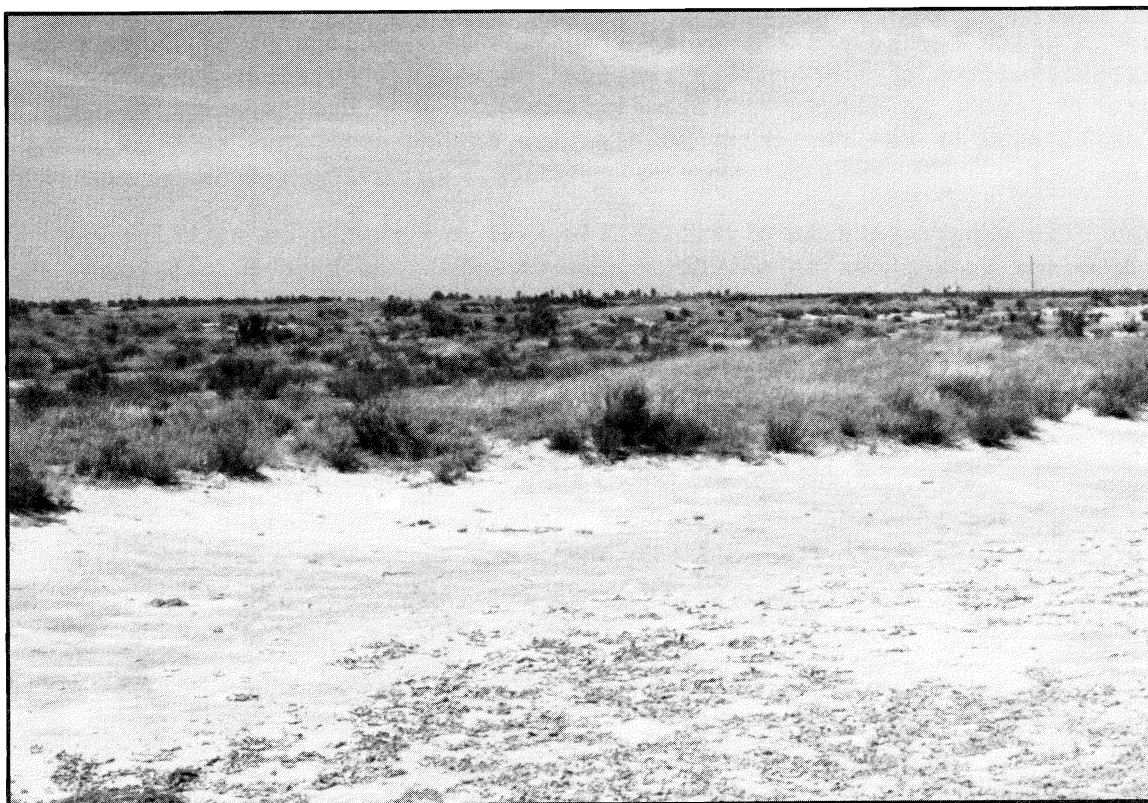
In summary, in both the Streptomyces spp. and the Pseudomonas spp. studied (results from Pseudomonas not discussed) there appears to be a physiological limit to the concentration of free amino acids used as osmotic solutes. Both types of bacteria strictly exclude  $\text{Na}^+$  and con-



concentrate  $K^+$ . The physiological response to salt stress has a definite energy cost resulting in part from the cost of synthesis of compatible solutes; the energy cost results in reduced growth efficiencies of the bacteria.

### **Effects on Microbial Nutrient Transformations**

Increasing salinity decreased rates of nitrification in all soils examined. However, salinity was less inhibitory to nitrification activity in the two saline soils tested than in the 2 nonsaline soils. There were no detectable differences in sensitivity to salinity in soils sampled at different times during the year. No specific ion toxicity resulting from  $Mg^{++}$  could be detected. In the naturally saline soils,  $K^+$  was generally more inhibitory to nitrification than was  $Na^+$ . These data indicate that nitrifiers indigenous to saline soils are more adapted to salinity (and  $Na^+$  toxicity) than those from nonsaline soils.



A "natural" saline site from which soil samples were taken for microbial studies.

Water-potential upshocks of 2.8 and 6.9 MPa had major impact on the soil microbial biomass. Biomass carbon was released by water upshocks, ranging from 17 to 70 percent of the total soil biomass, depending on the soil, the magnitude of upshock, and the method of calculation. A greater proportion of biomass carbon was released following a 6.9 MPa than a 2.8 MPa upshock. Biomass carbon release was measured both by a modified chloroform-fumigation method and by quantifying increases in soluble organic carbon in leachates of upshocked soils. Mineralization of biomass mobilized by water-potential upshock exceeded mineralization of biomass-carbon made available by preceding desiccation, thereby comprising a significant component of the pulse of respiration observed following wetting of dry soil. Water-potential upshocks associated with the wetting of dry soil appear to be a major catalyst for the release of nutrients present in soil as microbial biomass.

### **SIGNIFICANCE**

Our work on the physiological response of soil bacteria to salinity indicates that while microorganisms utilize a variety of strategies to adapt to soil salinity, the physiological mechanisms utilized by organisms that successfully tolerate soil salinity have real energy costs which reduce efficiency of growth. The development of heterotrophic microbial communities required to mediate nutrient transformations, such as nitrogen mineralization and urea hydrolysis, may be facilitated by the supply of energy-rich carbon compounds such as plant residues.

The experiments on nitrification indicate that even nitrifying organisms indigenous to saline soils are inhibited by the salt concentrations present in those soils. However, nitrifying populations from saline soils are significantly less sensitive to salinity (and  $\text{Na}^+$  toxicity) than nitrifiers from nonsaline soils. Nitrification in agriculturally saline soils should be expected to proceed if the soils have experienced saline conditions for long enough for adaptation of the microbial flora to occur.

Wetting of a dry soil causes a pulse of mineralization of nutrients tied up in the soil microbial biomass. Thus, drying-wetting cycles should enhance soil-nutrient availability (particularly N, S and P) to plants.

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#### **SUPPORTING PERSONNEL**

Tom Jones (graduate research assistant), Kwok Fong (SRA), Ariel Herrera (work-study undergraduate student), Tom Kieft (visiting microbiologist), Ken Killham (visiting microbiologist), Jean Larsen (graduate research assistant), Edith Soroker (graduate research assistant)



# IV. Boron

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## 12. Boron Interactions in Saline Soils as Affected by Sodium, Calcium, and Magnesium

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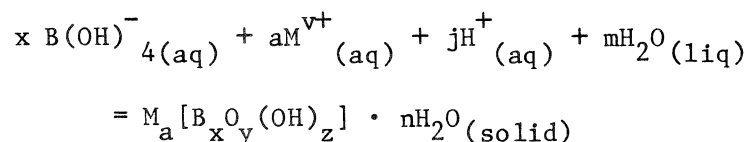
**PRINCIPAL INVESTIGATOR:** Shas V. Mattigod

In soils, the limits between essentiality and toxicity of boron for plants are rather low. Two recognized types of boron interactions in soils are exchange-adsorption and dissolution-precipitation of solids. Despite some evidence that dissolution-precipitation reactions of boron in soils do occur, the principal focus of innumerable studies has been the exchange-adsorption reactions. Therefore, one objective of this investigation was to develop a method for estimating the solubility of boron minerals that could lead to identifying alkali and alkaline earth boron minerals of limited solubility that may occur in saline soils. It has been established that alkaline earth cations form ion pairs with borate ion. However, the influence of these ion pairs on boron adsorption by soil constituents has not been studied. Therefore, the second objective was to examine the influence of ion pairs on boron adsorption to increase understanding of one of the important aspects of boron interactions in saline soils.

### PROCEDURES AND RESULTS

#### Borate Minerals

An empirical method was developed to estimate the  $\Delta G_f^0$  of hydrated borate minerals. It was found that for a reaction involving the formation of a hydrated borate mineral:



The free energy of reaction could be estimated by the following expression:

$$\begin{aligned} \Delta G_{r,209}^0 (\text{kJ/mol}) &= 124.26 - 82.37x + 2.95x^2 \\ r^2 &= 0.991 \end{aligned}$$

where  $x$  = number of boron atoms in the polyanion unit.

It was found that  $\Delta G^0_f$  of borate minerals could be estimated within  $\pm 10$  kJ of the experimental values. Using this method it was estimated that the boron minerals szaibelyite, ulexite, inderborite, and fabianite have limited solubilities and therefore may exist in saline soils.

### **Effect of Ion-Pair Formation on Boron Adsorption**

A series of experiments were conducted with well-characterized kaolinite as the adsorption surface. Initial boron concentrations between 2 and 10 mg/l and pH values between 6.0 and 10.5 were used in the Se experiments. Ionic strength was maintained at  $0.09 \pm 0.01$  using  $KClO_4$  or  $Ca(ClO_4)_2$  as background electrolyte. The kaolinite sample was pretreated to remove any surface oxide and hydroxide coatings.

Adsorption of boron in either medium showed similar dependence on pH and initial boron concentration in that maximum boron adsorption occurred between pH 8.5 and 9.0. Higher initial boron concentrations led to higher adsorption. At the same initial boron concentration, at pH values below 8.5, there was no significant difference between B adsorption in either medium. However, at higher pH values, B adsorption in  $Ca(ClO_4)_2$  medium was significantly enhanced. This was attributed to the fact that  $CaB(OH)_4^+$  ion pair contributed a significant fraction of soluble B. Therefore, in saline soils, the dominant cation would have a significant influence on B retained. Presence of significant concentrations of Ca and Mg would enhance B retention in saline soils.

### **SIGNIFICANCE**

The practical implication of the existence of boron-solid phases in saline soils is that complete reclamation of soils by leaching would be impossible to achieve and that continuous leaching needs to be maintained to keep solution levels of boron below toxic levels. Furthermore, these studies indicate that two important aspects of B interactions that need examination are the potential presence of B-solid phases of limited solubility and the enhanced adsorption of B at higher pH values due to Ca and Mg ion pairs with  $B(OH)_4^-$  ions.

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## **SUPPORTING PERSONNEL**

J. Frampton (postdoctoral researcher), C. H. Lim (postdoctoral researcher), J. Ervin (SRA)

## 13. Boron Reclamation and Regeneration

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**PRINCIPAL INVESTIGATORS:** F. T. Bingham and J. D. Rhoades

Before natively high boron (B) soils can be successfully used for agriculture, their soluble B contents must be reduced to nonphytotoxic levels. This is normally done by leaching the soil with low-B water. Soil B dissolves or desorbs and is transported below the root zone in drainage water. Dr. G. J. Hoffman, U.S. Water Management Laboratory, Fresno, developed an empirical equation to predict the soluble-B status of soils during reclamation; however, the soluble-B concentrations in reclaimed soils may not be stable. Dr. J. D. Rhoades and co-workers at the U.S. Salinity Laboratory, Riverside, found that soluble-B concentrations increased with time following the reclamation of high-B soils in the laboratory. They called this phenomenon "boron regeneration." A research team, led by Dr. F. T. Bingham, University of California, Riverside, reported evidence for B regeneration in a high-B soil reclaimed in the field; however, the observed increase in soluble B was not considered agronomically significant. Published research on B regeneration is limited to these 2 studies.

The continuing problem of B phototoxicity encountered by San Joaquin Valley growers prompted us to examine the phenomenon in laboratory column and batch experiments at UC Riverside.

### EXPERIMENTAL PROCEDURES

Seven high-B soils were used in the study. Four were natively high in B; 3 were artificially boronated with orthoboric acid. All soils were collected from inland desert valleys of California (San Joaquin, San Jacinto, and Imperial).

Subsamples of the soils were reclaimed in columns using several different leaching regimes. Calcium nitrate [ $\text{Ca}(\text{NO}_3)_2$ ] solution was used for leaching so that changes in native chloride ( $\text{Cl}^-$ ) levels could be monitored. The reclaimed soil columns were equilibrated for 30 days to permit B regeneration and were then releached.

Additional soil subsamples were reclaimed using a batch extraction system. The soils were shaken with  $\text{Ca}(\text{NO}_3)_2$  solution and centrifuged. The supernatants were removed, fresh  $\text{Ca}(\text{NO}_3)_2$  solution was added, and the extraction procedure was repeated until the soils were apparently reclaimed of B. The soils were then equilibrated for 30 days and re-extracted.

A third set of soil subsamples was reclaimed by reaction with an



organic resin that had a very high selective affinity for B. By separating the resin from the soils after known reaction times, the relative degree of B reclamation could be controlled. The resin-reclaimed soils were then reacted with deionized water and the release of B was monitored. The rate constants for B release were calculated using the Elovich reaction-rate equation.



Atomic adsorption spectrophotometer being used for analysis of trace elements.

## RESULTS

Results from the column-reclamation experiments indicated that soluble-B regeneration occurred, regardless of the leaching regime, the postreclamation equilibration conditions, or the native or artificial source of the B. In some cases B concentrations increased to levels that would be potentially phytotoxic to the cotton and sorghum crops commonly grown on these soils. Although B regeneration occurred in both natively high-B and B-amended soils, the data indicated that B which had been recently added to soil was more efficiently leached. In addition, the relative B increases due to regeneration were less in the B-amended soils.

The column experiment results suggested that each unreclaimed soil contained a finite concentration of reclaimable B. The regenerative ability of each soil diminished with depletion of the reclaimable B

component. Soils taken through a series of consecutive leaching and regeneration cycles eventually lost their ability to regenerate appreciable levels of soluble B. These observations indicated that B regeneration should be of greatest agronomic concern during the early stages of soil reclamation. Reclamation conditions that inhibited solid-liquid interactions, such as short contact time or incomplete leaching, promoted regeneration. On the other hand, regeneration was reduced by additional leaching and by low-moisture contents. The data also indicated that reclamation efficiency, defined as the amount of B removed per-unit depth of infiltrated reclamation water, was enhanced by allowing soluble B to regenerate between leachings.

The column and batch experiments were designed to demonstrate qualitatively the existence of several physico-chemical mechanisms for B regeneration. The sources of native  $\text{Cl}^-$  in the soils were found at much higher concentrations (5 to 32 times) and more readily soluble states than were sources of native B. One hundred percent of the  $\text{Cl}^-$  could be removed by consecutive batch extractions, while B could not be completely eliminated. Boron regeneration occurred in the batch-reclaimed soils;  $\text{Cl}^-$  did not. This evidence indicated that a mechanism for B regeneration was postreclamation dissolution or desorption of B sources that were resistant to leaching during reclamation. In the column systems the  $\text{Cl}^-$  concentrations in the column effluents rapidly dropped to nondetectable levels. Boron was always present in the column effluents. Both  $\text{Cl}^-$  and B regenerated in the columns, indicating that portions of the soil were being bypassed by the leaching water during reclamation. Subsequent diffusion of  $\text{Cl}^-$  and B from bypassed portions to leachable portions accounted in part for the regeneration phenomenon. The simultaneous presence of potentially regenerable sources of B in the leached-soil volumes (that is, the dissolution mechanism mentioned above) is also suggested by the column data. Chloride located in the bypassed soil regions appeared to be effectively isolated from the leaching solution. It is reasonable to presume that B in the bypassed regions was also isolated. Since B was always present in the column effluents, the contribution of a source of regenerable B outside of the bypassed soil volume was indicated.

The four natively high-B soils that were reacted with the B-specific resin for 25 hours were operationally defined as 100 percent reclaimed. Intermediate reaction times were used to produce soil subsamples that were zero to 85 percent reclaimed. The differentially reclaimed subsamples were then reacted with water and release of B was measured as a function of time. The data were analyzed using the Elovich reaction rate equation, which has the form

$$dq/dt = \alpha \exp(-\beta q)$$

where  $q$  is the amount of substance in solution at time  $t$ , and  $\alpha$  and  $\beta$  are rate constants during any one experiment. The equation indicates that the instantaneous rate of reaction is reduced by low-positive values of  $\alpha$  or high-positive values of  $\beta$ . For each of the four natively high-B soils tested, the calculated values of  $\alpha$  and  $\beta$  de-

creased and increased, respectively, as the relative degree of reclamation increased. These experimental results confirmed that the rate of postreclamation B dissolution (one of two mechanisms for B regeneration) was inversely related to the amount of B removed by reclamation processes. The potential for redevelopment of phytotoxic soluble-B concentrations will therefore be greatest during the early stages of reclamation of high-B soils. Both the rate and equilibrium concentrations of regenerated B will decline as the soils are progressively reclaimed by continued leaching events.

### **SIGNIFICANCE**

Results of the research program conducted at the University of California, Riverside, indicated that B regeneration is caused by at least two mechanisms: dissolution/desorption of solid-B sources resistant to leaching and redistribution of B from bypassed to leachable portions of reclaimed soil.

Reclamation conditions that inhibit leaching solution-soil interaction will promote B regeneration. Natively high B soils are less efficiently reclaimed and regenerate relatively more B than recently boronated soils. The rate and equilibrium concentrations of regenerated B will decline as high B soils are progressively reclaimed by continued leaching. The potential for redevelopment of phytotoxic soluble B concentrations is greatest during the early stages of soil reclamation.

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### **SUPPORTING PERSONNEL**

F. J. Peryea (graduate student)

## 14. Mechanisms of Boron Toxicity in California Crop-Plant Metabolism

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**PRINCIPAL INVESTIGATORS:** Carol J. Lovatt and W. M. Dugger

While good agricultural land is continually removed from crop production, existing cultivated land is taxed at increasingly higher rates. These 2 situations necessitate increased crop production per acre or development of agriculturally marginal land. Intensive cultivation of existing tillable soil and development of arid and semiarid land for crop production are both limited by the availability of good quality water. Many arid and semiarid regions of the world have soil and irrigation water with levels of boron too high to grow tree and herbaceous crops successfully. Excess boron in these areas is due to high levels of boron in soil or in irrigation water. Toxic levels of boron are very often found in saline soils as boron moves more slowly than NaCl and the removal of boron requires 3 times more water than the removal of NaCl. Approximately 1.9 million acres of agricultural land in the San Joaquin Valley have perched groundwater tables at depths of 0 to 10 feet. More than 3 million acres have reduced yield from salinity buildup and accumulation in the soil of other toxic elements including boron. Boron toxicity is also a problem in the Imperial Valley and inland desert areas due to the poor quality of water from local wells and from the Colorado River.

Boron has been demonstrated to be an essential micronutrient for a large number of vascular plant species. In spite of the diversity among higher plants, there is a small range between levels of soil boron causing deficiency and toxicity symptoms in plants. Thus, the range in boron concentration in the soil or culture medium that is optimal for growth is very narrow, approximately 0.01 to 4.0 mg B l<sup>-1</sup>.

In vascular plants, boron uptake is not regulated; it is carried passively in the transpiration stream and accumulates where the transpiration stream ends. Because boron is relatively immobile in the phloem, very little accumulating boron moves out of these tissues. For these reasons, leaves usually exhibit the first visible symptoms of boron toxicity: yellowing of the leaf tip, with chlorosis subsequently progressing along the leaf margin and then spreading into the blade. Necrosis of the chlorotic tissue follows, then leaf abscission. Thus, boron toxicity results in a loss of plant productivity. The rate at which boron accumulates to a toxic level in leaf tissue is a function of the concentration of boron to which the roots are exposed, the length of the exposure, and the rate of transpiration.

