An Abstract of the Thesis of

Isabella Cardona Cantrell for the degree of Master of Science in Botany and Plant Pathology presented on January 7, 2000. Title: Effects of Preinoculation with VAM Fungi Isolated from Different Sites on Plant Tolerance to Salinity in Soils Amended with Sodium Chloride.

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Abstract approved: _____

Robert G. Linderman

The hypothesis that inoculation of transplants with vesicular-arbuscular mycorrhizal (VAM) fungi before planting into saline soils would alleviate salt effects on growth and productivity was tested on lettuce (*Lactuca sativa* L.) and onion (*Allium cepa* L.). A secondary hypothesis was that the fungi isolated from a saline soil would be more effective than those from a nonsaline soil. VAM inocula from a high- and a low-salt soil were trap-cultured, their propagules quantified, adjusted, and added to a pasteurized growth medium in which seeds germinated and seedlings grew for a few weeks. These seedlings, once colonized by VAM fungi, were transplanted into saline soil. Seedlings were exposed to high concentrations of NaCl at the time of transplant; in this respect, our technique aimed to simulate conditions of high salinity prevalent in soils affected by NaCl. Preinoculated lettuce and onion transplants grown for 10 weeks had increased shoot biomass compared with nonVAM plants at all salinity (NaCl) levels tested. Leaves of VAM lettuce at the highest salt level were significantly greener than those of the non-VAM lettuce. NonVAM onions were stunted due to available P deficiency in the soil, but inoculation with VAM fungi alleviated P deficiency and salinity effects except at the highest salinity level; nevertheless, VAM onions were significantly larger at all salinity levels. Increasing the level of available P by weekly applications to nonVAM plants

partially alleviated the salinity effects on onion growth. VAM fungi from the saline soil site were not more effective in ameliorating the reduction on plant growth caused by salt than those from the nonsaline site. Colonization of roots and length of soil hyphae produced by the test fungi decreased with increasing salt. Results indicate that preinoculation of transplants with VAM fungi can effectively alleviate deleterious effects of saline soils on crop productivity.

Effects of Preinoculation with VAM Fungi Isolated from Different Sites on Plant Tolerance to Salinity in Soils Amended with Sodium Chloride

by

Isabella Cardona Cantrell

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented January 7, 2000
Commencement June 2000

Master of Science thesis of Isabella Cardona Cantrell presented on January 7, 2000.

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Isabella Cardona Cantrell, Author

Acknowledgements

I would like to thank Dr. Robert G. Linderman, my major professor, whose experience, skillful intelligence, and generosity made possible my research. Being the busy man he is, he made himself available to talk to me, to listen to me, to read and correct multiple drafts of this thesis, and to encourage me when I needed it the most.

My gratitude to Dr. Gabor Bethlenfalvay for awaking my interest and motivating my work in the world of mycorrhiza and for his suggestions to improve the manuscript of this thesis.

My gratitude to Dr. Paul Schreiner who patiently instructed me in many techniques used in my research and whose encouragement and friendship helped me through difficult times.

My gratitude to Dr. Joseph Marlow for his willingness to always help, his humor, and his assistance in complicated calculations.

My gratitude to Keiko Mihara who helped me finding materials and instruments and whose conversation and music made of my time at the microscope and laboratory a more enjoyable task.

My gratitude to Joyce Spain who patiently answered my questions, and led me through laboratory techniques.

My deepest gratitude to Ted Mackey, my fiancé, for his understanding and playful demeanor. Thank you for supporting my efforts, believing in me, and encouraging me every step of the way.

Thank you to Antonieta, my mother, who encouraged me to pursue an education in the USA while bearing my absence for all these years.

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Dedication

I want to dedicate this thesis to my grandmother, Josefina Alvarez Avila, who as a young girl was not taught how to read and write and later in life, she became head and support of her household. Her intelligence, love, and wisdom embraced me as long as she was alive...

Effects of Preinoculation with VAM Fungi Isolated from Different Sites on Plant Tolerance to Salinity in Soils Amended with Sodium Chloride

CHAPTER ONE.
INTRODUCTION AND LITERATURE REVIEW

Challenges and dilemmas

The purpose of agriculture is to provide food and fiber for human needs. The consumption of food and fiber increases with increasing population. According to the Bureau of the Census, world population is projected to reach 7.6 billion persons by the year 2020 (McDevitt, 1996). Assuming that the availability of arable land remains unchanged, increases in agricultural production of about 3 to 4% per year (Toenniessen, 1984) will be required to feed humans over the next 20 years due solely to the increase in population. The global demand for food, fiber, and bio-energy products (e.g. ethanol) is growing at a global annual rate of 2.5% in developed countries and 3.7% in developing countries (FAO, 1987). The food and fiber supply for our increasing population depends, among others factors, on the conservation of soil and water resources, and on the availability and incorporation of new land into agricultural production. About two-thirds of the increase in arable lands is expected to come from the expansion of irrigation. Irrigation plays a pivotal role in this increase in agricultural production, but paradoxically, many irrigation systems have failed to increase productivity and, with time, have rather transformed land into being unproductive, saline fallow. This transformation is known as secondary salinization, and it is as old as irrigated agriculture itself (Umali, 1993). Conditions that can lead to toxic levels of salt include, but are not restricted to, geochemical characteristics of soil parent material, quality and quantity of irrigation waters, or seawater intrusion (Todd, 1952).

Soluble salts are present in all soil parent materials, but it is only at high concentrations that these become a challenge for agriculture. Repeated drying and wetting may lead to excessive concentration of salts in the upper layers of the soil. Salts of different chemical

compositions are translocated upward with the water from deeper layers as this replaces, by capillarity, the water evaporated from the soil surface. As the water evaporates from the soil surface, it leaves the salts behind. If the parent material of an agricultural soil had a high level of soluble salts, and it is improperly irrigated, excessive salts could make most crop plant growth impossible. Irrigation and its consequences in salinization represent one of the dilemmas that human beings face at the end of the 20th century.

Earth's land surface is about 13.2 X 10^9 ha, about 7 X 10^9 ha are arable, and only 1.5 X 10^9 ha are cultivated. Of the cultivated lands, about 60% are either sodic or saline (Tanji, 1990). According to Umali (1993), the salt-affected area in the world is growing at a rate of 2 to 3 Mha/year. This rate is parallel to the expansion of irrigated land. If most of the human population on Earth is to avoid starvation within the next 30 to 50 years, land degradation must be significantly slowed, and we must learn to cultivate food in marginally saline soils.

