5-1982

The Interactive Effects of Water Salinity and Management on Symbiotic Nitrogen Fixation in Alfalfa

Thomas J. Keck
Utah State University

THE INTERACTIVE EFFECTS OF WATER SALINITY AND MANAGEMENT ON SYMBIOTIC NITROGEN FIXATION IN ALFALFA

by

Thomas J. Keck

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Soil Science and Biometeorology

(Soil Science)

Utah State University
Logan, Utah

1982

ACKNOWLEDGMENTS

I would like to express my special thanks to Dr. Jeff Wagenet. It was his enthusiasm which brought me back to Utah State for graduate work and his continued support which helped me see it through to completion.

In addition I wish to thank Dr. W. F. Campbell and Dr. J. J. Jurinak, the other members of my committee, whose help and support became especially important in the final months after Dr. Wagenet had moved on to Cornell University.

Thank you to both Mrs. Mary Veronica Kolesar and Dr. D. L. Turner for their counsel on the statistics used in this paper and also to Dr. D. A. Johnson of the Crops Research Lab for providing the use of his equipment in some of the analyses.

Finally to my wonderful parents, Mr. and Mrs. Harold Keck, and to my future wife, Mary, thank you for many things but most of all thank you for all your love.

Thomas J. Keck

Thomas J. Keck

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

ABSTRACT

Interactive Effects of Water Salinity and Management on

Symbiotic Nitrogen Fixation in Alfalfa

by

Thomas J. Keck, Master of Science

Utah State University, 1982

Major Professor: Dr. R. J. Wagenet
Department: Soil Science and Biometeorology

A greehhouse study was conducted to assess the interactive effects of three irrigation water salinity levels (1.0, 3.0, and 9.0 mmho/cm) and three quantities of water applied per irrigation (120, 240, 360 ml) on plant growth and nitrogen fixation by alfalfa (Medicago sativa L. cv. Resistador). Harvest dates corresponded to 10, 30, and 50 days after the initiation of salt and water treatments which were started after nodulation had been established in young plants.

Alfalfa top growth was limited by both salt and water stresses. Irrigation water salinity had a greater effect on top growth than root growth while root distribution was unaffected by either the quantity of water applied or by water salinity. The effects of salinity on plant growth were reduced in the presence of limiting moisture. The specific nodule activity (mmol C_2H_4/hr/g) of water stressed alfalfa plants was enhanced by increasing the quantity of water applied at each irrigation and was adversely effected by increased irrigation water salinity. In contrast, both nodulation and nodule growth were insensitive to salt stress and sensitive only to severe moisture stress. Alfalfa plants

continued to exhibit acetylene reducing capacity at the third harvest even under severe moisture and salt stress. The species apparently continues to fix nitrogen even though environmental stress is quite substantial.

(110 pages)

INTRODUCTION

Available soil nitrogen is the major limiting factor to crop production on much of the world's agricultural land. The high productivity of modern agriculture is dependent on the addition of synthetic nitrogen fertilizers, both to replace nitrogen removed by successive crops and to build up the level of available nitrogen in the soil. Petroleum shortages, experienced in the late 1970's, resulted in increased nitrogen fertilizer prices and the questions about their future availability. This spurred much interest and renewed research on the use of nitrogen fixing plants to supplement soil nitrogen. The availability and price of petroleum has since stabilized so that the future use of synthetic nitrogen fertilizers in agriculture is no longer being questioned. Symbiotic nitrogen fixation remains, however, a valuable complementary tool for managing nitrogen in modern agriculture. Especially important is the use of nitrogen fixing plants in areas of agriculture such as pastures or hay production where the economic returns from using synthetic fertilizers may be marginal.

The amount of agricultural land in the United States under irrigation has been continually expanding. Between 1974 and 1978 the area increased from roughly 41 million to over 50 million acres (Agricultural Statistics, 1981). Much of the increase has occurred in the arid regions of the west. For example, the area under irrigation in Colorado increased from 2.9 to nearly 3.5 million acres. Over the same time period, the acreage in Idaho increased from 2.85 to 3.5 million

acres (Agricultural Statistics, 1981). Crop production in arid regions is often hampered by salinity problems caused either by indigenous salts in the soil or by salts added with irrigation water. Salinity problems are expected to increase as more arid areas are brought under irrigation. Greater utilization of available water will require the use of more saline irrigation waters while more restrictions can be expected on the amount of salinity allowable in return flows.

Nationwide, 61% of all the hay produced in the United States during 1978 was either pure alfalfa or an alfalfa mix (Agricultural Statistics, 1981). Alfalfa is an especially important crop in arid areas because of both its ability to symbiotically fix nitrogen and its deep rooting habit which enables the species to continue extracting water from deep in the soil profile after water has become limiting in the surface soil layers. A large portion of the irrigated lands in the west are devoted to alfalfa production (Brown and Hayward, 1956). Effective utilization of symbiotic nitrogen fixation by alfalfa in these arid regions requires a thorough understanding of the effects of salinity on both alfalfa growth and its nitrogen fixing ability. The effects of salinity are largely dependent on the soil's moisture status. Thus, in studying the effects of salinity, it becomes necessary to study the interactive effects of salinity and water management. The present study is the first to assess the interactive effects of irrigation water salinity and water management on symbiotic nitrogen fixation.

OBJECTIVES

General

To determine, using laboratory lysimeters, the individual and interactive effects of saline irrigation water and its management on the growth and the nitrogen fixing ability of alfalfa.

Specific

1. To measure the nitrogen fixing activity of alfalfa root nodules using the acetylene reduction technique.
2. To assess the effects of saline water and its management on nodulation and rooting patterns in alfalfa.
3. To monitor the distribution of salt within the soil profile and relate its transient presence to alfalfa growth, nodulation, and nitrogen fixation.

LITERATURE REVIEW

Salt Effects on Nitrogen Fixation

The most prevalent effect of salinity is stunted plant growth (Maas and Hoffman, 1977). This may be accompanied by darker green leaf color and possibly thicker, more succulent vegetative growth. According to the above authors, salt-affected plants usually appear normal, yet both development and growth rates may be reduced. Only in extreme cases, or when specific ion toxicities occur does any obvious sign of plant damage appear. In the absence of obvious damage, the effects of salinity are often much more subtle. Plant response to salty soil or water is generally regarded as a response to the osmotic potential of the soil solution, irrespective of the type of salt present (Bernstein, 1961). The exact mechanisms by which plants respond to increased salinity are quite complex, with a comprehensive review of the physiological changes occurring in salt-affected plants beyond the scope of this study. A detailed bibliography of this literature is provided by Maas and Nieman (1978), with only excerpted, pertinent examples cited here for background.

Plant uptake of water occurs in response to the total water potential of the soil solution. Water becomes limiting once the total water potential drops below the level at which plants can adequately replace transpirational water loss. Under non-saline conditions, total soil-water potential is primarily a function of the matric potential, which depends only on soil-water content and soil type. However, under saline conditions, the total water potential is a function of the combined

influences of both the matric and osmotic potential energy, where the osmotic potential decreases (becomes more negative) as soil salinity increases. The removal of water from the soil, by either plant uptake or evaporation, concentrates salts in the remaining soil solution. Thus, as a salt-affected soil dries, both the osmotic and matric potentials of the soil water decrease (become more negative). Plants growing under saline conditions thereby exhibit symptoms indicative of reduced water availability at higher soil water contents than do plants grown under non-saline conditions. Plants can adjust to gradual increases in osmotic stress by increasing the concentration of ions and organic solutes in plant tissues, a process called osmoregulation (Maas and Nieman, 1978). The extent to which this adaptation is successful varies among plant species and may be a major factor in determining differences in salt tolerance (Maas and Nieman, 1978).

Many woody plant species, along with some herbaceous species, are susceptible to specific ion toxicities (Maas and Hoffman, 1977) with sodium and chloride the primary responsible ions, e.g., most fruit trees are especially sensitive to high soil concentrations of either sodium or chloride (Meiri and Shalhevet, 1973; Pearson, 1960). Injury results when the specific ion accumulates in the plant leaves. This injury can be minimized by even a low concentration of calcium (0.1 mMo/Ca^{+2}), which have been shown to limit the effects of high soil sodium upon otherwise susceptible bean plants (Lahaye and Epstein, 1971). Calcium had a dual effect of limiting sodium uptake by roots while at the same time enabling the plants to exclude sodium from the leaves. Thus, the particular ionic composition of the soil solution appears to be highly important in determining the occurrence of

specific ion toxicities. This may have far-reaching implications in certain studies where a single salt species, usually sodium chloride, has been used to assess plant response to salinity.

Nutritional imbalances resulting from high salinity levels in the soil may further complicate the interpretation of plant response to salinity. For example, high concentrations of sodium or sulfate may induce calcium deficiency (Maas and Nieman, 1978). Conversely, high calcium concentrations may induce either potassium (Bernstein, 1964) or phosphorus deficiency. It is important to recognize such interactive effects, as salt affected plants often exhibit symptoms which are similar to those of phosphorus deficiency (Hewitt, 1963). The high pH associated with high levels of basic cations in salt affected soils could also result in various micronutrient imbalances, such as iron or zinc deficiency.

Soil salinity may disrupt the symbiotic nitrogen fixing systems in several ways. First, salts can limit nodule formation, either by reducing the population or rhizobium in the soil or by impairing the ability of those rhizobia to infect root hairs (Pillai and Sen, 1966). Once root nodules have been formed, salinity may then limit subsequent nodule growth (Bernstein and Ogata, 1966; Subba Rao et al., 1972; Balasubramanian and Sinha, 1976a, 1976b) or may impair nodule nitrogen fixing capabilities (Wilson, 1970; Sprent, 1972). Disruption of the nitrogen fixing system thereby results from either a direct effect of salinity on root nodules or through the response of the host plant to salinity independent of particular effects on the host-symbiont relationship.

Inoculation of the host plant by rhizobium requires that an adequate population of the specific rhizobium species be present in the soil. Good survival of the free-living rhizobium is therefore essential for obtaining adequate nodulation. The effect of salinity on rhizobium varies not only among different species but also among different strains of the same species (Yadav and Vyas, 1971). Rhizobial growth, in general, appears to decrease with increasing salinity (Pillai and Sen, 1966), however the growth of certain strains of rhizobium may be stimulated by low concentrations of added salt (Yadav and Vyas, 1971). The type of salt present also influences the response. For example, $NaHCO_3$ inhibited the growth of the M-1 strain of Rhizobium melilotii at a concentration of 0.8% salt, while even 3.0% NaCl salt did not inhibit growth of the same Rhizobial strain (Yadav and Vyas, 1971).

The sensitivity of legume nodulation to increased salinity varies according to the host plant species, with often contradictory results among seemingly comparable studies. Nodulation of California common alfalfa was only slightly affected by an osmotic potential of -.54 M Pa (-5.4 bars) while the same salinity level strongly inhibited the nodulation of Lee soybeans (Bernstein and Ogata, 1966). Soil salinity had no effect on the nodulation of berseem (Trifolium alexandrinum) despite reducing the growth of free living Rhizobia trifolii at even lower salinities (Pillai and Sen, 1966). In contrast, nodulation was significantly reduced by salt stress in chickpea (Balasubramanian and Sinha, 1976a; Lauter et al., 1981), soybeans (Wilson, 1970), cowpea and mungbeans (Balasubramanian and Sinha, 1976b).

Variation in response to salinity depends in part on the timing of salt treatments. Salt stress applied at planting delays the onset of nodulation, as has been shown both in chickpea (Lauter et al., 1981) and in alfalfa (Subba Rao et al., 1972). The nodulation of California common alfalfa was only slightly affected by salinity (salt levels) once the nodules were well established (Bernstein and Ogata, 1966), however, in a separate study, when salinity treatments were required at planting, the nodulation of alfalfa was reduced at only 0.4% NaCl concentration (Subba Rao et al., 1972).

The most often observed legume response to salinity is a reduction in both nodule number and nodule mass (Bernstein and Ogata, 1966; Wilson, 1970; Subba Rao, et al. 1972; Balasubramanian and Sinha, 1976a). Salinity may inhibit the initiation and growth of nodules, yet nodules fully developed prior to stress are often quite salt tolerant (Wilson, 1970). The size of the individual nodules has been shown to increase with increasing salinity in chickpea, possibly due to less competition for available photosynthates (Balasubramanian and Sinha, 1976a).

Specific nodule activity, as determined by acetylene reduction, refers to the ability of root nodules to reduce acetylene (C_2H_4) to ethylene (C_2H_2). It is most often reported as µmoles ethylene produced per hour per gram nodule dry weight (µmoles C_2H_4/hr/g). Acetylene reduction rates are used as an indirect measure of the nitrogen fixing efficiency of root nodules.

The specific nodule activity of most legumes is reduced as salinity increases. As was true of other types of plant response to salt,

different species react differently in terms of specific nodule activity. For example, increasing salinity of added nutrient solutions (from EC = 3.0 dSm m^{-1} to EC = 15.0 dSm m^{-1}) decreased the acetylene reducing efficiency of mungbean root nodules, while the same range of salinities had no effect on the efficiency of cowpea root nodules (Balasubramanian and Sinha, 1976a). Low concentrations of salt also have been found to reduce the specific activity of detached soybean nodules (Sprent, 1972). The effect increased with increasing salt concentration and was independent of the type of salt used.

Soybean root nodules have been found to quickly recover once the salt stress is removed (Wilson, 1970). Recovery may be linked to the ability of root nodules to exclude sodium and chloride, or to the accumulation of phosphorus in the nodules during salt stress (Wilson, 1970). Soybean nodule recovery from salt stress was reduced by both prolonging the exposure time or increasing the salt concentration (Sprent, 1972).

The initial effects of salinity on nodule activity are hypothesized to be due to a disruption of cell metabolism at the nodule surface particularly via decreased oxygen uptake (Sprent, 1972). Thus, oxygen may limit the specific nodule activity in salt-stressed root nodules (Sprent, 1972), though this effect is indirect through the effects of salts on cellular metabolism.

Data from a recent study showed specific nodule activity of chickpea increasing with increasing salinity (Lauter et al., 1981), a finding in direct conflict with earlier research upon the same plant

(Balasubramanian and Sinha, 1976a). No apparent reasons can be found for this discrepancy. Lauter et al. (1981) did not include any explanation for the increase in specific nodule activity.

Nitrogen fixation can be limited by the effects of salinity on the host plant, independent of direct effects upon nodulation and nitrogen fixing processes. Stunted growth of the host plant reduces the supply of photosynthates to the root nodules. Since photosynthate supply is a major limiting factor in nitrogen fixation (Hardy and Havelka, 1976), this indirect effect can be quite important. In another study, defoliation of field grown white clover resulted in a decreased acetylene reduction rate within 24 hours. This effect was again attributable to the reduced photosynthate supply (Moustafa et al., 1969). Studies with other nitrogen fixing plants have revealed the same relationship. Growing C-3 plants in a carbon dioxide enriched atmosphere increases photosynthate production (Bannister, 1976). Nitrogen fixation by soybeans (a C-3 plant) was increased drastically by CO_2 enrichment in response to the increased photosynthate supply (Hardy and Havelka, 1976).

Salinity may indirectly limit nitrogen fixation by altering the water potential gradient within the plant, thereby affecting both the transport of photosynthate to the root nodules and the flow of fixed nitrogen out of root nodules (Maas and Nieman, 1978). Either of these effects might limit specific nodule activity.

Effects of Soil Water Stress on Nitrogen Fixation

Soil water stress, as well as excess soil water, will affect the nitrogen fixing ability of legumes (Sprent, 1973; Minchin and Pate, 1975;

Pankhurst and Sprent, 1975; Foulds, 1978; Gallacher and Sprent, 1978). Moisture stress occurs in the root nodules when the nodule begins losing water faster than the roots can resupply it (Sprent, 1973). A 50% loss in soybean root nodule activity resulted when the water potential of the nodule reached -6×10^5 Pa (Pankhurst and Sprent, 1975). It has been postulated in studies with soybeans that reduced nitrogen fixing activity is related to impaired diffusion of oxygen into the nodule (Pankhurst and Sprent, 1975). Nitrogen fixing activity of moderately stressed detached soybean nodules was completely restored when the partial pressure of oxygen was increased from 10^4 to 10^5 Pa (Pankhurst and Sprent, 1975). Even in severely stressed nodules, nitrogen fixing activity was partially restored by increasing the partial pressure of oxygen from 5×10^{-4} to 8×10^{-4} (Pankhurst and Sprent, 1975). Very low, but uniform, concentrations of oxygen are required by the bacteroid-containing cells of legume root nodules for the formation of ATP (Tjepkema, 1979).

Moisture stress may also inhibit the nodulation of legumes. Reduced nitrogen fixation in water stressed Vicia faba resulted from fewer nodules being produced and was not due to any reduction in specific nodule activity (Gallacher and Sprent, 1978). The specific activity of V. faba root nodules was not significantly different for any of the water treatments applied. Studies with Phaseolus vulgaris (Sprent, 1976) and Casuarina equisetifolia (Kant and Narayana, 1978) found reductions in nodule mass, nodule number, and nodule size resulted from increasing moisture stress. Moisture stress in Vicia faba retarded the growth of nodules that were initiated under conditions of adequate water (Gallacher and Sprent, 1978). The reduction in nodule number may be

due to fewer rhizobia available for infection (Foulds, 1971) or due to some effect on the infection process, or both. The effects of excess soil water on nitrogen fixation vary according to legume species. Flooding of Pisum sativum root nodules caused a greater reduction in fixation activity than drought stress (Minchin and Pate, 1975). In contrast, excess water applied to Vicia faba actually stimulated both nodule growth and fixing activity (Gallacher and Sprent, 1978). Reduced acetylene reduction by Vicia faba root nodules was observed when roots were incubated under water, yet nodule activity quickly returned to normal when the water was removed (Gallacher and Sprent, 1978). Plants do not need to be inundated by water since studies have demonstrated that even a thin layer of water on soybean root nodules could reduce nitrogen fixation (Sprent, 1969). Species differences in response to water may be due to such species-specific mechanisms as a more efficient oxygen diffusion system in Vicia faba or a larger internal gas volume in the nodules (Gallacher and Sprent, 1978). Thus, the supply of oxygen within root nodules appears to play an important role in determining the response to both water-stressed and water-logged conditions.

Alfalfa yield may be reduced by both soil water and soil stresses. Increased soil salinity results in smaller plants and a blue-green color in the vegetation (Brown and Hayward, 1956). These effects are greatly dependent upon the timing of the stress. Low concentrations of NaCl have been shown to delay nodulation and reduce nodule number when salt stress was applied at the time of planting (Subba Rao et al., 1972), yet nodulation was only slightly affected by salinity when plants were established prior to initiation of salt treatments (Bernstein and Ogata,

1966). No studies assessing salinity effects upon the acetylene reducing ability of alfalfa have been found in the literature.