While there are many reports in the literature relating the development of leaf symptoms to elevated levels of boron in leaves, there is very little information regarding the actual manner in which boron is toxic to plants. Therefore, our experimental approach was to screen

major metabolic pathways to determine their sensitivity to increasing concentrations of boron from optimal to toxic. The metabolic events examined include: (1) DNA, RNA, and protein synthesis; (2) stomatal conductance, transpiration, CO<sub>2</sub> fixation, and chlorophyll, starch, and glucose content; (3) de novo synthesis, salvage, and catabolism of pyrimidine and purine nucleotides; (4) phosphoribosyl-1-pyrophosphate availability; (5) activity and activation of phosphoglucomutase which catalyzes the interconversion of glucose 1-P and glucose 6-P; (6) in vivo synthesis of cell wall glucans; (7) activity of UDP-glucose pyrophosphorylase which catalyzes the synthesis of UDP-glucose.

Objectives 1 through 4 were examined using squash plants (Cucurbita pepo L. cv. Early Prolific Straightneck) cultured hydroponically in the presence of various concentrations of boron.

Since boron toxicity is a function of the concentration of available boron and the length of exposure to boron, our approach was to induce boron toxicity in hydroponically cultured squash plants in a relatively short period of time by adding higher concentrations of boron to the hydroponic nutrient solution than are normally encountered in the field. The classical early symptoms of boron toxicity include chlorosis of the leaf tip eventually spreading to the leaf margin and later to the entire blade; eventually, the yellow areas become necrotic. In this system, the symptoms of boron toxicity are first evident 96 hours after 5-day-old summer squash plants are transferred to nutrient solutions containing 40 mg B l<sup>-1</sup>. The validity of this approach was confirmed by mineral nutrient analysis of the leaves of the hydroponically grown squash plants. Initial symptoms of boron toxicity were first visible when the level of boron in the leaves was 750 µg B g<sup>-1</sup>. This compares favorably with levels of boron reported in the leaves of summer squash plants (Cucurbita pepo L., cv. Black Zucchini) grown for 2 to 3 weeks at concentrations of only 0.2 to 4 mg B l<sup>-1</sup> where no visible symptoms of boron toxicity were observed in plants with leaf levels of boron up to 693 µg B g<sup>-1</sup>; severe symptoms of boron toxicity were evident in plants with leaf levels of 895 µg B g<sup>-1</sup>.

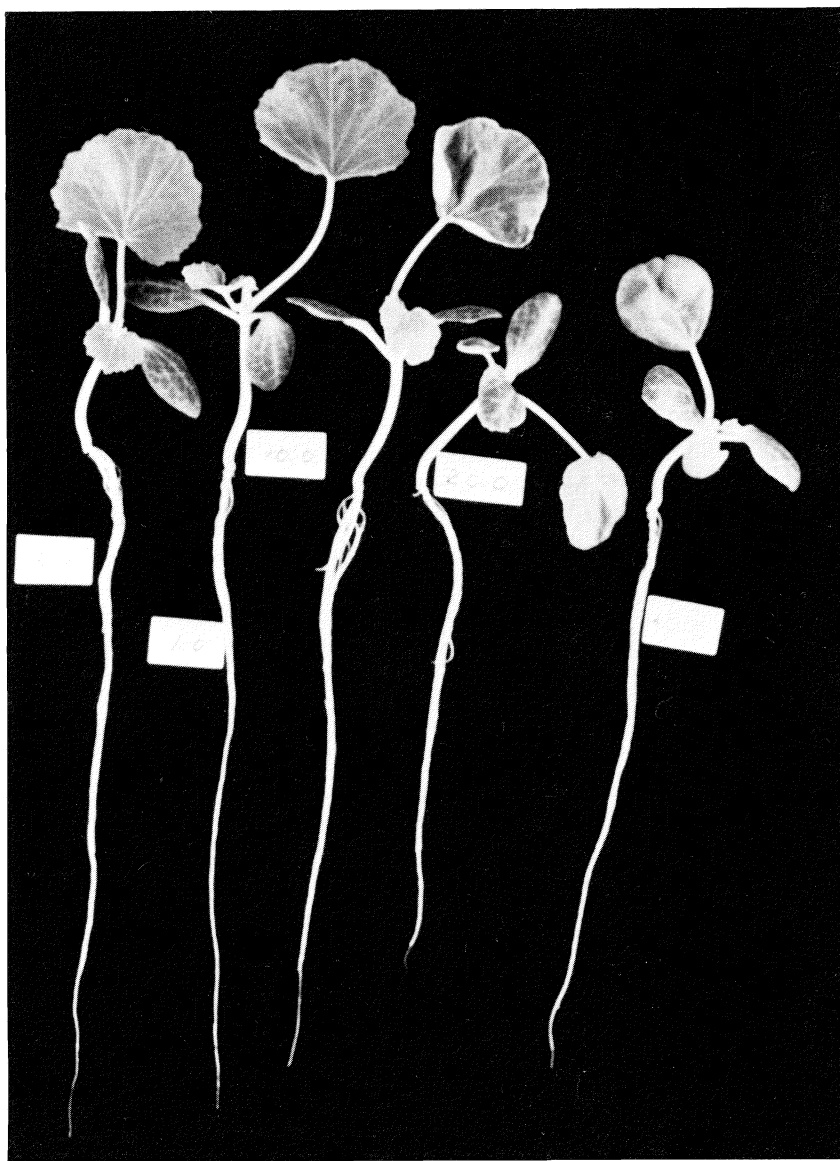
Objectives 5 through 7 were determined using cotton ovules (Gossypium hirsutum L. Acala SJ1) cultured in vitro at concentrations of boron that resulted in deficiency, optimal fiber growth, and toxicity.

## INFLUENCE OF EXCESS BORON ON SQUASH PLANT GROWTH AND METABOLIC PATHWAYS

### Plant Growth

A possible key to determining the mechanism(s) of boron toxicity is to determine its earliest symptom, since it is the one most likely to be associated with the primary effect that toxic levels of boron have on the metabolism of the plant. One of the earliest effects of boron toxic-

city was a reduction in the rate of root elongation and total root production. When 5-day-old squash plants were transferred to media containing excess boron, the rate of root elongation decreased with increasing length of exposure to boron and increasing concentrations of boron. By 72 h of boron excess, root elongation had essentially ceased for plants grown at 20 or 40 mg B l<sup>-1</sup>. It is not known whether inhibition of root growth was due to the concentration of boron in the total roots (370 µg g<sup>-1</sup> dry weight) or the amount of boron in the oldest leaf (650 µg l<sup>-1</sup> dry weight). In either case, shoot growth was less sensitive to excess boron than root growth since inhibition of shoot growth occurred only in plants grown for 72 h at 40 mg B l<sup>-1</sup>. Inhibition of both root and shoot growth occurred prior to the appearance of any visible symptoms of boron toxicity in the leaves.



Effect of increasing boron concentration on the growth of 5-day-old summer squash plants (Cucurbita pepo) at the end of 96 h. Boron ranged from 0.1 to 40 mg/L.

## DNA and Protein Synthesis

DNA synthesis in shoot apices was inhibited 50 and 70 percent after 24 h of 20 mg B l<sup>-1</sup> and 40 mg B l<sup>-1</sup> treatment, respectively. The loss in capacity to synthesize DNA parallels the decrease in available glucose in this tissue. Since neither protein content nor protein synthesis was affected by boron accumulation even after 72 and 120 h of 40 mg B l<sup>-1</sup> treatment, respectively, failure to incorporate [<sup>3</sup>H]thymidine into DNA is not likely due to a loss in the protein "machinery" necessary for normal nucleic acid synthesis.

DNA and protein syntheses in root apices were not significantly influenced by hydroponic culture in the presence of 20 or 40 mg B l<sup>-1</sup> for 72 h, the time at which inhibition of root growth occurs.

## Chlorophyll Content

Leaf chlorophyll content of healthy C. pepo seedlings increased with time under conditions of optimal boron nutrition. Additional boron prevented this normal increase in chlorophyll content and an actual loss in existing chlorophyll content was observed in plants hydroponically cultured for 96 h at 40 mg B l<sup>-1</sup>. The symptoms of boron toxicity were first visible at this time.

## Gas Exchange

In the control plants, CO<sub>2</sub> fixation did not increase with time in a manner that simply paralleled the increase in chlorophyll content; it appeared to follow a 48-h cycle. This pattern was also observed in the plants treated with 20 and 40 mg B l<sup>-1</sup>, but the rate of photosynthesis was depressed. The mg <sup>14</sup>CO<sub>2</sub> fixed dm<sup>-2</sup>h<sup>-1</sup> was about 30 percent less in the oldest leaf of plants grown in the presence of 40 mg B l<sup>-1</sup> for 72 h or longer (significant at the 5 percent level by Duncan's Multiple Range Test). There was no significant difference at the 5 percent level in rates of <sup>14</sup>CO<sub>2</sub> fixation between the control plants and those grown at 20 mg B l<sup>-1</sup> for any of the treatment periods. Thus, only in the plants grown in the presence of 40 mg B l<sup>-1</sup> was photosynthesis consistently reduced before the onset of the visible symptoms of boron toxicity.

Leaf conductance exhibited the same 48-h cycle as <sup>14</sup>CO<sub>2</sub> fixation in both the treated and untreated plants. For the oldest leaf of plants grown at 40 mg B l<sup>-1</sup>, leaf conductance to water vapor was 30 percent less than that of the same leaf in the control plants at the 5 percent level (Duncan's Multiple Range Test). Transpiration followed the same 48-h cycle as leaf conductance and was reduced by excess boron, but not significantly at the 5 percent level.

## Carbohydrate Content

Starch starch content of leaves from healthy C. pepo plants increased with time under conditions of optimal boron nutrition.

Additional boron prevented the normal accumulation of starch in leaves developing during boron intoxication. The oldest leaf of squash seedlings grown at 20 or 40 mg B l<sup>-1</sup> for 72 h had 30 percent less starch than control plants of the same age; carbohydrate stored as starch was reduced 40 percent after 96 h of treatment at 20 or 40 mg B l<sup>-1</sup> (p < 0.05 by Student's t-Test).

Glucose content of healthy leaves also increased with time, and the accumulation of boron resulted in the failure of the leaf glucose content to increase in a normal manner. Concomitant with the appearance of leaf symptoms typical of boron toxicity in C. pepo seedlings hydroponically cultured at 40 mg B l<sup>-1</sup> for 96 h or 20 mg B l<sup>-1</sup> for 120 h, the glucose content of the oldest leaf was significantly reduced (p < 0.05).

The amount of glucose in the shoot apex was reduced by 50 percent or more in plants grown at 20 or 40 mg B l<sup>-1</sup> for only 24 h. The starch content of the shoot apices was too low to be reliably measured.

Unlike leaves, where the glucose concentration of the tissue increased during development, the level of glucose remained constant in the apical 3-cm segments of developing roots of healthy C. pepo seedlings. The content of glucose in root apices was reduced at the 5 percent level for all treatments at 20 or 40 mg B l<sup>-1</sup>. However, the loss in available glucose did not impact on starch synthesis until the plants had been cultured for 48 h at 40 mg B l<sup>-1</sup>. At this time, the glucose content of roots grown at 40 mg B l<sup>-1</sup> was 60 percent less than the control plants.

### **Purine and Pyrimidine Biosynthesis**

Measurements of the capacity of shoot apices to synthesize de novo the purine and pyrimidine nucleotides necessary for normal DNA synthesis provided evidence suggesting that the etiology of the reduction in DNA synthesis observed during boron intoxication was due to reduced de novo synthesis of adenine nucleotides. Incorporation of NaH<sup>14</sup>CO<sub>3</sub> into total adenine nucleotides, riboside, and base was reduced 40 percent (p < 0.05) after only 24 h hydroponic culture in the presence of 40 mg B l<sup>-1</sup>. De novo pyrimidine nucleotide biosynthesis was reduced 50 percent after 48 h culture at 40 mg B l<sup>-1</sup>. Preliminary results suggest that the salvage of both uracil and adenine are reduced after 24 h of treatment at 40 mg B l<sup>-1</sup>.

In root apices of plants grown at 40 mg B l<sup>-1</sup> for 24 h, de novo purine synthesis was reduced 35 percent (p < 0.001), de novo pyrimidine synthesis was reduced 50 percent after 48 h at 40 mg B l<sup>-1</sup>. The salvage of adenine and uracil also decreased prior to inhibition of shoot and root growth.



## Phosphoribosylpyrophosphate (PRPP) Availability

PRPP is the only substrate common to the de novo synthesis and salvage of both purine and pyrimidine nucleotides. The synthesis of PRPP is directly related to glucose availability. Preliminary results suggest that a loss in available PRPP may be the cause of reduced nucleotide de novo synthesis and salvage.

## BORON'S INFLUENCE ON GROWTH OF COTTON OVULES IN CULTURE

Fiber growth and ovule body growth (dry weight/20 ovules) was determined for cotton ovules cultured in vitro for 14 days at various levels of boron. Without added boron, there is a minimum growth of fiber at the top of the ovules, with most of the growth confined to undifferentiated callus growth of the ovule body. The carryover of boron in the ovule at time of transfer to the culture medium evidently accounts for the observed fiber growth at the "zero added" level.

With increasing levels of boron added to the culture media, there are progressive increases in fiber dry weight and decreases in ovule body dry weight, up to 500  $\mu\text{M}$  boron. The data, when "zero added" fiber growth is subtracted, fits a hyperbolic curve.

Interestingly, the total dry weight produced by the growth of the ovules over the 14 days (fiber plus ovule body growth) does not change a great deal over the range of boron investigated. However, the percent of total dry weight contributed by the ovule body increased as the boron concentration was increased above 1 mM; the percent of total dry weight contributed by the fiber decreased. At the higher levels of boron, very little or no proliferated callus growth of the ovule body occurred, as was observed when boron was deficient. Rather, the ovules were enlarged but retained their normal shape with fiber growth occurring over the entire surface of the ovule. Evidently, boron has some control in the kind of growth occurring in this tissue.

In a series of papers published several decades ago, Eaton showed that Acala cotton exhibited best overall growth at a 10 mg B  $\text{l}^{-1}$  level, but the dry weight of seed cotton produced at 25 mg B  $\text{l}^{-1}$  was about 120 percent of that produced at 10 mg B  $\text{l}^{-1}$ . At both concentrations, injury from accumulated levels of boron in the leaves occurred.

## In Vivo Synthesis of Cell Wall Glucose

The generally accepted substrate for the biosynthesis of cell wall glucose is UDP-glucose; however, it is not incorporated into cellulose to any extent when supplied to intact or detached cotton fibers. Exogenously supplied UDP- $^{14}\text{C}$ glucose does become incorporated into glucans that are soluble in acetic-nitric reagent (callose, hemicellulose and short-chain  $\beta$ -1,4-linked oligosaccharides). Fibers grown with boron

in the medium incorporate about the same amount of [ $^{14}\text{C}$ ]glucose ( $\text{nmol mg}^{-1}$ ) as fibers grown without added boron in the medium. There does not appear to be a difference in the amount of [ $^{14}\text{C}$ ]glucose incorporated into those products extracted by hot water (simple sugars and pectins). On the other hand, there was an increase in the labeled products extracted with chloroform-methanol (lipid) and acetic-nitric reagent from the fibers cultured without boron. This was reversed in the case of [ $^{14}\text{C}$ ]glucose incorporated into the acetic-nitric reagent-insoluble products (cellulose). Expressed on a specific-activity basis ( $\text{nmol incorporated/mg of cellulose}$ ), the difference is apparent; after 48 h, these values were 250 and 40 for the fibers grown, with and without added boron. If boron has this effect on cellulose biosynthesis, was it on the synthesis of the substrate used in the biosynthetic process or on a later step in the process, perhaps on cellulose synthetase?

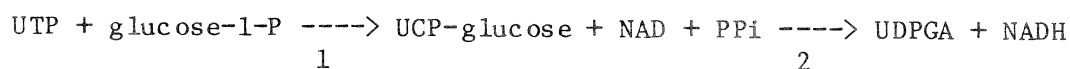
Although this question cannot be answered from the data reported here, several points are indirectly related to the question. There is no significant difference in the percent of fiber weight that is cellulose between the cultures, with or without boron. However, the quantity of cellulose synthesis is much less in the cultures without boron. The utilization of glucose for cellulose synthesis between 10 and 21 days is the same for cultures with and without boron ( $\text{nmol of glucose h}^{-1} \text{ mg fiber}^{-1}$ ) to produce the cellulose that is made. Apparently, once the glucose is utilized to start the cellulose biosynthetic process, the subsequent steps will proceed with about the same efficiency in the presence or absence of boron. This may mean that the boron effect on cellulose synthesis is on the synthesis of the substrate for cellulose synthetase (UDP-glucose) or on the synthesis of glucose-1-phosphate from glucose.

### UDP-Glucose Pyrophosphorylase and Glucan Synthetase

In a study to locate glucan synthetase activity in cotton fiber extracts, glucan synthetase activity was located in the pellet obtained from the first centrifugation of the crude extract. No significant glucan synthetase activity was found in the supernatant.

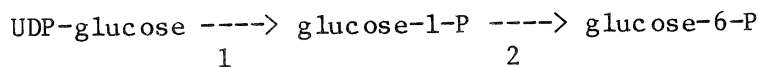
An attempt to extract both UDP-glucose pyrophosphorylase and glucan synthetase at the same time proved not possible because each enzyme must be extracted at a different pH. Additional studies may provide a compromise that will make this possible.

To verify whether UDP-glucose pyrophosphorylase activity was really being measured in the coupled assay reported previously, the assay was run in the opposite direction to previous investigations. The reaction:



- 1 = plant UDP-glucose pyrophosphorylase
- 2 = commercial UDP-glucose dehydrogenase

is a coupled enzyme assay for determining UDPG pyrophosphorylase activity. When assayed in the reverse direction, glucose-1-P was formed from UDP-glucose by the reaction:



1 = plant UDP-glucose pyrophosphorylase

2 = commercial phosphoglucomutase

These preliminary experiments demonstrate that both glucan synthetase and UDP-glucose pyrophosphorylase can be extracted from cotton fibers and assayed successfully. Work must now be done on purifying the enzymes to study boron's effects on these enzymes.

### Activity and Activation of Phosphoglucomutase (PGM)

Phosphoglucomutase (PGM), which catalyzes the interconversions of glucose-1-P and glucose-6-P and thus regulates the flow of glucose through the glycolytic and pentosephosphate pathways, was partially purified from pea and mung bean. The plant enzyme was only slightly phosphorylated (approximately 20 to 25 percent). PGM from mung bean seedlings, pea seedlings, and imbibed pea seeds was activated by both Glc 1,6-P<sub>2</sub> and Fru 2,6-P<sub>2</sub>. The K<sub>a</sub> values for activation by the bisphosphates varied by a factor of approximately 10 when PGM from mung beans and pea seedlings were compared. When pea seed PGM activation was examined, the K<sub>a</sub> values of the 2 bisphosphates differed by a factor of 4, with Glc 1,6-P<sub>2</sub> having the smaller of the 2 K<sub>a</sub> values.

Fru 1,6-P<sub>2</sub> was also used to activate pea seed PGM. The K<sub>a</sub> value for Fru 1,6-P<sub>2</sub> was virtually the same as that obtained for Fru 2,6-P<sub>2</sub>; however, the V<sub>max</sub> for the reaction when Fru 1,6-P<sub>2</sub> was used was half that obtained when using Fru 2,6-P<sub>2</sub>.

While Glc 1,6-P<sub>2</sub> has been known for several decades to be a highly effective activator of PGM from both plant and animal sources, the activation of PGM by Fru 2,6-P<sub>2</sub> has much current interest because of our developing knowledge of the role of Fru 2,6-P<sub>2</sub> in plants.