Soil salinity

The effects of salt on soil structure have been well documented (Barzegar et al., 1996 and Crescimanno et al., 1995). Soil structure, with its hierarchical orders of domains, clusters, microaggregates, and macroaggregates, results from biotic and abiotic phenomena (Oades, 1993). The aggregates of soils irrigated with sodic water slake in a nonstepwise manner through the hierarchical ladder eliminating macropores and reducing soil permeability to water. Plant roots cannot penetrate the massive soil, and poor access to soil water and nutrients decreases plant growth and yield.

Most crop plants are glycophytes (Gk. glyco= sugar or sweet, and -phyte= a plant with a specified character or habitat) and respond with reduced growth and death to the presence of excessive salt. Soil salinity affects the uptake of nutrients (Marschner, 1995) and reduces plant growth. Bernstein (1961, 1963) proposed that salt has both ionic and osmotic effects on plants. Membrane functioning or internal solute balance can be disturbed under high ionic concentrations; however, specific ions, such as chloride, may be

toxic, even if its concentration in the soil solution is lower in relation to other ions. High concentrations of certain ions in soil solution may displace other nutrient ions (e.g. heavy nitrogen fertilization intensifies copper and zinc deficiencies. In the same way, excess sodium or potassium may adversely affect manganese uptake). As the concentration of salts increases in the soil solution, the osmotic potential of the soil becomes more negative, and absorption of water by plant roots is reduced. In the case of osmotic stress, the plant's cells respond by eliminating water or synthesizing organic compounds (McCue and Hanson, 1992), and thereby adjust the osmotic differential. When the plant is unable to osmotically adjust, death follows desiccation. Plants that are nutritionally balanced can more readily adjust to transitory increases in ionic concentrations of soil solution and survive osmotic stress.

Solutions and remedies: do they work?

Efforts to make salt-affected lands productive have been and continue to be made. Researchers have investigated food, fuel, and fodder crops whose yields are little-affected when irrigated with highly saline water (Aronson, 1985, 1989; Epstein, 1983; Gallagher, 1985; Glenn and O'Leary, 1985). Other efforts involved: (1) developing salt-resistant crops by classical breeding programs (Shannon, 1984; Ramage, 1980); (2) deciphering the genetic basis for salt tolerance in plants (Apse et al., 1999); (3) adopting the use of halophytes as crop plants (O'Leary, 1984); (4) leaching out excessive salts in soils (Hamdy, 1990a and 1990b); and (5) desalinizing seawater to use for irrigation (Lee, 1972; Muralev et al., 1997). Although these approaches have been successful, most are beyond the means of the developing parts of the world.

Vesicular-arbuscular mycorrhizal (VAM) fungi

Vesicular-arbuscular mycorrhizas are the most common symbiosis under ground. VAM fungi form a mutualistic symbiotic association with plant roots that can enhance plant growth and health. In this mutualistic symbiosis, fungi are completely dependent on the plant for organic carbon. VAM fungi associate with roots of a wide variety of plants. They can enhance uptake of nutrients of low mobility in the soil solution such as P, Zn, and Cu (Linderman, 1992). VAM fungi are able to exploit sources of P in soil not otherwise available to plants due to low solubility of the source (e.g., rock phosphate or ferric and aluminum phosphates) or low level of available P (Ojala et al., 1983). In addition, VAM fungi can hydrolyze organic forms of P by altering pH in localized sites, producing organic anions as chelating agents, and by producing surface or soluble phosphatases (Smith and Read, 1997). These fungi can ameliorate the negative effects of environmental stresses such as drought (Sylvia and Williams, 1992) and salinity (Hirrel and Gerdemann, 1980; Poss et al., 1985; Ojala et al., 1983; Pond et al., 1984) and provide protection against some soil pathogens (Newsham et al., 1994 and 1995).

While some plants produce similar leaf biomass with or without VAM fungi (e.g. lettuce), others respond strongly to the symbiotic association (e.g. onion). Most often, the difference depends on how plants acquire nutrients from the soil solution. Plants with extensive, fibrous root systems may be less responsive to VAM because they are capable of acquiring sufficient nutrients from the soil solution by themselves. Plants with smaller, coarse roots are highly responsive to VAM. Furthermore, P nutrition is essential to plant growth, and researchers have shown that growth differences between VAM and nonVAM plants can be minimized by providing additional P fertilizer to the noninoculated plants (Hirrel and Gerdemann, 1978).

Structural features of VAM

VAM fungal hyphae, grow inside the roots and through the soil forming an interface between plant roots and soil. The external hyphae absorb nutrients from the soil while the internal hyphae and arbuscules (shrub-like structures) act in the exchange of nutrients between the symbionts. Spheric or ovoid structures with thin walls, called vesicles, are considered storage organs and are found within or between cortical root cells. Hyphal germ tubes emerge from germinating spores or emerge from colonized root fragments in the soil. Hyphae and spores, as well as colonized fragments of roots, are often called VAM fungal propagules because they are vehicles of new colonization. The majority (about 80%) of the species presently described form both arbuscules and vesicles (Smith and Read, 1997). The remainder do not form vesicles and are called simply 'arbuscular' mycorrhizal fungi.

VAM and salinity

There is accumulating evidence that the VAM relationship results in increased nutrition and protection from various stresses in plants. Protection against salt stress is well documented. Hirrel and Gerdemann (1980) reported that VAM-colonized onions and bell peppers were more salt tolerant than noncolonized plants. The advantage that plants have from the symbiotic association with VAM fungi often results in greater yields of crop plants even under saline conditions (rice: Sharma et al., 1988; tomato and onion: Poss et al., 1985; bell pepper: Hirrel and Gerdemann, 1980). Poss et al. (1985) observed that dry weight of tomato plants grown in highly saline soil was significantly greater compared to that of noninoculated plants. Ojala et al. (1983) indicated that improved nutritional status of onion plants due to VAM fungi was at least partially responsible for increased plant growth under saline conditions. Hirrel and Gerdemann (1980) speculated that differences between VAM and nonVAM plants grown under salt stress might result from improved P nutrition. Later, Poss et al. (1985) concluded that the salt-tolerance mechanism in onion is

primarily related to P nutrition. Thus it has been proposed that increased phosphorus (P) nutrition by VAM plants was responsible for increased salt tolerance.