Alfalfa yield under water-stressed conditions decreases linearly as a function of decreasing evapotranspiration (Bauder et al., 1978); Sammis, 1981). Thus, increasing the quantity of water applied should result in a corresponding increase in plant growth until water is no longer limiting. Flooding, on the other hand, results in a drastic reduction in alfalfa of both top and root growth (Thompson and Fick, 1980).

Alfalfa yield is particularly sensitive to salt concentrations in the upper portion of the soil profile (Francois, 1981). Proper water management should insure that salts are leached to deeper soil depths. Within this constraint, maximum yield can be produced under a broad range of leaching fractions (Bernstein and Francois, 1973). Changes in the salinity of the irrigation water appear to be more important than differences in drainage water salinities, as change in irrigation water quality from 1 mmho/cm to 2 mmho/cm resulted in a 10% reduction of alfalfa yield (Bernstein and Francois, 1973) over a broad range of leaching fractions. Since alfalfa response to salinity was similar for both nitrogen fertilized and fixation-dependent plants (Bernstein and Ogata, 1966), it can be hypothesized that nitrogen fixation by alfalfa is less affected by salinity than is overall plant growth.

Acetylene Reduction Measurement of Nitrogen Fixation

Nitrogen fixation, the conversion of N_2 gas to NH_4^+, occurs in the root nodules of legumes due to the presence of the enzyme nitrogenase.

Hardy and Knight in 1967 (Hardy et al., 1968) first proposed the use of an acetylene reduction method to assess the nitrogen fixing activity of legumes. The method has since become widely accepted and has several variations depending upon the investigator. The technique estimates nitrogen fixation by using an analogous reaction, the reduction of acetylene (C_2H_2) to ethylene (C_2H_4), which is also catalyzed by nitrogenase.

The first step in the acetylene reduction procedure is the collection of a nodule sample. This sample may be of detached nodules, nodulated root sections, the entire root system or in some instances, the whole plant. The sample is then incubated for one hour in a gas-tight container filled with 10% acetylene atmosphere (Hardy et al., 1968). After one hour, a sample of gas is removed from the incubation vessel and is either injected immediately into a gas chromatograph or is stored in a small air-tight container (vaccutainer for later analysis (Johnson and Rumbaugh, 1981). The gas is analyzed to determine the amount of ethylene produced during the incubation period, with the results most often reported in µmol C_2H_2/hr/g nodule dry weight.

Unfortunately, sample preparation may have a large effect on the results obtained by acetylene reduction. It is important to adhere strictly to a set procedure in order to obtain reliable, reproducible results. Some of the pitfalls to be avoided have been mentioned in other studies. For example, detached root nodules do not maintain a constant fixation rate over the entire incubation period (Hardy et al., 1968; Burris, 1974) resulting in lower acetylene reduction rates than

either nodulated roots or entire root systems. Fixation rates are highly temperature dependent (Hardy et al. 1968; Waughman, 1972), making it important to maintain incubation temperatures either at existing soil temperatures, or at a standardized temperature if comparisons are to be made (Waughman, 1972). Additional variation may result from washing soil off the root nodules with water. Studies with soybeans (Sprent, 1969) have shown that the thin coat of water surrounding the nodules after washing may reduce acetylene reduction activity by limiting the diffusion of oxygen into the nodule. The level of ambient light to which the plants are exposed prior to sampling (Bergerson, 1970), as well as the time of day and season of sampling, effect the values obtained. When all of the above factors are held constant, variations are still possible in replicate samples from the same plants (Waughman, 1972).

Additional problems arise if acetylene reduction data are used in conjunction with measured nodule weights or numbers to provide estimates of system nitrogen balance. When this type of calculation is made, both the specific nodule activity and the mass of nodules per volume of soil must be determined. The tiny size and extreme spatial variability of nodule distribution within the root zone prevent the use of these numbers except within very large confidence limits. Additionally, both seasonal and dirunal fluctuations of nitrogen-fixing rates must be considered when extrapolating acetylene reduction values taken at any specific time into integrated yearly reduction rates (Hardy et al., 1968; Wheeler, 1969). Finally, acetylene reduction rates are only an indirect measure of nitrogen fixing ability and must be converted to

obtain actual nitrogen fixation rates. It is often assumed that the ratio of nitrogen fixed to acetylene reduced is 3/1. This is the ratio of electron transfer between the two reactions:

$$\text{(Nitrogen fixation)}\quad N_2 + 6e^- \longrightarrow 2\,NH_4^+$$

$$\text{(Acetylene reduction)}\quad C_2H_2 + 2e^- \longrightarrow C_2H_4$$

The reduction of acetylene to ethylene is the much more efficient reaction of the two (Burris, 1974). Thus, the actual ratio may vary from 3/1 to as high as 4.5/1 (Hardy et al., 1968; Bergersen, 1970; Burris, 1974). Accurate conversion of acetylene reduction rates to nitrogen fixation requires that the exact ratio be determined using ^{15}N isotopes (Burris, 1974). Despite these problems, acetylene reduction has gained acceptance as a valuable tool for environmental studies of nitrogen-fixing systems. Once the samples are prepared, the actual analysis is straightforward, accurate, and precise (Burris, 1974). Precise measurements may be of little value, however, unless strict control is exercised in sample preparation and all other aspects of analytical work.

MATERIALS AND METHODS

Water and Salt Treatments

A greenhouse study was conducted in which alfalfa plants were grown in lysimeters under several salt and water regimes. Treatments consisted of a 3 x 3 factorial arrangement of irrigation water salinity (1.0, 3.0, 9.0 mmho cm^{-1}) and the quantity of water applied at each irrigation (120, 240, and 360 ml). These water quantities were equivalent depths of 2.3, 4.7, and 7.0 cm for the three water treatments, respectively. The frequency of watering was the same at any one time over all treatments, but varied during the course of the experiment in response to increased evapotranspiration as the alfalfa increased in size and greenhouse temperatures became warmer. The initial interval between irrigations was four days, which decreased to two days before the final harvest. Saline irrigation waters were artificially constructed by adding dry calcium chloride ($CaCl_2 \cdot 2H_2O$) to tap water using the approximate relationship 10 EC = me/liter (United States Salinity Laboratory, 1954). The establishment of the desired water salinities was confirmed by measurement of electrical conductivity.

Lysimeter Construction

A total of 120 lysimeters were constructed from polyvinylchloride (PVC) irrigation pipe 8.25 cm in diameter and 54.8 cm in length (Fig. 1). Each piece of pipe was cut along one side and then resealed with fiberglass prior to filling with soil. This allowed for the easy removel from the lysimeter of the intact soil profile at the time of harvest.

Fig. 1. Construction of the support framework. (a) Securing screens over drainage holes. (b) Lysimeters set into grooves at the base and supported above by the framework.

The filled lysimeters were mounted on a wooden base into which grooves had been cut to match the diameter of the pipe and through which holes had been cut to provide drainage. A support structure of iron bracing and wood held the lysimeters rigidly in place during the course of the study.

Soil Preparation

Soil was collected from the 1 to 3 meter depth of a site in Cache Valley, Utah, which had been classified as belonging to the Hillfield-Timpanogos Association. This soil was a coarse-silty, mixed mesic Calcicxerollic Xerochrept with increasing sandiness by depth.

Table 1. Chemical and physical properties of the 1 to 3 meter depth of of a coarse-silty, mixed mesic Calcicxerollic Xerochrept

pH (saturation paste)	7.53
EC (saturation extract)	0.42
Ca^{++} (me/liter)	1.60
Mg^{++} (me/liter)	0.85
Na^{+} (me/liter)	0.45
K^{+} (me/liter)	0.30
Cl^{-}	0.42
$NaHCO_3$ extractable P (mg-P/g)	8.5
Sand (%)	71.3
Silt (%)	22.2
Clay (%)	6.5
Saturated water content (g/g)	0.358
Saturated hydraulic conductivity (cm/hr)	0.32

Potassium phosphate fertilizer was added to the soil at a rate of 40 μg-P/g to prevent any phosphorus deficiency from arising during the experiment. No nitrogen fertilizers were added to the soil. The soil was passed through a 6-mm sieve and then packed into the lysimeter to an average bulk density of 1.38 g/cm.

Establishment of Alfalfa

The soil in all lysimeters was prewetted to near saturation and allowed to drain for several days in order to establish a field capacity water content in the lysimeter prior to planting. Inoculation of the alfalfa seed (Medicago sativa var. resistador) was accomplished using a commercially available Rhizobium culture (Rhizobia melilotii in a sugar slurry (Personal communication, Dr. W. F. Campbell, Utah State University, 1980). Ten seeds were planted in each lysimeter on April 1, 1980 (day 1). Subsequent thinnings reduced the number of plants per lysimeter to six by day 10, four by day 20, two by day 30, and one by day 40 (Fig. 2).

The development of iron chlorosis was evident in the alfalfa leaves on day 27, and was remedied by foliar application of iron chelate. Salt and water treatments were initiated 32 days after application of the chelate, at which time several lysimeters were sampled to insure that an acceptable level of nodulation had been established.

Randomization of Treatments

All plants received equal watering with tap water until the start of salt and water treatments on day 60. Treatments were completely randomized using 99 lysimeters from the original 120. This provided

Fig. 2. Establishment of a uniform stand of alfalfa plants. (a) Young plants shortly after emergence (6 plants per lysimeter). (b) Healthy alfalfa plants prior to the initiation of salt and water treatments (1 plant per lysimeter).

11 replications for each treatment from which three were randomly selected at each of three harvest dates (70, 90, and 110 days after planting).

Sampling

Twenty-seven lysimeters were destructively harvested on day 70. Care was taken to prepare all samples in a controlled environment to eliminate sample variations due to differences in environmental factors at the time of sampling. The work area used was both cool and out of direct sunlight to minimize dessication of samples during preparation.

The first step in sampling was to sever the top growth at the root collar, record its fresh weight and store for later analysis. The lysimeter was then opened along the pre-cut side (Fig. 3). The intact soil profile was removed and split into top (0-16 cm), middle (16-32 cm), and bottom (32-48 cm) sections. Each section was spread on a tray and the roots picked from the soil for a five-minute time period. This amount of time was sufficient to allow for a thorough job of removing roots from the soil, yet short enough to minimize any dessication of the root samples. Soil samples were also taken at this time and stored in moisture cans for later analysis.

Acetylene Reduction Analysis

Immediately after being removed from the soil, the roots were placed into 60 ml syringes which were used as incubation chambers for the acetylene reduction analysis (Fig. 4). The syringe plunger was then placed back into the syringe to the 48 ml mark. Commercial acetylene, which had been passed through H_2SO_4 and distilled H_2O

Fig. 3. Removal of the intact soil profile from a lysimeter. (a) Cutting along the pre-cut side of the lysimeter. (b) Soil profile being slid out of the cut lysimeter.

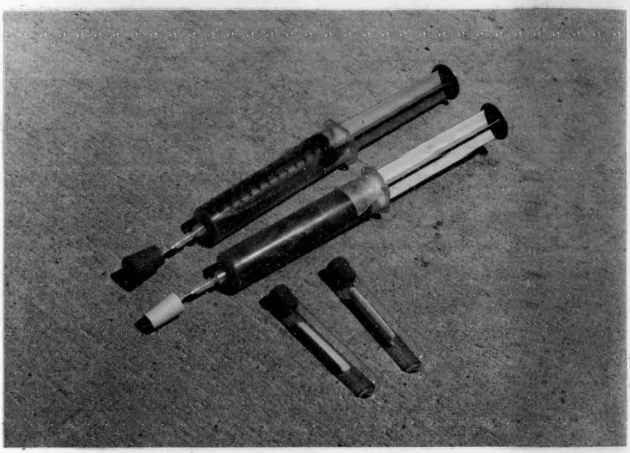


Fig 4. Preparing roots for acetylene reduction analysis. (a) Placing roots into 60 ml syringe incubation vessel. (b) Syringes containing roots during incubation and vaccutainers used to store gases for later analysis.

traps was used. The scrubbed acetylene was stored in an automobile inner tube and transported to the greenhouse. Six milliliters of acetylene were drawn into the syringe from the inner tube, with an additional 6 ml of air added to make a final volume of 60 ml of gas in the syringe. The syringe needle was then capped with a rubber stopper. This resulted in a 10% acetylene atmosphere in the syringe.

After a one-hour incubation period, one 12-ml gas sample was taken from each syringe and stored in a 12-ml vaccutainer. All 27 lysimeters were harvested in this manner between the hours of 1100 and 1600 on each of the three harvest dates.

Gas samples were periodically taken to determine minor ethylene contamination in the acetylene with these background levels later subtracted from the acetylene reduction values. All root samples were kept in the syringes and stored in a cold room (4°C) for further analysis.

Gas Chromatograph Methods

The stored gas samples were taken to the laboratory and analyzed for acetylene reduction by gas chromatograph. An airtight 100-µl syringe was used to remove gas subsamples from the vaccutainers and inject them into the gas chromatograph. Nitrogen carrier gas was passed through a 183 cm length by 0.3 outside diameter stainless steel column that was packed with 80 to 100 mesh Poropak N. Retention times for ethylene and acetylene were approximately 1.48 and 2.62 min, respectively. A 100 µl/liter external standard was used to convert the area under the curve into the actual amount of ethylene per injection. The total amount of ethylene produced in the original 60-ml

syringe was later obtained by subtracting the background ethylene level and multiplying by a volume conversion factor of 600 (100 µl to 60 ml).

Other Analyses

Top growth samples were placed in a drying oven (80°C) for 24 hrs and reweighed to obtain the dry weight of plant tops. Nodules were hand-picked from the roots and counted before they were placed in the drying oven for 24 hours and weighed. The dry weight of root growth was determined in the same manner as top growth after the nodules had been removed.

The mass water content of soil samples was determined gravimetrically. Chemical analyses included pH (saturation paste) and Na, K, Ca, Mg, Cl, and EC (saturation extract). Na and K were measured by flame emission, Ca and Mg by atomic absorption spectroscopy, and chloride by titration. Total water potentials were calculated from the sum of calculated matric and osmotic potentials. Matric potentials were estimated from soil water characteristic curves developed by pressure plate outflow methods. Osmotic potentials (OP) were calculated from the relationship (United States Salinity Laboratory, 1954)

$$OP\ (bars) = -0.36\ EC$$

where the EC is in mmho/cm.

Statistical Analysis

Statistical analysis was accomplished using the Rummage Statistical Package developed at Brigham Young University. Analysis of variance

for a three-way interaction of quantity water applied each irrigation, irrigation water salinity, and harvest date was performed along with Duncan's Multiple Range Test for comparisons between means. A separate statistical analysis was made for the data from each sample depth and for the combined data (overall depths) for the following plant parameters: top growth, root growth, acetylene reduction, nodule dry weight, nodule number, and specific nodule activity. Data were not collected at the final harvest for the last three of these parameters. In these cases (nodule dry weight, nodule number, and specific nodule activity) only the first and second harvests were included in the statistical analysis. Three soil parameters (mass water content, saturation extract electrical conductivity, and calculated total water potential) were statistically analyzed by depth.

RESULTS AND DISCUSSION

Top Growth

Analysis of variance indicated that the quantity of water applied per irrigation (W) and harvest date (H) were the primary statistically significant factors (α = 0.05 throughout the discussion) affecting the top growth (Table 2). Irrigation water salinity (S) as well as W x S and W x H interactions also exerted a significant influence. There were no significant differences among treatments means at the time of the first harvest (Fig. 5). This is not surprising, as water treatments had been initiated only 10 days previously. The effect of water quantity (W) became significant by the second harvest, that is increasing water quantity (W) resulted in significantly higher yields (Fig. 6). The negative effect of increasing irrigation water salinity (S) was statistically significant only at the highest level of applied water (W). Trends established at the time of the second harvest were more apparent at the third harvest (Fig. 7). Insufficient water limited top growth at both the low and medium water levels while the effect of salinity was insignificant except at the highest level of applied water.

The increased effect of irrigation water salinity with increasing amounts of applied water is often explained as a response to a greater quantity of total added salts. The high salt-high water treatment applied approximately 270 me/liter of salt at each irrigation as compared to 90 me/liter and 180 me/liter for the high salt-low water and

Table 2. Analysis of variance for entire lysimeter of top growth, root growth, acetylene reduction, nodule number, nodule dry weight and specific nodule activity.

Source of Variation	Degrees of Freedom	F-ratio	Source of Variation	Degrees of Freedom	F-ratio	Source of Variation	Degrees of Freedom	F-ratio
	Top Growth			Root Growth			Acetylene Reduction	
Water	2	80.50*	Water	2	14.80*	Water	2	28.97*
Salt	2	12.70*	Salt	2	2.12	Salt	2	0.63
Harvest	2	115.17*	Harvest	2	71.59*	Harvest	2	47.91*
WxS	4	4.81*	WxS	4	1.21	WxS	4	4.51*
WxH	4	17.29*	WxH	4	3.42*	WxH	4	20.91*
SxH	4	2.45	SxH	4	1.87	SxH	4	6.98*
WSH	8	1.13	WSH	8	0.53	WSH	8	1.75
Error	54	--	Error	54	--	Error	54	--
	Nodule Number			Nodule Dry Weight			Specific Nodule Activity	
Water	2	5.35*	Water	2	11.94*	Water	2	31.96*
Salt	2	0.34	Salt	2	1.46	Salt	2	9.34*
Harvest	1	35.63*	Harvest	1	53.03*	Harvest	1	79.39*
WxS	4	2.09	WxS	4	1.98	WxS	4	0.84
WxH	4	1.25	WxH	4	3.48*	WxH	4	33.56*
SxH	2	0.44	SxH	4	1.55	SxH	4	0.06
WSH	4	0.87	WSH	4	0.57	WSH	4	0.36
Error	36	--	Error	36	--	Error	36	--

*Denotes significance at $\alpha = 0.05$ level.