In addition, preliminary results with partially purified phosphoglucomutase suggest that boron inhibits this enzyme from both plant and animal sources. This observation is consistent with the work of Loughman (1961, Nature 191:1399-1400), which demonstrated that boric acid inhibited phosphoglucomutase of pea seeds. These results, together with those demonstrating boron's effect on the incorporation of [<sup>14</sup>C]glucose into various glucan fractions, suggest that boron may regulate the diversion of glucose-1-phosphate into different synthetic pathways.

Although these initial results require more work, the following conclusions can be drawn: (1) PGM from both plant and animal sources

can be activated by Fru 2,6-P<sub>2</sub> as well as by Glc 1,6-P<sub>2</sub>; (2) plant PGM is isolated primarily in a dephosphorylated state, which would suggest that, in vivo, plant PGM may be subject to regulation by biophosphates; (3) Fru 2,6-P<sub>2</sub> levels in plant tissues are sufficient to activate the PGM reaction; and (4) the PGM reaction may not be as specific for Glc 1,6-P<sub>2</sub> determinations as was once thought.

### SIGNIFICANCE

Our study has begun to identify the probable etiology of boron toxicity. At the whole plant level, excess boron initially causes the failure of leaves to accumulate normal levels of chlorophyll. This results in a reduced rate of CO<sub>2</sub> fixation and, subsequently, in a significant loss in available carbohydrate in all plant tissues. Studies of nucleotide metabolism in Cucurbita pepo shoot and root apices and glucan synthesis in cotton ovules (Gossypium hirsutum) cultured in vitro both suggest that boron plays a role in allocating glucose-1-P for the synthesis of UDP-glucose or glucose-6-P, thus influencing the synthesis of glucans and the flow of glucose through the glycolytic and pentosephosphate pathways. High levels of boron favor glycolysis and cell wall synthesis but inhibit the pentosephosphate pathway. This results in reduced levels of phosphoribosylpyrophosphate which, in turn, limits purine and pyrimidine de novo synthesis and salvage. Reduced availability of purine and/or pyrimidine nucleotides is consistent with the inhibition of DNA synthesis observed in shoot and root apices of boron intoxicated plants.

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## **SUPPORTING PERSONNEL**

C. Galloway (graduate student)



## V. Soil Chemical Factors

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### 15. Carbonate and Sulfate Chemistry and Mineralogy in Salt-Affected Soils

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**PRINCIPAL INVESTIGATORS:** K. K. Tanji and L. D. Whittig

Control and management of dissolved mineral salts in plant-root zones are required to sustain successful irrigated agriculture in arid and semiarid regions. Much is known about the causes and effects of soil salinity and about salinity management, but knowledge of specific conditions and processes is still inadequate to permit quantitative assessment of the net effects of interactive variable factors that regulate salt balance and salt movement in dynamic soil-water-plant systems. This inadequacy limits our ability to design and manage reliable salinity control systems and to predict long-term effects of management practices on salt balance in plant-root zones.

Considerations of carbonate and sulfate chemistry in salt-affected soils and in soils receiving saline waters are central to managing salinity in agricultural lands. The present investigations were aimed toward enhancing knowledge of the nature of soil carbonates and gypsum, their interaction chemistry, and their contributory and regulatory influence on soil salinity under the dynamic conditions imposed by irrigation management. Research included: (1) laboratory studies on carbonate precipitation and solubility and related effects on cation adsorption selectivity in Ca-, Mg-, Na-bentonite systems; (2) field and laboratory studies of soil and environmental factors that influence carbonate and sulfate chemistry under irrigation management; and (3) kinetic studies and refined modeling for carbonate and sulfate systems.

#### **CARBONATE CHEMISTRY IN Ca-Mg-Na-BENTONITE SYSTEMS**

##### **Carbonate Precipitated from Ca-Mg-Bentonites**

**Experimental design:** Series of bentonite systems were preconditioned with appropriate solutions to produce clays with exchangeable Ca:Mg ratios ranging from 99:1 to 7.5:92.5. Portions of each system were seeded with calcite, and both seeded and unseeded systems were equilibrated with deionized water or  $\text{NaHCO}_3$ . Equilibrium solution concentrations of Ca, Mg, Na,  $\text{HCO}_3$ , and Cl and also pH served as input

data to the WATEQF solution chemistry computer program for calculations of ion strength, ion pairs, ion activities, and carbonate-ionic activity products (IAP).

**Results:** The experiments clearly demonstrated that solubility characteristics of calcium carbonate in the Ca-Mg-bentonite systems were influenced by incongruent properties of freshly precipitated carbonate surfaces. The data further showed that Mg inhibited crystallization and increased solubility of calcium carbonate and that high concentrations of Mg in the  $\text{NaHCO}_3$ -bentonite precipitation medium induced crystallization of Mg-calcite. In some systems, Mg was precipitated in forms which were not identified by either x-ray diffraction analysis or IAP characteristics.

### Ca-Mg-Na Exchange in Bentonite Systems

**Experimental design:** Bentonites saturated with Ca and Mg at 5 different ratios were equilibrated with NaCl solution for 1 week. Solution extracts were then analyzed for Ca, Mg, Na,  $\text{HCO}_3$ , and pH. The activity ratio of Ca to Mg in the NaCl solutions were calculated using the chemical analyses of the equilibrium solutions and the WATEQF solution chemistry computer program. Separation factors,  $\alpha_{\text{Na}_{\text{Ca+Mg}}^{\text{Na}}}$ , of the Na-Ca-Mg exchange were determined for the systems.

**Results:** Preference of the bentonite for Ca over Mg was not affected by the presence of exchangeable Na. In systems with alkaline earth carbonates as the only precipitate,  $\alpha_{\text{Na}_{\text{Ca+Mg}}^{\text{Na}}}$  was a function of ionic strength and equivalent fraction of soluble Na. However, precipitation of the carbonates altered the distribution of exchangeable ions. Where carbonates precipitated, Na was found between the interlayers and Ca and Mg occupied the outside surfaces of the clay. As the pH of the equilibrium solution increased and additional precipitation of alkaline earth carbonates and magnesium hydroxide occurred,  $\alpha_{\text{Na}_{\text{Ca+Mg}}^{\text{Na}}}$  increased with decrease in ionic strength. Apparently, this increase in preference for Na was due to the restriction on interlayer Na to diffuse into the equilibrium solution because of hydroxide coating of the crystallites. x-ray diffraction data suggested that some Mg-hydroxy interlayers may have formed in the bentonite treated with the solution having the highest concentration of Mg and with solution pH of 8.05.

## KINETICS OF CARBONATE AND SULFATE CHEMISTRY

### Kinetic Dissolution of Sulfate Minerals

**Experimental design:** The rates of dissolution of 2 mineral forms of gypsum, selenite ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) and phosphogypsum (a fertilizer industry by-product), were studied under controlled laboratory conditions. Samples of each of 3 size fractions (20-40 mesh, 40-80 mesh, and >80 mesh) of the 2 materials were suspended in deionized water at 25°C. Suspensions were stirred with a magnetic bar and the pH, electrical



conductivity, and Ca concentration for each were recorded at intervals over time periods ranging from 15 to 70 min.

**Results:** Initial rates of dissolution were most rapid for materials with the highest specific surface area and rates were much more rapid for phosphogypsum than for selenite. Citing data from 40-80 mesh materials for illustration, the pH of the selenite solution was initially 5.5 and it increased asymptotically to pH 6.1 over a 70-min period. In contrast, the pH of the phosphogypsum solution was initially 5.5 but it decreased abruptly to about pH 4.0 in less than 2 min. The latter abrupt decrease in pH indicated the formation of  $H_3PO_4$ . The Ca concentration in the selenite solution increased asymptotically to >50 ppm over the 70-min period, whereas in the phosphogypsum solution it fluctuated around 500 ppm from very shortly after initial mineral-water contact. Under the conditions established for these studies, the dissolution of 40-80 mesh selenite was nearly complete within 30 min, whereas for phosphogypsum, the dissolution was essentially complete in less than 10 min.

It appears that dissolution of the sulfate mineral forms involves 2 steps. An initial rapid dissolution rate, related to specific mineral surface area, is followed by a slower rate. This 2-step mechanism was most evident for selenite.

### **Kinetic Dissolution of $CaCO_3$ (Iceland Spar)**

**Experimental design:** An improved experimental setup was developed to study the dissolution kinetics of  $CaCO_3$ . A 1- $\ell$  polyethylene reaction vessel containing  $CaCO_3$  suspended in deionized water is immersed in a water bath with temperature control to within  $\pm 0.2^\circ C$ . Carbon dioxide gas of varying concentration is introduced into the vessel through a gas diffusor and the suspension is mixed with an air-driven magnetic stirrer.

For the dissolution studies, Iceland Spar was ground and separated into <40, 40-80 and >80 mesh fractions with respective surface areas of 0.05, 0.09 and 0.30  $m^2/g$ . The initial concentrations of Iceland Spar for each dissolution run was 3.75 g/ $\ell$ , which was well above the equilibrium solubility of  $CaCO_3$  for the  $pCO_2$  levels selected for the experiments. Gases with  $pCO_2$  levels of 0.03, 1.0 and 4.6 KPa, respectively, and preconditioned to saturation with water vapor and to the same temperature as the reaction vessel, were passed through the system at the rate of 3  $\ell/min$  for 1 hr prior to and during each dissolution run. The time course of reaction was monitored every 10 sec with a temperature probe, a pH electrode, and a Ca-selective ion electrode.

**Results:** The dissolution of  $CaCO_3$  (Iceland Spar) was influenced by mineral-specific surface area,  $pCO_2$ , and reaction time, as indicated by Ca activity ( $a_{Ca}$ ) and pH data, and the influences of the variables were interdependent. Independent data plots equating  $a_{Ca}$  with each of the variables clearly indicated these relationships and further demonstrated differential responses of particular systems to change in 1 or more of the variables.

The  $a_{Ca}$  in solutions reacting with the 0.06 and 0.09  $m^2/g$  samples were nearly the same at all 3  $pCO_2$  levels after any particular time of reaction. A slow, progressive increase in  $a_{Ca}$  of relatively low magnitude was registered for these systems throughout reaction periods of up to 200 min. The relatively low  $a_{Ca}$  values, together with the slowly continuing increases through the duration of the dissolution monitoring periods, clearly indicated the relatively slow rates of dissolution of these materials and the lack of attainment of equilibrium within the reaction periods. The 0.30  $m^2/g$  Iceland Spar, in contrast to the coarser materials, exhibited a much faster dissolution rate which was markedly influenced by  $pCO_2$ .

The pH of solutions typically increased parabolically with time for the systems under study, with 1 exception. For the 0.30  $m^2/g$  material with  $pCO_2$  of 0.03 KPa, the pH rose rapidly in early stages of dissolution to near pH 10 and then gradually decreased exponentially to about pH 8.5. This abrupt change in pH was interpreted as due to aqueous  $CO_2$  (that is,  $H^+$ ) becoming rate limiting under conditions of relatively low  $pCO_2$  and relatively high mineral specific surface area so that a readjustment of the carbonate chemistry equilibria took place, accumulating  $CO_3^{2-}$  and  $OH^-$ . And with a continued supply of aqueous  $CO_2$ , and hence  $H^+$ , pH approaching equilibrium values were finally established.

### **Modeling Kinetics of Dissolution of Carbonate Minerals**

**Theoretical considerations:** An attempt was made to improve on carbonate chemistry prediction capabilities by modeling dissolution kinetics in the  $CO_2$ - $H_2O$ - $CaCO_3$  system. Current soil-solution chemistry models assume equilibrium conditions, and solute transport models for soil-water systems assume local equilibrium for carbonate chemistry. There is increasing evidence that equilibrium conditions for carbonate chemistry seldom prevail.

The interactive chemistry of carbonates in aqueous systems is complex. Thus, the dissolution kinetics of carbonate minerals involves several interdependent and time-varying processes. For the 3-phase system under investigation the carbonate chemical reactions involving 10 solute species within the bulk aqueous phase are very fast. In contrast, physicochemical reactions at the interface between gaseous and liquid as well as between liquid and solid phases are rate limiting. Between the bulk gaseous and aqueous phases is a thin liquid film through which exchange of  $CO_2$  must occur by diffusion. At the immediate surface of the bulk solid phase, surface reactions occur such as adsorption of  $Ca^{2+}$  and  $CO_3^{2-}$  related to precipitation and desorption of  $Ca^{2+}$  and  $CO_3^{2-}$  related to dissolution. Dissolution and precipitation, in turn, are significantly influenced by the transport of  $Ca^{2+}$  and  $CO_3^{2-}$  to and away from the mineral surface. This transport is controlled by diffusion against the chemical concentration or by a combination of diffusion and hydrodynamic forces. The latter involves the hydrodynamic movement within the liquid phase due to convective forces, formation, bubbling and dissolution of soil gases, and, in laboratory studies, hydrodynamics due to stirring with magnetic bars.

For the rate model under consideration, carbonate reactions in the bulk aqueous phase are assumed to be sufficiently rapid to warrant assumption of instantaneous equilibrium. The 10 solute species indicated above are those considered in equilibrium calculations. The diffusion of  $\text{CO}_2$  across the gas-liquid interface as well as dissolution and precipitation of  $\text{CaCO}_3$  are considered to be time dependent.

The 10 solute species are defined by ionic equilibria, including equilibrium constants for hydration of aqueous  $\text{CO}_2$ , first and second dissociation constant for  $\text{H}_2\text{CO}_3$ , ion product of  $\text{H}_2\text{O}$  and dissociation constants for ion pairs  $\text{CaHCO}_3^+$ ,  $\text{CaCO}_3^0$ , and  $\text{CaOH}^+$ . In addition, the mathematical model for the aqueous phase involves simultaneous calculations for C mass balance, Ca mass balance, charge balance, ionic strength, and ion activity.

The model for the dissolution and precipitation at the solid-aqueous interface is

$$\partial \text{Ca}_T / \partial t = K_9 * M * K_{sp} / f_2^2 - K'9 * M * m_{ca} m_{co3} \quad (1)$$

The time rate of change of total aqueous Ca is related to dissolution and to precipitation of  $\text{CaCO}_3$ .  $K_9$  and  $K'9$  are the respective reaction rate constants,  $M$  is the initial concentration of  $\text{CaCO}_3$ ,  $K_{sp}$  is the solubility product constant for calcite,  $f_2$  is the divalent ion activity coefficient, and  $m_{ca}$  and  $m_{co3}$  are the analytical concentrations of  $\text{Ca}^{2+}$  and  $\text{CO}_3^{2-}$ .

The model for the exchange of  $\text{CO}_2$  gas across the gas-liquid interface is a combination of Fick's First Law of Diffusion and Henry's Law, that is,

$$\partial C_T / \partial t = K_8 * S/V * (K_H * p\text{CO}_2 - C_{\text{CO}_2}) + \partial \text{Ca}_T / \partial t \quad (2)$$

The rate of change of total aqueous carbon is related to the concentration gradient of  $\text{CO}_2$  across the liquid interface, the conductivity function, and  $\partial \text{Ca}_T / \partial t$ .  $K_8$  is the mass transfer coefficient of  $\text{CO}_2$  and is related to the diffusion coefficient of  $\text{CO}_2$  and  $\text{H}_2\text{O}$  and thickness of the film;  $S$  and  $V$  are, respectively, the surface area and volume of the solution phase; the product of  $K_H$  (Henry's Law constant) and  $p\text{CO}_2$  gives the theoretical concentration of dissolved  $\text{CO}_2$  at that  $p\text{CO}_2$ , and  $C_{\text{CO}_2}$  is the initial or calculated dissolved  $\text{CO}_2$ .

Equations (1) and (2) are solved by the finite difference method and  $\partial \text{Ca}_T / \partial t$  and  $\partial C_T / \partial t$  serve as input values to the instantaneous equilibrium calculations at each time step.  $p\text{CO}_2$  can be fixed or can be varied abruptly at any time in the simulation period. At the moment  $K'9$ , reaction-rate constant for precipitation, is assumed to be the same as the dissolution-rate constant. This model is applicable not only to conditions very far removed from equilibrium, where one is concerned

mainly with forward kinetics, but also to near-equilibrium conditions, where both forward and backward kinetics must be considered.

**Tests of the model:** A number of controlled laboratory experiments have been performed to test the validity of certain assumptions of the model, to refine the sensitivity of model parameters, and to identify factors that limit the model's applicability. Testing to date has demonstrated the positive potential of the model to simulate dissolution of  $\text{CaCO}_3$  and the sensitivity of model parameters, including the mass-transfer coefficient for  $\text{CO}_2$ , dissolution rate constants and  $p\text{CO}_2$ . The testing has shown that the specific surface area of  $\text{CaCO}_3$  is an important variable and that a functional model must include certain kinetic consideration.

## **SOIL AND ENVIRONMENTAL FACTORS RELATED TO CARBONATE AND SULFATE CHEMISTRY AND MINERALOGY**

### **Field-Site Selection and Plot Management**

Field aspects of these investigations were carried out over a 3-year period on plots selected, designed, and managed by D. E. Rolston and co-investigators for their study of "Crop Response to Temporarily- and Spatially-Variable Soil Salinity Profiles" at the University of California West Side Field Station. The plots were designed to provide delivery of variable quantities of irrigation water and salts through parallel line-source sprinklers. The plots were cropped to sorghum during summer and to wheat during late winter/early spring.

Soil solutions and soil gases were collected periodically with sampling probes implanted at 30-, 60-, 90-, and 120-cm depths at regularly spaced locations along transects normal to and parallel with sprinkler lines. Soil temperatures and soil moisture were also monitored periodically in conjunction with gas and solution sampling. Soil samples from each sampling location were also characterized with respect to chemical and mineralogical properties. Sampling was designed to include seasonal and diurnal periods, varying temperature, moisture and salinity regimes and cropped and noncropped conditions. Analyses included determinations of soil-moisture contents and temperature,  $\text{CO}_2$  contents and isotopic composition of carbon and oxygen in the  $\text{CO}_2$  gas, and ionic composition and pH of soil solutions.

### **Special Sampling Techniques**

**Soil solutions:** Degassing of  $\text{CO}_2$  in a vacuum-extracted soil solution is a significant problem; it affects the chemistry of the soil solution as well as the isotopic fractionation factor between gaseous  $\text{CO}_2$  and dissolved carbonate species. A field method was designed to minimize the degassing of  $\text{CO}_2$  from soil solutions extracted under vacuum. To obtain more chemically representative soil solutions, soil gases from an identical soil depth as the ceramic suction cup for soil solution were recirculated through collected soil solutions. Soil gases

from a nearby gas probe were slowly bubbled through fritted glass into the collected soil solution for 1 hour with the aid of a peristaltic pump. The pH measurements taken of the soil solutions prior to and after soil gas bubbling showed decreases in pH from 0.2 to 0.8 units, with an average of about 0.7, depending on the prevailing CO<sub>2</sub> concentrations.

**Soil gases:** Extensive sampling of soil gases at a location remote from the laboratory required a system of sampling which would permit storage of samples for as long as several days without loss of gas from the sample container. This was accomplished by collecting and storing gas samples in pre-evacuated blood serum collection bottles capped with rubber stoppers. A small-diameter copper tube capped with a rubber stopper was implanted to the appropriate sampling depth. Sampling was then accomplished with a double-pointed needle inserted through stoppers on the implanted copper tube and the collection bottle. Gas was thus drawn into the collection tube. When sampling was completed, the pressure in the bottle was equalized with the outside pressure. This prevented loss of gas from the bottle as might occur under differential pressure.