More recent evidence (Ruiz-Lozano et al., 1996) indicates that VAM fungi operate physiological changes in plants beyond improved P nutrition, and that these changes are important in the adaptation of VAM plants to conditions of salt stress. Among these changes brought about by VAM fungi in plant physiology are increased photosynthetic rates (Ruiz-Lozano et al., 1996), increased chlorophyll content (Tsang and Maun, 1999) and tissue elemental composition (Pfeiffer and Bloss, 1988). The latter may influence or mediate the osmotic adjustment necessary to overcome salt effects.

Experimental factors involving VAM effects and salt stress

In reviewing and comparing published work on the benefits that VAM fungi give to plants grown in saline soils, a number of factors which could have influenced the experimental results should be considered in order to assess the valid extension of their interpretation and the agricultural applicability of the techniques.

a) Equalizing inoculum. In earlier investigations, VAM fungi were introduced into previously autoclaved or pasteurized experimental soil at the time of transplanting bare-root seedlings (Hirrel and Gerdemann, 1980; Poss et al., 1985; Ojala et al., 1983). Pond and Menge (1984), Ojala et al. (1983), and Poss et al. (1985) added VAM fungal inocula to their experimental pots without accounting for possible differences in the number of viable propagules present in the volume of trap culture soils added. Number of viable VAM propagules present in trap cultures determines the level of root colonization, which in time can affect plant growth responses to salinity. As researchers compare plant responses to different VAM fungal species or fungal mixes, erroneous conclusions on the performance of a particular VAM fungal species (or mixture) can be achieved if trap-culture soils contained radically different numbers (by order of magnitude) of viable propagules and resulted in different colonization rates

b) Salt supply. Another experimental factor that could have affected the outcome was the time after which plants were exposed to saline conditions. Salinization of soil by adding NaCl solutions did not start until two to three weeks after transplantation (Poss et al., 1985; Ojala et al., 1983; Pond et al., 1984; Copeman et al., 1996), or even after six weeks after emergence (Tsang and Maun, 1999). Delaying salt exposure was thought to give plants time to overcome transplant shock and to become colonized by VAM fungi without additional stress. Saline solutions used in these works were of increasing concentrations (Copeman et al., 1996) and were added regularly while allowing leaching (Ojala et al., 1983), or of constant concentrations while avoiding leaching (Hirrel and Gerdemann, 1980). It is conceivable that by the time salt was added the seedlings had already grown beyond the developmental stage in which they were more susceptible to salt injury independent of their VAM status. Mass (1986) reported that certain crop plants (such as barley, corn, cowpea, rice, sorghum, and wheat) are most sensitive to salt during early seedling stages and that with time they become increasingly tolerant. Tsang and Maun (1999) harvested their leguminous plants only 3 weeks after the start of salt treatment, but they were 9 weeks old. The developmental change that brings salt tolerance to the maturing seedlings could mask VAM effects. More importantly, in an agricultural field, seedlings are exposed immediately after transplanting to a set level of salt that is rarely adjustable; therefore, exposing seedlings to salt gradually (to minimize osmotic shock and allow plant adjustment) is an experimental technique that does not simulate the conditions in an agricultural field.

c) Effects of salt on VAM fungi. With the exception of Pond et al. (1984), all other reports had the objective to measure plant responses, but they did not observe the effects of salt on the fungal symbionts. In this symbiosis, both plant and VAM fungi are important.

d) Preinoculation. Inoculation with VAM fungi prior to salt stress bypasses the effects that salt could have on spore germination (Juniper and Abbot, 1993), and on post-germination events such as localization and colonization of host roots in a saline soil. To date, no previous research has preinoculated plant starts with VAM fungi in plugs that are transplanted in salinized soil.

Work plan

Based on reports in the literature and the desire to examine VAM effects on salt tolerance of plants under high salt levels, we considered of paramount importance in our experiments, to estimate and equalize the number of viable propagules in each VAM fungal mixture so that comparisons between plant responses were independent of differences in the amount of root colonization by the VAM fungal sources used. We added equalized VAM fungal inocula to the growth medium in which seeds were germinated and the symbiosis established before salt exposure (preinoculation). We considered appropriate to allow two to four weeks for VAM to establish and to expose seedlings to saline (from NaCl) conditions in the same way as if they had been transplanted into a salt-affected field. Delaying seedling salt exposure for more time could conduce to a moderate degree of tolerance reached by the developing seedlings independent of their VAM status. We intended to separate P effect from VAM effect (Experiment 3) by providing P fertilizer in increasing concentrations to noninoculated onions growing under different salt levels. Similar growth responses observed between onions inoculated with VAM without additional P fertilization (Experiment 2) and nonVAM onions fertilized with inorganic P (Experiment 3), would be evidence supporting the hypothesis that VAM fungi enhance plant growth under saline conditions by increasing the concentration of P in plant tissues, and that it is through improved P nutrition that VAM plants are able to tolerate salt stress. We decided to also observe the effects that salt could have on VAM fungi by estimating soil VAM hyphal length of both inocula

We tested the following hypotheses:

a) Onion and lettuce plants preinoculated with VAM fungi survive and yield better than noninoculated plants under saline conditions; and

b) Plants colonized by VAM fungi from a saline soil grow better than plants colonized by VAM fungi from a nonsaline soil when grown in saline soil.

VAM inocula from a high- and a low-salt soil with comparable number of propagules were used to inoculate seedlings before they were transplanted into saline soil. Soils were treated with NaCl solutions of different concentrations. Daily watering replaced a portion

of the holding water capacity to prevent leaching. All pots received weekly application of fertilizer, but not P. With the exception of percent root colonization and leaf color of lettuce plants, all other plant responses and soil parameters were measured at harvest (10 weeks after transplant).

CHAPTER TWO.
EFFECTS OF PREINOCULATION WITH VAM FUNGI ISOLATED FROM DIFFERENT SITES ON PLANT TOLERANCE TO SALINITY IN SOILS AMENDED WITH SODIUM CHLORIDE.