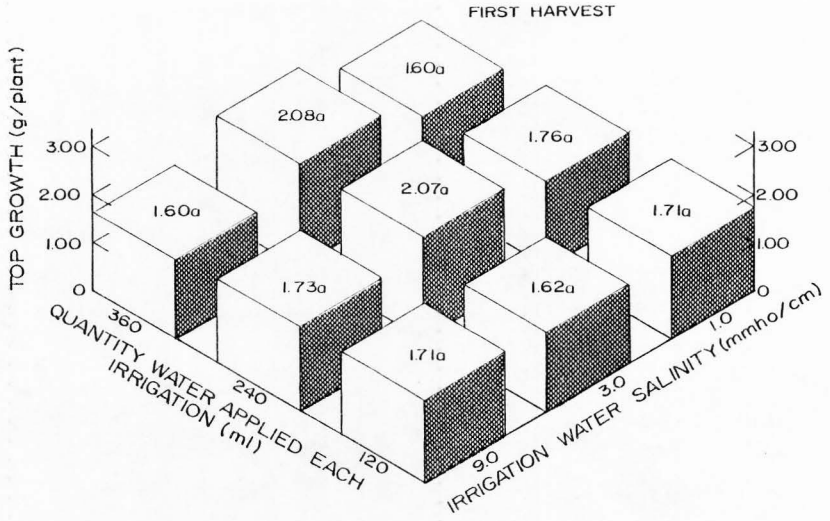


Fig. 5. Alfalfa top growth (g) at the first harvest. (Treatment means followed by the same letter are not significant at $\alpha = 0.05$.)

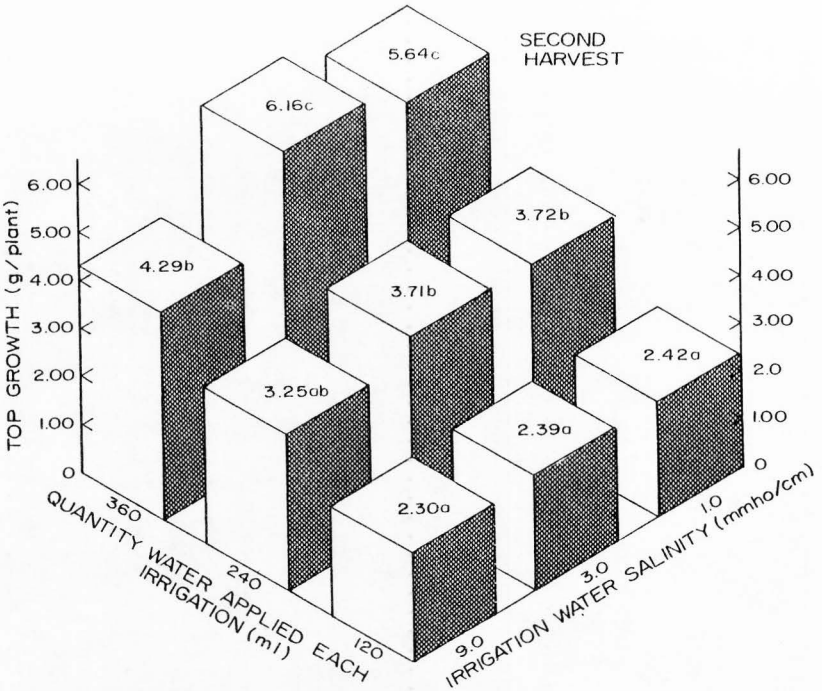
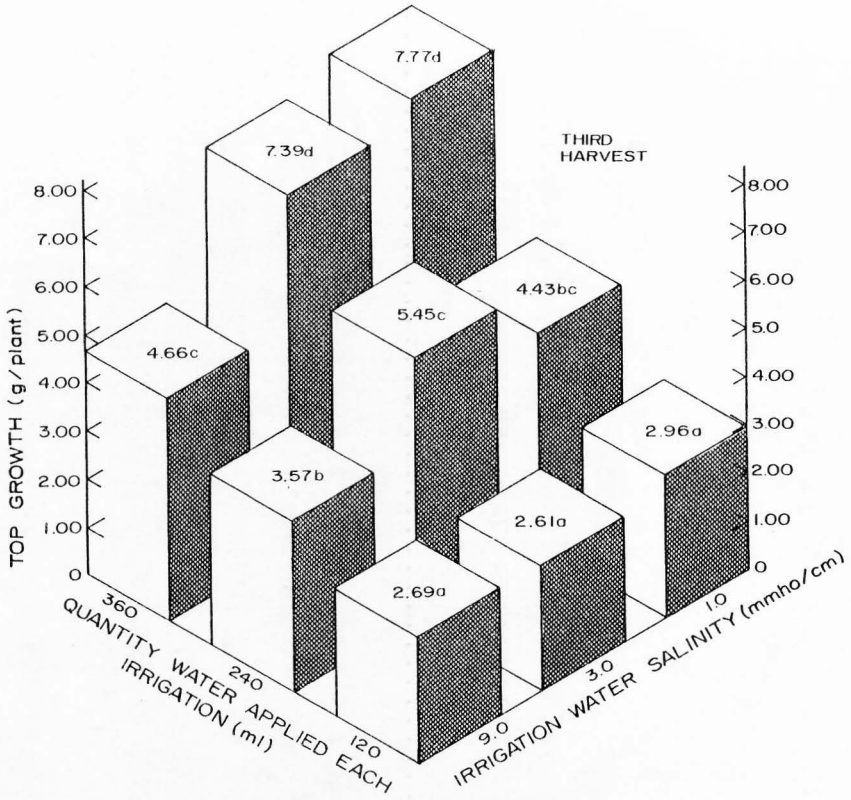


Fig. 6. Alfalfa top growth (g) at the second harvest. (Treatment means followed by the same letter are not significant at $\alpha = 0.05$.)


THIRD HARVEST
TOP GROWTH (g/plant)
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0
7.77d
7.39d
4.66c
5.45c
4.43bc
3.57b
2.96a
2.69a
2.61a
360
240
120
QUANTITY WATER APPLIED EACH IRRIGATION (ml)
9.0
3.0
1.0
IRRIGATION WATER SALINITY (mmho/cm)

high salt-medium water treatments, respectively. Alfalfa growth is generally considered to be related to salt concentration in the soil profile and not to the total amount of salt present (Bower et al., 1969; Bernstein and Francois, 1973; Francois, 1981), with the salinity of the upper portion of the root zone the critical factor affecting the response (Francois, 1981). The electrical conductivities of the saturation extracts from the 0-16 cm depth (Table 3) at both the second and third harvests were equally high for the high salt treatments over all levels of applied water. Thus, the greater amount of salt added in the high salt-high water treatment did not result in a greater amount of salt being accumulated in the top portion of the lysimeter. From these data, it is obvious that total added salt was not the single factor affecting alfalfa response in the study. A more plausible explanation may be that the insufficient amount of water applied in both the low and medium water treatments biased the salt stress effects on alfalfa yield. Moisture stress particularly in the low water treatments was so extreme that it virtually eliminated any measurable salt effect. That is, the adverse matric potential prevented the expression of the osmotic effect. These data suggest that alfalfa grown under dryland conditions may show a greater relative yield decrement in the presence of a high concentration of soil borne salt during wet years than dry years.

Root Growth

Significant effects on root growth resulted from the quantity of water applied per irrigation, the harvest date and from a W x H interaction (Table 2). These results were consistent over all three sample

Table 3. Means of saturation extract electrical conductivity (mmho/cm) by depth and harvest.

Factor		Depth 0-16 cm First harvest	0-16 cm Second harvest	0-16 cm Third harvest	16-32 cm First harvest	16-32 cm Second harvest	16-32 cm Third harvest	32-48 cm First harvest	32-48 cm Second harvest	32-48 cm Third harvest
W1	S1	1.62a	1.99a	2.23a	1.16a	2.58ab	4.13b	1.08a	1.16a	1.35a
	S2	2.79ab	3.93abc	5.77c	1.35a	6.76c	10.84d	1.83a	1.55a	1.25a
	S3	6.40c	10.40d	12.59d	3.13a	14.23f	15.23f	1.12a	4.57b	17.80e
W2	S1	1.58a	1.66a	1.87a	1.88a	3.71ab	6.01b	1.23a	1.87a	2.90a
	S2	3.01ab	4.09ab	4.55bc	3.61a	8.21cd	13.11e	1.42a	4.01a	10.36c
	S3	7.48c	12.25d	13.70d	7.50c	10.23d	17.03f	1.50a	16.48e	20.73f
W3	S1	1.65a	1.87a	1.80a	2.04a	2.37ab	4.57b	1.67a	4.75b	6.88b
	S2	3.31ab	2.65ab	4.78bc	3.51a	7.10c	13.81e	3.42ab	13.02c	17.20e
	S3	7.47c	12.43d	12.04d	8.63c	16.03f	11.02d	5.56b	18.73e	14.25d
Water										
W1		3.60a	5.44b	6.87bc	1.88a	7.87c	10.07d	1.34a	2.43a	6.80c
W2		4.02a	5.10bc	6.71c	4.33b	7.38c	12.05e	1.38a	7.45c	11.33d
W3		4.14ab	5.65bc	6.20c	4.73b	8.50c	9.80cd	3.55b	12.17d	12.78d
Salt										
S1		1.62a	1.84a	1.97a	1.70a	2.89a	4.90b	1.33a	2.59ab	3.71b
S2		3.04ab	3.56bc	5.04c	2.83a	7.36b	12.59c	2.22a	6.19c	9.60d
S3		7.11c	11.69d	12.77d	6.42b	13.50cd	14.43d	2.73a	13.26d	17.60e

*Means within any depth followed by the same letter are not significantly different at $\alpha = 0.05$.

depths (Table 4). Only in the 0-16 cm depth was there any significant effect related to salinity and that was through a S x H interaction. This agrees with other reported studies in which the effects of salinity were to suppress top growth more than root growth (Maas and Hoffman, 1977).

The trend of reduced root growth with decreasing quantity of applied water became apparent by the second harvest, but it was not until the third harvest that this trend became statistically significant (Fig. 8). Root growth at the third harvest was significantly depressed under the low water treatments for both low and medium irrigation water salinities (Fig. 9). The negative effect of salinity on root growth as with top growth, was evident only in cases in which water was not severely limiting. There was no apparent effect of irrigation water quality upon root growth in the low water treatments.

The greater affect of irrigation water salinity on top growth than root growth should result in higher root-to-shoot ratios with increasing salt stress. Calculated root-to-shoot ratios do not indicate that this was the case (Table 5). Although statistical analysis was not accomplished on these data, there appears to be a slight increase in the ratio with increasing moisture stress, as is indicated by comparing across W levels for a given S level. Plants under moisture stress would be expected to invest greater energy in expanding their root systems at the expense of top growth (Personal communication, Dr. William Campbell, 1981).

Table 4. Analysis of variance for root growth and acetylene reduction (2 cases) by depth.

Depth	Root Growth			Acetylene Reduction					
				Harvest 1 and 2			Harvests 1, 2 and 3		
	Source of Variation	Degrees of Freedom	F-ratio	Source of Variation	Degrees of Freedom	F-ratio	Source of Variation	Degrees of Freedom	F-ratio
0-16 cm	Water	2	11.64*	Water	2	8.46*	Water	2	7.91*
	Salt	2	2.35	Salt	2	2.36	Salt	2	1.16
	Harvest	2	73.08*	Harvest	1	19.16*	Harvest	2	0.11
	WxS	4	0.95	WxS	4	9.46*	WxS	4	7.55*
	WxH	4	3.36*	WxH	2	12.76*	WxH	4	16.44*
	SxH	4	3.11*	SxH	2	6.72*	SxH	4	9.56*
	WSH	8	1.14	WSH	4	3.09*	WSH	8	3.34*
	Error	54	--	Error	36	--	Error	54	--
16-32 cm	Water	2	11.29*	Water	2	13.32*	Water	2	13.22*
	Salt	2	0.64	Salt	2	3.12	Salt	2	2.75
	Harvest	2	40.60*	Harvest	1	18.00*	Harvest	2	1.41
	WxS	4	1.43	WxS	4	1.01	WxS	4	1.04
	WxH	4	2.88*	WxH	2	3.86*	WxH	4	0.86
	SxH	4	0.38	SxH	2	1.21	SxH	4	0.94
	WSH	8	0.28	WSH	4	1.43	WSH	8	1.53
	Error	54	--	Error	36	--	Error	54	--
32-48 cm	Water	2	11.40*	Water	2	20.13*	Water	2	19.59*
	Salt	2	2.61	Salt	2	6.68*	Salt	2	6.20*
	Harvest	2	35.13*	Harvest	1	19.45*	Harvest	2	9.96*
	WxS	4	1.13	WxS	4	2.79*	WxS	4	2.58
	WxH	4	4.14*	WxH	2	11.65*	WxH	4	10.10*
	SxH	4	1.00	SxH	2	2.50	SxH	4	1.76
	WSH	8	0.25	WSH	4	2.12*	WSH	8	2.17
	Error	54	--	Error	36	--	Error	54	--

*Denotes significance at the $\alpha = .05$ level.

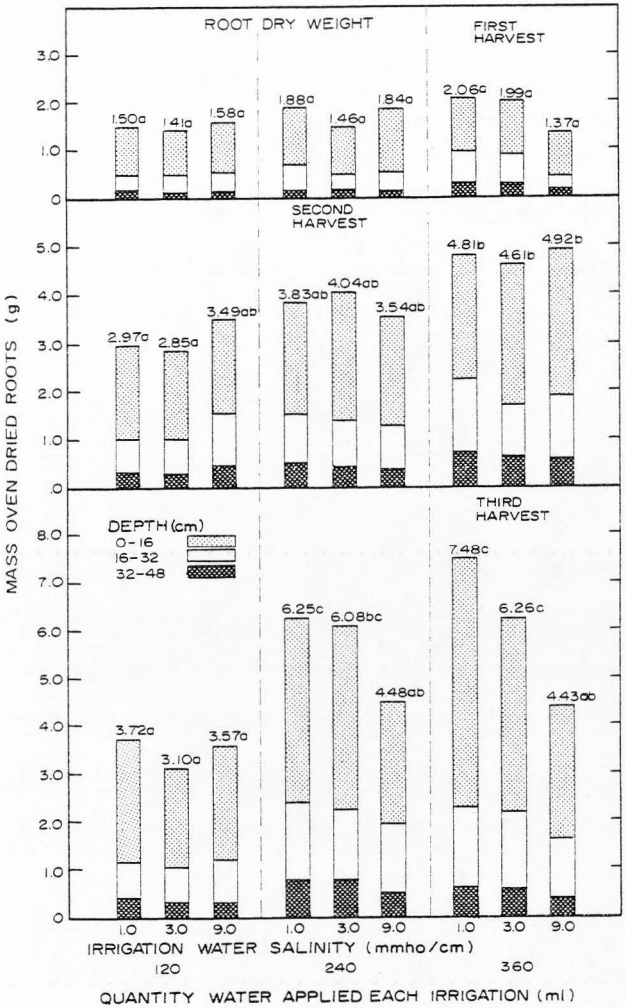


Fig. 8. Root dry weights (g) at three harvest dates, resulting from salt and water treatments. (Treatment means followed by the same letter are not significant at α = 0.05.)

Fig. 9. Root dry weights (g) at the third harvest. (Treatment means followed by the same letter are not significant at $\alpha = 0.05$.)

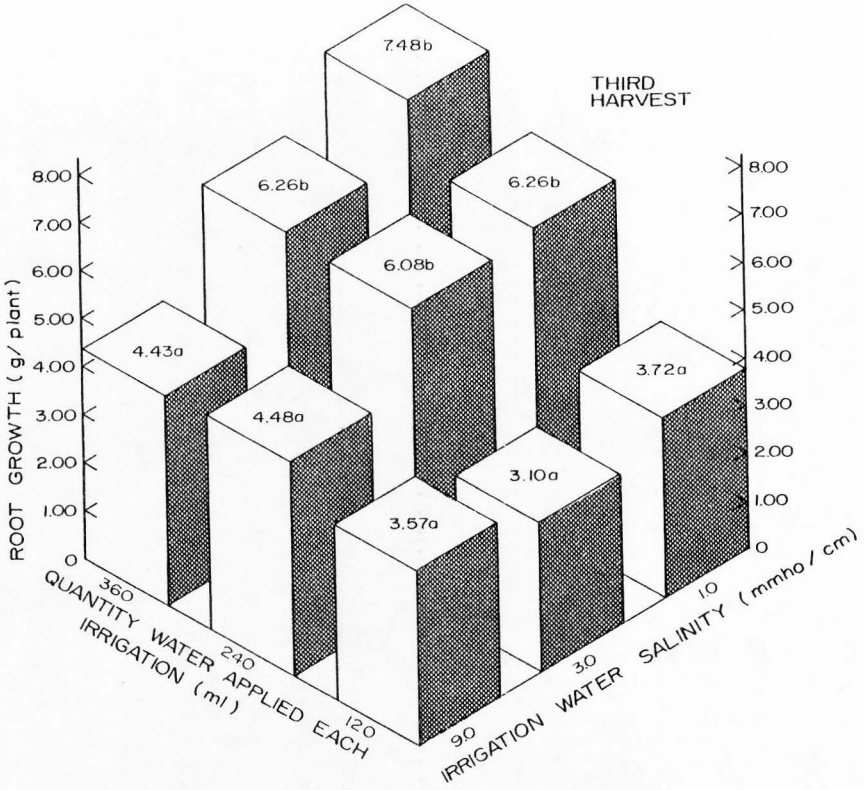

THIRD HARVEST
7.48b
6.26b
6.26b
6.08b
4.43a
4.48a
3.72a
3.10a
3.57a
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0
ROOT GROWTH (g/plant)
360
240
120
QUANTITY WATER APPLIED EACH IRRIGATION (ml)
90
30
10
IRRIGATION WATER SALINITY (mmho/cm)

Table 5. Measured root:shoot ratios as a function of water (W), salt (S), and harvest date

Treatment	Harvest		
	First	Second	Third
W1 S1	0.80	1.22	1.25
S2	0.87	1.19	1.19
S3	0.93	1.52	1.33
W2 S1	1.05	1.03	1.41
S2	0.70	1.09	1.11
S3	1.04	1.09	1.25
W3 S1	0.93	0.85	0.96
S2	0.95	0.77	0.84
S3	0.85	1.15	0.95

Specific Nodule Activity

Specific nodule activity is defined as the rate at which root nodules reduced acetylene per gram nodule dry weight (μmol C_2H_4/g/hr). Determinations were made of the specific nodule activity for both the first and the second harvests. Acetylene reduction by the final harvest was an order of magnitude lower than observed at the previous harvests. It was observed, though no count was made, that at the time of the third harvest, the roots sill contained numerous nodules, particularly at the high water treatments. Therefore, the marked decreases in acetylene reduction values at the third harvest was due probably to greatly reduced nodule activity resulting from cumulative treatment effects, as explained below. Specific nodule activity at the third harvest was not determined because of the lack of individual treatment effects on measured acetylene reduction rates.

Specific nodule activity was significantly affected by the quantity of water applied per irrigation, irrigation water salinity, harvest date and by a W x H interaction (Table 2). The same four factors were significant at all three depths sampled (Table 6).