Soil gases were sampled for isotopic analyses as follows: a large-volume (2 l) collection bottle was evacuated to a standard pressure and closed with an air-tight stopcock. A fine capillary tube adjusted by length and diameter to provide a calculated slow and steady flow rate in response to known differential pressure was connected via tygon tubing to the collection bottle and the implanted copper gas sampling tube. The stopcock in the collection bottle was opened for a time sufficient to collect approximately 200 ml of soil gas. The collection of a relatively small amount of gas in a large evacuated bottle had little effect on the pressure within the bottle and, hence, little effect on gas flow rate.

### **Seasonal Variations in Soil CO<sub>2</sub>**

The CO<sub>2</sub> content of soil gas at 4 sampling depths per location were monitored periodically over 1 1/2 years for both fallow and cropped soil. The CO<sub>2</sub> contents of soil gas under fallow conditions ranged from 0.1 to 0.7 percent. These data, coupled with concurrent soil-water and temperature data, indicated that soil temperature was the dominant parameter controlling the CO<sub>2</sub> contents, with minimum CO<sub>2</sub> levels coincident with minimum soil temperatures. The major source of CO<sub>2</sub> under fallow conditions was most probably from soil-microbial activity.

A complex set of factors was responsible for wide seasonal variations in soil CO<sub>2</sub> contents under irrigated and cropped conditions. Depending upon seasons, the CO<sub>2</sub> contents reflected influences of soil temperature and moisture status, the type of crop grown and the stage of crop maturity. Along a transect perpendicular to the water and salt application lines, the mean CO<sub>2</sub> contents ranged from less than 1 percent to more than 5 percent. The CO<sub>2</sub> contents were consistently higher during growth of summer sorghum than during growth of winter-spring wheat. Soil CO<sub>2</sub> content was seldom higher than 2 percent during the wheat season. The highest levels of soil CO<sub>2</sub> also occurred in the

regions of best crop growth. The vertical distribution of soil CO<sub>2</sub> during crop growth also clearly reflected the extent of root development. Seasonal and depth variations in soil CO<sub>2</sub> content clearly demonstrated these relationships and documented the importance of plant root respiration as the major source of soil CO<sub>2</sub>.

### Isotopic Composition of CO<sub>2</sub> Gas

**Theoretical considerations:** Ratios of the stable isotopes <sup>13</sup>C and <sup>12</sup>C, and <sup>18</sup>O and <sup>16</sup>O, in soil CO<sub>2</sub> can be expected to be highly variable, depending upon the interplay of a number of factors which allow differential isotope fractionation in response to isotope mass. Differentiation and evaluation of the various mechanisms involved in carbon and oxygen isotope fractionation are viewed as keys to understanding carbonate source-sink relationships in dynamic soil systems.

Stable isotope fractionation in gas, liquid, or solid phases is commonly evaluated in terms of δ°/oo, the ratio of the heavy to the light isotopes, defined as:

$$\delta \text{ (}^\circ/\text{oo)} = \frac{\text{Ratio in sample} - \text{Ratio in standard}}{\text{Ratio in standard}} \times 1000$$

for example,

$$\delta^{13}\text{C} \text{ (}^\circ/\text{oo)} = \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}} - (^{13}\text{C}/^{12}\text{C})_{\text{standard}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} \times 1000$$

**Results:** Without consideration for the separate mechanisms involved in carbon isotope fractionation, the isotopic composition of soil CO<sub>2</sub> was highly variable.

When some of the factors affecting carbon isotope composition of soil CO<sub>2</sub> were considered individually, the scattered data became more interpretable. In a separate greenhouse experiment, sorghum plants were grown under carefully controlled conditions in solution culture. At specific times throughout a 59-day growing period, sorghum plants were removed from the solution culture and the plant roots were allowed to respire in a CO<sub>2</sub>-free container. The isotopic composition of root-respired CO<sub>2</sub> from the sorghum (a C<sub>4</sub> plant) registered a δ<sup>13</sup>C (°/oo) value of near -11 throughout growth. In a similar experiment with wheat (a C<sub>3</sub> plant), the δ<sup>13</sup>C (°/oo) values were consistently near -25.

The δ<sup>13</sup>C (°/oo) values for soil CO<sub>2</sub> during growth of sorghum ranged from near -16 at the beginning of growth to near -12.5 as the crop matured. Clearly, the isotopic composition of the soil CO<sub>2</sub> was influenced by the root-respired CO<sub>2</sub>. As the growing season advanced, this influence intensified, but the δ<sup>13</sup>C (°/oo) value failed to reach the

-11 value registered for the solution culture experiment. In all probability, this was due to influence of microbial decomposition of organic matter in the field situation.

The  $\delta^{13}\text{C}$  (‰) for soil  $\text{CO}_2$  ranged from -16 to -21 during growth of wheat in the field. The data clearly showed the approach toward the experimentally determined  $\delta^{13}\text{C}$  (‰) values for wheat-respired  $\text{CO}_2$  as the crop matured.

$\delta^{13}\text{C}$  (‰) values were determined for carbon of soil  $\text{CO}_2$  collected at different times and depths across the field plot, including a location outside of the cropped area. The  $\delta^{13}\text{C}$  (‰) values for the non-cropped location were near -18, representing the value for  $\text{CO}_2$  evolved by microbial decomposition of soil organic matter. Data from the cropped sites showed a progression of  $\delta^{13}\text{C}$  (‰) values toward values expected for sorghum-respired  $\text{CO}_2$  as the crops matured.

Appreciable variations in  $\delta^{13}\text{C}$  for  $\text{CO}_2$  collected immediately before and for 7 days after an irrigation demonstrated dynamic changes due to interactions between the gas and liquid phases.

**Experimental procedure:** Since soil gases obtained from the field are mixtures of  $\text{N}_2$ ,  $\text{O}_2$ ,  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  vapor, etc., it was necessary to cryogenically isolate  $\text{CO}_2$  from other gases. Stable isotope concentrations in purified soil  $\text{CO}_2$  were determined by mass spectrometry. In addition to the isotopic analyses of  $\text{CO}_2$  collected in gas from field locations, isotopic determinations were also made for  $\text{CO}_2$  produced in laboratory experiments on plant-root and microbe respiration.

### Isotopic Equilibrium Between Soil $\text{CO}_2$ and $\text{H}_2\text{O}$

Since gas-phase  $\text{CO}_2$  and liquid-phase  $\text{H}_2\text{O}$  coexist in continually varying proportions in soil systems, investigations were conducted to determine whether oxygen isotopic equilibrium exists between  $\text{CO}_2$  and  $\text{H}_2\text{O}$  in soils under variable environmental conditions. Soil and gas samples were collected concurrently from 30-, 60-, 90-, and 120-cm depths in the field. Sampling sites included irrigated and nonirrigated and cropped and noncropped soils. Soil temperatures were recorded with each sampling. Water was extracted from soil samples by azeotropic distillation and  $\text{CO}_2$  was extracted from soil gases by a system of cryogenic traps. The  $^{18}\text{O}/^{16}\text{O}$  ratios in the  $\text{H}_2\text{O}$  and  $\text{CO}_2$  were determined with a mass spectrograph. By taking into account the temperature dependence of the  $\text{CO}_2$ - $\text{H}_2\text{O}$  isotopic fractionation factor, the data showed that the  $\delta^{18}\text{O}$  of soil  $\text{CO}_2$  was directly correlated with the  $\delta^{18}\text{O}$  of soil water. This indicated that the soil  $\text{H}_2\text{O}$  and soil  $\text{CO}_2$  exist in both chemical and isotopic equilibrium. This also demonstrated the fact that the  $\delta^{18}\text{O}$  of soil  $\text{CO}_2$  can be used to predict  $\delta^{18}\text{O}$  of soil water in dynamic soil systems.

## Environmental Factors Affecting the $\delta^{13}\text{C}$ of Plants

**Experimental procedure:** The observation that the  $\delta^{13}\text{C}$  of soil  $\text{CO}_2$  varied considerably as a function of type and stage of maturity of growing plants across the plot transect prompted an examination of  $\delta^{13}\text{C}$  of tissue of plants growing along the transect. Plants (sorghum or wheat, depending upon season) were sampled at 3-m intervals along a transect normal to the sprinkler lines. Purified  $\text{CO}_2$  isolated from gases produced by combustion of the plant tissue was analyzed for stable carbon isotopes.

**Results:** Contrary to expectations, the  $\delta^{13}\text{C}$  of the plant tissues (leaves as well as whole plants) was not uniform across the transect. For sorghum, the  $\delta^{13}\text{C}$  (‰) values ranged from about -11 to -13. For wheat, values ranged from about -17 to -24. Interestingly, for sorghum ( $\text{C}_4$  plant), the  $\delta^{13}\text{C}$  (‰) values of near -11 were obtained from healthy plants in the center of the plot, whereas the values of near -13 were from stressed plants along the margins of the irrigated plot. Conversely, for wheat ( $\text{C}_3$  plant) the  $\delta^{13}\text{C}$  (‰) values near -20 or less were obtained from stressed plants at the plot margins and the values of near -24 were from the healthy plants at the center of the plot. These data demonstrate the fact that environmental stress (presumably water stress) affects carbon-isotope fractionation of these plants. The reason for the seemingly opposite effects on the 2 crop plants is not readily apparent at this time.

### Solid-Solution Equilibrium

For 1 full year, soil solutions were collected from selected locations across the experimental field plot. Nine different sampling dates included sorghum and wheat growth periods as well as between-crop periods. Soil solutions could not be extracted at some depths at some locations on particular sampling dates. However, a sufficient number of solution samples were collected to provide representative data. The pH, alkalinity, and electrical conductivity of soil solutions were determined in the field as soon as possible after extraction. Ionic composition of solutions was determined in the laboratory.

The analytical data served as input to the WATEQF water-equilibrium model for prediction of saturation index for the minerals calcite and gypsum. The results showed that the soil solutions at all profile locations throughout the year were supersaturated with respect to calcite. With the exception of a period following a relatively wet winter season, soil solutions were also supersaturated with respect to gypsum.

### SIGNIFICANCE

The research has demonstrated the influences of major rate-controlling factors affecting the dissolution of gypsum and calcite under controlled laboratory conditions. Specific surface area is a



major factor in regulating the kinetics of dissolution of gypsum; specific surface area and  $p\text{CO}_2$  are major factors regulating the kinetics of dissolution of calcite. Further, the research has established improved methods for soil solution and gas sampling for study of carbonate and sulfate chemistry in salt-affected soils and has demonstrated the applicability of carbon and oxygen-stable isotope chemistry for identification of and interactions between major  $\text{CO}_2$  sources and sinks in dynamic soil systems under irrigation and cropping management.

Improvements over existing carbonate chemistry models have been advanced by modeling kinetics in the  $\text{CO}_2\text{-H}_2\text{O-CaCO}_3$  system. The improved model incorporates mass transfer coefficients for  $\text{CO}_2$ , carbonate dissolution rate constants and  $p\text{CO}_2$  with other parameters to improve carbonate chemistry prediction capabilities. The modeling efforts, with continuing refinements aided by field data and laboratory experiments, contribute to assessment of long-term effects of soil salinity and to design of practices for salinity control and management.

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#### **SUPPORTING PERSONNEL**

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## 16. Competitive Exchange and Adsorption of Ionic Solutes in Low-Quality Waters during Transport through Soil

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**PRINCIPAL INVESTIGATOR:** Garrison Sposito

The principal objectives of this research project were:

(1) To carry out laboratory experiments on ternary cation exchange reactions in arid-zone soils and to determine whether these reactions can be characterized solely in terms of data for exchange reactions involving only 2 cations simultaneously.

(2) To carry out theoretical studies on ion adsorption during transport in soil to determine adsorption equations with a sound chemical and physical basis and to develop methodologies for measuring the parameters in these equations.

(3) To develop a computer model of solute transport in an arid-zone soil which combines the transfer-function approach of Professor W. A. Jury with the chemical speciation algorithms in the GEOCHEM program and to validate this model with field-scale solute transport data.

### **Ternary Cation Exchange Reactions**

Research on objective (1) involved studies of Na-Ca-Mg exchange in suspensions containing either specimen montmorillonite or illite, or montmorillonitic or illitic soil separates, and an electrolyte background of  $50 \text{ mol}_c\text{m}^{-3}$ . The electrolyte background usually comprised perchlorate salts, but chloride salts were used in some binary exchange experiments with specimen montmorillonite.

The exchange of calcium and magnesium for sodium at 298 K on Wyoming bentonite was investigated in perchlorate and chloride background media maintained at pH 7.0 and at a total cation exchange concentration of  $50 \text{ mol}_c\text{m}^{-3}$ . In the perchlorate medium, the apparent total adsorbed metal charge was independent of the exchanger composition and equal to  $0.97 \pm 0.06 \text{ mol}_c\text{kg}^{-1}$  (average for Na-Ca and Na-Mg exchanges). In the chloride medium, the apparent total adsorbed metal charge increased with the amount of calcium or magnesium adsorbed, tending to a value near  $1.3 \text{ mol}_c\text{kg}^{-1}$  as the clay became saturated with the bivalent metal cation. These results were interpreted as evidence for the adsorption of  $\text{CaCl}^+$  or  $\text{MgCl}^+$  complexes in the exchange experiments conducted in the chloride medium. A detailed analysis of the exchange data led to the conclusion that a Ca-montmorillonite or Mg-montmorillonite suspension in a chloride background consists of quasicrystals with bivalent cations adsorbed on the internal surfaces and monovalent metal-chloride complexes adsorbed on the external surfaces.

Exchange isotherms were prepared for  $\text{Na}^+$  in  $\text{Na}^+ \rightarrow \text{Ca}^{2+}$  and  $\text{Na}^+ \rightarrow \text{Mg}^{2+}$  exchange reactions, and for  $\text{Mg}^{2+}$  in  $\text{Ca}^{2+} \rightarrow \text{Mg}^{2+}$  exchange reactions at 298 K on Wyoming bentonite suspended in a  $50 \text{ mol m}^{-3}$  perchlorate background. These isotherms were essentially congruent with the appropriate thermodynamic nonpreference exchange isotherms. It was concluded from this fact that there is essentially no difference in the affinity of montmorillonite clay for  $\text{Ca}^{2+}$  vs.  $\text{Mg}^{2+}$ . However, exchange isotherms prepared for  $\text{Na}^+$  in  $\text{Na(I)} \rightarrow \text{Ca(II)}$  and  $\text{Na(I)} \rightarrow \text{Mg(II)}$  exchange, as well as published ones for  $\text{Mg(II)}$  in  $\text{Ca(II)} \rightarrow \text{Mg(II)}$  exchange on montmorillonite suspended in a  $0.05 \text{ M}$  chloride background, consistently indicated a slight preference on the clay for  $\text{Ca(II)}$  over  $\text{Mg(II)}$ . This preference for  $\text{Ca(II)}$  is concluded to be the result of the formation of  $\text{CaCl}^+$  complexes, which are more stable thermodynamically and have a greater affinity for the clay than do  $\text{MgCl}^+$  complexes.

The exchange of magnesium for calcium at 298 K on Wyoming bentonite was investigated in a  $50 \text{ mol m}^{-3}$  perchlorate background maintained at pH 7. The charge fraction of  $\text{Na}^+$  in the aqueous solution phase was controlled at the values of 0.74 or 0.87 during the exchange experiments, resulting in average exchangeable sodium percentages (ESP) of either 16 or 36 percent on the clay. The Vanselow selectivity coefficient for  $\text{Ca} \rightarrow \text{Mg}$  exchange was equal to 1.0 and was independent of exchanger composition at both ESP values. This result implied that, under the conditions of the exchange experiments, the clay exhibited equal affinities for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , regardless of the quantity of adsorbed sodium present.

The binary cation exchange processes,  $\text{Na} \rightarrow \text{Ca}$ ,  $\text{Na} \rightarrow \text{Mg}$ , and  $\text{Ca} \rightarrow \text{Mg}$ , were investigated at 298 K for a montmorillonitic soil separate (principally clay and silt) suspended in a  $50 \text{ mol m}^{-3}$  perchlorate background at pH 6, 6.6, or 7. The resulting exchange isotherms indicated some preference on the soil for  $\text{Ca}$  and  $\text{Mg}$  over  $\text{Na}$ , and for  $\text{Ca}$  over  $\text{Mg}$ . These preferences were attributed primarily to organic matter associated with the soil separate, since previous studies had shown that pure montmorillonite exhibits essentially no preference among the 3 cations under the same condition as in the present experiments. The exchange isotherms at pH 6, 6.6, and 7 were congruent for all 3 binary exchange reactions, showing that, at these pH values, proton competition did not affect the exchange selectivity of the soil. The total adsorbed metal charge,  $Q_0$ , however, increased with the pH value, as expected from the presence of organic matter in the soil, and varied with the surface composition of the exchanger. The exchange of  $\text{Mg}$  for  $\text{Ca}$  also was investigated in a  $50 \text{ mol m}^{-3}$  perchlorate background at pH 5, 6, and 7 with the charge fraction of sodium in the aqueous solution phase controlled at 0.70 or 0.85, resulting in average ESP ranging from 5 to 25 percent on the soil. Exchange isotherms for magnesium indicated some preference on the soil for calcium over magnesium. This preference was attributed primarily to the presence of organic matter associated with the soil separate. However, the exchange isotherms showed no effect of pH in the range between 5 and 7, and no effect of ESP in the range between 0 and 25 percent.

The Vanselow selectivity coefficient was calculated for  $\text{Mg} \rightarrow \text{Ca}$  exchange using 7 sets of cation exchange data wherein the pH value

ranged from 5 to 7 and the ESP ranged from 0 to 25 percent. The selectivity coefficient was observed to decrease slightly as the fraction of adsorbed bivalent metal cation charge accounted for by Ca increased. There was no effect of pH on the selectivity coefficient, but, at low Ca saturation of the soil, the coefficient increased somewhat as the ESP increased. This behavior was interpreted as consistent with a preferential adsorption of Na by the montmorillonite component of the soil.

Binary and ternary cation exchange reactions involving Na, Ca, and Mg on Silver Hill illite suspended at pH 7 in a 50 mol m<sup>-3</sup> perchlorate background were investigated. The binary exchange experiments indicated a preference of the clay mineral for Ca and Mg over Na and for Ca over Mg. The principal ternary exchange data, on the other hand, indicated no selectivity difference between Ca and Mg in the presence of adsorbed Na at a nominal ESP of 25. No differences among the values of the total adsorbed metal charge,  $Q_0$ , were observed in a set of binary and ternary Na  $\rightarrow$  M (M = Ca and/or Mg) exchange experiments carried out at the same time. There was, however, a systematic increase in the value of  $Q_0$  on a time scale of months in Na-illite suspended in NaClO<sub>4</sub>. The increase in  $Q_0$  was produced by adsorbed Na<sup>+</sup> that could be displaced by NH<sub>4</sub><sup>+</sup> but not by Ca<sup>2+</sup> or Mg<sup>2+</sup>. The quantity of this "quasi-exchangeable" Na present in the principal ternary exchange experiments was determined to be about 13 percent of the value of  $Q_0$ , an amount concluded to be sufficient to cover the high-energy exchange sites in the illite and thereby relegate Ca-Mg exchange to sites of relatively low selectivity, as observed experimentally.