Abstract

VAM inocula from a high- and a low-salt soil were trap-cultured, their propagules quantified, adjusted, and added to a pasteurized growth medium in which seeds germinated and seedlings grew for a few weeks. Inoculation of lettuce and onion seedlings with VAM fungi prior to salt exposure bypassed the inhibitory effects that salt could have on spore germination and the ability of VAM fungal hyphae to locate and colonize plant roots in a saline soil environment. The establishment of the mutualistic symbiosis prior to salt exposure gives the plants physiological advantages that could represent increased yields under saline conditions. These seedlings, once colonized by VAM fungi, were transplanted into saline soil. Seedlings were exposed to moderate to high concentrations of NaCl at the time of transplant; in this respect, our technique simulated conditions of high salinity prevalent in soils affected by NaCl. Salt exposure of the seedlings occurred in the same way as if they had been transplanted into a salt-affected field. Preinoculated lettuce and onion transplants grown in salinized soil for 10 weeks had increased shoot biomass compared with nonVAM plants at all salinity (NaCl) levels tested. Leaves of VAM lettuce at the highest salt level were significantly greener than those of the nonVAM lettuce. NonVAM onions were stunted due to available P deficiency in the soil, but inoculation with VAM fungi alleviated P deficiency and the salinity effects. VAM onions were significantly larger at all salinity levels. Increasing the level of available P by weekly applications to nonVAM plants partially alleviated the salinity effects on onion growth. VAM fungi from the saline soil site were not more effective in alleviating salt stress than those from the nonsaline site. Colonization of roots and length of soil hyphae produced by the tested fungi decreased with increasing salt. Results indicate that preinoculation of

transplants with VAM fungi can effectively alleviate deleterious effects of saline soils on crop productivity.

Introduction

Several studies have shown that VAM alleviates salt stress, but few have offered an explanation on the mechanisms involved. Although some researchers considered an improved, balanced plant nutrition, especially phosphorus nutrition, as the mechanism responsible for increased salt-tolerance in VAM plants (Pfeiffer and Bloss, 1988; Dickson et al., 1999; Poss et al., 1985; Hirrel and Gerdemann, 1980), others indicated that the increased tolerance is due to changes in physiological processes (e.g., photosynthetic, transpiration, and stomatal conductance rates, and water use efficiency) of VAM plants (Ruiz-Lozano et al., 1996; Tsang and Maun, 1999). Independently of the mechanism involved, our results show that VAM ameliorate the growth reduction that soil salinity causes in plants and that preinoculation with VAM represents a practical technique in growing VAM crops in saline soil environments.

In this study, special emphasis was given to: 1) Obtaining inoculum soils with comparable numbers of VAM propagules to control the amount of colonization of plant roots so that plant responses to the two VAM fungal sources could be compared independently of the degree of colonization. 2) Inoculating seedlings with VAM fungi before salt exposure to reduce/eliminate the effects that salt could have on spore germination and establishment of symbiosis. 3) Exposing the VAM seedlings to a set, steady level of salinity as it would have occurred if they had been transplanted in a field affected by salt. Quantification and equalization of inoculum potential (Hirrel and Gerdemann, 1980), and delayed salinization (Poss et al., 1985; Pond et al., 1984; Ojala et al., 1983) were integrated in the experimental conditions of previous works, but they did not simulate salt-field conditions in which transplants are exposed to high, fixed salt levels at once.

Plant responses measured were fresh and dry mass of shoots and roots, VAM root colonization (percent as well as length colonized per pot), shoot length, number, color (in lettuce only) and diameter (in onion only), and tissue elemental composition. Other measurements included final soil electrical conductivity (EC) and pH, and soil hyphal length. The objective of Experiments 1 and 2 were to determine if VAM fungi increased salt-tolerance in lettuce and onion plants, and if a VAM fungal source from a saline soil was more efficient than that from a nonsaline soil. The objective of Experiment 3 was to determine if applications of inorganic P to nonVAM plants would mitigate the reduction of growth caused by salt and to compare results obtained by P application against those obtained with VAM fungal inoculation.

Materials and Methods

Inoculum soils and trap cultures

VAM inocula used in these experiments were obtained (1) from a playa in the High Desert of southeastern Oregon in Harney County 25 miles south of Burns, Oregon; and (2) from the flood plains of the Willamette River at the Vegetable Farm of Oregon State University in Corvallis, Oregon. Soils from both sites were analyzed for nutrient contents and VAM fungal propagules. The "Burns" soil was a sandy loam (Gee and Bauder, 1986) with a pH of 8.8, and an organic matter content of 4.6%. It contained (mg kg^{-1}): P ($NaHCO_3$-extractable), 11; NH_4-N, 2.1; NO_3-N, 4.8; K, 2691; B, 7.9; Cu, 3.46; Fe, 8; Mn, 9.3; Cl, 1567; SO_4-S, 187; and Zn, 2.74. Other nutrients were (meq/100g) Ca, 32; Mg, 2.8; and Na, 14; and a Sodium Adsorption Ratio (SAR), 3.40. The electrical conductivity of the soil paste extract (Orion conductivity meter, Model 142) was 16 dS/m. The "Veg Farm" soil was a silt loam with a pH of 6.3 and an organic matter content of 5.6%. It contained (mg kg^{-1}): P (NH_4F-extractable), 67; NH_4-N, 3.6; NO_3-N, 3.8; K, 382; B, 0.3; Cu, 3.04; Fe, 12; Mn, 10.4; Cl, 26; SO_4-S, 4.5; and Zn, 20.6. Other nutrients were

(meq/100g) Ca, 15; Mg, 4.9; and Na, 0.12; SAR, 0.038. The electrical conductivity of the soil paste extract was 0.2 dS/m.

The initial search (assayed by wet sieving) for viable spores of VAM fungi in the Burns soil yielded <2 spores g^{-1}soil, and for the Veg Farm 6 spores g^{-1} soil. Nevertheless, roots (mostly of annual grasses) from the Burns site and corn (*Zea mays* L.) from the Veg Farm sites were highly colonized by VAM fungi. These soils were brought into the greenhouse to be used as inocula and were mixed with previously pasteurized (90 °C for 1 h. with aerated steam) sand at soil:sand ratios of 100:0, 50:50, or 25:75, in order to dilute potential salinity effects. Onions (*Allium cepa* L. cv White Bunching) and sudan grass (*Sorghum bicolor* L.) were planted to develop trap cultures. Long-Ashton nutrient solution (Hewitt, 1952) without phosphorus (P) was added once per week. After 4 months in the greenhouse, the roots were assayed for percent colonization using the grid-line intercept method (Giovanetti and Mosse, 1980). Colonization was not influenced by sand dilution, and onion roots were more heavily colonized than sudan grass roots. Within all sand:soil dilutions, colonization percent ranged from 21 to 54 and from 13 to 33 in onion and sudan grass, respectively. The soil and roots from these trap cultures were used for the experiments to follow.

Estimation of VAM fungal inoculum potentials.