A dramatic water effect can be observed (Fig. 10) in the specific nodule activity rates measured under high water treatments at the first harvest date. It is interesting to note that the quantity of water applied exerted a significant effect on specific nodule activity long before any differences among treatments became apparent in either shoot or root growth. Specific nodule activity drops off sharply once the nodules begin to lose water faster than it is supplied by the roots (Sprent, 1973). The acetylene reducing activity of soybean root nodules has been shown to decline rapidly during initial water stress (Pankhurst and Sprent, 1975). The results of this study are consistent with these observations. Thus, even short term moisture stresses may be sufficient to temporarily disrupt the activity of alfalfa root nodules.

A significant salt effect at the first harvest was apparent only with the low water treatments. It was observed that the specific nodule activity was greatly reduced by the low water-high salt treatment as compared to all other treatments. Increased salinity in the 0-16 cm and 16-32 cm depths (Table 3) may have further restricted water availability at this low water level. Decreased acetylene reduction due to dessication under moisture stressed conditions has been attributed to reduced oxygen diffusion into the nodules (Pankhurst and Sprent, 1975). Salinity has also been shown to depress oxygen uptake by root nodules (Sprent, 1972). Thus, the influence of salinity on specific nodule

Table 6. Analysis of variance for nodule number, nodule dry weight, and specific nodule activity by depth.

Depth	Nodule Number			Nodule Dry Weight			Specific Nodule Activity		
	Source of Variation	Degrees of Freedom	F-ratio	Source of Variation	Degrees of Freedom	F-ratio	Source of Variation	Degrees of Freedom	F-ratio
0-16 cm	Water	2	2.53	Water	2	4.41*	Water	2	10.99*
	Salt	2	1.44	Salt	2	2.43	Salt	2	5.58*
	Harvest	1	37.87*	Harvest	1	31.08*	Harvest	1	35.39*
	WxS	4	4.13*	WxS	4	4.51*	WxS	4	1.79
	WxH	2	2.41	WxH	2	4.22*	WxH	2	14.34*
	SxH	2	0.30	SxH	2	0.90	SxH	2	0.36
	WSH	4	0.44	WSH	4	0.45	WSH	4	0.43
	Error	36	--	Error	36	--	Error	36	--
16-32 cm	Water	2	3.45*	Water	2	7.44*	Water	2	14.50*
	Salt	2	0.34	Salt	2	0.24	Salt	2	8.61*
	Harvest	1	9.35*	Harvest	1	9.17*	Harvest	1	36.81*
	WxS	4	0.60	WxS	4	0.55	WxS	4	0.67
	WxH	2	2.75	WxH	2	3.95*	WxH	2	6.80*
	SxH	2	1.01	SxH	2	1.50	SxH	2	1.26
	WSH	4	1.22	WSH	4	1.72	WSH	4	0.95
	Error	36	--	Error	36	--	Error	36	--
32-48 cm	Water	2	5.15*	Water	2	5.56*	Water	2	27.11*
	Salt	2	1.00	Salt	2	0.32	Salt	2	8.32*
	Harvest	1	9.61*	Harvest	1	10.86*	Harvest	1	57.00*
	WxS	4	0.81	WxS	4	0.99	WxS	4	2.52
	WxH	2	2.17	WxH	2	0.84	WxH	2	17.56*
	SxH	2	0.07	SxH	2	0.12	SxH	2	3.00
	WSH	4	1.55	WSH	4	0.84	WSH	4	1.45
	Error	36	--	Error	36	--	Error	36	--

*Denotes significance at the $\alpha = .05$ level.

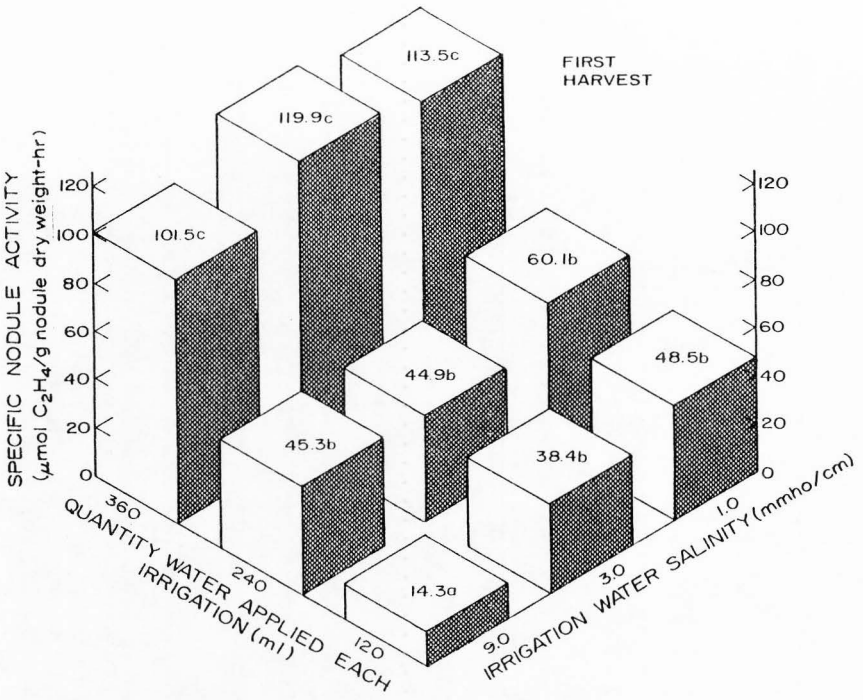


Fig. 10. Specific nodule activity (μmol C_2H_4/g/hr) at the first harvest. (Treatment means followed by the same letter are not significant at $\alpha = 0.05$.)

activity in the low water treatments may not be solely an osmotic effect but an additive effect of both water and salt stresses on oxygen uptake by the root nodules. Whether reduced oxygen uptake by salt-stressed root nodules results from any osmotic adjustments within the nodules or whether it is due to some other physiological mechanism is not apparent from the literature.

A drastic reduction in specific nodule activity was measured at the second harvest with high water treatments across all salinity levels (Fig. 11). Specific nodule activity under medium and low water treatments, by contrast, was only slightly reduced from first harvest levels. Salt effects became more apparent at the second harvest with significant differences being found under both medium and low water quantities. Specific nodule activity in the low water-high salt treatment was reduced to almost zero a response again produced by the inseparable, but combined, effects of water and salt stress.

Flooding has been reported to depress nitrogen fixing activity in various legume species in a number of studies

(Sprent, 1969, 1971; Mague and Burris, 1972; Minchin and Pate, 1975). It can be postulated that the measured reduced specific nodule activity of high water treatments at the second harvest resulted from excess water and resulting saturated soil conditions. This was not the case, however, since the soil water contents (Table 7) were well below the saturated mass water content of 0.36. The alfalfa plants were utilizing most of the water applied even at the high water treatments. If any effect of excess water was being imposed, it may be through some unmeasured mechanism, as the maintenance of thin water films on the nodules (Sprent, 1969).

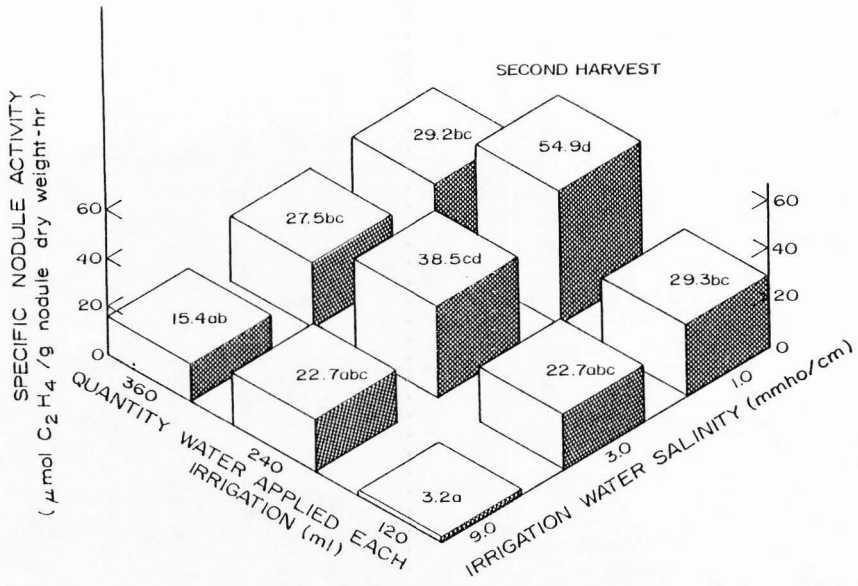


Fig. 11. Specific nodule activity (μmol C_2H_4/g/hr) at the second harvest. (Treatment means followed by the same letter are not significant at $\alpha = 0.05$.)

Table 7. Means of mass water content (g water/g soil) by depth and harvest.*

Factor		Depth								
		0-16 cm			16-32 cm			32-48 cm		
		First harvest	Second harvest	Third harvest	First harvest	Second harvest	Third harvest	First harvest	Second harvest	Third harvest
W1	S1	0.09a	0.11ab	0.07a	0.07a	0.08a	0.07a	0.07a	0.06a	0.06a
	S2	0.11ab	0.11ab	0.10ab	0.10a	0.09a	0.10a	0.10a	0.06a	0.06a
	S3	0.12ab	0.14bc	0.13bc	0.08a	0.14b	0.15b	0.07a	0.08a	0.15b
W2	S1	0.09a	0.10ab	0.07a	0.09a	0.11a	0.08a	0.07a	0.07a	0.07a
	S2	0.11ab	0.10ab	0.07a	0.11ab	0.13ab	0.10a	0.08a	0.09a	0.10a
	S3	0.15bc	0.17c	0.17c	0.16bc	0.20c	0.20c	0.13b	0.20c	0.21c
W3	S1	0.11ab	0.08a	0.07a	0.11ab	0.09a	0.07a	0.09a	0.10a	0.08a
	S2	0.22c	0.08a	0.08a	0.14b	0.10a	0.13ab	0.12ab	0.13ab	0.15b
	S3	0.17c	0.16c	0.17c	0.20c	0.19c	0.19c	0.20c	0.21c	0.22c
Water										
	W1	0.11a	0.12a	0.10a	0.08a	0.10ab	0.11b	0.08a	0.06a	0.09a
	W2	0.11a	0.12a	0.10a	0.12b	0.14cd	0.13bc	0.10ab	0.12bc	0.13cd
	W3	0.17b	0.11a	0.10a	0.15d	0.12bc	0.13bd	0.14d	0.15cd	0.15d
Salt										
	S1	0.10a	0.10a	0.08b	0.09ab	0.09ab	0.07a	0.08ab	0.07a	0.07a
	S2	0.15c	0.09a	0.08b	0.12c	0.11bc	0.11bc	0.10b	0.09ab	0.10b
	S3	0.15c	0.16c	0.16c	0.15d	0.18e	0.18e	0.13c	0.17d	0.19d

*Means within any depth followed by the same letter are not significantly different at $\alpha = 0.05$.

Legume phenology or development stage also influences the nitrogen fixing rate. Reproductive sinks, such as flowering or the setting of seed, compete with root nodules for available photosynthate (Hardy and Havelka, 1976). If the rate of plant development in this study (unmeasured) was delayed in the lower water treatments relative to the high water treatment, the reduced fixing rates observed at the second harvest might be related to the onset of reproductive growth. Ontogeny in salt-affected plants if often reduced (Maas and Nieman, 1978), however moisture stress in most cases results in plants flowering and setting seed earlier than in unstressed plants (Personal communication, Dr. William Campbell, 1982). It is recommended that future studies should be designed to better correlate legume development stage with the effects of water and salt upon nitrogen fixation.

Oxygen is required by the root nodule for ATP production (Tjepkema, 1979). Insufficient oxygen sharply reduces the nitrogen fixing activity of root nodules. The greater mass of roots produced under the more favorable soil moisture conditions of the high water treatments may be responsible for the decrease in measured acetylene reduction rates through the increased respirational demand upon oxygen within the incubation vessel.

The acetylene reduction rate of heavily nodulated roots has been observed to drop sharply after 60 minutes (Hardy et al, 1968). Sprent (1971) reported having to replace both oxygen and acetylene after 1 hour during measurement of acetylene reducing activity, to prevent both gases from becoming limiting. The respiratory oxygen demand of a fairly large root mass, relative to the incubation vessel size, could

cause the acetylene reduction reaction to stop, thus having the effect of producing artificially low estimates of nitrogen fixation. The mass of roots assayed in this study was greater in the high water treatments than in the low or medium water treatments. Whether this extra mass was sufficient to limit oxygen during incubation is not known. Attempts to prove or deny the possibility have not been successful (Personal communication, Dr. William Campbell, 1982).

Nodule Dry Weight

Nodule dry weight was significantly affected both by the quantity of water applied at each irrigation and by harvest date (Table 2). A significant water by harvest (W x H) interaction was also measured. A water by salinity interaction (W x S) was significant at the 0-16 cm depth (Table 6). The irrigation water salinity (S), while not significant at $\alpha = 0.05$, was found to be significant at $\alpha = 0.10$ for both the combined analysis and the 0-16 cm depth.

The highest irrigation water salinity had an initially positive effect on nodule growth in cases where water was not limiting (Fig. 12). This positive effect may be related to the addition of calcium stimulating nodule initiation (Lowther and Loneragan, 1968), since the soil used in this study had a low concentration of solute calcium (Table 1). The literature indicates that calcium requirement for the nodulation of subterranean clover was higher than for the growth of either the host plant or the Rhizobium (Loneragan and Dowling, 1958). Base fertility elements are considered essential for maximum nodulation by alfalfa (Miles, 1969), and any lack could have limited nodulation in this study.

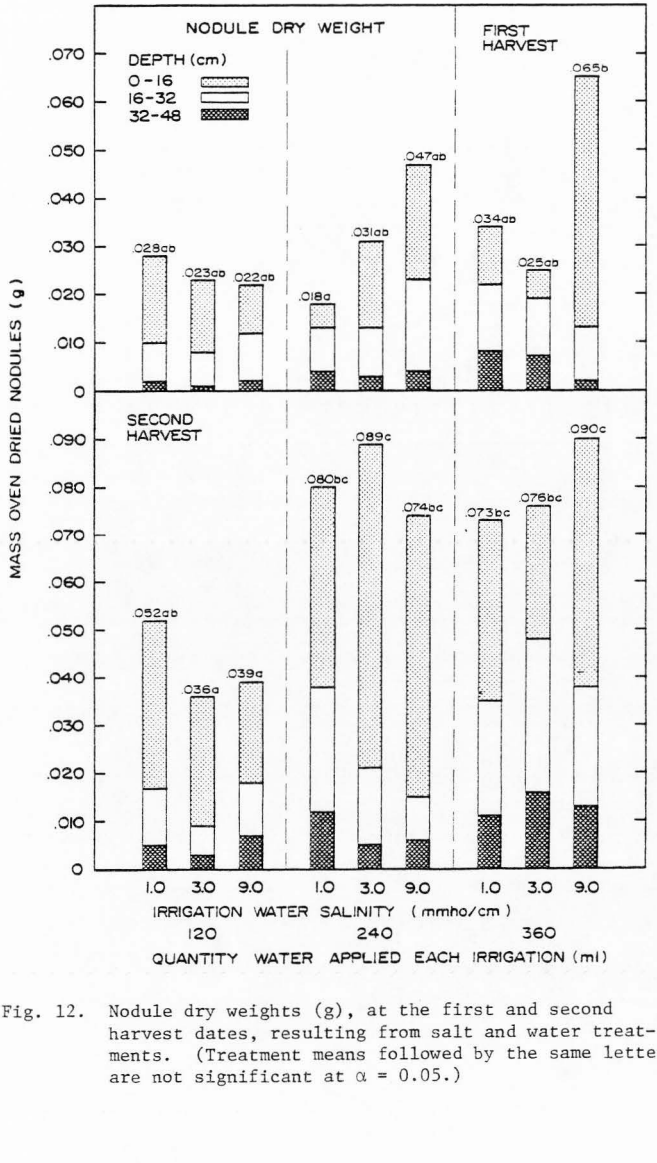


Fig. 12. Nodule dry weights (g), at the first and second harvest dates, resulting from salt and water treatments. (Treatment means followed by the same letter are not significant at $\alpha = 0.05$.)

The data indicate that the initial effects of added calcium salts stimulated nodule growth until salinity reached a threshold value beyond which no calcium response was measured. Increasing salinity at the second harvest had no effect on nodule dry weight, suggesting the threshold had been surpassed. The grain yield in barley, though not related to nodulation, has exhibited a similar positive threshold response in yield to increasing salinity up to a moderate salt concentration (Wagenet et al., 1980).

No significant effect on nodule dry weight related to the quantity of water applied at each irrigation was measured by the first harvest date (Fig. 12). Nodule dry weight was significantly depressed by the second harvest at only the lowest water treatments. No difference in nodule dry weight existed between medium and high levels of applied irrigation. This suggests that nodule growth in alfalfa was sensitive only to severe moisture stress and relatively insensitive to the moderate levels of stress.

Nodule Number

The number of nodules reflects the ability of the Rhizobia-plant root symbiosis to successfully establish a healthy relationship. Significant differences in numbers were found due to water quantity and harvest date (Table 2). A water by salinity (W x S) interaction was statistically significant only in the upper (0-16 cm) profile (Table 6). The high water-high salt treatment had the greatest number of nodules at both the first and second harvests (Fig. 13). The number of nodules were essentially equal for the other eight treatments at the first harvest date. Both medium and high (3.0, 9.0 mmho/cm, respectively)

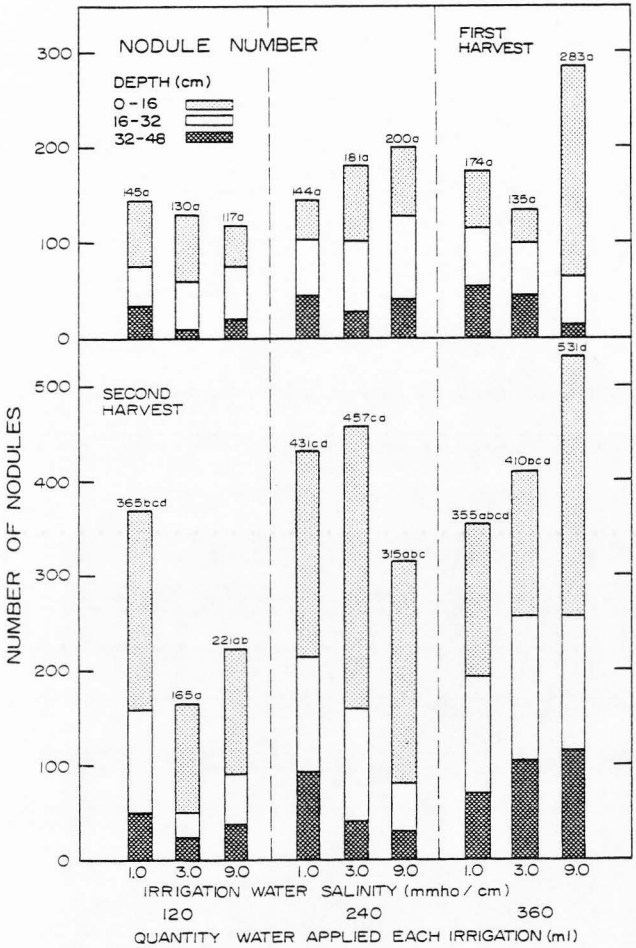


Fig. 13. Number of root nodules at the first and second harvest dates, resulting from salt and water treatments. (Treatment means followed by the same letter are not significant at $\alpha = 0.05$.)