Binary and ternary Na-Ca-Mg exchange reactions also were investigated at pH 7 and in a 50 mol m<sup>-3</sup> perchlorate background for an illitic soil separate (principally silt and clay). The binary exchange isotherms indicated preference for Ca and Mg over Na and for Ca over Mg. The ternary exchange data (Ca-Mg exchange at ESP = 23 percent) were virtually congruent with the binary Ca-Mg exchange data, showing no effect of adsorbed Na. The time-dependent development of "quasi-exchangeable" Na observed with specimen illite did not occur in the illitic soil. Plots of the Vanselow selectivity coefficient for Mg  $\rightarrow$  Ca exchange at zero ESP were nearly identical for the illitic soil separate and specimen illite, showing a gradual decrease to unit value as the charge fraction of adsorbed Ca increased.

### **Adsorption during Transport in Soil**

Research on this objective focused on fundamental studies of the "adsorption term" in the standard convection-dispersion equation (CDE) used in soil physics to describe solute transport. These studies revealed some of the limitations of conventional models of solute adsorption incorporated with the CDE.

The macroscopic transport equation for a reactive solute at low concentration in a homogeneous, saturated, porous medium was derived on the basis of a rigorous cumulant expansion applied to the equation of mass balance for the solute both in the liquid phase and in the solid

phase. The derivation included a detailed consideration of the molecular-kinetic picture of adsorption. The commonly neglected fact that all adsorption phenomena in natural porous media also are exchange phenomena was emphasized, as was the important distinction between uniform and nonuniform adsorbing surfaces. For the uniform case, where there is only one type of adsorption site on the solid matrix, the expression for the macroscopic transport equation is identical in form with the standard convection-dispersion equation incorporating a first-order kinetic adsorption model. For the nonuniform case, where there is more than one type of adsorption site, the macroscopic transport equation derived and the conventional dispersion-convection equation were no longer identical.

A rigorous, mathematical representation of a sorption isotherm as a Stieltjes transform was employed to prove a theorem about the "2-surface" Langmuir equation. This theorem states that, if the distribution coefficient for an ion sorbed by a soil is a finite, decreasing function of the amount sorbed,  $q$ , and extrapolates to zero at some finite value of  $q$ , then the sorption isotherm can always be represented mathematically by a 2-surface Langmuir equation. Since the proof of this theorem does not depend on the chemical mechanism of ion sorption, it follows that the adjustable parameters in the 2-surface Langmuir equation cannot be interpreted in terms of surface reactions without additional, independent evidence that only adsorption on 2 kinds of surface site actually is involved in the ion sorption reaction.

The thermodynamic theory of ternary exchange equilibria was developed. It was shown that the analogs of well-known thermodynamics expressions for binary exchange systems can be derived for ternary systems as well. The subregular model of ternary cation exchange systems, applied recently to  $\text{NH}_4$ -Ba-La exchange on montmorillonite, was examined critically within a thermodynamic context. It was found that the subregular model for a ternary system cannot be expressed solely in terms of parameters that depend only on data obtained for binary systems, in contradiction with claims made recently in the literature. The subregular model was shown to contain 1 parameter that requires ternary exchange data for its evaluation. A calculation of this ternary constant for  $\text{NH}_4$ -Ba-La exchange on montmorillonite indicated that its value was zero within experimental precision. Nonetheless, in general it must be concluded that the behavior of ternary exchange systems cannot be predicted from data on binary exchange systems alone.

### **Computer Model of Solute Transport through Soil**

The transfer-function model (TFM) of solute transport through unsaturated soil, initiated by W. A. Jury, was generalized to describe the movement of a solute that may undergo any kind of chemical or biological transformation as it moves in a soil unit of arbitrary size through which the water flow may vary in space and time. The generalized TFM was shown to be related closely to the law of mass balance for a solute as interpreted in the context of probability theory. The principal

quantity in the TFM is the solute lifetime probability density function (pdf). Methods for determining this function experimentally were described and its relation to the solute travel-time pdf defined by Jury was shown. Since the generalized TFM is independent of any particular mechanism of solute transport through soil, it was concluded that all mechanistic models of solute movement consistent with the law of mass balance must also be consistent with the TFM.

The 2-component CDE model (mobile/immobile zone or rapid/slow adsorption) was developed as an example of the TFM. It was shown that the 2-component CDE model can be reformulated as an integral equation for the "fast" solute component which has the same form and interpretation as the generalized TFM integral equation, specialized to (1) steady water-flow conditions and (2) solute input or loss restricted to the entrance or exit surface of a field-soil unit. The travel-time pdf for a solute according to the 2-component CDE model then was calculated analytically as a Laplace transform. Numerical inversion of the transformed pdf is carried out for several different sets of values of the four adjustable parameters in the CDE model (Peclet number, "fast" solute fraction, retardation factor, and intercomponent transfer coefficient). The effects of convection, dispersion, and linear sorption processes, as well as the influence of transfer into the "slow" solute component, were illustrated by numerically simulated travel-time pdf. It was concluded that the fractional transport volume (mobile-zone volume/soil volume) is the most significant physical parameter in the model in terms of impact on the shape of the travel-time pdf.

## SIGNIFICANCE

The most important conclusions in respect to soil salinity that can be drawn from the results obtained in this project are as follows:

(1) Under conditions of relatively high electrolytic conductivity ( $EC \approx 5 \text{ dS m}^{-1}$ ), Ca-Mg exchange behavior is independent of ESP in the range 0 to 25 percent at pH values near neutrality. This conclusion applies to montmorillonitic and illitic soils.

(2) Soils high in illite or high in montmorillonite/organic matter units will tend to show preference for Ca over Mg on the exchange complex. This preference decreases to no preference as the charge fraction of Ca in the soil increases for any ESP in the range 0 to 25 percent. Soils high in montmorillonite but low in organic matter should exhibit little or no preference for Ca over Mg at any charge fraction of Ca. The presence of chloride ions will enhance the preference for Ca in these soils.

(3) Sorption isotherm equations applied to soils should be interpreted as model curve-fitting expressions with predictive capability under well-defined conditions but with no a priori surface chemical significance.

(4) Conventional solute transport models based on a partial differential equation can be interpreted probabilistically through the Jury transfer-function model. This model will permit the prediction of solute movement at the field scale based on straightforward calibration methods. However, it does not lend itself immediately to coupling with a chemical speciation model like GEOCHEM because it is not mechanistic in nature.

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#### **SUPPORTING PERSONNEL**

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## VI. Field-Scale Studies

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### 17. Crop Response to Temporally and Spatially Variable Soil-Salinity Profiles

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**PRINCIPAL INVESTIGATORS:** D. E. Rolston, J. W. Biggar, R. J. Miller,  
D. R. Nielsen, and F. E. Broadbent

Research on soil salinity has made great strides in the past several decades toward understanding the effects of salinity on various crops. There is, in general, however, a lack of conceptual knowledge on how best to monitor and analyze soil water and soil salinity within field profiles. Crop response to spatial and temporal variability of soil-water and soil-salinity distributions has not been adequately investigated or understood. Several quantitative techniques are available or are being proposed to predict the root-zone salinity and the leaching required to maintain optimum yield of various crops. Field verification of these quantitative techniques is required to improve our abilities to relate crop tolerance, water quality, and leaching requirements to protect the soil and related environments while maintaining acceptable crop returns. This project made use of new methodology to reach those goals and to evaluate the spatial variability of soil characteristics influencing water and salt distribution within soil profiles. These new techniques are based upon geostatistical methods relying on regionalized variable analysis and other approaches. They allow the natural spatial and temporal variability of the soil and crop response to be evaluated and related to measurable parameters of the soil profile.

#### EXPERIMENTAL APPROACH

##### Experimental Design

In the research conducted at the University of California West Side Field Station, the irrigation system consisted of 2 lines of sprinklers placed on the field in a direction generally parallel to prevailing winds. One sprinkler line was used to apply low-salt water and the other high-salt water. The 2 lines were arranged so that overlap between the lines resulted in uniform water application between the lines but nonuniform salt application from one line to the other. Both water and salinity applications were nonuniform on either side of the 2 sprinkler lines. The area irrigated by the 2 lines was 45 x 50 m

or 0.23 ha. Various schemes were used for yield measurement including harvesting 1.0, 3.0, and 10.0 m<sup>2</sup> plots. The average ECs of the low- and high-salt waters for the 1981, 1982, and 1983 seasons were 1.5 and 4.0 dS/m, respectively. The ECs for the 1984 season were 1.5 and 6.0 dS/m, respectively.

During summer, sorghum was planted in rows parallel to the sprinkler lines. The crop was irrigated 1 to 3 times per week to satisfy 110 percent of ET requirements in the section of the field between the sprinkler lines. During winter, wheat was planted in the same configuration as the sorghum. The wheat was used primarily to extract winter rainfall and to increase soil salinity, although the crop was carried to full maturity in 1985. Irrigations were sometimes required for the wheat crop during April and May.

Water content was measured from 3 transects of neutron probe pipes spaced 1.5 m apart and perpendicular to the sprinkler lines. Measurements were taken at several depths once a week. Soil solution was also occasionally extracted from 2 transects of samplers, 1 perpendicular and the other parallel to the sprinkler lines.

Various in situ methods have been proposed by Rhoades and colleagues for assessing soil salinity, using differently configured electrical conductivity devices. In this experiment the Wenner array and the 4-electrode probe were utilized for study. The latter took the form of buried probes and a portable design developed by Hanson. In addition to measurements in the main plot, a calibration plot was developed where uniform profile concentrations of salt were established by leaching with drainage water to steady-state conditions.

Each year a 3-m strip across the field perpendicular to the line source sprinkler was fertilized with <sup>15</sup>N-depleted ammonium sulfate at the same N rate as the unlabeled fertilizer used for the remainder of the field. At intervals during the growing season, leaf or whole-plant samples of sorghum were collected and their total N and <sup>15</sup>N content determined. Similar samples of the unfertilized wheat crop have been analyzed to measure residual effects of fertilizer applied to the preceding sorghum crop.

Air, soil surface, and crop-canopy temperatures were measured at several locations along transects perpendicular to the sprinkler lines. These measurements were used to evaluate the effects of salinity and water stress on crop response.

The soil profile was extensively sampled at least once a year in October and occasionally in June as well. Samples were removed from 5 depths at locations consisting of 5 transects perpendicular to the sprinkler lines with 22 sites within each transect and from 9 transects parallel to the sprinkler lines with 25 sites within each transect during 1983 and 1984. Somewhat different sampling schemes were used for the initial, the 1981, and the 1982 samplings. These samples were analyzed for pH, EC, Cl, CO<sub>3</sub>, HCO<sub>3</sub>, B, SO<sub>4</sub>, Na, Ca, Mg, and K in extracts from saturated paste.

Crop yield was determined from 5 transects across the field perpendicular to the sprinkler lines. Each sampling consisted of a 1 x 4 m strip.



Sorghum growth adjacent to the high-salt sprinkler line. Low-salt sprinkler line is to the left of the photograph.

### **Availability of Field to Other Research Projects**

The field experimental site at the West Side Field Station was designed to also provide a field location for several other projects funded by the Kearney Foundation. The projects by Lauchli and Epstein, Tanji and Whittig, and Broadbent depended upon this field experiment for data. Substantial time and resources were dedicated to coordinating and managing the experiment and the data collection of the many investigators depending upon the field experiment.

## **GENERAL RESULTS**

### **Geostatistical Techniques**

A major research objective was to develop geostatistical techniques to evaluate and manage field-soil salinity. Several statistical

approaches are being used, including autoregressive and moving average models, bivariate models using a first-order state-space concept with an expectation maximization algorithm, polynomial plus autoregression equations based upon partial autocorrelation analysis, and multivariate statistical analysis.

An example of these statistical approaches is the use of polynomials and autoregression equations for analyzing yield as a function of distance within several transects perpendicular to the sprinkler lines. A fourth-order polynomial was fitted to the data to remove the spatial trend. The residuals representing the stochastic part of the data were described by an autoregressive model of order 2. The combination of the fourth-order polynomial (deterministic due to applied water) and the autoregressive model (stochastic) describe yield as a function of distance with a high degree of certainty. The stochastic component includes the effects on yield of soil variability, genetic variation, cultural practices, past history of the field, and measurement errors.

Another example of a statistical approach applied to this research is the use of a state-space model. The state-space model can be used for smoothing or estimating and forecasting a relatively short, nonstationary series of observations. This model was used to estimate missing observations of soil-water content, using joint analysis of observed surface-water content and soil-surface temperatures from the sorghum field irrigated by the double-line source sprinkler system. The parameters of the model indicated the degree of spatial correlation between the 2 measured parameters, an important factor in understanding the mechanisms that create the spatial variation.

### **Crop Response to Salt and Water Stress**

With each succeeding cropping year, root-zone salinity increased with depth and the maximum yield of sorghum in general decreased. Since the maximum average root-zone salinity did not cover the expected range of salinity required to ascertain the total crop-yield decrement curve, only a portion of it, independent of matric potential effects, can be evaluated. Depending upon the manner in which the average root-zone salinity is determined, the threshold value for sorghum agrees with published values. This observation is made at this time, however, without specifically removing the possible effects of interacting stresses created by other constituents and variations in soil-water content. The contribution of the various factors to total stress will be evaluated after analyses of the 1984 soil samples are completed.

As might be expected, the crops responded differentially in the regions where both salinity and water content could be limiting. Assuming that equal quantities of water were applied external to the midregion of the line sources, an assumption based on measured amounts of water applied, it was observed for at least 1 year that yield on the high salt side equalled that on the low salt side for that region of the irrigated area in which sufficient water was present to carry the crop to maturity. This suggests that the presence of salinity at certain stages of growth may be beneficial to ultimate production for

conditions where water alone might otherwise be the limiting factor in production. It also brings into question the equivalence of solute and matric potential effects on crop response. While EC of the saturation extract is useful for predicting crop response to water quality and crop tolerance to salinity, the opportunity exists to examine crop response based on predicted values of the root-zone salinity, using chemical models and the effects of specific constituents.

### **Spatial Variations of Soil Chemical Properties**

The experimental design was purposely chosen to impose salt and water gradients to examine the nature of the resultant distributions in the root zone of an actively growing crop. During the experiment, the pattern of accumulation of various constituents was in general agreement with the imposed salinity content of the water. That is, the concentration of the various constituents accumulating in the profile was lowest on the low-salt side and increased to a maximum on the high-salt side. On the latter side, the maximum values occur in the region outside the high-salt line where water application and crop extraction combine to optimize salt application and minimize leaching. Exceptions to this observation include  $\text{HCO}_3^-$  and  $\text{CO}_3^-$  which were more uniform with the variations about an average value normal to the line sources from low to high salt.

As might be expected, the variations in different constituents were of a different nature. As time progressed with each succeeding year, the concentrations at any particular location increased with depth. Surface soils were salinized most rapidly and responded most quickly to variations in winter rainfall.

Early in the experiment, it was observed that spatial variations in the exchangeable sodium percentage (ESP) and sodium-adsorption ratio (SAR) differed. Spatial fluctuations in the ESP were quite large when compared with the SAR, and the correlation between the SAR and ESP was not high but improved with depth. The lack of correlation may reflect variations in chemical reactions involving cation exchange, precipitation and dissolution, and clay minerals. Other investigations have reported results that suggest that spatial variations of EC appear to be greater in wet soils and that such variations tend to be less important in drier soils. These experiments tend to agree with that observation and show that under treatments of high salinity the coefficient of variation for some constituents is less.

Relationships between constituents and their impact on soil and plant response are often based on observations derived from the saturation extract and often larger water contents. This may be quite satisfactory, even though it is recognized the crop is functioning in a much drier environment. While it is not possible to calculate the actual environment, some indications of the chemical environment at the field water content can be obtained by utilizing chemical simulation programs that include speciation, precipitation and dissolution, and exchange. Calculations utilizing a chemical model have indeed revealed a chemical environment quite different from the saturation extract. The spatial

distribution of constituents based on lower water contents is different from 1 based on saturation extract values. That fact could affect the interpretation of plant absorption relative to soil-solution concentrations as well as predictions of solute transport by various models.

### **Temperature and Plant Water Stress Index**

Considerable interest has recently developed in the use of infrared techniques for temperature measurement of crop canopies and soil temperatures. The nondestructive rapid technique could provide a simple and effective method of scheduling irrigations or otherwise evaluating plant stress caused by water and possibly salt.

Results of this experiment clearly demonstrate that plant temperature and soil-water regime are related. It was also shown that soil-moisture and soil-surface temperatures are related. Of particular interest was the testing and use of a plant-water stress index (PWSI). The index as proposed requires measuring the vapor-pressure deficit as well as air and canopy temperatures. Because of the wide range in quantity of applied water and the corresponding canopy that developed, there was a wide range in both soil and plant-canopy temperatures which changed with the season as well as with soil-water content. Soil-surface temperatures across the experiment ranged from 28° to 60°C, and corresponding canopy temperatures from 32° to 42°C on a particular day. Salt appeared to affect soil temperatures significantly. On the other hand, a range in soil EC of 3 to 6 dS/m apparently did not greatly change plant-canopy temperature. Results of several hundred measurements, however, did demonstrate that plant-canopy temperature and soil-water regime are related as are soil-surface temperature and water content. Use of a PWSI that responds to soil salinity as well as to water content would appear beneficial. Results from 1983 showed the PWSI was well correlated with mean soil salinity on some days but poorly correlated on others. Measurements conducted in 1984 greatly improved on 1983 results and it now appears that not only is the previously poorly-defined base line improved but the PWSI consistently reflects response of the crop to irrigation and corresponding changes in soil salinity.

### **Uptake of <sup>15</sup>N Labeled Fertilizer**

A typical pattern of N uptake by the sorghum crop shows steep gradients corresponding to gradients in applied salt and applied water, alone and in combination. A much smaller gradient in N uptake has been found, where there was a salinity gradient but no water gradient. Utilization of nitrogen by sorghum was spread over a longer growth period in the region of salt stress than where salt was not applied, which partially compensated for the depressing effect of salt on N uptake.

The fraction of the total N in the sorghum crop derived from the labeled fertilizer was related to moisture stress and salt stress, alone



and in combination, with the result that little fertilizer N was utilized in the areas of greatest stress. Wheat crops grown in winter reflected the larger amounts of residual N near field edges.

### **Analysis in Progress**

Soil samples following the 1984 sorghum crop and 1985 wheat crop are being analyzed, and data will be included with previous years to assess better the spatial and temporal variations of constituents.

Because of the nonstationarity of yield, chemical and physical properties of the soil, various statistical techniques that recognize that fact are required and some have already been described. This is different from other line-source experiments where stationarity has been assumed a priori. The experiment not only provides a measure of the drift that is observed in soil properties and how that characteristic may influence chemical distribution and yield, but it also provides a means of examining various methods of data analyses. These include using an intrinsic random function of order  $k$  to kriging a data set of textures and CEC. As an adjunct, pattern analyses involving univariate and multivariate techniques have been applied to a limited set of data involving several chemical and physical parameters. In addition to water content, salinity helps determine the pattern of plant response. Application of these techniques will be extended to a wider range of chemical parameters, including predicted values based on chemical models.

### **SIGNIFICANCE**

The line-source sprinkler system for conducting agronomic field experiments has been used for several years. We developed a unique design using a double-line source with one line applying low salt and the other high-salt water. The overlap between the lines resulted in uniform water application between the 2 but nonuniform salt application from 1 line source to the other. This configuration allowed us to develop a range of soil-water content and soil salinity in a continuous pattern within a reasonably small experimental area. Thus, this design resulted in a maximum treatment effect with a minimum of irrigation management cost.

The use of the double-line source sprinkler system offered opportunities and challenges for statistical approaches to handling the data. Polynomial and autoregression equations were used for analyzing yield as a function of distance along transects perpendicular to the sprinkler lines. This approach allowed the sorghum yield to be described by a polynomial component accounting for deterministic water and salt application and a stochastic component accounting for measurement errors, effect of soil variability on yield, cultural practices, and genetic variation.