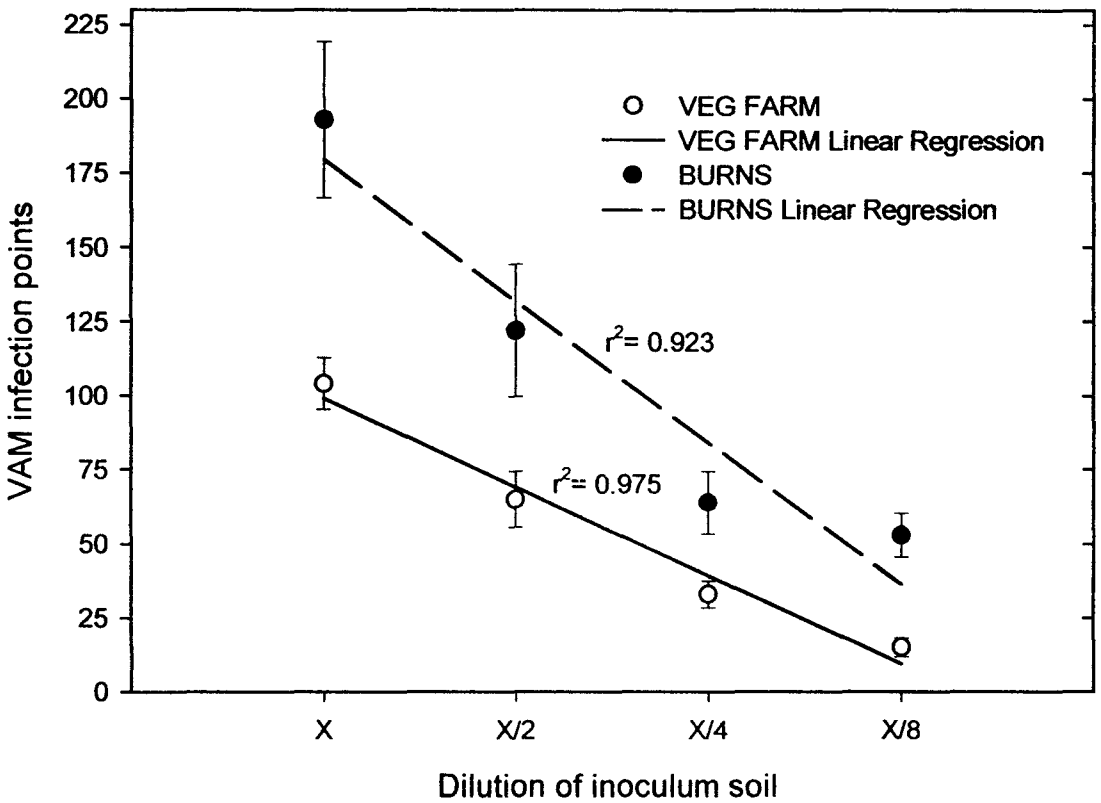
Soils to be used as inocula were assayed for VAM propagules, including spores, extraradical hyphae, and root fragments that had been colonized. Inoculum potentials for soils from Burns and the Veg Farm were estimated by the infection-unit method (Franson and Bethlenfalvay, 1989) modified as follows: inoculum soil from each site was mixed with a pasteurized sandy-loam Newberg-series soil (pH 6.3, 14.6 ppm NH_4–N; 1.4 NO_3-N; 27 ppm NH_4F-extractable P; 587 ppm total P; therefore, high fixing capacity; and SAR, 0.051) at ratios of 100:0, 50:50, 25:75 and 12.5:87.5. The Newberg-series soil was used as the base for all experiments. Tubes (2.5 cm upper diameter by 12 cm long, slightly tapered, 40 cc; Ray Leach Cone-Tainers, Aurora, OR) were filled with these mixtures of

the dilution series. Six replicate tubes per dilution level were used. Sudan grass seeds were surface-sterilized, germinated, and selected for uniformity before transplanting in tubes containing Newberg (base) soil. Seedlings were grown in the greenhouse. After 15 days, roots were harvested and washed on a sieve (1-mm openings), cleared (5% KOH v:v, 20 min at 90 °C), and stained (0.05% trypan blue in a mixture of lactic acid:glycerol:water 1:2:1 v:v:v for 15 min at 90 °C), to assess root colonization by VAM fungi.

The complete root system was examined for infection. Each infection unit represented a viable propagule that had germinated and colonized the sudan grass roots. Infection points were counted and divided by the amount of inoculum soil contained in the tube. A linear relationship was observed between the amount of trap-culture soil (dilution level) and the number of infection points (Figure 2.1). The Burns and Veg Farm soils were estimated to contain 6.97 and 2.83 propagules cc^{-1} of trap culture soil, respectively.

It was important to have the same inoculum densities for the two soils at the beginning of the experiments in saline soils to be able to compare plant responses to each inoculum independently of colonization percent at transplant time. Equivalent number of propagules in the two inocula was accomplished by diluting the Burns soil with pasteurized, base soil. Canadian peat moss was added (15% by volume) to all treatments in order to increase water retention and cohesiveness of the growth medium. Another infection-unit assay was then conducted to confirm propagule equivalence. Dilution resulted in similar mean root colonization percentages that ranged from 1 to 4 for the Veg Farm and from 2 to 7 for the Burns soil.

Figure 2.1 Number of VAM infection points in the roots of 15-day-old sudan grass seedlings as correlated with dilution levels of trap-culture soils of two different VAM fungal sources, Burns and Veg Farm.



Experiment 1: Effects of preinoculation with VAM fungal inocula on lettuce grown in saline soil.

Plug stage inoculation

Plug flats with 25-cc volume cells were filled with the Burns or the Veg Farm VAM fungal mixtures with similar number of propagules. A non-inoculated control treatment was prepared using only the base soil. All treatments received Canadian peat moss (15% by volume).

Lettuce (*Lactuca sativa* L. cv Black-seeded Simpson) were sown and thinned to one plant per cell by cutting shoots to avoid disturbance of the developing fungal network, and allowed to grow on a mist bench for 19 days. Roots were assayed for colonization 18 days after seeding. Prior to transplant into salinized soils, colonization of lettuce-seedling roots was 18 or 13% for the Veg Farm or the Burns inoculum, respectively. No VAM colonization was observed in roots of nonVAM seedlings. At this time, lettuce seedlings inoculated with the Burns VAM inoculum appeared to be slightly larger than those inoculated with the Veg Farm VAM inoculum and control seedlings, but this apparent difference was not quantified.