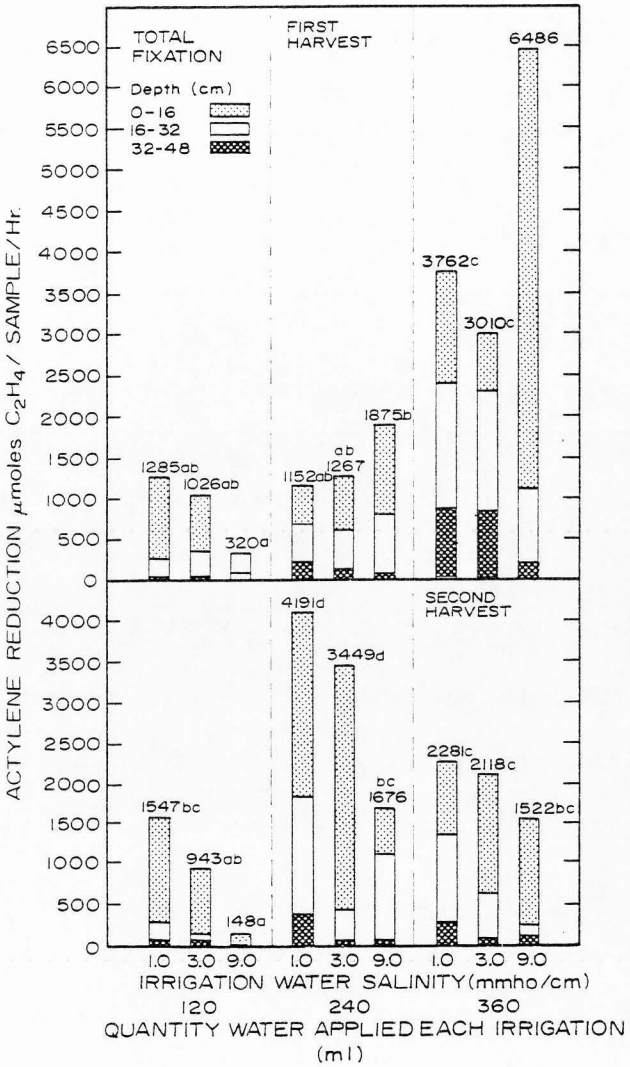
irrigation water salinities depressed nodule formation under the low water treatments by the second harvest. Very little difference was noted between the medium and high water treatments at this time, indicating that nodule formation on the roots of alfalfa is quite resistant to total soil moisture stress. This agrees with the field studies of Johnson and Rumbaugh (1981) where Medicago sativa was found nodulated in semi-arid environments while other legume species were not.

Total Acetylene Reduction

Total acetylene reduction is the amount of acetylene reduced by the entire root sample for a given sampling depth during the 1 hour incubation period. This is essentially an integrated measure of nodulation, nodule growth and the specific nodule activity. Significant differences in total acetylene reduction occurred in response to the quantity of water applied each irrigation, harvest date, and W x S, W x H, and S x H interactions (Table 2). The harvest date effect was only significant when data from all three harvests were included in the analysis (Table 4) and largely reflects the overriding effect of the reduction in nodule activity by the third harvest. Data from Lauter et al. (1981) showed a similar substantial drop in the specific nodule activity of chickpea root nodules by the time of their third harvest.

Total acetylene reduction was significantly greater in the high water treatments under all salinity levels at the first harvest date (Fig 14). This reflects the positive effect that increased water quantity had on specific nodule actifity. The enhanced nodulation and nodule growth at the first harvest coupled with the effects of favorable

Fig. 14. Total acetylene reduction rates (μmol C_2H_2/sample/hr), at the first and second harvest dates, resulting from salt and water treatments. (Treatment means followed by the same letter are not significant at $\alpha = 0.05$.)


TOTAL FIXATION
Depth (cm)
0-16
16-32
32-48
FIRST HARVEST
SECOND HARVEST
ACTYLENE REDUCTION µmoles C_2H_4/ SAMPLE/Hr.
1285ab
1026ab
320a
1152ab
1267 ab
1875b
3762c
3010c
6486
1547bc
943ab
148a
4191d
3449d
1676 bc
2281c
2118c
1522bc
1.0 3.0 9.0 1.0 3.0 9.0 1.0 3.0 9.0
IRRIGATION WATER SALINITY(mmho/cm)
120 240 360
QUANTITY WATER APPLIED EACH IRRIGATION
(ml)

moisture conditions resulted in the high water-high salt treatment having a substantially higher total acetylene reduction rate than any of the other treatments.

The above trends were both reversed by the second harvest. The drop in the specific nodule activity of high water treatments was evident here through a similar drop in the total acetylene reduction rate of these treatments. Likewise, the acetylene reduction rate of the high water-high salt treatment was greatly reduced due to the combined effects of lower specific nodule activity and the loss of any initial enhancement of nodulation by salinity. Irrigation water salinity was, by the second harvest, exerting a significant negative effect on acetylene reduction under both the low and medium water quantities as a result of reduced specific nodule activity. Low total acetylene reduction rates under the low water treatments indicate reduced nodulation and nodule growth as well as reduced specific nodule activity of alfalfa under water stressed conditions.

Interestingly, the high water-high salt treatment had, by the third harvest, the highest acetylene reduction rate though nodule activity was severely restricted in all treatments by this time.

Soil Parameters

Soil salinity profiles. Soil salinity as measured by the electrical conductivity of the saturation extract, varied primarily in response to irrigation water salinity (Table 8). The effect of the amount of applied water became much more pronounced with the increasing depth in the soil profile. Salts accumulated at different depths in the soil as a function of the imposed water management regime. Harvest date also had a dominant

Table 8. Analysis of variance for mass water content, saturation extract electrical conductivity and calculated total water potential by depth.

Depth	Mass Water Content			Saturation Extract Electrical Conductivity			Calculated Total Water Potential		
	Source of Variation	Degrees of Freedom	F-ratio	Source of Variation	Degrees of Freedom	F-ratio	Source of Variation	Degrees of Freedom	F-ratio
0-16 cm	Water	2	1.84	Water	2	0.23	Water	2	0.53
	Salt	2	27.30*	Salt	2	212.02*	Salt	2	49.81*
	Harvest	2	4.46*	Harvest	2	18.87*	Harvest	2	27.18*
	WxS	4	1.80	WxS	4	0.86	WxS	4	0.83
	WxH	4	3.49*	WxH	4	0.36	WxH	4	0.66
	SxH	4	3.37*	SxH	4	7.83*	SxH	4	2.07
	WSH	8	0.96	WSH	8	0.35	WSH	8	0.34
	Error	54	--	Error	54	--	Error	54	--
16-32 cm	Water	2	19.16*	Water	2	6.57*	Water	2	2.31
	Salt	2	84.01*	Salt	2	229.31*	Salt	2	23.41*
	Harvest	2	0.49	Harvest	2	165.60*	Harvest	2	95.65*
	WxS	4	4.05*	WxS	4	0.96	WxS	4	2.69*
	WxH	4	3.52*	WxH	4	6.05*	WxH	4	3.73*
	SxH	4	3.49*	SxH	4	18.69*	SxH	4	8.38*
	WSH	8	0.41	WSH	8	7.11*	WSH	8	1.87
	Error	54	--	Error	54	--	Error	54	--
32-48 cm	Water	2	32.47*	Water	2	83.00*	Water	2	12.29*
	Salt	2	62.48*	Salt	2	175.98*	Salt	2	17.11*
	Harvest	2	2.32	Harvest	2	160.54*	Harvest	2	80.33*
	WxS	4	5.86*	WxS	4	12.84*	WxS	4	7.92*
	WxH	4	1.11	WxH	4	12.73*	WxH	4	3.67*
	SxH	4	3.26*	SxH	4	33.52*	SxH	4	3.60*
	WSH	8	1.09	WSH	8	14.94*	WSH	8	6.49*
	Error	54	--	Error	54	--	Error	54	--

*Denotes significance at $\alpha = .05$ level.

effect on soil salinity levels, especially at the middle (16-32 cm) and bottom (32-48) cm depths. A salinity by harvest (S x H) interaction was significant at all three depths.

Soil Water Content

Mass water content also varied between treatments primarily in response to irrigation water salinity (Table 8). The significance of the water quantity effect increased with depth as did the significance of a W x S interaction.

Mass water content measured at harvest (Fig. 15) is a point measurement and as such is only a relative indication of the overall moisture status of the soil throughout the irrigation cycle. Since harvests were made near the end of irrigation cycles, the values of mass water content more nearly represent the level to which plants depleted soil moisture between irrigations. Salt build-up in the soil largely determined these levels through the osmotic effect on total water potential. Thus, increased salinity of the irrigation water resulted in higher mass water contents, especially at the second and third harvest, irrespective of the quantity of water applied (Fig. 16). Drainage occurred in the high water-high salt treatment and in the medium water-high salt treatment, once salinity had built up in the soil. No drainage occurred in either of the other two high water treatments. Similar results have been reported by Wagenet et al. (1980). They showed that increasing salinity resulted in an increased leaching fraction by limiting water use of barley.

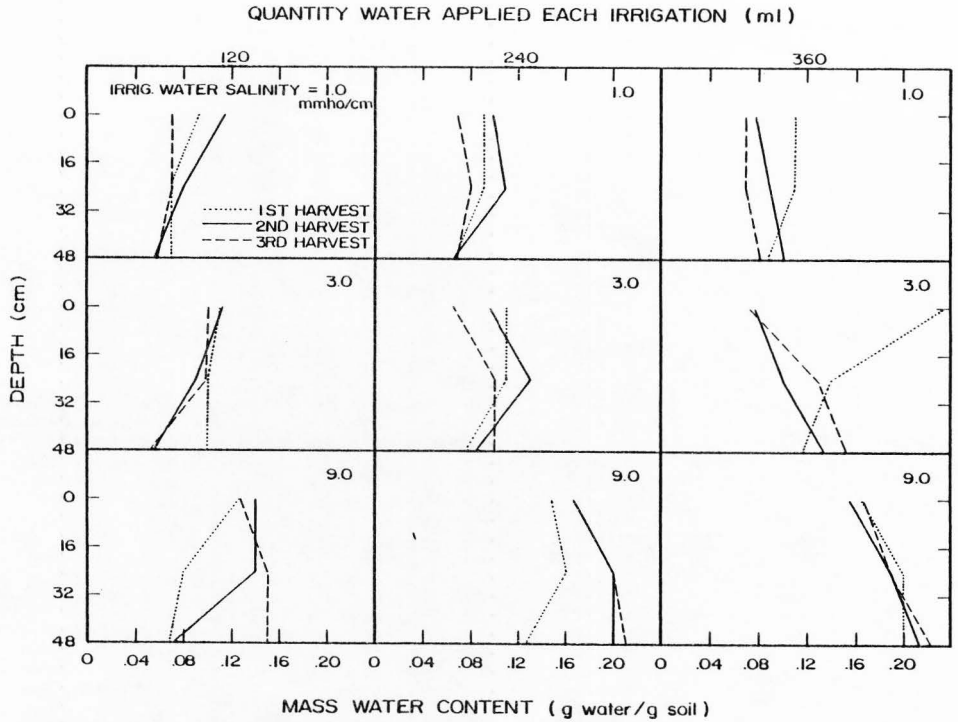


Fig. 15. Soil moisture profiles, at three harvest dates, resulting from salt and water treatments.

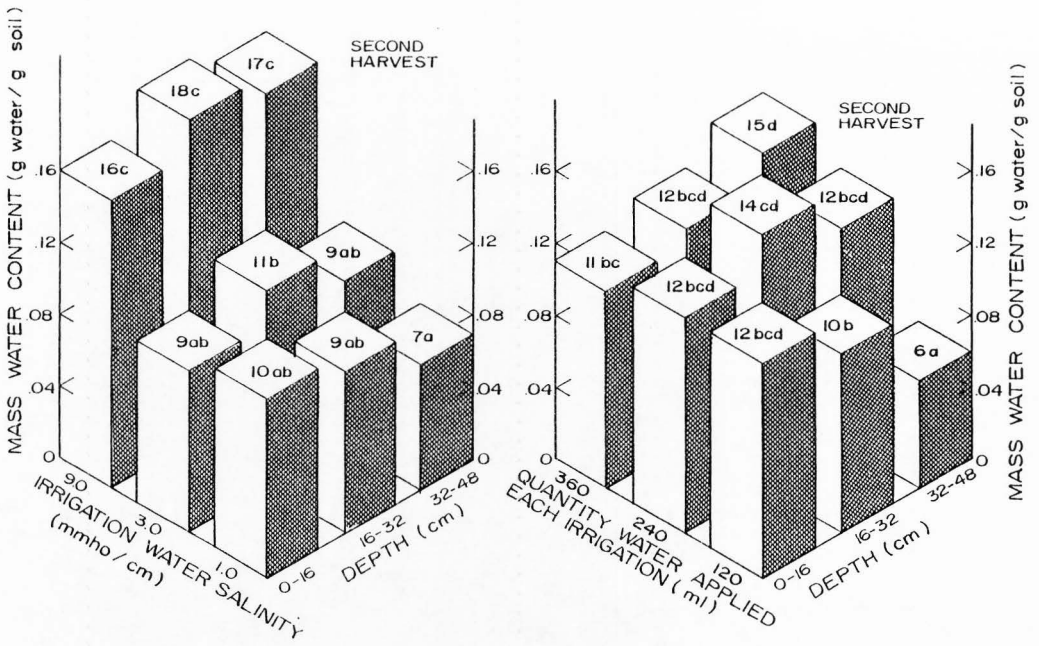


Fig. 16. Comparative effects of irrigation water salinity (mmho/cm) and the quantity of water applied at each irrigation (ml) on the mass water content (g water/g soil) by depth at the second harvest. (Treatment means followed by the same letter within a given graph are not significant at $\alpha = 0.05$.)

Calculated Total Water Potentials

The total water potential (ψ_T) delimits the amount of water available to plants in the soil. Available water has been traditionally defined as the quantity of water held in the soil between -1/3 bar ψ_T (field capacity) and -15 bars ψ_T (permanent wilting point). Water held at tensions above -1/3 bar ψ_T will drain from the soil under the influence of gravity and so be only available for plant uptake for a short period of time immediately after entering the soil. Water held in the soil at tensions below -15 bar ψ_T is considered unavailable since the rate at which it can be obtained by most plants is insufficient to compensate for transpirational losses. Total water potential under non-saturated conditions result from the additive effects of the matric (ψ_m) and the osmotic (ψ_s) potentials (equation 1).

$$\psi_T = \psi_m + \psi_s$$

The matric potential exerts the predominant effect so long as the soil is non-saline. Increasing salinity, however, increases the significance of the osmotic potential.

The analysis of variance for calculated total water potentials (Table 8) indicates that irrigation water salinity, harvest date, and not the quantity of water applied per irrigation were the primary statistically significant experimental factors. Harvest date and salinity were significant at all three depths sampled. Water quantity effects, along with W x S and W x H interactions became significant with depth, but were generally less strongly exhibited than S or H. All possible combinations of factors were significant at the

lowest depth. This is the same pattern observed in the statistical analysis of electrical conductivity. The shape of the calculated total water potential profiles (Fig. 17) illustrates this point by essentially mirroring the shape of soil salinity profiles (Fig. 18). This pattern reflects the increasing significance of the osmotic component on total water potential with increasing salinity.

Plant Moisture Status

Most of the alfalfa plants by the third harvest were surviving under extreme salt-stressed and/or moisture-stressed conditions. The leaves on plants subjected to the highest irrigation water salinity had become noticeable thickened and somewhat waxy to the touch (Fig. 19). Plants under the low- and medium-water treatments had lost many of their leaves and were dying back at the top (Fig. 20). Yet, not a single plant had died as a result of the water and salt treatments. Insufficient applied water at both the low- and medium-water treatments confounded the effects of irrigation water salinity on plant growth from that of water quantity. Increasing salinity did, however cause a significant reduction in plant growth in the high water treatment but in no case did salinity have any affect on survival as noted in earlier studies (Brown and Hayward, 1956). Acetylene reduction capacity, though greatly reduced by the third harvest, was still measurable despite the highly unfavorable soil moisture and salinity conditions. Medicago sativa has exhibited acetylene reducing capacity in the field under severe moisture stress (Johnson and Rumbaugh, 1981), and results of the present study substantiate this tolerance of the alfalfa-Rhizobium symbiosis to environmental stress.

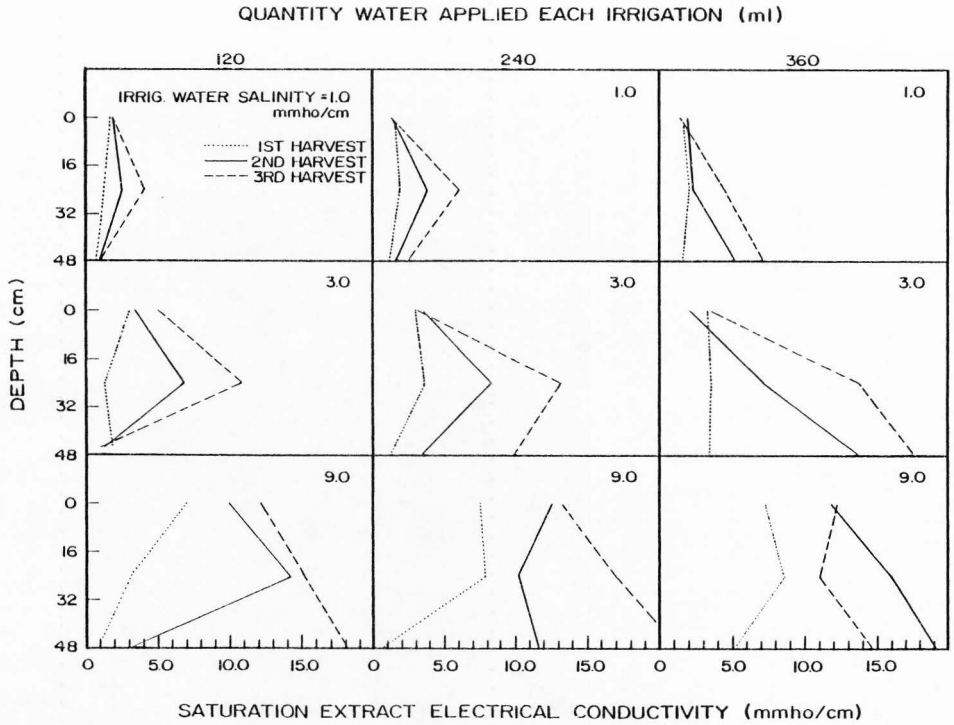


Fig. 17. Soil salinity (mmho/cm) profiles, at three harvest dates, resulting from salt and water treatments.