The state-space method was used to examine the relationship between jointly measured series of observations separated by distance.

This is the first time that this method has been applied to data from agronomic field experiments. The method provides another technique for smoothing or estimating, and forecasting. The series does not have to be stationary, observations need not be evenly spaced, and a relatively short series can be used. This approach provides a means of identifying those different soil parameters that are related spatially or temporally. Being able to make such identifications allows greater opportunity to understand the mechanisms that create the variability.

Although N-mineralization and nitrification are only slightly affected by salinity and sodicity within the range which higher plants can tolerate, N uptake is affected by both salinity and moisture gradients, and although we found evidence that the period over which sorghum assimilates N is prolonged in salinized soil, fertilizer N use efficiency was decreased by soil salinization. Fertilizer N levels used on saline and/or sodic soils should be reduced accordingly.

The results demonstrated that plant-canopy and soil-surface temperature are related to the soil-water regime. On the other hand, a range in soil EC of 3 to 6 dS/m did not greatly change plant-canopy temperature, indicating small effects of salinity on transpiration.

The spatial variations in the exchangeable-sodium percentage (ESP) and sodium-adsorption ratio (SAR) were found to differ. The SAR changed spatially in a smooth manner, whereas the variability of ESP was large.

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#### **SUPPORTING PERSONNEL**

G. Cheng (SRA), T. N. Nakashima (SRA), C. Bergens (SRA), D. Louie (SRA), J. MacIntyre (SRA), F. Morkoc (graduate student), G. Kluitenberg (graduate student)

## 18. Spectral and Cospectral Analyses of Crop and Soil Observations in Relation to Infiltration and Soil Salinity

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**PRINCIPAL INVESTIGATOR:** D. R. Nielsen

### Objectives

(1) To improve existing field technology to link the behavior of crop plants with soil properties, particularly those that relate to the management of salt-affected soils,

(2) To develop criteria and economically explicit procedures for using classical and/or geostatistical sampling schemes for irrigated agricultural fields.

### Synopsis

Several field experiments were analyzed using spectral and cospectral analyses as well as other geostatistical methods. Each analysis has been recorded in Ph.D. dissertations or in technical journals cited in publications listed below.

### PUBLICATIONS

- Bazza, M. 1985. Modeling of soil surface temperature by the method of two-dimensional spectral analysis. Ph.D. Dissertation, University of California, Davis.
- Mechergui, Michael. 1984. Stochastic modelling of the water table in the vicinity of drainage tiles. Ph.D. Dissertation, University of California, Davis.
- Nielsen, D. R., and J. Bouma. 1985. Soil spatial variability. Pudoc, Wageningen, The Netherlands. 243 pp.
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## **SUPPORTING PERSONNEL**

Patrick Aina (postgraduate researcher), Mohamed Alemi (postgraduate researcher), Mohamed Bazza (research assistant), Mingliang Chen (postgraduate researcher), Peter J. Greminger (postgraduate researcher), S. Hajrasuliha (postgraduate researcher), Masaaki Kiuchi (postgraduate researcher), Tassos Louisakis (postgraduate researcher), Mohamed Mechergui (research assistant), T. Miyazaki (postgraduate researcher), Yashvir K. Sud (postgraduate researcher), Michael Sully (research assistant), Patricia M. Tillotson (research assistant)

## 19. On-Farm Field and Crop Variability: Kings County

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**PRINCIPAL INVESTIGATORS:** J. Oster, B. Hanson, and S. Kite

Farmers in Kern, Kings, and Fresno counties are constructing on-farm evaporation ponds for drainage water and salt disposal. Pond areas range from 10 to 20 percent of the irrigated area served, and depend primarily on the spatial variation of water infiltration within irrigated fields. Five percent or less of an irrigated area would be sufficient to evaporate the drainage water required to assure salinity control. Except for the experience in sizing on-farm evaporation ponds and estimates of drainage volumes based on field water budgets, infiltration variability has not been characterized: infiltration is a difficult parameter to measure. One purpose of this project is to determine whether the new methods for measuring soil conductance can be used to characterize infiltration variability.

The hypotheses of this project are: (1) soil-salinity variability results primarily from water-infiltration variability, and (2) new resistivity and electromagnetic methods facilitate rapid measurement of soil-salinity variability and, if hypothesis 1 is valid, of infiltration-variability, also.



Instrument used to measure soil salinity distribution across fields.

## EXPERIMENTAL PROCEDURES

Six fields (table 1) were selected for this study. Preliminary soil-conductance and crop-quality measurements were conducted at 30-m intervals along three transects in fields 1 through 3, 5 and 6. Similar data were obtained along a single transect in field 4. Calibration relationships between soil-conductance and saturation-extract electrical conductivity were established for each field, based on soil samples obtained at selected sites along the transects. The spectral transmittance of aerial photographs (both regular color and infrared) of each field was determined, on a grid of about 1 m, using computer technology available at UCD. The degree of correlation between spectral-transmittance soil conductance and crop quality was determined. Based on these preliminary studies more detailed soil-conductance measurements were made along one transect in fields 1 and 4. In addition, infiltration and water advance rates were measured along the transect for field 4.

Table 1: Crop and geographic description of selected fields.

| Field | Crop    | Area<br>(ha) | Location (Kings Co.) |        |
|-------|---------|--------------|----------------------|--------|
| 1     | Alfalfa | 33           | T.19.S               | R.21.E |
| 2     | Cotton  | 33           | T.19.S               | R.21.E |
| 3     | Alfalfa | 33           | T.19.S               | R.21.E |
| 4     | Cotton  | 65           | T.19.S               | R.19.E |
| 5     | Alfalfa | 20           | T.19.S               | R.22.E |
| 6     | Alfalfa | 13           | T.19.S               | R.22.E |

The research was only funded during the last year of the Kearney mission and the data have not been fully analyzed. Consequently, the results reported here are preliminary.

## RESULTS

(1) Crop quality, soil conductance, and spectral transmittance were correlated with one another ( $0.3 < R < 0.9$ ). Crop quality and transmittance correlated best with the average soil conductance at 30- and 60-cm depths. Including deeper depths decreased the correlation. Along one furrow, the lowest soil conductances occurred where the irrigation water advance rate was the slowest.

(2) Spectral transmittance of aerial photographs can be obtained quickly on a scale as small as 1 m with camera/computer facilities available at UCD.

(3) Soil-conductance measurements require from 2 to 6 minutes per site for a crew of 2. Consequently, a 400-m transect at a 10-spacing requires from 0.5 to 1.5 hours of work.

(4) Estimates of infiltration variability may be possible from either soil-conductance measurements or from spectral transmittance provided field-specific soil conductance and associated spectral transmittance data are correlated. The ratio,  $EC_{iw}/EC_{dw}$ , which is approximately equal to the leaching fraction, and its spatial variation would be used to characterize the corresponding infiltration variation. The advantage of the spectral transmittance method is that each number represents an area from 1 to 10 m<sup>2</sup> and the data for a 30-ha field can be digitized by the computer/camera system in less than 1 minute.

(5) The following aerial distributions of leaching fractions, a relative measure of infiltration uniformity, are based on soil conductance measurements: (a) Less than 1 percent of the values along a transect in a cotton field corresponds to leaching fractions greater than 0.25, or 1 percent per 0.25; 33 percent corresponds to leaching fractions greater than 0.10, or 33 percent per 0.1. (b) The corresponding pairs of numbers for an alfalfa field are 8 percent per 0.25 and 38 percent per 0.10. Similar assessments for the other fields in this study are currently underway. Because of the good correlation between soil conductance and spectral transmittance for several fields, use of this technique to assess irrigation uniformity appears to have some potential for fields with sufficient salinity to affect crop growth.

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#### **SUPPORTING PERSONNEL**

D. Monson (SRA), R. Strohman (SRA), W. Wildman (soils specialist)



## 20. Estimating Mean Value and Variance of Solute Concentrations and Mass Emissions during Leaching in the Field

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**PRINCIPAL INVESTIGATORS:** William A. Jury and Lewis H. Stolzy

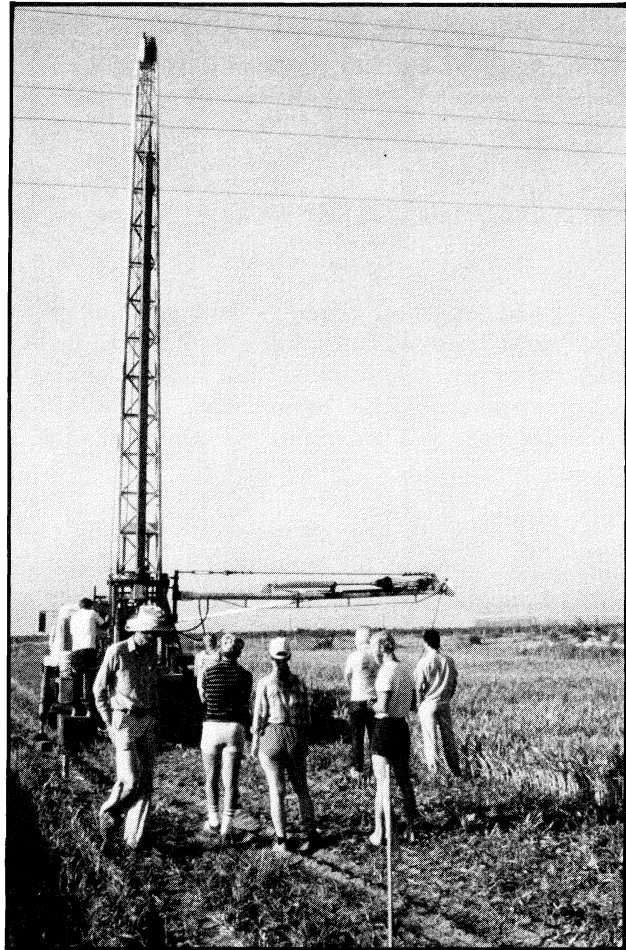
Although virtually all salinity research is applicable to agricultural operations in the field, most of the fundamental soil physics research on transport has taken place in the laboratory. This laboratory research has been used to develop guidelines for saline water management and to develop theoretical models of water and chemical transport.

In our research project conducted over the last 7 years, with partial support of the Kearney Foundation, we have focused on conducting field-scale experiments on transport and developing field-scale models of the solute transport process. Our goal has been to incorporate the natural variability present in the field in the description of the transport process and into the management guidelines that emerge from this description.

At the outset we distinguished between transport problems. The first focused on describing the movement of water-tracer chemicals, such as chloride or bromide, under field conditions. This problem had immediate applications to the transport of mobile agricultural constituents, such as nitrates. The second part of the program, developed during the latter stages of the Kearney Foundation support, focused on superimposing this water-tracer variability with simple representations of solution-solid phase interactions, which retarded the movement of chemicals adsorbing to the solid interfaces. Among the agricultural chemicals affected by adsorption are virtually all pesticides applied to the soil surface and in addition, cationic constituents, such as calcium, magnesium, and sodium, which adhere to the negatively charged clay-mineral surfaces common to most agricultural soils. Thus, the implications of the adsorption research eventually could affect characterization of all exchange-related problems, such as soil sodicity, as characterized through the exchangeable sodium relation or other similar indices.

The objectives of the 3-year Kearney Foundation project were: (1) to investigate the mechanisms governing transport of nonadsorbing chemicals on the field scale; (2) to characterize the spatial variability of solute concentrations and mass emissions during leaching; and (3) to represent theoretically and study experimentally the superposition of adsorption and transport in a spatially variable situation where each parameter may take on different values over the field.

Soil sampling in the field to monitor chemical movement in the profile.



## RESULTS

The project's first milestone was in the successful application of a transfer function model to the description of nonadsorbing chemical transport on the field scale. The transfer function formulation consists of a description of solute transport expressed as a travel-time probability density for a soil volume of interest (for example, a crop-root zone). In this formulation, the solute travel time between the surface-entry point and the exit point in the soil is viewed as a random process which is defined by a travel-time probability density function. All that is required to apply the theory is a measurement of the average concentrations exiting from the volume at a given depth in the field.

We made this measurement with a network of solution samplers located at 16 points within a sandy-loam field at Etiwanda, California. The mean concentration obtained by averaging the 16 sampler readings, when properly normalized, forms the travel-time probability density function. The concentration at this exit depth arising from any future input of solute to the surface may then be expressed as a superposition of the input concentration function and the travel-time density function expressed as a convolution integral.

The model was successfully applied to the description of solute transport over the top 3 m of this same sandy soil. This transfer-function expression provided a superior description to the convection-dispersion equation on this data set, thus casting doubt on the validity of conventional laboratory methodology when it is applied to field conditions. An advantage of this formulation is that it does not require a physical picture of the transport process within the soil volume nor does it require any transport-coefficient measurements other than the travel time distribution function.

A by-product of this experiment and the other measurements made in the field during the 5-year history of solute-transport studies was an accurate characterization of the lateral variability of solute concentrations during vertical transport processes. In a comprehensive analysis of these experiments and others throughout the literature, we concluded that the coefficient of variability of solute transport during dynamic events in which the concentrations changed suddenly commonly manifest coefficients of variation between 60 and 130 percent of the sample mean value. This characteristic variation may be used to design future experiments in which a certain desired precision of estimate of the field mean value is required. For example, with this characteristic estimate of the coefficient of variation, one can design the number of samples in advance of a field study using the desired level of precision as the optimizing factor. As a rough guideline, we estimated that for any future experiments conducted on our Etiwanda field we would need approximately 25 samples at a given depth to come within 30 percent of the field mean value of concentration during a dynamic event, such as solute leaching or infiltration of a front of chemical.

In the latter stages of the Kearney Foundation study we developed an extension of the transfer-function model to allow for both water-flow variability and for variability in solute adsorption. To illustrate the theoretical model, we used a simple linear-partitioning model employing a partition coefficient that was constant at a given location but that varied randomly throughout the field. At the same time, the travel time of a mobile, nonadsorbing solute also varied randomly according to a probability distribution we had measured earlier in the field. We analyzed a number of cases theoretically and found that prediction of the earliest arrival time in groundwater for a soil-applied chemical depended critically on the interaction between adsorption and water-flow variability. A theoretical paper was produced which analyzed predictions made by different models, which were calibrated on the same data set at the soil surface and were subsequently used to predict travel times to groundwater. Depending on the model, and whether it took parameter variability as well as mean value into account, radically different predictions were made for travel times to groundwater lying far beneath the soil surface. For example, in a model scenario, the conventional convection-dispersion model with constant adsorption predicted almost 500 years for the travel time of the leading edge of an adsorbing solute front to a groundwater table 100 m below the surface, while the stochastic transfer-function model, using the same data but taking variability into account, predicted that the earliest arrival would occur after only 15 years. These significant differences could play an important role in emerging groundwater protection strategies under consideration by state and federal agencies.

The final step in our characterization of solute concentration and flux variability was to conduct a field experiment on the comparison between adsorbed and nonadsorbed chemical transport at the Etiwanda field site. This experiment, just completed and analyzed preliminarily at the time of conclusion of the Kearney-funded study, eventually showed that pesticide transport variability drastically exceeded the variability of the nonadsorbed tracer (chloride) when applied simultaneously at the Etiwanda field site. While approximately 80 percent of the pesticide applied remained near the soil surface in the top 20 cm, the remaining 20 percent of applied pesticide was erratically distributed between 20 cm and 180 cm, in a manner that indicated that the adsorption mechanism was not operating.

An extensive analysis of the soil structure and the experimental data led us to conclude that this erratic movement could not be explained exclusively on the basis of pore bypass involving macropores; more likely, it involved the presence of a soluble pesticide complex that did not interact with the adsorption sites. Such behavior does not appear in traditional laboratory methods of measuring adsorption but can play a critical part in downward transport under natural field conditions. Because pesticide regulation strategy is now moving towards monitoring the zone immediately below the soil surface, the presence of pesticides or other organic chemicals at this depth is considered an indication that the chemical is not suitable for use because of environmental hazards. This research, which should be followed up by further studies, may indicate that such behavior is more widespread than previously indicated. The work may also apply to inorganic chemicals commonly found in irrigation water, such as calcium, magnesium, and sodium, undergoing exchange adsorption, or could apply to fertilizer amendments, such as phosphorus and potassium, which interact with the solid phase as well.

## **SIGNIFICANCE**

The research conducted under the Kearney Foundation study allowed us to participate in several large-scale field experiments looking at the spatial variability of chemical movement. Analysis of these experiments provided motivation for developing a theoretical transfer-function model which does not require a detailed set of measurements to be made of hydrologic properties which may vary substantially under field conditions. The research also provided repeated estimates of solute concentration variability from which management guidelines for expected solute concentrations could be formulated.

Extension of the spatial variability research into adsorbed chemical transport revealed a hitherto unexpected degree of variability compared to the water-flow variability which has implications for characterization of adsorption under transport conditions in the field.

#### **PUBLICATIONS PRODUCED UNDER KEARNEY FOUNDATION**

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#### **SUPPORTING PERSONNEL**

Tim Collins and Hesham Elabd (graduate students), Ted Szuszkiewicz and Paul Sternberg (SRAs), Gary Lopatynski (laboratory assistant)

## 21. Designing Subsurface Drainage Systems for Irrigated Cropland to Minimize Salinity Effects

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**PRINCIPAL INVESTIGATOR:** A. C. Chang

Proper water management is essential to the success of any crop production. Neither excess nor deficient soil water conditions during the growing season are desirable. If they occur for extended periods of time, the soil physical environment created by the adverse soil water conditions not only is undesirable for plant growth but also limits the accessibility of the field. Trafficability of the crop-growing fields ensures the timeliness of cultivating, planting, and harvesting.

Irrigation and drainage are by far the most important practices in providing the soil moisture conditions most conducive to crop production. In semiarid climate, often both irrigation and drainage are needed to maintain the optimum plant growth. A properly installed subsurface drainage system will alleviate the waterlogging caused by irrigation and, subsequently, prevent salinization of the soil. Customarily, tile drains are designed to maintain a specific water table level beneath the irrigated field which is receiving a given water input. Ideally, a subsurface drainage system for irrigated fields should be related not only to the underlying water table but also the soil salinity and the crop yield.

A water management simulation model (DRAINMOD) for shallow water table soils has been developed. The model is based on a water balance at midpoint between 2 parallel drains. It was intended for comprehensive water management analyses on the scale of a farm or a production field where most water management facilities are designed or installed as a single unit. Since its development, the model has been verified with data from at least 20 site years of field scale drainage experiments in North Carolina and Ohio. The model in its entirety was adapted by the U. S. Department of Agriculture, Soil Conservation Service, for evaluating the water management schemes of high water table soils in humid regions. It enables the users to examine the effects of various water management alternatives and to select the most suitable drainage system design.

The fundamental concept of water balance is universally applicable; the model, however, was tested only against field data from the humid climate. If this model is also judged adequate for irrigated cropland in the semiarid climate, it would provide a new approach to design subsurface drainage systems. A functional water balance can become the basis of a salt balance for irrigated cropland. A water/salt balance model would permit selection of drainage systems based on water table and soil salinity.

Objectives of this study are:

- (1) to test the model against field data from tile-drained irrigated cropland of semiarid climate,
- (2) to compare the outcome of designing a subsurface drainage system using the water balance model with those obtained by following the conventional drain-design procedures, and
- (3) to develop a salt balance for the model for the salinity of the tile-drained irrigated cropland.