Soil treatments, transplanting, and plant growth conditions

Base Newberg mineral soil was steam-pasteurized (90 °C, 1 h, twice at interval of 24 h), air-dried, potted (500 g/pot), and treated with a 19.5mM NH_4NO_3 (1.56 g of NH_4NO_3 /L). Each pot received 180 mL of this solution to bring the soil to field capacity and provide 100 ppm-N. The electrical conductivity (EC) of this solution was 2.66 dS/m (hereafter referred to as EC=2). Sodium chloride (NaCl) was added to the N-fertilizer solution to raise the EC of the remaining three solutions to 4, 8, and 12 dS/m, respectively (0.7, 4.0, and 5.1 g NaCl/L equivalent to 12.1, 68.9, and 87.9mM NaCl). These solutions were applied only once at the beginning of the experiment on 13 replicate pots for each salt level treatment.

The soil was allowed to dry for two days before transplanting plugs into it. Whole plugs containing one seedling (19-day old) were transplanted into the saline soils and maintained in a greenhouse in Corvallis, Oregon from the end of April to the beginning of July 1999. Daily watering began two days after transplant. A water-retention curve (generated by the Soil Physical Characterization Lab at OSU) was used to calculate volumetric water at field capacity, and deionized water was added to keep soil at only 80% field capacity to prevent leaching. Pots were weighed every day and lost water was replaced. Long-Ashton nutrient solution (25 mL/pot) without P was added once per week from week 3 through week 10. As plants showed differences in growth, pots were weighed twice a day to reduce the drying and maintain 80% field capacity as long as possible. Sunlight was supplemented with metal halide lamps that provided 800 par (photosynthetic active radiation) at pot level. Temperatures ranged from 17 to 32 °C, and the humidity from 40 to 90%.

Plant growth responses

Leaf length, leaf number, and plant height. In order to establish a growth curve, the length of the more expanded leaf of each plant was measured using a plastic plate with concentric circles and a radial scale. The plate was suspended at 10 cm above the rim of the pot while measuring to avoid crushing the leaves, and the scale was adjusted to correspond with a scale laid directly on the leaf. Leaves were counted. The height of each plant was measured from the base to the highest leaf. These responses were recorded from weeks 1 to 6. Beyond that time, measurements were impracticable.

Leaf color. At 7 and 9 weeks after transplant, color of lettuce leaves was determined with a SPAD 502 (Minolta) meter to quantify chlorophyll content nondestructively. An average of five measurements from the distal half of a young fully expanded leaf was calculated from 10 replicate plants.

Shoot and root mass. Ten randomly chosen plants from the 13 in each treatment were harvested. Lettuce shoots were removed after 58 d of growth in saline soils, and weighed.

Roots were washed to remove all soil. Leaves and roots were oven-dried for 48 h at 60 ° C, ground to 20 mesh, and weighed.

Tissue elemental composition. Lettuce leaf tissue was analyzed for nutrients by the Central Analytical Laboratory of Oregon State University. Total C and N were assayed with a LECO CNS 2000 combustion analyzer. Na, P, K, Ca, Mg, Mn, Fe, Cu, B, and Zn concentrations were obtained with a Perkin-Elmer Optima 3000 Inducted Coupled Plasma Analyzer. Chloride contents were obtained by water extraction in a Waters Capillary Ion Analyzer.

VAM root colonization. VAM colonization was assessed after 7 and 9 weeks. Soil cores were removed to retrieve lettuce root samples and assess VAM colonization 7 weeks after transplant. Ten pots were sampled randomly from the 13 total in each treatment, and all were cored to equalize soil and root disturbance. The holes were filled with pasteurized Newberg soil. Root segments in the cores were washed on a 1-mm sieve, collected, cleared, and stained. Stained roots were examined under a dissecting scope at 40X magnification and colonization was assessed by the grid-line intercept method (Giovanetti and Mosse, 1980). All VAM structures found (hyphae, arbuscules, and vesicles) were counted.

At the end of the experiment, 10 randomly chosen replicates per treatment were harvested. Soil was gently broken and shaken over a 1cm sieve to retrieve roots. Soil was bagged and allowed to air dry for 4 d. Roots from each pot were gently washed on the sieve, thoroughly rinsed, and gently pressed between paper towels. Root fresh weights were recorded. A subsample was weighed, recorded, and used for VAM colonization, and total root length assessments. Total root length and the VAM-colonized fraction of inoculated plants was estimated. The remaining roots were oven-dried for 48 h at 60 °C, and dry weights determined.

Soil and hypha assays

Electrical conductivity and soil pH. After retrieving roots, soil was bagged and allowed to air dry for 4 d in the greenhouse in the shade before determining electrical conductivity of each replicate pot at the end of the experiment. Soil pastes of each sample were prepared and extracted with a vacuum pump to measure the final electrical conductivity. Soil pH was determined using 1:2 w:w soil:deionized water suspensions. Remaining soils were refrigerated (4 °C) until hyphal length assays were done.

Extraradical hyphal length. Soil-hyphae were extracted using the membrane-filter technique described by Hanssen et al. (1974) and modified as follows. At the end of the experiment, a 5-g soil sample from 6 pots within each soil treatment was weighed. Two samples were combined to make 3 pooled 10-g samples. Pooled samples were suspended in 95 mL of Phosphate Buffer Saline solution (0.01 M KH_2PO_4, pH 7.6) in bottles. The suspensions were agitated for 20 min at 250 rpm on a mechanical shaker, and aliquots of 10 mL were taken 10 seconds after opening the bottles and transferred to vials containing 5 mL of glycerol and lactic acid (70:30 v:v). Vials were stored at 4 °C until hyphal counts were determined. Contents of each vial were placed in a 150-mL beaker, diluted with deionized water to a final volume of 100 mL and sieved (38 μm) to eliminate small particles. Hyphae were transferred from the sieve back into the beaker by rinsing with 50 mL deionized water. Final volume was brought to 100 mL and stirred while a 10-mL subsample was removed by pipette. A membrane filter (GN 6, 0.45 μm pore size, 47 mm diameter with a 3 mm grid, Gelman Scientific, Ann Arbor, Mich.) was placed on a filter holder attached to a vacuum apparatus. The filter was moistened with deionized water, wetted with 10% ethyl alcohol, and rinsed before placing the 10-mL aliquot of suspended hyphae on it. After filtering the water, a small amount of trypan blue (5% in a mixture of lactic acid:glycerol:water, 1:2:1 by volume) was added to cover the filter for 10 min. The filter was rinsed and hyphae resuspended to assure even distribution on the membrane before final filtering. A drop of destaining solution (60% glycerol, 2% HCl, and 38% deionized water) was placed on the inside of a small (48 mm diameter) Petri dish. The membrane was removed from the filter holder and placed inverted against the bottom of a

Petri dish with the hyphae between the membrane and the dish. Air bubbles were removed gently and a cover was placed on the dish. Intercepts were observed and counted under stereo microscope at 40X magnification. Hyphal length was calculated by the grid-line intersect method (Giovanetti and Mosse, 1980) adjusting for soil moisture to report in a dry soil-weight basis (Gardner, 1986).