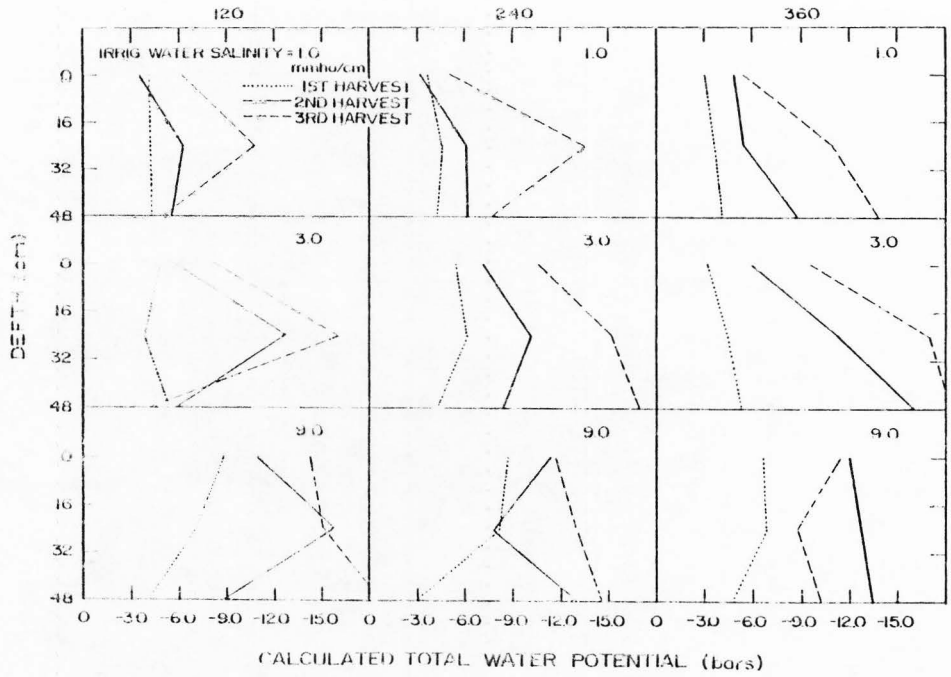


Fig. 16. Calculated total water potentials (bars) by depth, at three harvest dates, resulting from salt and water treatments.

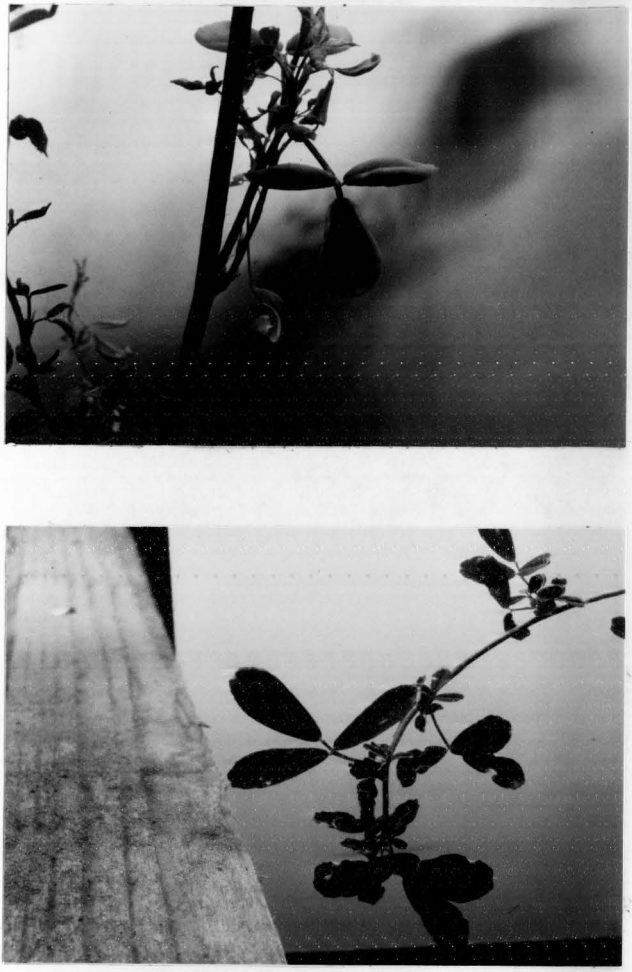


Fig. 19. Comparison between the leaves of salt-affected (a) and non-salt affected (b) alfalfa plants.

Fig. 20. Comparisons of salt and water treatments. (a) Lysimeter #13 - high water-high salt treatment. (b) Lysimeter #6 (middle of photograph) high water-medium salt treatment and Lysimeter #5 medium water-low salt treatment.

SUMMARY

A greenhouse experiment was conducted to study the interactive effects of saline irrigation water and water management on the forage yield and symbiotic nitrogen fixation of Medicago sativa L. var. Resistador. Treatments consisted of a 3 x 3 factorial arrangement of irrigation water salinity (1.0, 3.0, and 9.0 mmho/cm) and the quantity of water applied per irrigation (120, 240, and 360 ml). One alfalfa plant was established in each of 120 lysimeters (8.25 x 54.8 cm) prior to the start of salt and water treatments, initiated 60 days from planting. Twenty-seven lysimeters, three per treatment, were destructively sampled on each of three harvest dates (70, 90, and 110 days from planting) and the following plant parameters were determined: plant tip growth, acetylene reduction rate, root nodule number, root nodule mass and specific nodule activity. Soil samples taken at the time of harvests were analyzed for both mass water content and the electrical conductivity of a saturation extract from which total water potentials were calculated.

Both shoot and root growth were adversely affected by salinity and water stresses. Reduced growth was somewhat more pronounced in the top growth. The response in plant growth to salinity increased with increasing quantity of water applied. Salinity reduced the nitrogen fixing capacity of alfalfa primarily by reducing specific nodule activity. The initiation and growth of root nodules appeared to be unaffected by increasing salinity. Increasing water stress also reduced specific nodule activity while the initiation and growth of

nodules was only limited by extreme moisture stresses. Measured acetylene reduction rates reflected the integrated treatment effects on nodule initiation, nodule growth and specific nodule activity. Continued additions of saline irrigation waters without leaching depressed the osmotic potential of the soil solution which then limited the level to which plants could deplete soil moisture.

CONCLUSIONS

The following conclusions were reached as a result of this study.

1. Alfalfa top growth was limited by both salt and water stresses.

2. Irrigation water salinity had a greater effect on top growth than on root growth.

3. The effect of salinity on both root and top growth was reduced in the presence of limiting moisture.

4. Root distribution was unaffected by either the quantity of applied water or by irrigation water salinity.

5. Increased irrigation water salinity had an adverse effect on specific nodule activity.

6. Specific nodule activity of water stressed alfalfa plants was enhanced by increasing the quantity of water applied at each irrigation.

7. Nodulation and nodule growth were sensitive only to severe moisture stress and appeared to be insensitive to salt stress.

8. Added calcium salt may have had an initially positive effect under the high water treatment on both nodulation and nodule growth.

9. Total acetylene reduction measurements reflected the integrated effects of water and salt treatments on nodulation, nodule growth and specific nodule activity.

10. Irrigation water salinity determined the level to which plants depleted soil moisture during the irrigation cycle.

11. Plant response to soil water conditions was largely a function of plant available water (as determined by total soil water potential) and not the measured soil water content.

12. Alfalfa continued to exhibit acetylene reducing capacity even under severe moisture and salt stress. The species apparently continues to fix nitrogen even though environmental stress is quite substantial.

RECOMMENDATIONS FOR FUTURE STUDY

This study indicated the need for research in the following areas:

1. The applicability of incubating roots and soil together instead of picking roots from the soil.

2. Studies of salt and water stress on symbiotic nitrogen fixation need to be correlated with stages of plant phenology.

3. Further study should investigate the possibility that a large root mass, relative to the volume of the incubation cylinder, could deplete either oxygen or acetylene during the incubation period.

4. The effects of other salt species on the results should be investigated.

5. The present study examined the effects of saline irrigation water on previously nodulated alfalfa. Further work should include the effects of salt treatments initiated prior to nodulation.

6. Field studies investigating the interactive effects of salt and water stress on symbiotic nitrogen fixation in alfalfa are needed to extrapolate greenhouse findings to field conditions.

LITERATURE CITED

Agricultural Statistics, USDA. 1981. U. S. Government Printing Office, Washington, D.C.

Balasubramanian, V., and S. K. Sinha. 1976a. Nodulation and nitrogen fixation in chickpea (Cicer arietinum L.) under salt stress. J. Agric. Sci. 87:465-466.

Balasubramanian, V., and S. K. Sinha. 1976b. Effects of salt stress on growth, nodulation, and fixation in cowpea and mungbeans. Physiol. Plant. 36:197-200.

Bannister, P. 1976. Introduction to physiological plant ecology. John Wiley and Sons Co., New York.

Bauder, J. W., A. Bauer, J. M. Ramirez, and D. K. Cassel. 1978. Alfalfa water use and production on dry land and irrigated sandy loam. Agron J. 70:95-99.

Bergerson, F. J. 1970. The qualitative relationship between nitrogen fixation and the acetylene-reduction assay. Aust. J. Biol. Sci. 23:1015-1025.

Bernstein, L. 1961. Osmotic adjustment of plants to saline media. I. Steady state. Am. J. Bot. 48:909-918.

Bernstein, L. 1964. Effects of salinity on mineral composition and growth of plants. Plant Anal. and Fert. Problems 4:25-45.

Bernstein, L., and L. E. Francois. 1973. Leaching requirement studies: Sensitivity of alfalfa to salinity of irrigation and drainage waters. Soil Sci. Soc. Am. Proc. 37:931-943.

Bernstein, L., and G. Ogata. 1966. Effects of salinity on nodulation, nitrogen fixation, and growth of soybeans and alfalfa. Agron. J. 58:201-203.

Bower, C. A., G. Ogata, and J. M. Tucker. 1969. Root zone salt profiles and alfalfa growth as influenced by irrigation and leaching fraction. Agron. J. 61:783-785.

Brown, J. W., and H. E. Hayward. 1956. Salt tolerance of alfalfa varieties. Agron. J. 48:18-20.

Burris, R. H. 1974. Methodology. 13-33. In A. Quispel (ed.). The biology of nitrogen fixation. North Holland Publishing Co., Amsterdam.

Foulds, W. 1971. Effect of drought on three species of Rhizobium. Plant and Soil 35:665-667.

Foulds, W. 1978. Response to soil moisture supply in three leguminous species. II. Rate of $N_2(C_2H_2)$ fixation. New Phytol. 80:847-855.

Francois, L. E. 1981. Alfalfa management under saline conditions with zero leaching. Agron. J. 73:1042-1045.

Gallacher, A. E., and J. I. Sprent. 1978. The effect of different water regimes on growth and nodule development of greenhouse grown Vicia faba. J. Exp. Bot. 29:413-423.

Hardy, R. W. F., and U. D. Havelka. 1976. Photosynthate as a major factor limiting nitrogen fixation by field grown legumes, with emphasis on soybeans. p. 421-439. In P. S. Nutman (ed.). Symbiotic nitrogen fixation in plants. Cambridge Univ. Press, Cambridge.

Hardy, R. W. F., R. D. Holsten, E. K. Jackson, and R. C. Burns. 1968. The acetylene-ethylene assay for N_2 fixation: Laboratory and field evaluation. Plant Physiol. 43:1185-1207.

Hewitt, J. E. 1963. The essential nutrient elements: Requirements and Interactions in plants. p. 137-360. In F. C. Steward (ed.). Plant physiology, a treatise. Vol. III. Academic Press, New York.

Johnson, D. A., and M. D. Rumbaugh. 1981. Nodulation and acetylene reduction by certain rangeland legume species under field conditions. J. of Range Mgmt. 34:178-181.

Kant, S., and H. S. Narayana. 1978. Effect of water stress on growth, nodulation, and nitrogen fixation in Casuarina equisetifolia. Annals of Arid Zone 17:216-221.

Lahaye, P. A., and E. Epstein. 1971. Calcium and salt toleration by bean plants. Physiol. Plant 25:213-218.

Lauter, D. J., D. N. Munns, and K. L. Clarkin. 1981. Salt response of chickpea as influenced by N supply. Agron. J. 73:961-966.

Loneragan, J. F., and E. J. Dowling. 1958. The interaction of calcium and hydrogen ions in the nodulation of subterranean clover. Aust. J. Agric. Res. 9:464-472.

Lowther, W. L., and J. F. Loneragan. 1968. Calcium and nodulation in subterranean clover (Trifolium subterraneum L.). Plant Physiol. 43:1362-1366.

Maas, E. V., and G. J. Hoffman. 1977. Crop salt tolerance--current assessment. J. of the Irrig. and Drain Div., Proc. Am. Soc. of Civil Eng. 103(IR2):115-134.

Maas, E. V., and R. H. Nieman. 1978. Physiology of plant tolerance to salinity. 277-299. In Crop tolerance to suboptimal land conditions: Proceedings of a symposium. Madison, Wisc.

Mague, T. H., and R. H. Burris. 1972. Reduction of acetylene and nitrogen by field grown soybeans. New Phytol. 71:275-286.

Meiri, A., and J. Shalhevet. 1973. Crop growth under saline conditions. In B. Yaron, E. Danfors, and Y. Vaadia (eds.). Springer-Verlag, New York.

Miles, A. D. 1969. Alfalfa as a range legume. J. Range Mgmt. 22:205-207.

Minchin, F. R., and J. S. Pate. 1975. Effects of water, aeration, and salt regime on nitrogen fixation in a nodulated legume--Definition of an optimum root environment. J. Exp. Bot. 26:69-96.

Moustafa, E., R. Ball, and T. R. O. Field. 1969. The use of acetylene reduction to study the effect of N-fertilization and defoliation on N-fixation by field-grown white clover. N. Zealand J. Agric. Res. 12:691-696.

Pankhurst, C. E., and J. I. Sprent. 1975. Effects of water stress on the respiratory and nitrogen-fixing activity of soybean root nodules. J. Exp. Bot. 26:287-304.

Pearson, G. A. 1960. Tolerance of crops to exchangeable sodium. USDA Inf. Bull. 216.

Pillai, R. N., and A. Sen. 1966. Salt tolerance of Rhizobium trifolium. Indian J. Agric. Sci. 36:80-84.

Sammis, T. W. 1981. Yield of alfalfa and cotton as influenced by irrigation. Agron. J. 73:323-328.

Sprent, J. I. 1969. Prolonged reduction of acetylene by detached soybean notules. Planta 88:372-375.

Sprent, J. I. 1971. Effects of water stress on nitrogen fixation in root nodules. Plant and Soil. Special Volume 225-228.

Sprent, J. I. 1972. The effects of water stress on nitrogen-fixing root nodules. III. Effects of osmotically supplied stress. New Phytol. 71:451-460.

Sprent, J. I. 1973. Growth and nitrogen fixation in Lupinus arboreus as affected by shading and water supply. New Phytol. 72:1005-1022.

Sprent, J. I. 1976. Nitrogen fixation by legumes subjected to water and light stresses. p. 405-420. In P. S. Nutman (ed.). Symbiotic nitrogen fixation in plants. Cambridge Univ. Press, Cambridge.

Subba Rao, N. S., M. Lakshmi-Kumari, C. S. Singh, and S. P. Magu. 1972. Nodulation of lucerne (Medicago sativa L.) under the influence of sodium chloride. Indian J. of Agric. Sci. 42:384-386.

Thompson, T. E., and G. W. Fick. 1980. Growth response of alfalfa to duration of soil flooding and to temperature. Agron. J. 73:329-332.

Tjepkema, J. 1979. Oxygen relations in leguminous and actinorhizal nodules. p. 175-186. In J. C. Gordon, C. T. Wheeler, and D. A. Perry (eds.). Symbiotic nitrogen fixation in the management of temperate forests. Forest Research Lab., Oregon State Univ., Corvallis.

United States Salinity Laboratory. 1954. Diagnosis and improvement of saline and alkali soils. Agric. Handbook No. 60, USDA

Wagenet, R. J., W. F. Campbell, A. M. Bamatraf, and D. L. Turner. 1980. Salinity irrigation and fertilization effects on barley growth. Agron. J. 72:969-974.

Waughman, G. J. 1972. The effect of varying oxygen tension, temperature, and sample size on acetylene reduction by nodules of Alnus and Hippophae. Plant and Soil 37:521-528.

Wheeler, C. T. 1969. The diurnal fluxuation in nitrogen fixation in the nodules of Alnus flutinosa and Myrica gala. New Phytol. 68: 675-682.

Wilson, J. R. 1970. Response to salinity in Glycine VI--Some effects of a range of short-term salt stresses on the growth, nodulation, and N-fixation of Glycine wightii. Aust. J. Agric. Res. 21:571-582.

Wilson, R. J., K. P. Haydock, and M. F. Robins. 1970. The development of time of stress effects in two species of Glycine differing in sensitivity to salt. Aust. J. Biol. Sci. 23:537-551.

Yadav, N. K., and S. R. Vyas. 1971. Response of root-nodule rhizobia to saline, alkaline, and acid conditions. Indian J. Agric. Sci. 41:875-881.