## INVESTIGATION APPROACH AND RESULTS

### Description of the Model

The mathematical basis of the water-management simulation model is the water balance of a shallow water table soil at midpoint between 2 parallel drains (extending from the ground surface to the impervious layer). The water balance equation of a given time interval  $\Delta t$  may be written as:

$$\Delta V_a = D + ET + DS - F \quad (1)$$

where  $\Delta V_a$  = the change of air volume in the soil profile. Air volume denotes the void volume of the soil that is not occupied by water,

$D$  = water removed due to artificially induced subsurface drainage,

$ET$  = evapotranspiration of the soil profile,

$DS$  = water loss due to deep seepage, and

$F$  = infiltration at the ground surface.

In this equation, the infiltration ( $F$ ) is the sum of water applications (precipitation and irrigation), surface runoff, and the change in depression storage. Terms on the right hand side of Eq. (1) are either entered as inputs or evaluated by defined mathematical relationships. Assuming the water held in the unsaturated zone of the soil profile is hydrostatically in equilibrium with the underlying water table, the moisture distribution of the soil may be expressed in terms of the air volume. The change in air volume calculated by Eq. (1), in turn, reflects the fluctuation of water table for  $\Delta t$ .

The simulation starts with the water balance at the ground surface to determine the amount of infiltration and proceeds with calculation of drainage (D) and deep seepage (DS) based on the initial conditions. The evapotranspiration (ET) is given as input data. The resulting  $\Delta V^a$  enables the model to define the water table at the end of  $\Delta t$ . Using the approach of successive steady-state equilibrium and with given climatological data, soil-water characteristics, crop-growing information and physical dimensions of the drainage system, the model is able to determine day-by-day responses of the water table and the soil moisture regime above the water table for defined water application schemes.



Collector ditch for drainage water from irrigated fields.

### **Model Verification**

Data from two drainage experiments in California were selected for testing the performance of DRAINMOD under irrigated cropland conditions. The San Joaquin Valley Drainage Investigation was initiated in 1959. Experimental sites selected for this study covered a large part of the San Joaquin Valley and represented a wide range of soil classifications, drain spacings, and cropping practices. Four sets of data from this study were selected for testing. The second set of data was obtained from a drainage experiment conducted in the Imperial Valley of California by researchers of the Imperial Valley Conservation Research Center, ARS, USDA, at Brawley.



The result indicated that the calculated water balance at each test site agreed well with that observed in the field. Statistical analyses comparing predicted daily water-table elevations with the observed water table of the corresponding growing season showed 6 to 23 cm of standard error for seasonal water-table fluctuations of 150 to 200 cm. The model was not capable of predicting the transport of water in the soil profile. Because the simulated water table responded to water applications in the same manner as the observed water table and the extents of water table fluctuations predicted were similar in magnitude as those measured, the model was judged adequate for examining the effect of various water-management alternatives and to select appropriate drainage systems. The model testing also demonstrated that water loss by deep seepage is a significant component in soil-water balance for the irrigated cropland.

### **Comparison of Drain Design Procedures**

For the computer model to be useful in assessing the performance of and in designing subsurface drainage systems, it is essential that results obtained by model simulation are compared with those using conventional drain design procedures. The dynamic equilibrium procedure outlined in the Bureau of Reclamation Drainage Manual was used to represent the conventional design approach. Because of discrepancies in estimating specific yields of the soil and in distributing the water inputs, the lateral spacings calculated by these two approaches were dissimilar. If the same values of specific yields and water inputs were used in both calculations, the dynamic equilibrium equation produced essentially the same results as the model simulation. Since procedures used in DRAINMOD provided more accurate estimation of specific yields and more realistic water inputs, it was felt that drain spacings derived from the model simulation were more reliable.

The effect of deep seepage on lateral spacings of drains was also analyzed. If the hydraulic conductivity of the impervious layer was less than 1/10,000 of that of the soil profile above it, the calculated lateral spacing was not affected by the deep seepage. When the hydraulic conductivity of the impervious layer became greater than 1/1,000 of that of the soil profile, the lateral spacings might be relaxed by accounting for the seepage loss.

### **Salt Balance**

In the salt balance, a crop year (starts in September and ends in August of the following year) was divided into two seasons, noncrop growing season (September to March) and crop-growing season (April to August). The soil salinity of the effective root zone was determined by water/salt balance of each season.

The water balance of the effective root zone for a season is written as:

$$D_N = I + R - ET + \Delta SW \quad (2)$$

where  $D_N$  = net drainage below the root zone

$I$  = irrigation

$R$  = effective precipitation

$ET$  = evapotranspiration

$\Delta SW$  = change in soil moisture

of the season considered. The corresponding salt balance was:

$$V_S \times EC = I \times EC_I - D_N \times EC_D \quad (3)$$

where  $EC_I$  = salinity of irrigation water

$EC_D$  = salinity of drainage

$EC$  = change in soil salinity

$V_S$  = saturation volume of the soil

Assuming there is no loss of salts due to chemical precipitation and plant absorption and no increase of salts due to mineral dissolution, the salinity of the drainage may be calculated

$$EC_D = \frac{[I \times EC_I + V_S \times EC_0]}{[I + R - ET + SW]} \quad (4)$$

where  $SW$  = initial soil moisture content of the effective root zone

$EC_0$  = initial soil salinity of the effective root zone

Substituting Eqs. (2) and (4) into (3) and solving for  $EC$ ,

$$EC = \frac{I \times EC_I - (I + R - ET + SW) \times \frac{[I \times EC_I + V_S \times EC_0]}{[I + R - ET + SW]}}{V_S} \quad (5)$$

All terms of Eq. (5) may be obtained from the water balance or are the initial conditions of the salt balance.

The salt balance has been incorporated into the model and tested.

## SIGNIFICANCE

The model was developed with the basic principle of water and salt balance of the soil profile. It demonstrated that deep seepage, an often neglected component in drainage design, may be important in the water balance for the irrigated cropland of the semiarid climate. The usefulness of this investigation lies on the application of the model as a tool in evaluating the water-management alternatives and in designing drainage systems. The model will be used to evaluate drainage performance in the western U.S. in a regional research project proposal to be submitted by WRCC-54. Currently, it is being used to analyze the data of two long-term drainage experiments from Egypt. The confidence on the model simulation will grow with more and more uses.

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- Persaud, N., and A. C. Chang. 1983. Estimating soil temperature by linear filtering of measured air temperature. Soil Sci. Soc. Am. J. 47:841-47.
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## SUPPORTING PERSONNEL

N. Persaud (postdoctoral researcher), J. Frampton (postdoctoral researcher)



# VII. Economic Considerations

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## 22. Economic Analysis of Salinity and Drainage Problems in the San Joaquin Valley

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**PRINCIPAL INVESTIGATOR:** Keith C. Knapp

The project investigated agricultural water management under saline and/or limited drainage conditions. Methods and results for the specific topics addressed are given in the following sections. Further research needs are discussed in the last section.

### **Optimal Quantities of Seasonal Irrigation Water**

Production functions were developed for several crops. These relate crop yield, quantity, and quality (EC) of drainage water to seasonal quantity and quality (EC) of irrigation water. The production functions consist of 4 main components. These are: (1) soil salinity as a function of leaching fraction and salt concentration of the irrigation water, (2) relative yield as a function of soil salinity, (3) a relation between yield and evapotranspiration, and (4) a probability distribution for B where B is the depth of water infiltration at any point in the field expressed as a fraction of the average applied depth in the field as a whole. Given a quantity and quality of irrigation water applied to the field as a whole, the probability distribution of B is used to calculate depths in various parts of the field. The first 3 relations are then solved simultaneously to determine crop yield and quantity and quality of drainage water in each part of the field. Summing over all parts of the field gives yield and quantity and quality of drainage water for the field as a whole.

For crops with a nonlinear relation between yield and evapotranspiration (for example, cotton, wheat, and barley), relations 2 and 3 are based on vegetative growth or shoot weight, instead of yield, and an additional relation between yield and vegetative growth or shoot weight is developed. Several different probability distributions for B were used. The production function model under uniform water applications and an empirical test for fescue are described in [9]. Applications to alfalfa, cauliflower, celery, corn, cotton, cowpea, lettuce, oats, sugarbeets, tomatoes, and wheat are given in [8]. The production function under nonuniform water applications is described in [4].

The production functions were used in [4] to calculate optimal (profit-maximizing) seasonal irrigation quantities and associated yields, net income, and drainage quantities for corn and cotton under a range of conditions. Alternative levels were considered for irrigation water salinity, irrigation uniformity, salt tolerance, and price of irrigation water under several different drainage scenarios. Increasing the salt concentration of the irrigation water implied substantial increases in optimal water quantities for corn, small increases or no change for cotton up to 5 dS/m, but more substantial increases when water with an EC of 11 dS/m was used. Decreasing the uniformity of applied water from a CUC (Christiansen Uniformity Coefficient) of 100 to 72 increased optimal water quantities by as much as 100 percent when drainage was not limiting. The effects of decreases in application uniformity were much smaller when the cost of disposing the drainage water was significant.

The nature of the drainage situation has a significant impact on optimal water quantities, yields, and net income. Going from a situation of unlimited natural drainage to one where all drainage water must be disposed of in an evaporation pond reduced optimal water quantities by as much as 63 percent and net income levels by as much as 58 percent in some cases. The reductions are greater for nonuniform irrigations than for uniform irrigations. Improvement in crop-salt tolerances had relatively small impacts in most--but not all--of the cases analyzed. Increasing the price of irrigation water from \$1 to \$3/ha-cm implied significant reductions in optimal water quantities for cotton under nonuniform water applications. The conclusion is that variations in these parameters over a plausible range can imply substantial changes in profit-maximizing water quantities, yields, net incomes, and other variables. Blanket statements as to required or optimal water quantities in a given region for a particular crop are likely to be unreliable.

### **Reuse of Agricultural Drainage Water for Irrigation**

Reuse of agricultural drainage water for irrigation was investigated in [6]. Six crops in 2 areas were considered. The production function assumed steady-state soil salinities, Maas and Hoffman coefficients for yield reduction, and constant ET. Two water sources were considered, 1 freshwater and 1 drainwater, with specified salt concentrations. Profit-maximizing quantities of water from both sources were then calculated for each crop under alternative water prices.

Drain water was not reused for the most sensitive crops (lettuce and grapes) in any situation considered. Some drain water was reused on navel oranges in the Coachella Valley. However, the increase in returns net of water costs due to reuse was less than 3 percent, so it seems unlikely that it would pay to reuse drainage water in this case when other factors are considered. Drain water was reused on the field crops in most situations considered. However, the increase in net returns was small for the lowest freshwater prices considered, so reuse would probably not be profitable in this case either when other factors were considered. The increase in returns net of water costs for the

field crops was much more substantial when freshwater was expensive (a 21 percent increase was obtained in 1 instance) and so drain water reuse may well be profitable in this case.

Optimal mixing of saline and nonsaline waters was investigated in [5] using the production function described in the previous section. Isoquants were generated for the crops discussed in [8]. These show alternate combinations of saline and nonsaline water which give a specified yield level. Given the isoquants, an optimal (cost-minimizing) mix of water from the two sources can be found. For 1 crop (lettuce) it did not pay to mix water for any of the relative yield or EC values of saline water considered. For the majority of crops (alfalfa, cauliflower, celery, corn, oats, sugarbeets, and tomatoes) mixing would be optimal, depending on the relative water prices for saline waters with the lower EC values considered ( $<6$  dS/m) but not for the high EC value (11 dS/m). For cotton, cowpeas, and wheat, mixing could be optimal, depending on relative water prices for all EC values considered. Thus, the degree to which waters of varying quality should be mixed depends on the crop, the desired yield level, the salt concentrations of the waters being considered, and the relative prices of the irrigation water. Depending on the situation, it may be optimal to use no saline water, a blend of nonsaline and saline waters, or saline water only.

### **Interseasonal Management under Saline Conditions**

Interseasonal irrigation management under saline conditions is addressed in [2]. Production functions and dynamic salt balance relations are estimated econometrically for alfalfa and cotton, using field experiment data. These relate yield and ending soil salinity, respectively, to soil salinity at the beginning of the season, quantity and quality of irrigation water applied during the season, and pan evaporation. Good results were obtained from the analysis.  $R^2$  values were generally high (.8-.9) for at least 1 set of functional forms for each crop and the functions generally behaved as expected. The estimated functions were then incorporated into a dynamic programming model. This model calculates optimal water quantities and qualities for each crop over a sequence of years. (Only a single water quality was considered for alfalfa.) For comparison purposes a so-called 1-year decision rule was also computed. This assumes that decisions are made to maximize current year profits without regard to the future.

For cotton optimal water quantities increase as initial soil salinities increase. This behavior is as expected. For alfalfa optimal water quantities first decrease, then increase as initial soil salinity increases, and water quantities in the 1-year rule were greater than those in the optimal rule for low initial soil salinity levels. The alfalfa results are contrary to intuition and stem from anomalies in the field experiment data which were noted in the original reports describing the experiments. Use of the poor-quality water on cotton was not economically justified within a plausible range of water prices. Analysis of the dynamic time paths for soil salinity suggests convergence to a unique steady state within 10 to 12 years for both

crops. In comparison to the 1-year rule, crop revenues net of water costs were approximately 20 percent greater for alfalfa under the optimal rule but negligible for cotton under the optimal rule. Thus, consideration of the interseasonal effects of salinity may be warranted for some crops in areas with negligible leaching due to rainfall.

### **Irrigation Scheduling**

A production function with dated inputs of water quantity and quality is estimated for cotton in [3]. The production function is based on a daily model of crop growth over the season and consists of 4 relations. The first relation gives daily ET as a function of soil moisture, soil salinity, and pan evaporation. The second relation is an equation of motion for soil moisture. The third relation describes soil salinity over time and the fourth gives yield as a function of ET throughout the season.

The production function was estimated using field-experiment data for cotton. After specifying a functional form, unknown parameters for the ET function were estimated using a maximum likelihood technique. Daily ET and soil moisture are then simulated over the growing season for each plot in the field experiment using the estimated relations. Several functions relating crop yield to seasonal ET were then estimated. A piston-flow model for predicting soil salinity after irrigation was tested against the data and found to work well. After empirical specification the model can be used to predict crop yield, ending soil salinity, and quantity and quality of drainage flows throughout the season, given initial soil salinity, initial soil moisture, and quantity, quality, and timing of irrigations throughout the season.

A dynamic programming model for determining optimal quantities and qualities of irrigation water throughout the season has been constructed. The model is set up on a daily basis and thus considers irrigation timing as well as quantities and qualities applied at individual irrigations. The intraseasonal optimization model will be incorporated into an interseasonal model and then used to address the questions discussed in previous sections.

### **On-Farm Management of Drain Water**

A linear programming model was developed to determine optimal (profit-maximizing) management strategies in situations where natural drainage and access to off-farm disposal facilities are limited. An individual farm is considered. Several crops can be grown; each can be irrigated with different quantities and qualities of irrigation water. Drain water from 1 cropping activity can be used to irrigate other crops. The water table is maintained below levels causing yield losses on the farm by installing drain lines in fields and then disposing the effluent through reuse or evaporation from the pond. The



volume of effluent can also be reduced by reducing water applications and/or by changing cropping patterns.

The model was applied to a representative farm with conditions typical of a Kern County area with drainage problems. The production functions used are from [8]. Salt-removal costs are based on a Bureau of Reclamation study in which salt residue is transported by rail to the Delta area and then by barge to the ocean. The model and results are summarized in [1]. A more complete description is given in [7].

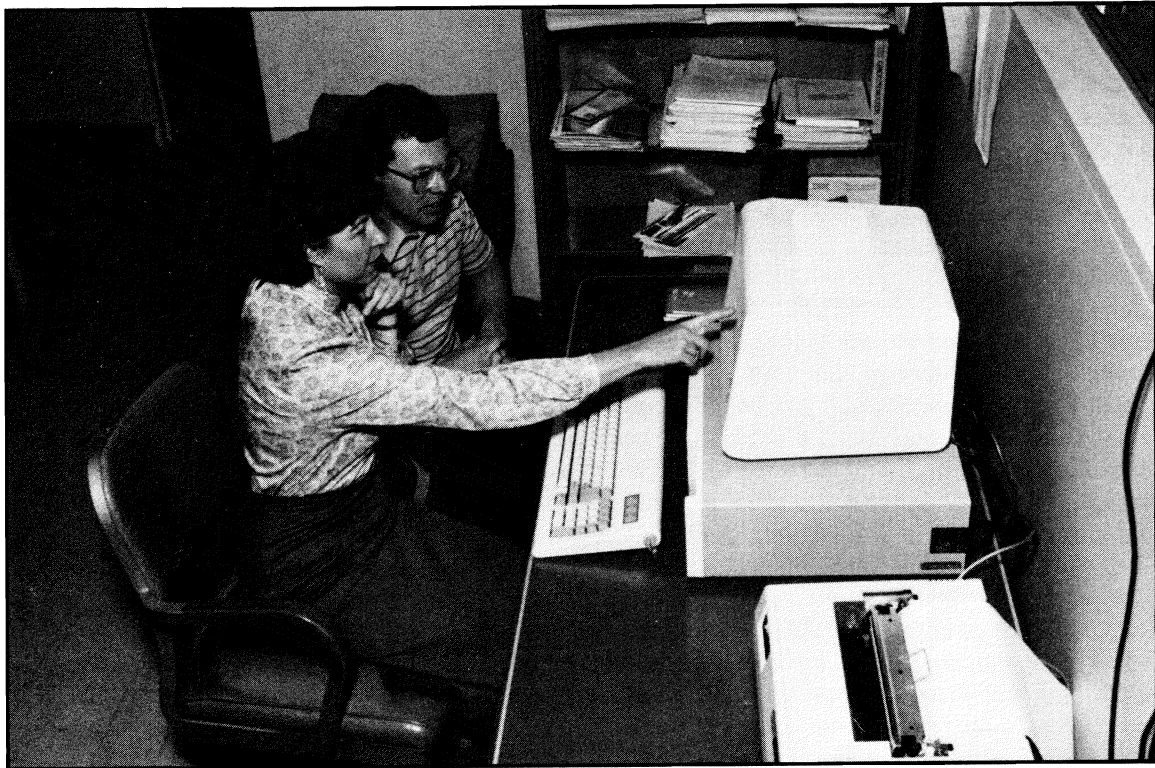
In general, results suggest that it pays to reduce water applications significantly compared to what they would be in a situation where drainage is not limiting. Relatively high yields are maintained; however, drainage flows are reduced considerably, resulting in a much smaller evaporation pond than would otherwise be needed. There is very little change in the cropping pattern compared with the unlimited drainage situation. Although reuse was optimal, when considering only the variable costs, it did not reduce the pond size by very much in absolute terms, and benefits from reuse did not significantly outweigh capital costs in the situation considered. Returns to land and management were positive, indicating that long-run operation was viable, depending on the opportunity costs of these 2 inputs.

Alternative levels of access to an external facility for disposal of drainage water were also considered. Results suggest that a moderate fee on discharges to the facility could reduce flows by 63 percent or more. This option should be seriously considered if the marginal cost of capacity expansion is significant. Lateral underground flows of drainage water to the farm were computed under plausible values for water-table levels and hydraulic conductivities. Effects were generally small. Optimal management policies remained the same on a per-unit area basis; the pond increased by approximately 1 percent of the farm area, and returns to land and management decreased by 7 percent. Another sensitivity run to decrease the pond-evaporation rate in accordance with the lowest pan-evaporation rate in the data set, also had relatively little effect on returns to land and management.