Experimental design and statistical analysis

The experiment was a factorial with three VAM fungal treatments (Veg Farm and Burns source sites, and nonVAM) and four levels of salt (EC of 2.66, 4.0, 8.0, and 12.0 dS/m) for a total of 12 treatments. Thirteen replicate lettuce plants per treatment were grown; however, only 10 randomly chosen replicates were measured or harvested. Data were analyzed as for a Complete Randomized Design (CRD). ANOVA tools were used to find treatment differences when the data did not violate their assumptions. Barlett's Test was used to detect heterogeneous variances ($p< 0.05$). Orthogonal contrasts were used to compare plant responses such as leaf length and color, root fresh mass, and number of leaves per plant of VAM against those of nonVAM lettuce plants. Only when the interaction VAM fungal source X salt was significant were the same orthogonal contrasts done at each salt level. Otherwise, only significant main effect(s) were (was) discussed. In all comparisons throughout the present study, actual p-values pertaining to comparisons between treatment means were reported to allow the reader to evaluate statistical significance of the differences. These same tools and criteria were used for comparison between the plant responses to the two VAM fungal mixtures. When responses of plants inoculated with one of the VAM fungal inoculum sources were compared against the nonVAM (unplanned comparisons), Multiple Pair-wise Comparison Tukey's tests were used (e.g., leaf color). Logarithmic transformations were performed when useful in correcting uneven variances, and results were back-transformed for interpretation purposes. This strategy was used in the analysis of dry root mass, and nitrogen, potassium, and sodium concentrations in leaves of lettuce plants. For interpretation of the analysis

performed on log transformed data, it was necessary to take the antilogarithm of the estimate of the mean in the log scale; this does not give an estimate of the mean on the original scale but of the median. The mean of the logged values is not the log of the means. Assuming that log-transformed data have symmetric distributions, the antilogarithm of the mean of the log values is the median on the original scale of measurement. Therefore, interpretation of comparisons between fungi-salt treatments after log transformation is related to the medians of the population and not means (Ramsey and Schafer, 1997).

In order to analyze tissue elemental composition, elements were divided as follows: a) those for which two-way ANOVA resulted in a significant VAM fungal source X salt interaction, and b) those for which such interaction was not significant (p-value< 0.05). When the VAM fungal source X salt interaction in the full model (n=36) was significant, the VAM main effect was used as the one-way ANOVA factor (n=9), and differences between nonVAM and VAM responses and between Burns- and Veg Farm-inoculum source responses were tested by orthogonal contrasts within each salt level.

Kruskal-Wallis nonparametric test was used to analyze percent root colonization, total root length, colonized root length of lettuce plants, and electrical conductivity. The Kruskal-Wallis analysis of variance replaces all observation values by their ranks in a single combined sample and applies a one-way ANOVA on the rank-transformed data (Ramsey and Schafer, 1997). A series of these tests was done to include all fungal treatments while eliminating the salt variable. If p-values were less than 0.05, the test was interpreted as indicating that at least one out of the three fungal treatments was different in the complete data set. To determine which fungal treatment was different, another series of tests was performed at each individual salt level including the three fungal treatments (n=30). If the p-value was less than 0.05, the test was interpreted as indicating that at least one out of the three fungal treatments within that salt level was different. Finally, to determine whether the responses to either VAM fungal inoculum were different within that salt level, another series of tests was done including only the data pertaining to the two VAM fungal mixtures (n=20).

Identification of VAM fungal species in inoculum sources

Several species of VAM fungi have been identified in previous work that used the Veg Farm VAM inoculum source (R.P. Schreiner, personal communication). These were: *Glomus mosseae* Gerd.& Trappe; *G. aggregatum/intraradices* Schenck & Smith; *Acaulospora trappei* Ames & Linderman; *Entrophospora infrequens* Ames & Schneider; unknown clear *Glomus*, and unknown yellow *Glomus*.

The VAM fungi species identified in the Burns VAM fungal trap cultures were *Glomus intraradices* and three other unidentified different spore morphotypes.

Experiment 2: Effects of preinoculation with VAM fungal inocula on onions grown in saline soil.

Inoculation and growth conditions

Onion seeds were planted in the same growth media and treated in the same way as described for Experiment 1; however, initial colonization was assessed 28 d after sowing, and seedlings were transplanted into saline and control soils 30 d after sowing. Colonization of onion seedling roots prior to transplant was 30 and 22 % for the Veg Farm and the Burns inocula, respectively. As with lettuce, onion seedlings inoculated with Burns VAM fungi appeared to be slightly larger at the plug stage than seedlings in the other two VAM treatments, but the apparent difference was not quantified.

Soil treatments, fertilization, root assessment for colonization, final EC and pH, were the same as for Experiment 1. The only differences were that for the onions, the bulb weights were included with the leaf weights, and those plants were harvested after 76 d of growth in saline soils. VAM colonization was not determined for onion roots except at harvest. Also, use of the SPAD 502 was not possible in onions since it damaged leaf tissue due to the necessary pressure of the clip to read light differentials.

Experimental design and statistical analysis

The experiment was a factorial with three VAM fungal treatments (Veg Farm, and Burns source sites, and nonVAM) and four levels of salt (EC of 2.66, 4.0, 8.0, and 12.0 dS/m) for a total of 12 treatments. Thirteen replicate onion plants per treatment were grown; however, only 10 randomly chosen replicates were measured or harvested. Uneven variances among treatments within each salt level precluded use of ANOVA tools since log transformations did not correct heterogeneous variances. Statistical analysis of these data sets was done using the Kruskal-Wallis nonparametric test using the same criteria as described above for the lettuce experiment. Other responses analyzed with this test were VAM percent root colonization, colonized root length, total root length, and final soil EC. Tissue elemental composition of onion was performed after pooling the 10 onion replicates of each fungal-salt treatment. At high salt treatments there was only enough plant material to perform one tissue analysis per treatment. In the absence of replicate tests for each treatment, no statistical analysis was performed.