APPENDICES

Appendix A

Mean Comparison Tables for Plant Parameters

Table 9. Means for entire lysimeter of top growth, root growth and acetylene reduction for the three harvests as functions of water and salt treatments*

		Top growth (g/plant)			Root growth (g/plant)			Acetylene reduction (μmol C_2H_2/plant-hr)		
		Harvest			Harvest			Harvest		
		1	2	3	1	2	3	1	2	3
W1	S1	1.87a	2.42ab	2.96ab	1.50a	2.97ab	3.72bc	1285ab	1541bd	238ac
	S2	1.62a	2.39ab	2.61ab	1.41a	2.85ab	3.10bc	1026ab	943abc	286ac
	S3	1.71a	2.30ab	2.69ab	1.58a	3.49bc	3.57bc	320ac	148c	174c
W2	S1	1.73a	3.72c	4.43cd	1.88a	3.83bc	6.26d	1152ab	4191g	45c
	S2	2.07a	3.17c	5.45de	1.46a	4.04bc	6.08d	1267ab	3449fg	236ac
	S3	1.76a	3.25bc	3.57c	1.84a	3.54bc	4.48c	1875bd	1676bd	333ac
W3	S1	2.21a	5.64e	7.77f	2.06a	4.81c	7.48d	3762fg	2281de	193c
	S2	2.08a	6.16e	7.37f	1.99a	4.61c	6.26d	3010ef	2118de	176c
	S3	1.60a	4.29cd	4.66d	1.37a	4.92c	4.43c	6486h	1522bd	1084bc
Water										
	W1	1.73a	2.37ab	2.75b	1.50a	3.11b	3.46b	877ab	878ab	233a
	W2	1.85a	3.56c	4.49e	1.73a	3.80bc	5.60de	1431bc	3105d	205a
	W3	1.97a	5.36d	6.61f	1.81a	4.78cd	6.06e	4419e	1973c	484a
Salt										
	S1	1.94a	3.93c	5.06d	1.81a	3.87b	5.82d	2066d	2671de	159a
	S2	1.92a	4.09c	5.15d	1.62a	3.83b	5.15cd	1768cd	2170d	233a
	S3	1.69a	3.28b	3.64bc	1.59a	3.98b	4.16bc	2894e	1115bc	530ab

*Means of the same parameter followed by the same letter are not significantly different at $\alpha = 0.05$.

Table 10. Means of root dry weight (g) by depth and harvest*.

Factor		Depth 0-16 cm First harvest	0-16 cm Second harvest	0-16 cm Third harvest	16-32 cm First harvest	16-32 cm Second harvest	16-32 cm Third harvest	32-48 cm First harvest	32-48 cm Second harvest	32-48 cm Third harvest
W1	S1	1.03a	1.96abc	2.57bc	0.30a	0.70abc	0.75bc	0.17a	0.32ab	0.40ab
	S2	0.94a	1.82ab	2.06bc	0.36ab	0.74abc	0.73abc	0.11a	0.28ab	0.30ab
	S3	1.05a	1.93abc	2.45bc	0.40ab	1.10cd	0.90bc	0.13a	0.46b	0.30ab
W2	S1	1.20a	2.30bc	3.87de	0.53abc	1.03cd	1.62e	0.15a	0.50bc	0.78c
	S2	0.98a	2.66bc	3.85de	0.31a	0.95cd	1.45de	0.17a	0.43ab	0.78c
	S3	1.30a	2.26bc	2.53bc	0.40abc	0.91cd	1.46de	0.14a	0.37ab	0.49b
W3	S1	1.09a	2.56bc	5.20	0.66abc	1.53de	1.66e	0.30a	0.72c	0.62bc
	S2	1.10a	2.88bc	4.07	0.62abc	1.11cde	1.61e	0.28a	0.62bc	0.58bc
	S3	0.92a	3.01cd	2.79bc	0.28a	1.33cde	1.24cde	0.17a	0.59bc	0.41bc
Water										
W1		1.01a	1.90b	2.36bc	0.35a	0.85b	0.79b	0.14a	0.35b	0.33b
W2		1.15a	2.41bc	3.42de	0.42a	0.96b	1.51c	0.15a	0.43bc	0.68d
W3		1.04a	2.82cd	4.02	0.52a	1.32c	1.50c	0.25a	0.64e	0.54cd
Salt										
S1		1.11a	2.27b	3.88c	0.50a	1.09b	1.35b	0.21a	0.51bc	0.60c
S2		1.01a	2.45b	3.32c	0.43a	0.94b	1.27b	0.19a	0.44bc	0.56bc
S3		1.09a	2.40b	2.59b	0.36a	1.11b	1.19b	0.15a	0.47bc	0.40b

*Means within any depth followed by the same letter are not significantly different at $\alpha = 0.05$.

Table 11. Means of acetylene reduction (μmol C_2H_4/sample/hr) by depth and harvest*

Factor		Depth								
		0-16 cm			16-32 cm			32-48 cm		
		First harvest	Second harvest	Third harvest	First harvest	Second harvest	Third harvest	First harvest	Second harvest	Third harvest
W1	S1	1014abc	1274bcde	198abc	242ac	246abc	40ab	28a	22a	1a
	S2	667abcd	822abcd	214abc	340acd	112ab	66ab	19a	10a	8a
	S3	137ab	83a	162abc	162ab	10a	2a	22a	55ab	11a
W2	S1	382abc	2372ef	16a	543acde	1456fg	29ab	227abc	363c	6a
	S2	655abcd	3008f	206abc	490acd	383abd	22ab	122abc	58ab	9a
	S3	1066abc	1654de	333abc	721bcdef	17a	1a	88ab	5a	1a
W3	S1	1355bcde	1180abcd	21a	1521g	324cdefg	98ab	886d	278bc	74ab
	S2	702abcd	763abcd	143ab	1454fg	1258eg	32ab	854d	96ab	1a
	S3	5353	844abcd	1084abc	931defg	559abcde	1a	202abc	119abc	1a
Water										
W1		606ab	727ab	191a	248abc	123a	36a	23ab	29ab	7a
W2		701ab	2345c	185a	585bcd	619cd	17a	146ab	142ab	5a
W3		2470c	929b	416ab	1302	880d	44a	648	165b	26ab
Salt										
S1		917bc	1609d	78a	769c	841c	56a	381d	221bc	27a
S2		675bc	1531d	188ab	761c	585bc	40a	332cd	55a	6a
S3		2186	860c	526abc	605c	195ab	1a	104ab	60a	4a

*Means within any depth followed by the same letter are not significantly different at $\alpha = 0.05$.

Table 12. Means of nodule dry weight (g) by depth and harvest.*

Factor		Depth							
		Combined		0-16 cm		16-32 cm		32-48 cm	
		First harvest	Second harvest	First harvest	Second harvest	First harvest	Second harvest	First harvest	Second harvest
W1	S1	.028ab	.052bc	.017a	.036abc	.008a	.012a	.002a	.005a
	S2	.023ab	.036ab	.015a	.027ab	.007a	.006a	.001a	.003a
	S3	.022ab	.039ab	.009a	.021ab	.010a	.011a	.002a	.007ab
W2	S1	.018a	.080cde	.005a	.042bc	.009a	.026bc	.004a	.012ab
	S2	.031ab	.089de	.017a	.069d	.010a	.016ab	.003a	.005a
	S3	.047abc	.074cde	.024ab	.059cd	.019	.009a	.004a	.006a
W3	S1	.034ab	.073cde	.012a	.039bc	.014ab	.024bc	.008ab	.011ab
	S2	.025ab	.076cde	.006a	.028ab	.012a	.032c	.007ab	.016b
	S3	.065bcde	.090e	.052cd	.051cd	.011a	.025bc	.002a	.013ab
Water									
W1		.024a	.043b	.014a	.028ab	.009a	.010a	.002a	.005a
W2		.032ab	.081c	.015a	.057c	.013a	.017a	.003a	.008ab
W3		.041ab	.080c	.023a	.039b	.012a	.027	.006a	.013b
Salt									
S1		.027a	.069c	.011a	.039bc	.010a	.021b	.005ab	.009b
S2		.026a	.069c	.013a	.042c	.010a	.018b	.004ab	.008ab
S3		.044b	.068c	.028b	.044c	.013a	.015ab	.003a	.009b

*Means of the same parameter followed by the same letter are not significantly different at $\alpha = 0.05$.

Table 13. Means of nodule number by depth and harvest.*

	Depth							
	Combined		0-16 cm		16-32 cm		32-48 cm	
Factor	First harvest	Second harvest	First harvest	Second harvest	First harvest	Second harvest	First harvest	Second harvest
W1 S1	145a	369cdef	69ab	211cde	43ab	109bcde	33abc	69abcd
S2	130a	165ab	70ab	115abc	51abc	27a	9a	23ab
S3	117a	221abcd	40a	131abc	56abc	54abc	20ab	37abc
W2 S1	144a	431ef	40a	216cde	60abc	123cde	44abc	92cde
S2	181abc	457ef	79ab	298e	74abcd	118cde	28ab	41abc
S3	200abc	315abcde	72ab	235cde	87abcde	51abc	41abc	30ab
W3 S1	174abc	354bcef	59ab	162bcd	62abc	123cde	54abcd	70bcde
S2	135a	410def	35a	153abcd	55abc	153e	45abc	104de
S3	283abcde	531f	218cde	274de	51abc	142de	14ab	115e
Water								
W1	130a	252b	60a	152bc	50a	63a	21a	36a
W2	175ab	401c	64a	250d	74a	97ab	38a	54a
W3	198ab	432c	104ab	196cd	56a	139b	37a	96
Salt								
S1	155a	385b	56a	196b	55a	118b	43ab	70b
S2	149a	344b	61a	189b	60a	99ab	27a	56ab
S3	200a	356b	110a	213b	65a	82ab	25a	60b

*Means within any depth followed by the same letter are not significant at $\alpha = 0.05$.

Table 14. Means of specific nodule activity (μmol C_2H_4/g nodule dry weight-hr) by depth and harvest*

	Depth							
	Combined		0-16 cm		16-32 cm		32-48 cm	
Factor	First harvest	Second harvest	First harvest	Second harvest	First harvest	Second harvest	First harvest	Second harvest
W1 S1	48.5cd	29.3bc	65.3cd	35.3abc	30.7abc	20.7ab	21.1abc	3.9a
S2	38.4cd	22.7abc	37.3ab	31.0abc	57.3bc	12.3a	11.0ab	1.4a
S3	14.3ab	3.2a	17.a	3.8a	15.0ab	0.7a	10.1ab	5.9a
W2 S1	60.1d	54.9cd	84.8de	73.9cd	53.3bc	51.6bc	54.3c	24.0abc
S2	44.9cd	38.5cd	42.8b	45.2bc	51.8bc	23.7ab	36.5bc	9.2ab
S3	45.3ab	22.7ab	55.2cd	28.3ab	36.7abc	1.9a	20.6abc	0.8a
W3 S1	113.5e	29.2bc	104.3e	28.2ab	115.2d	31.5abc	119.1d	22.5a
S2	119.9e	27.5bc	120.1e	29.5ab	124.1d	36.4abc	123.7d	12.9a
S3	101.5e	15.4ab	110.5e	16.8ab	63.3c	16.5ab	46.9c	6.6a
Water								
W1	33.7b	18.4a	40.1ab	23.4a	34.3b	11.2a	14.1a	3.7a
W2	50.1c	38.7bc	60.3b	49.1b	47.2b	25.7ab	37.1	11.3a
W3	116.d	24.0ab	111.6	24.9a	100.9	28.1ab	96.6	14.0a
Salt								
S1	74.0a	37.8c	84.2d	45.8bc	66.4c	34.6b	64.8c	16.8ab
S2	67.7a	29.6c	66.7cd	35.3ab	77.7c	24.1ab	57.1c	7.8a
S3	53.7b	13.8d	61.1c	16.3a	38.3b	6.3a	25.9b	4.4a

*Means within any depth followed by the same letter are not significantly different at $\alpha = 0.05$.

Appendix B

Data Files

Table 15. Data file for the entire lysimeter.

WSH	Top growth	Root growth	Ace reduction	Nodule dry wt	Nodule number	SNA
231	2.08	2.47	2575	0.065	364	039.6
211	1.46	1.85	1580	0.022	166	071.8
221	2.43	1.09	1670	0.046	194	036.3
121	1.05	0.74	1310	0.025	093	052.4
331	1.04	0.57	6577	0.068	246	096.7
111	1.89	1.75	1072	0.019	097	056.4
181	2.15	1.76	0505	0.032	149	015.8
111	1.73	1.56	0825	0.035	214	023.8
211	1.88	1.70	0348	0.007	088	049.7
131	1.85	1.53	0298	0.020	104	014.9
121	1.96	1.91	1600	0.032	155	050.0
321	2.04	2.48	2912	0.028	155	104.0
311	2.48	2.02	4278	0.039	153	109.7
331	1.46	2.55	5462	0.049	183	111.5
311	2.27	2.33	3985	0.038	182	104.9
231	1.90	2.29	1886	0.026	117	072.5
211	1.84	2.08	1528	0.026	179	058.8
131	1.12	1.46	0158	0.013	097	012.2
321	2.09	1.88	3888	0.027	109	144.0
311	1.89	1.82	3023	0.024	188	126.0
321	2.12	1.62	2231	0.020	142	111.6
221	1.94	1.82	0873	0.027	250	032.3
231	1.31	0.75	1163	0.049	119	023.7
121	1.85	1.57	0167	0.013	141	012.8
111	2.00	1.19	1957	0.030	124	065.2
331	2.30	0.99	7419	0.077	420	096.4
221	1.84	1.48	1258	0.019	099	066.2
322	6.22	5.71	2797	0.083	352	033.7
112	2.53	3.67	1277	0.045	213	028.4
122	2.43	3.57	2533	0.044	213	057.6
332	4.48	5.69	0280	0.073	325	003.8
312	6.56	4.66	2334	0.084	443	027.8
212	4.74	3.84	5930	0.118	619	050.3
332	4.86	6.45	2903	0.123	877	023.6
212	3.33	2.46	3261	0.076	473	042.9
232	2.29	3.03	0974	0.038	207	025.6
232	3.48	4.25	3113	0.100	384	031.1
222	3.95	3.52	4410	0.115	587	038.3
322	5.79	4.63	1574	0.068	355	023.1
112	2.25	2.03	2033	0.057	463	035.7
332	3.53	2.62	1382	0.074	392	018.7
212	3.10	4.20	3382	0.047	202	071.6
132	2.54	3.76	0257	0.054	316	004.8
312	5.32	5.77	3672	0.084	337	043.7

Table 15. Continued

WSH	Top growth	Root growth	Ace reduction	Nodule dry wt	Nodule number	SNA
222	3.54	4.13	2054	0.075	470	027.4
222	3.65	4.46	3883	0.078	315	049.8
322	6.48	3.49	1983	0.077	522	025.8
112	2.49	3.22	1313	0.055	430	023.9
312	5.04	4.01	0837	0.052	283	016.1
122	2.90	2.60	0169	0.046	125	003.7
232	3.98	3.35	0940	0.083	354	011.3
132	1.94	2.86	0017	0.023	171	000.7
132	2.41	3.85	0171	0.041	177	004.2
122	1.83	2.39	0128	0.019	158	006.7
223	6.39	7.55	0.95			
213	5.62	5.37	0094			
233	4.25	3.46	0565			
213	4.15	6.69	0013			
323	6.85	6.83	0111			
123	3.23	4.45	0402			
333	6.76	6.09	0875			
313	6.92	6.88	0343			
313	7.51	9.11	0128			
133	2.78	3.37	0340			
113	3.03	1.71	0582			
333	3.08	2.33	2077			
233	3.27	4.45	0206			
223	4.27	6.20	0429			
233	3.19	5.53	0229			
313	8.88	6.46	0108			
213	3.53	6.73	0029			
323	8.03	7.83	0130			
133	2.48	4.09	0089			
223	5.70	4.49	0085			
113	2.65	4.50	0048			
113	3.21	4.96	0084			
133	2.81	3.25	0092			
333	4.14	4.88	0301			
123	2.55	3.18	0239			
123	2.05	1.66	0218			
323	7.30	4.13	0286			

Table 16. Data file for 0-16 cm sampling depth

WSH	Mass water	EC_e	Water potential	Root dry wt	Ace reduction	Nodule dry wt	Nodule number	SNA
231	0.109	07.54	11.06	1.71	0814	0.0157	073	051.8
211	0.093	01.52	03.81	1.31	0754	0.0092	064	082.0
221	0.084	03.14	07.03	0.83	1475	0.0416	150	035.5
121	0.135	03.93	04.94	0.54	1132	0.0232	080	048.8
331	0.225	09.64	06.36	0.54	6574	0.0655	227	100.4
111	0.095	01.45	03.60	1.06	0875	0.0090	043	097.3
131	0.110	06.77	09.98	1.17	0273	0.0169	071	016.2
111	0.083	01.72	04.68	1.18	0577	0.0200	082	028.9
211	0.089	01.54	04.03	1.05	0161	0.0016	021	100.5
131	0.127	06.38	08.08	1.08	0079	0.0073	034	010.8
121	0.096	03.28	06.31	1.21	0793	0.0156	062	050.8
321	0.136	03.64	04.59	1.30	0910	0.0108	053	084.3
311	0.116	01.64	03.05	1.14	1366	0.0122	043	112.0
331	0.144	06.39	07.05	1.58	3050	0.0226	068	134.9
311	0.107	01.63	03.36	1.21	2124	0.0177	079	120.0
231	0.117	06.51	09.01	1.50	1312	0.0150	047	087.4
211	0.101	01.68	03.67	1.23	0231	0.0035	035	066.0
131	0.133	06.03	07.31	0.89	0060	0.0023	015	026.1
321	0.382	02.62	01.01	1.13	0766	0.0048	020	159.6
311	0.119	01.68	03.00	0.93	0575	0.0071	054	081.0
321	0.135	03.67	04.66	0.87	0430	0.0037	033	116.3
221	0.124	02.89	04.25	1.26	0323	0.0068	052	047.6
231	0.210	08.38	05.96	0.69	1072	0.0405	097	026.5
121	0.108	01.16	02.69	1.07	0077	0.0062	068	012.4
111	0.097	01.70	03.88	0.86	1591	0.0228	082	069.8
331	0.150	06.38	06.73	0.65	6436	0.0670	360	096.1
221	0.107	03.00	05.20	0.85	0167	0.0037	035	045.2
322	0.081	02.73	06.60	4.01	0563	0.0141	072	040.0
112	0.100	02.18	04.44	2.10	0788	0.0225	104	035.0
122	0.100	04.05	07.13	2.24	2173	0.0268	109	081.1
332	0.129	15.50	18.12	3.44	0201	0.0534	202	003.8
312	0.081	01.80	04.95	2.57	0898	0.0416	192	021.6
212	0.101	01.65	03.63	2.45	3611	0.0704	327	051.3
332	0.174	11.06	09.56	3.87	1217	0.0573	403	021.2
212	0.095	01.71	04.00	2.10	1943	0.0429	256	045.3
232	0.202	11.63	08.55	2.06	0961	0.0275	134	035.0
232	0.150	17.40	17.32	2.81	3091	0.0880	295	035.1
222	0.100	03.04	05.68	2.49	3488	0.0878	320	039.7
322	0.079	02.95	07.18	2.69	1024	0.0412	164	024.9
112	0.087	01.94	04.80	1.50	1900	0.0482	293	039.4
332	0.189	10.72	08.50	1.71	1114	0.0436	218	025.5
212	0.111	01.61	03.18	2.35	1563	0.0125	066	125.0
132	0.135	09.61	10.99	2.22	0154	0.0290	184	005.3
312	0.083	01.85	04.90	3.01	2201	0.0546	207	040.3