## CONCLUSIONS

Several topics were identified as needing further work. Non-uniformity of water applications clearly affects optimal water applications, yields, and drainage flows. While uniformities can be characterized for particular irrigation systems, there are substantial difficulties in relating them to yield and other variables of interest. One topic of potential importance in analyzing drain water management is the issue of lateral flows. When lateral flows are relatively small, management by individual operators may be feasible. When they are not, more coordinated action may be necessary in the absence of a valley-wide drainage system. Finally, agricultural production is subject to substantial risk and uncertainty. Prices received fluctuate substantially, weather conditions change, water availability cannot

always be guaranteed, and there is significant uncertainty as to the actual production function in any given situation. Risk and uncertainty have not been adequately accounted for in analyzing agricultural water use.



Computers serve as a useful tool in evaluating alternative management strategies.

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## **SUPPORTING PERSONNEL**

Ariel Dinar (postdoctoral research agricultural economist), J. Letey (professor of soil physics)



## VIII. Co-sponsored Research

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### 23. Use of Saline Drainage Water for Irrigation: Field Evaluation in the Imperial Valley

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**PRINCIPAL INVESTIGATORS:** J. D. Rhoades and F. T. Bingham

The projected increase in cropland for the final quarter of this century is only 10 percent; yet world demand for food is expected to double between 1975 and 2000, according to a United Nations World Food Conference report. If these projections are valid, world agriculture must produce a greater yield per-unit land area than ever before, and limited land and water resources must be used more efficiently. Furthermore, the unevenness in year-to-year production related to variations in weather patterns must be attenuated.

Irrigated agriculture takes on special importance in this regard because it yields more per-unit area and depends less on the vagaries of weather. Expansion of irrigated agriculture would contribute significantly towards achieving and stabilizing world food and fiber needs. Expanding irrigation, however, runs headlong into competition for ever more limited water supplies. By reassessing the standards for suitability of water (and land) for irrigation, the available supplies can indeed be expanded significantly. In the past, very conservative standards have been used. Waters generally classified as too saline for irrigation are used successfully today to grow crops without hazardous long-term consequences to crops or soils, even with the use of "conventional" farming practices. The adoption of new crop/water management strategies will further facilitate use of saline waters for irrigation and crop production. Irrigated agriculture could be expanded considerably by adopting such strategies. Considerable saline water is available, including drainage waters from irrigation projects and shallow groundwaters, in many places in the world, including California. Saline waters can be used to aid California in its water conservation efforts and to develop new supplies for irrigation. Estimated water demands in the San Joaquin Valley, for example, are 14.6 million acre-feet per year, and normal year supplies average only about 12.9 million acre-feet. The average annual groundwater overdraft is 1.7 million acre-feet, about three-fourths of which occurs in the southern valley. Annual water demands in the valley are projected to grow to 16.8 million acre-feet by the year 2000. Without new supplies, the overdraft will double to 3.6 million acre-feet.

Paradoxically, at the same time the lower valley has a serious water deficit, disposal of excess drainage water has become a problem. Shallow water tables are developing in a number of San Joaquin Valley

basin locations, and by the year 2000, a projected 1 million acre-feet of drainage water per year will require disposal. The average salt concentration of this drainage water ranges from 4,000 to 5,000 mg/L. Construction of a drainage system to the San Francisco Bay, the only outlet from the San Joaquin Valley, is both expensive and controversial.

In the Imperial Valley, annual discharge of drainage water to the Salton Sea amounts to about 1.5 million acre-feet; its average salt concentration is about 3,500 mg/L. In this valley, as in the San Joaquin Valley, reuse of drainage water could reduce disposal problems and increase the amount of water available for irrigation.

### **STRATEGY CONCEPT**

Many brackish waters, which are not now used for irrigation because they are deemed too salty, can be used effectively with properly adapted management practices. In fact, whether inadvertent or planned, such reuse is common in many places; however, farmers could carry reuse much further by successively irrigating a sequence of crops of increasing salt tolerance. This approach has little appeal to most farmers, because it restricts them to salt-tolerant crops, and requires special management practices and equipment to obtain a good stand on saline land. Use of saline waters for irrigation would probably be more acceptable if these limitations could be circumvented by substituting saline (drainage) water for normal (low-salinity) water to irrigate certain crops in the rotation when they are in a suitably tolerant growth stage and to use the normal water at other times. The timing and amount of substitution possible varies with the quality of the two waters, the cropping pattern, climate, certain soil properties, and the irrigation system. Whatever salt buildup occurs in the soil from irrigating with the brackish water can be alleviated in the subsequent cropping period when a more sensitive crop is grown with the low-salinity irrigation water.

Soil does not usually become unduly saline from use of brackish water for a part of a single irrigation season and often not when this practice is continued for several seasons. The maximum soil salinity in the root zone that results from continuous use of brackish water does not occur when such water is used for only a fraction of the time. Furthermore, yield of the sensitive crop is not likely to be significantly reduced if proper preplant irrigations and careful management are used during germination and seedling establishment to leach salts out of the seed area and shallow soil depths. Subsequent in-season irrigations will leach the salts farther down in the profile, ahead of the advancing root system, and reclaim the soil before the brackish water is used again to grow a suitably tolerant crop. This cyclic use of low- and high-salinity water prevents the soil from becoming excessively saline, while permitting substitution of brackish for better quality water for a substantial fraction of the irrigation water used over the long period.

Implementation of this strategy would not only increase water conservation and protect the soil for crop production, it also could minimize the drainage disposal (and associated) problems (where drainage waters are the saline waters recycled for use for irrigation) as well as the salt loading of our water supplies.

### **Evidence of the Credibility of Proposed Strategy**

The suggested strategy for facilitating use of brackish drainage water for irrigation is being evaluated in: (1) a field experiment that has been underway since 1978 near Lost Hills in the San Joaquin Valley, (2) a 40-acre field experiment begun in 1982 on a cooperator's farm in the Imperial Valley, and (3) in a controlled lysimeter facility at Riverside begun in 1983 which simulates the two field projects. This report summarizes the findings to date of the Imperial Valley project.

Two cropping patterns are being tested at the Imperial Valley location: a successive-crop and a block rotation. The successive-crop rotation consists of wheat, sugarbeets, and melons. Colorado River water (900 mg/L total dissolved salts) is being used in the preplant and early irrigations of wheat and sugarbeets and for all irrigations of melons. The remaining irrigations are made with the Alamo River (drainage water of 3,500 mg/L total dissolved salts).

The block rotation consists of cotton (a salt-tolerant crop) for 2 years, followed by wheat (intermediate salt tolerance), and then by alfalfa (more sensitive) for a block of several years. Drainage water is substituted for a substantial part of the normal water in the irrigation of cotton in this rotation. Beginning with the wheat crop, only Colorado River water is used. The hypothesis is that wheat should withstand the salinity initially present in the soil from irrigating the cotton with brackish water and should yield well when irrigated with Colorado River water; desalinization of the soil is expected to be sufficient from irrigating wheat with Colorado River water so that alfalfa can be subsequently grown without loss of yield.

To date, one and two-thirds cycles have been completed in the successive-crop rotation (wheat, sugarbeet, and melon crops), and 3 crops of the block rotation have been completed; 2 cotton crops, 1 wheat crop, and 3 cuttings of alfalfa have been harvested.

No yield losses occurred with any of the crops (wheat, sugarbeets and cotton) where Alamo River water was substituted for Colorado River water, after seedling establishment relative to the control treatment (sole use of Colorado River water for irrigation), or in any of the rotation crops grown subsequently on these brackish-water irrigated fields when irrigated with Colorado River water. The percentages of drainage water substitution for normal irrigation water in the test were 76 and 63 in wheat, 82 and 75 in sugarbeets, and 63 and 47 in cotton during the first and second cycles, respectively.



Cotton irrigated with saline drainage water yielded 2.5 bales per acre in Imperial Valley study.

### **CONCLUSION**

Use of saline water for irrigation can increase the resource base for irrigated agriculture and can help meet the increasing biomass needs of the world. Such use can be made more practical when used cyclically with low-salinity waters. When the saline water is a drainage water, such use could convert a "waste" product into an asset, reducing the volume of drainage water needing export, reducing the deleterious impact of drainage disposal, and increasing the amounts of water available for irrigation and crop production. As with anything, the advocated strategy will not be appropriate for all conditions, but it should be given careful consideration.



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## 24. Salt Tolerance of Mature Plum Trees

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**PRINCIPAL INVESTIGATORS:** Glenn J. Hoffman, Peter B. Catlin, R. Scott Johnson, Lee Francois, David A. Goldhamer

As water conservation continues to improve and the competition for fresh water intensifies, the salt content of irrigation water will increase. In California's Sacramento-San Joaquin Valley, where a preponderance of Prunus species is grown, the anticipated future needs for water are likely to lead to a relaxation of water-quality standards in the Sacramento-San Joaquin Delta with a corresponding increase in salinity of irrigation waters. Among the first crops to suffer yield reductions as irrigation waters become more saline will be deciduous fruit trees. Not only have fruit trees been shown to be sensitive to osmotic stress, but they are reported to be especially sensitive to chloride and prone to foliar damage when sprinkler-irrigated. Because of their reliance upon irrigation to meet water requirements, fruit growers will be among the first confronted with the hazards of increasing salinity.

Although deciduous fruit trees are acknowledged to be sensitive to salinity, few results substantiate the claim, none under field conditions. Of the thousands of publications on the effects of salinity on agricultural crops, only 42 deal with deciduous fruit trees and only 1 reported yield response. In the early 1940s, Hayward and colleagues conducted a sand-tank experiment with 72 peach trees beginning with 1-year-old seedlings and continuing for 5 years. A significant number of fruit were harvested only during the last year. Fruit yields were reduced from 37 to 60 percent when soil salinity, dominated by chloride salts, had an electrical conductivity of the soil saturation extract ( $EC_e$ ) of less than 3 dS/m. Yields were not reduced significantly by sulfate salts at a similar salinity level. The researchers warned, however, that over a longer period of time it seemed probable that effects would be even more severe at comparable levels of salinity. Except for this experiment, all of the salt-tolerance data on deciduous fruit trees, such as given by Maas and Hoffman, are based on the fresh weight of scions from young trees. Bernstein and coworkers reported the average growth reduction for 2 rootstocks of Santa Rosa plum on a saline soil having an  $EC_e$  of 5.1 dS/m was 55 percent after 3 years; for prune, almond, apricot, and peach, reductions in growth were 60, 70, 85, and 55 percent, respectively.

Commercial production of Prunus crops, valued in 1981 at more than \$500 million for U.S. consumption and more than \$500 million for export, is limited largely to California. The objectives of this field study are to: (1) determine the salt tolerance of mature plum trees, (2) identify and quantify any toxic effects of chloride or sodium in the soil solution, (3) identify and quantify foliar damage from chloride or sodium by sprinkling, (4) evaluate the water relations of and salt distribution within mature plum trees, and (5) monitor the salt and

water distribution within the root zone caused by a mini-sprinkler irrigation system.

## EXPERIMENTAL PROCEDURES

The experiment, located at the Kearney Agricultural Center in Fresno County near Parlier, consists of 650 plum trees transplanted in the spring of 1966. The 2.3-ha orchard consists of 50 rows of trees with 13 trees per row. One-third of the rows contain the cultivar "El Dorado" and two-thirds are "Santa Rosa." Tree spacing is 5.6 by 6.4 m. The rootstock is "Mariana." The soil is classified as Hanford sandy loam (Typic Xerorthents) developed from recently deposited alluvium of granitic origin from the Sierra Nevada mountain range. Little profile development is evident, and the soil has an average bulk density of  $1.57 \text{ Mg/m}^3$  through a depth of 120 cm.

The experimental design consists of 6 treatments replicated 5 times. Each replicate consists of 2 rows, each row containing 5 trees of the Santa Rosa cultivar. From the total of 650 trees, 300 are experimental; the remainder serve as border trees around the edge of each plot. Six salinity treatments are imposed by irrigating with waters having electrical conductivities of 0.2, 1, 2, 4, 6, and 8 dS/m. Water for the 0.1 dS/m treatment is the normal irrigation water for the research center and serves as the nonsaline control. The other water qualities are generated by adding NaCl and  $\text{CaCl}_2$  in equivalent amounts to increase the electrical conductivity of the irrigation water to the desired level.

Irrigation water is applied by mini-sprinklers that wet the soil surface beneath the tree canopy without wetting the foliage or trunk. The amount applied is in excess of evapotranspiration to achieve a high leaching fraction. The annual evapotranspiration for deciduous trees on the east side of the San Joaquin Valley is approximately 950 mm. The irrigation system was installed during the fall of 1983 and treatments were imposed in March 1984.

The influence of sprinkling the foliage with 6 different concentrations of saline water was evaluated on Santa Rosa trees that served as a border to the main experiment. Each saline water was sprayed on 4 randomly selected trees 14 times from early May to late August 1985. The average electrical conductivities of the 6 spray waters were 0.2, 1.1, 2.2, 3.3, 4.5, and 6.9 dS/m. Approximately 40 L of water were sprayed on each tree to wet the canopy completely during each application. Leaves were sampled just before each spraying and 2 weeks after the final spraying, and analyzed for Cl, Na, Ca, Mg, K, and P.

## INITIAL RESULTS

Fruit yield in 1983, the year before the experiment began, was 33.6 kg per tree. Four months after the salinity treatments were imposed in

1984, fruit yields averaged 79.6 kg per tree, more than twice the yield of 1983. Salinity had no significant effect on yield in 1984. Either improved water management and/or weather conditions were thought to be the cause of increased production. Although the irrigation waters differed in salt content, the same amount of water (1230 mm of irrigation and rainfall) was applied to all treatments. The salinity level of the most saline treatment increased from an average electrical conductivity of the soil-saturated extract of 0.5 dS/m in February 1984 to 8.6 dS/m by September when 10 percent of the leaves in the high-salt treatment were visually damaged.

Salts were leached to pre-experimental conditions to a depth of 60 cm during the winter of 1984-85 when 260 mm of rain fell. By May 1985, however, soil salinity levels were comparable to those measured in July and September 1984. The harvest in June 1985 was comparable to the 1984 harvest, except the yield of the 8 dS/m was reduced to half of the control. Yields of the other treatments were equal to or greater than the control treatment. By mid-April, less than 6 weeks after the beginning of the irrigation season in 1985, foliar damage equal to what had taken 6 months to occur in 1984 was evident in the most saline treatment; no damage was noted on the 3 least saline treatments. Foliar damage in September 1985, although not documented yet, appears to be more severe than in September 1984. Damage to the 6 dS/m treatment in 1985 appears similar to the damage on the 8 dS/m treatment in 1984 and many of the trees in the 8 dS/m treatment are severely damaged and near death. Projecting into 1986, yields in the 6 dS/m treatment may be half those of the control and yields in the 8 dS/m treatment will be near zero.

Fruit size in 1984 decreased slightly as salinity increased; the effect was more pronounced in 1985 with the fruit mass for the control treatment being 30 percent larger than the 8 dS/m treatment (69 vs. 52 g). The major factor limiting fruit yield in 1983 was the number of fruit harvested. In 1984, 1510 fruit were harvested from each tree on the average, 1230 in 1985, but only 500 in 1983. In 1985, 30 percent less fruit was harvested from the most saline treatment compared to the control (1110 vs. 760).

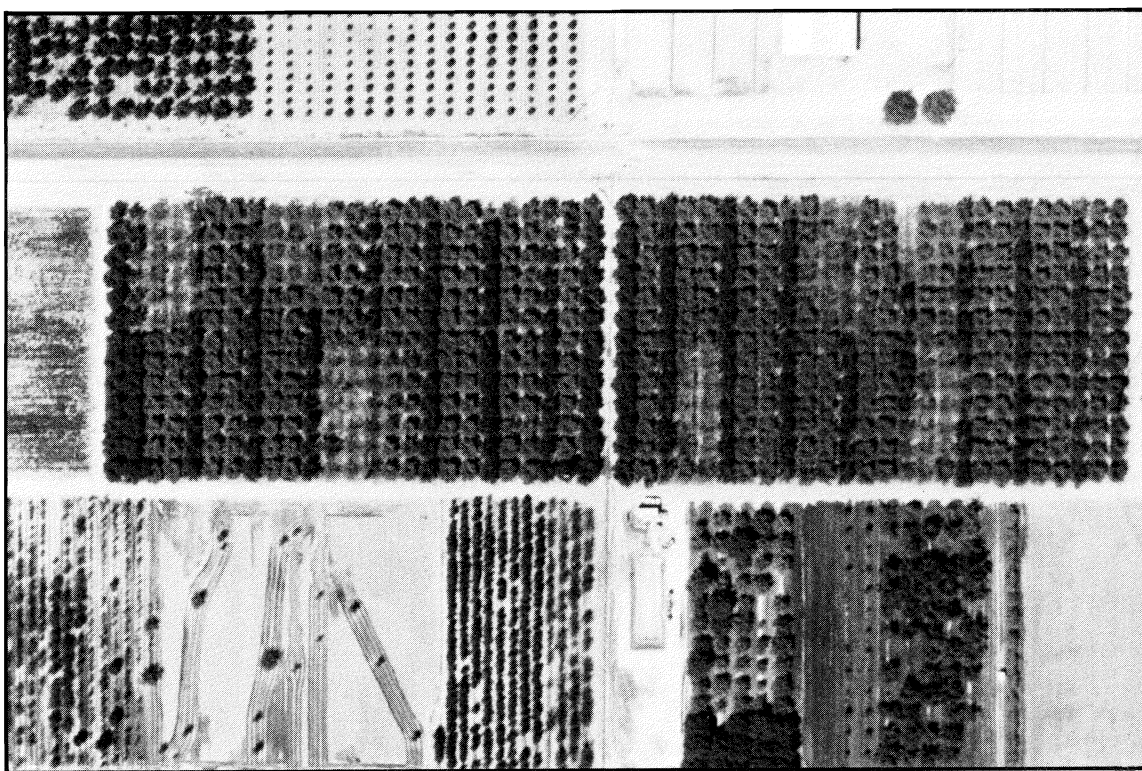
Other measures of tree response to salinity also began to appear in 1985. The amount of solar radiation striking the ground through the tree canopy in July 1985 indicates the leaf canopy on the 8 dS/m treatment is only 2/3 of the control. Annual regrowth after the trees are topped showed a linear decrease in shoot growth as salinity increased with a 75 percent growth reduction in the most saline treatment. Analyses of leaves sampled in 1984 indicate that chloride, but not sodium, is accumulating in the leaf tissue. Chloride concentrations increase more than 30-fold from low to high salinity while sodium is nearly constant with increasing salinity.

Foliar application of saline water has produced severe visual leaf damage with water having an electrical conductivity of 3 dS/m or higher. Leaf tissue analyses of samples taken in June after 5 spray applications show increases of 25- and 70-fold in the concentration of sodium and

chloride, respectively, for spray water having an electrical conductivity of 6.9 dS/m compared to 0.2 dS/m. Calcium increased slightly with water salinity but Mg, P, and K were not affected. Salinity level of the spray water did not influence yield in 1985 but pronounced differences are expected in 1986.

### SIGNIFICANCE

Waters having a salinity level of as much as 8 dS/m could be used the first year without yield reduction. After 1 year, however, the plum trees responded adversely to the highest salinity treatment. It is anticipated that the responses now evident at salinities of 8 dS/m will begin at lower levels of salinity in future years. Continuation of the research during the next few years will provide, for the first time, information on sequential effects of salinity on mature deciduous fruit trees.



Aerial view of plots and pool used to mix salt water. Note lack of foliage on high-salt water treatments.



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