Table 16. Continued

WSH	Mass water	EC_e	Water potential	Root dry wt	Ace reduction	Nodule dry wt	Nodule number	SNA
222	0.089	06.25	11.64	2.83	1877	0.0659	377	028.5
222	0.105	02.99	05.30	2.65	3660	0.0542	198	067.5
322	0.080	02.27	05.86	1.94	0703	0.0298	222	023.6
112	0.136	01.85	02.70	2.27	1134	0.0360	236	031.5
312	0.081	01.95	05.22	2.11	0440	0.0193	087	022.8
122	0.104	03.81	06.50	1.82	0169	0.0380	100	004.5
232	0.170	07.71	06.96	1.91	0909	0.0616	275	014.8
132	0.140	12.18	13.23	1.55	0016	0.0190	136	000.8
132	0.136	09.37	10.66	2.02	0081	0.0156	072	005.2
122	0.112	03.93	06.13	1.41	0124	0.0165	136	007.5
223	0.072	04.87	11.76	4.31	0124			
213	0.066	02.05	06.68	3.49	0036			
233	0.161	15.80	14.61	2.03	0565			
213	0.066	02.00	06.57	4.33	0003			
323	0.070	04.18	10.67	3.87	0063			
123	0.078	04.19	09.57	2.53	0402			
333	0.152	17.10	16.77	3.81	0874			
313	0.063	01.77	06.36	4.43	0031			
313	0.066	01.68	05.88	6.58	0007			
133	0.119	11.66	15.07	2.21	0309			
113	0.070	02.41	07.03	1.41	0555			
333	0.164	09.11	08.48	1.53	2078			
233	0.166	11.90	10.79	2.50	0205			
223	0.074	04.43	10.58	4.25	0411			
233	0.195	13.40	10.18	3.07	0229			
313	0.066	01.95	06.47	4.58	0025			
213	0.072	01.55	05.12	3.78	0008			
323	0.074	04.61	10.93	5.76	0086			
133	0.154	13.90	13.57	2.79	0087			
223	0.069	04.36	11.21	2.98	0084			
113	0.071	02.41	06.93	2.90	0026			
113	0.064	01.88	06.51	3.40	0014			
133	0.127	12.20	14.68	2.35	0089			
333	0.184	09.90	08.08	3.02	0301			
123	0.099	06.40	10.63	2.25	0239			
123	0.137	06.77	07.84	1.40	0001			
323	0.090	05.54	10.37	2.57	0281			

Table 17. Data file for 16-32 cm sampling depth

WSH	Mass water	EC_e	Water potential	Root dry wt	Ace reduction	Nodule dry wt	Nodule number	SNA
231	0.123	08.09	10.40	0.624	1589	0.0414	206	038.4
211	0.087	01.69	04.38	0.413	0724	0.0106	068	068.3
221	0.090	03.15	06.55	0.125	0177	0.0040	040	044.3
121	0.147	01.22	01.82	0.087	0179	0.0017	013	105.3
331	0.256	09.34	05.36	0.023	0003	0.0025	019	001.2
111	0.070	01.13	04.40	0.483	0161	0.0081	040	019.9
131	0.073	02.71	07.33	0.446	0202	0.0112	053	018.1
111	0.065	01.12	04.73	0.253	0227	0.0104	056	021.8
211	0.087	01.88	04.70	0.506	0145	0.0040	046	036.2
131	0.083	03.26	07.35	0.319	0206	0.0118	060	017.5
121	0.071	01.37	04.82	0.587	0751	0.0148	078	050.8
321	0.138	03.55	04.42	0.770	1318	0.0100	057	131.8
311	0.109	01.95	03.70	0.638	2038	0.0202	073	100.9
331	0.155	08.27	08.23	0.605	1815	0.0204	078	089.0
311	0.106	02.04	03.95	0.832	0887	0.0094	033	094.4
231	0.123	07.91	10.16	0.552	0482	0.0080	033	060.3
211	0.092	02.08	04.73	0.670	0760	0.0137	067	055.4
131	0.093	03.43	06.76	0.432	0077	0.0083	056	009.3
321	0.132	03.55	04.66	0.543	2001	0.0139	039	144.0
311	0.120	02.13	03.50	0.514	1638	0.0109	080	150.3
321	0.153	03.44	04.41	0.535	1043	0.0108	075	096.6
221	0.132	03.92	05.06	0.386	0548	0.0173	148	031.7
231	0.241	06.49	04.02	0.040	0091	0.0080	021	011.3
121	0.072	01.47	04.96	0.402	0090	0.0057	061	015.8
111	0.089	01.24	03.55	0.163	0337	0.0067	034	050.3
331	0.177	08.28	07.12	0.203	0976	0.0098	057	099.6
221	0.102	03.77	06.58	0.430	0746	0.0094	033	079.3
322	0.107	07.29	10.97	1.212	2203	0.0426	137	051.7
112	0.069	02.57	07.47	1.055	0436	0.0130	053	033.5
122	0.080	06.40	13.30	1.017	0332	0.0097	042	034.3
332	0.161	18.50	17.05	1.651	0071	0.0162	086	004.4
312	0.085	02.38	05.68	1.499	0820	0.0254	155	032.3
212	0.116	04.22	06.25	0.974	2264	0.0374	183	060.5
332	0.198	15.30	11.41	1.764	1395	0.0410	261	034.0
212	0.099	03.60	06.55	0.866	0451	0.0134	100	033.7
232	0.226	08.73	05.74	0.627	0007	0.0046	030	001.4
232	0.176	08.46	07.31	1.098	0020	0.0094	070	002.2
222	0.127	08.22	10.17	0.653	0757	0.0199	205	038.0
322	0.095	07.80	13.23	1.203	0491	0.0250	160	019.7
112	0.064	02.06	06.92	0.350	0128	0.0073	127	017.5
332	0.216	14.30	09.73	0.570	0211	0.0190	079	011.1
212	0.111	03.31	05.38	1.258	1653	0.0273	086	060.5
132	0.130	15.50	18.00	1.136	0005	0.0144	066	000.3

Table 17. Continued

WSH	Mass water	EC_e	Water potential	Root dry wt	Ace reduction	Nodule dry wt	Nodule number	SNA
312	0.089	02.25	05.18	1.893	1441	0.0280	104	051.5
222	0.126	07.42	09.33	0.920	0170	0.0080	074	021.2
222	0.129	09.00	10.87	1.286	0223	0.0188	076	011.8
322	0.102	06.21	10.02	0.914	1081	0.0287	161	037.7
112	0.116	03.12	04.89	0.698	0173	0.0158	146	011.0
312	0.087	02.47	05.68	1.198	0211	0.0198	109	010.6
122	0.090	07.33	13.23	0.534	0001	0.0076	022	000.1
232	0.190	13.50	10.57	0.999	0023	0.0116	052	002.0
132	0.138	12.68	13.95	0.868	0001	0.0030	032	000.1
132	0.141	14.50	15.51	1.292	0024	0.0150	064	001.6
122	0.191	06.55	11.87	0.682	0004	0.0016	018	002.6
223	0.109	15.90	10.52	2.280	0046			
213	0.075	06.99	15.33	1.320	0063			
233	0.181	18.80	15.32	1.020	0001			
213	0.070	05.82	14.04	1.680	0010			
323	0.106	13.10	18.96	2.120	0046			
123	0.111	10.52	14.73	1.300	0001			
333	0.172	16.70	12.75	1.760	0001			
313	0.071	05.75	13.70	1.660	0091			
313	0.071	04.17	10.50	1.761	0121			
133	0.130	14.00	16.33	0.900	0001			
113	0.067	03.36	09.40	0.250	0027			
333	0.189	08.32	06.67	0.660	0001			
233	0.178	15.40	12.86	1.480	0001			
223	0.100	09.34	14.75	1.320	0018			
233	0.227	16.90	10.88	1.870	0001			
313	0.079	03.79	08.71	1.570	0083			
213	0.080	05.21	11.15	1.860	0013			
323	0.116	13.10	17.28	1.670	0044			
133	0.173	15.10	12.97	1.100	0002			
223	0.105	14.10	20.54	0.760	0002			
113	0.068	04.52	11.71	1.070	0022			
113	0.071	04.50	11.17	0.940	0070			
133	0.152	16.60	16.29	0.670	0003			
333	0.214	10.04	06.98	1.290	0001			
123	0.106	13.30	19.23	0.720	0001			
123	0.096	08.70	14.44	0.180	0195			
323	0.154	15.23	14.81	0.050	0005			

Table 18. Data file for 32-48 cm sampling depth

WSH	Mass water	EC_e	Water potential	Root dry wt	Ace reduction	Nodule dry wt	Nodule number	SNA
231	0.070	01.30	04.75	0.144	0172	0.0082	085	021.0
211	0.073	01.28	04.51	0.128	0101	0.0018	034	056.3
221	0.080	01.25	04.03	0.136	0018	0.0004	004	045.0
121	0.154	01.05	01.54	0.116	0001	0.0001	001	000.1
331	0.268	02.90	01.65	0.006	0001	0.0001	001	000.1
111	0.067	01.08	04.50	0.216	0035	0.0020	014	017.4
131	0.066	01.06	04.52	0.142	0030	0.0038	025	007.9
111	0.062	01.03	04.75	0.126	0020	0.0042	076	004.9
211	0.075	01.15	04.13	0.141	0043	0.0010	021	042.6
131	0.069	01.13	04.47	0.125	0013	0.0009	010	014.0
121	0.066	03.26	09.32	0.113	0056	0.0017	015	032.8
321	0.104	03.23	05.70	0.412	0685	0.0071	050	096.4
311	0.088	01.76	04.45	0.247	0874	0.0068	037	128.6
331	0.142	07.23	08.01	0.366	0597	0.0057	037	104.7
311	0.085	01.55	04.27	0.292	0974	0.0104	070	093.6
231	0.076	01.74	05.18	0.247	0092	0.0032	037	028.7
211	0.075	01.27	04.36	0.182	0538	0.0084	077	064.0
131	0.070	01.16	04.46	0.132	0022	0.0026	026	008.3
321	0.106	03.16	05.47	0.215	1121	0.0082	050	136.7
311	0.101	01.71	03.72	0.371	0811	0.0060	054	135.1
321	0.142	03.86	04.59	0.219	0758	0.0055	034	137.9
221	0.075	01.38	04.57	0.175	0002	0.0026	050	000.7
231	0.253	01.46	00.95	0.019	0001	0.0001	001	012.0
121	0.067	01.18	04.71	0.098	0001	0.0008	012	000.1
111	0.084	01.14	03.62	0.170	0029	0.0007	008	041.1
331	0.181	06.56	05.58	0.142	0007	0.0002	003	036.0
221	0.079	01.63	04.77	0.203	03.45	0.0054	031	063.9
322	0.134	13.00	14.73	0.486	0031	0.0266	143	001.2
112	0.056	01.24	05.78	0.511	0054	0.0092	056	005.9
122	0.053	01.83	07.68	0.322	0028	0.0073	062	003.9
332	0.172	19.40	16.66	0.603	0008	0.0030	037	002.6
312	0.087	04.95	09.79	0.591	0616	0.0173	096	035.6
212	0.061	01.66	06.31	0.409	0056	0.0100	109	005.6
332	0.219	19.70	13.18	0.817	0292	0.0243	213	012.0
212	0.075	01.75	05.28	0.498	0867	0.0200	117	043.4
232	0.244	13.80	08.28	0.341	0006	0.0056	043	001.1
232	0.172	14.63	12.69	0.338	0001	0.0024	019	000.5
222	0.077	04.30	09.90	0.373	0.65	0.0072	062	022.9
322	0.126	12.52	15.15	0.738	0059	0.0022	031	026.7
112	0.056	01.02	05.21	0.184	0006	0.0015	043	004.0
332	0.250	17.10	09.97	0.341	0058	0.0111	095	005.2
212	0.067	02.21	06.93	0.593	0166	0.0072	050	023.0
132	0.061	01.87	06.81	0.408	0098	0.0104	066	009.5

Table 18. Continued

WSH	Mass water	EC_e	Water potential	Root dry wt	Ace reduction	Nodule dry wt	Nodule number	SNA
312	0.109	04.79	07.46	0.868	0031	0.0018	076	017.0
222	0.101	02.45	04.77	0.378	0007	0.0014	019	001.7
222	0.082	05.29	11.01	0.524	0001	0.0052	041	000.1
322	0.125	13.53	16.46	0.641	0199	0.0182	139	010.9
112	0.056	01.22	05.72	0.258	0005	0.0030	048	001.8
312	0.099	04.51	07.88	0.706	0187	0.0126	087	014.8
122	0.054	01.17	05.79	0.239	0001	0.0001	003	000.1
232	0.195	21.00	15.79	0.440	0008	0.0093	027	000.8
132	0.078	02.90	07.19	0.442	0001	0.0006	003	002.0
132	0.095	08.95	14.97	0.540	0066	0.0105	041	006.3
122	0.058	01.64	06.58	0.291	0001	0.0006	004	000.1
223	0.079	07.09	14.72	0.960	0025			
213	0.063	02.02	06.94	0.560	0009			
233	0.196	19.70	14.76	0.410	0001			
213	0.065	03.36	09.69	0.680	0001			
323	0.130	19.40	22.32	0.840	0002			
123	0.063	01.43	05.59	0.620	0001			
333	0.188	18.40	14.44	0.520	0001			
313	0.073	06.28	14.37	0.790	0221			
313	0.075	05.33	12.15	0.770	0001			
133	0.125	16.00	19.31	0.460	0031			
113	0.066	01.21	04.85	0.050	0001			
333	0.211	10.66	07.52	0.140	0001			
233	0.197	19.90	14.83	0.470	0001			
223	0.111	11.70	16.26	0.630	0001			
233	0.244	22.60	13.48	0.590	0001			
313	0.104	09.02	13.72	0.310	0001			
213	0.071	03.31	08.75	1.090	0008			
323	0.136	16.20	17.88	0.400	0001			
133	0.174	19.00	16.14	0.200	0001			
223	0.100	12.30	19.01	0.750	0001			
113	0.053	01.73	07.41	0.530	0001			
113	0.061	01.12	05.04	0.620	0001			
133	0.143	18.40	19.21	0.230	0001			
333	0.246	13.70	08.17	0.570	0001			
123	0.054	01.29	06.11	0.210	0001			
123	0.058	01.02	05.04	0.080	0022			
323	0.170	16.00	13.97	0.510	0001			

Appendix C

Control Statements for Rummage Program

Example of control statements used to run the Rummage program.

```
 100   NOTE TOTALS DATA
 200   MODEL Y=W+S+H+WS+WH+SH+SWH+E
 300   FIXED W C1 3 'WATER'
 400   FIXED S C2 3 'SALT'
 500   FIXED H C3 2 'HARV'
 550   LABEL C4 'MASSW' C5'EC' C6'WAPOT' C7'ROOT' C8'TOFIX' C9'NODMS' C10'NOD#' C11'EFFI'
 600   NO PLOT
 700   LAST
 800   ESTIMATE W (L)
 900   ESTI S (L)
1000   ESTI H
1100   ESTI WS (L)
1200   ESTI SH (L)
1300   ESTI WH (L)
1400   ESTI WSH (L)
1500   TAPE 25
1600   DEPTH.DAT
1700   DEPENDENT VARIABLES ARE C4-C11
1800   FREAD C1-C11
1850   (3F1.0.X.F3.3.X.F4.2.X.F3.2.X.F4.0.X.F.4.X.F3.0.X.F4.1)
2000   STOP
```

Appendix D

Sample Calculation of LSD Values

Sample calculation of LSD values used by the Rummage Program to compare treatment means:

Formula used for equal sample sizes:

$$LSD = t_{dfE}^{\alpha/2}\sqrt{\frac{2\ MSE}{n}}$$

't' = 't' test statistic

α = significance level (alpha)

dfE = degrees of freedom for the mean square error

MSE = mean square error from ANOVA table

n = sample size

Example: LSD calculation for comparing treatment means of alfalfa top growth

MSE = 0.47 with 45 degrees of freedom

n = 3 for any W x S x H

From cumulative "t" distribution table

$$t_{54}^{0.05/2} = 2.004$$

$$LSD = 2.004\sqrt{\frac{2*0.47}{3}}$$

$$LSD = 1.12$$

Therefore, the top growth means of any two W x S x H combinations are significantly different at the $\alpha = 0.05$ level only if they differ by more than 1.12.

Appendix E

Calculation of Salt Added to Irrigation Waters

Sample calculation of calcium chloride salt needed to increase the salinity of tap water to the desired electrical conductivity:

Example:

Desired $EC_{Irr\ water}$ = 9.0 mmho/cm

$EC_{Tap\ water}$ = 0.27 mmho/cm a5 25°C

Salt added (meq/liter) χ10 (Desired EC_{IW} - $EC_{Tap\ water}$)

Salt added (meq/liter) = 10 (9.0 - 0.27) ≃ 87.3 meq/liter

Equivalent weight of calcium chloride = 55.49 mg/meq

Salt added (g/gal) = 87.3 meq/liter * 55.49 mg/meq *

1 g/1000 mg * 3.785 liter/gallon

= 18.34 g CaCl/gal of tap water

These calculations were used to approximate the amount of calcium chloride saltneeded. Additional salt was added to obtain the exact EC_{IW} levels as measured by an electrical conductivity meter.

Appendix F

Calibration of the Gas Chromatograph

Calibration of the gas chromatograph:

Standard gas used = 100 ppm C_2H_4

Major component = Nitrogen

Formula weights = C_2H_4 = 28

N_2 = 28

100 ppm C_2H_4 = 100 g C_2H_4/1,000,000 g Gas (N_2)

100 g C_2H_4 * 1 mole/28 g = 3.57 moles C_2H_4

1,000,000 g N_2 * 1 mole/28 g * 22.4 liter/mole = 800,000 liters

100 ppm C_2H_4 = 4.46×10^{-6} moles/liter = 4.46×10^{-6} µmoles/µL

Thus, a 100 µliter standard injection contains 4.46×10^{-4} µmoles C_2H_4 and the conversion factor for calibration equals:

CF = 4.46×10^{-4}/Average area for Standard Injection

Example Calibration:

Area = 29,349 for 100 ppm Ethylene std.

CF = 4.46×10^{-4}/29,349

CF = 1.5196×10^{-8}

Area readings for subsequent samples are then multiplied by the conversion factor to obtain acetylene reduction rates in µmol/C_2H_4/100 µL/hr.