Experiment 3: Effects of phosphorus fertilization on onion under salt stress.

This experiment was conducted in a greenhouse in Corvallis, Oregon from mid August to the beginning of December 1999. Temperature ranged from 8 to 12°C at night and from 12 to 29°C during the day. Sunlight was supplemented for the first twelve weeks with metal halide lamps that provided 800 par (photosynthetic active radiation) at pot level.

Seedlings in plugs

Steam pasteurized Newberg soil was amended with Canadian peat moss (15% v:v) and used as the growth medium for onion seedlings. Flats were placed on a mist bench for

three weeks until seedlings were transplanted into salinized soil. Air-steam pasteurized, air-dried Newberg soil was potted (500 g/pot) and treated once with the salt-fertilizer solutions.

Soil treatment solutions and plant growth conditions

a) An NH_4NO_3 (7.0 mM) solution was prepared (3.12 g NH_4NO_3/L), and 500 mL was diluted with 500 mL of deionized water to make it equivalent to the N-solution used in Experiment 1; 180-ml volume was added to each N-control pot.
b) A pentophosphate (P_2O_5) stock solution was prepared by mixing 9.53 g P_2O_5 in a liter of deionized water (67.1 mM). Aliquots of 0, 10, 20, and 30 mL of this P-solution were placed in 2-L bottles. A 500-mL volume of the concentrated NH_4NO_3 solution (a) was added and the volume was brought to 1 L with deionized water. A 180-ml volume was added to each P-control pot.

For the NaCl treatments, the final volume was adjusted with deionized water after adding this salt. Each 180-ml aliquot of the resulting solution provided 15 (7.5 mg P kg^{-1} soil), 30 (15 mg P kg^{-1} soil), or 45 ppm-P (22.5 mg P kg^{-1} soil), to the 500 g of soil in each pot correspondingly in addition to 100 ppm N. Pacovsky et al. (1986) used similar methods to add P. Electrical conductivity and components of each solution are given in Table 2.1.

Table 2. 1. Preparation of soil treatment solutions with P, N, and NaCl and their electrical conductivities

Treatment	NH4NO3 (ml)	P stock (ml)	NaCl (g)	d-water	EC (dS/m)
P0Na0	500	0	0	500	2.72
P1Na0	500	10	0	490	3.17
P2Na0	500	20	0	480	3.64
P3Na0	500	30	0	470	4.04
P0Na1	500	0	2.72	*	8.00
P1Na1	500	10	2.55	*	8.04
P2Na1	500	20	2.34	*	8.04
P3Na1	500	30	2.03	*	8.00
P0Na2	500	0	5.05	*	12.03
P1Na2	500	10	4.65	*	12.04
P2Na2	500	20	4.46	*	12.07
P3Na2	500	30	4.27	*	12.04

* enough to bring total volume to 1L.

Treatment solutions were added to the potted soil and allowed to dry for 2 d before transplanting. Onion seedlings were transplanted and watered to only 80% field capacity to avoid leaching. Long-Ashton solution without P was provided once a week after week 3. On week 7 and thereafter, Long-Ashton nutrient solution containing 0, 15, 30, or 45 ppm P (25 mL/pot) was added weekly to each corresponding pot. Plants were allowed to grow in a greenhouse for 16 weeks and harvested to obtain fresh/dry root and shoot mass. No further examinations were made.

Experimental design and statistical analysis

The experiment was a factorial with three NaCl treatments (EC < 4, 8.0, and 12.0 dS/m) and four P treatments (0, 7.5, 15, and 22.5 mg P kg^{-1} of soil or 0, 15, 30, and 45 ppm P) for a total of 12 treatments, each with five replicate pots. The collected data were

analyzed using Kruskal-Wallis nonparametric tests to find treatment differences. Each salt level was analyzed separately and salt was the independent variable. If the Kruskal-Wallis p-value was less than 0.05, at least one out of the four P levels within that salt level was assumed to be statistically different. Consecutive Kruskal-Wallis tests were performed excluding the dry weight data of the highest P level (the most likely to be significantly different) until the p-value was greater than or equal to 0.05 or until the only remaining data were those of the two lower P levels.

CHAPTER THREE.
RESULTS

Experiment 1

Shoot fresh and dry mass of lettuce

Differences in the several post-harvest lettuce plant responses measured, and discussed below, were independent of root-colonization percentages because these were not statistically different between the two inocula at the end of the experiment (Table 3. 1).

Plants grown in soil of high salt contents had lower shoot fresh and dry mass than those grown in soil with low salt level (Figure 3.1 and 3.2). Higher variances were associated with treatments with lower mean masses (high salt); therefore, the fresh and dry masses of leaves were not suitable for log transformation to correct for heterogeneous variances. A two-way ANOVA indicated interaction between VAM fungi source and salt main effects. This meant that the fresh and dry mass of the fungal treatments changed with salt. This interaction is not invalidated by heterogeneous variances because of the robustness of the F-test (Milliken, 1984) and the small p-value of the interactions (0.0006 and 0.0055 fresh and dry mass, respectively).

Because one of our interests was to study plant responses to VAM fungi at different salt concentrations, one-way ANOVAs were done in each salt level where the variances between the three fungal treatments were comparable. As Figure 3.1A shows, fresh masses of VAM lettuce shoots were significantly different from those of noninoculated ones at all salt levels (one-way ANOVA $p= 0.003$ at EC 2; $p= 4.36 \times 10^{-5}$ at EC 4; $p= 0.002$ at EC 8; $p= 3.74 \times 10^{-5}$ at EC 12).

The means of nonVAM fresh shoots were smaller than the means of the combined VAM fresh shoots by 5.6% at EC 2; by 8.7% at EC 4; by 9.3% at EC 8; and by 32.5% at EC 12.). Tukey's test ($p= 0.05$) was used to compare the means of the Burns and Veg

Table 3.1 Percent colonization of lettuce roots by two VAM fungal mixtures from two different sites and extraradical hyphal lengths at the end of the experiment. Plants were inoculated with VAM fungi from Burns (high salt soil) or Veg Farm (low salt soil) and grown in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). Values are percent root colonization and hyphal length means of 10 replicate plants and (+/- SE). Within each parameter at each salt level, VAM fungal treatment means followed by the same letter are not significantly different (at $p < 0.05$) as determined by Kruskal-Wallis nonparametric test for percent root colonization (*or Tukey's test when appropriate) and by orthogonal contrasts for hyphal length.

Week 7

EC(t) (dS/m)	Burns % Colonization		Veg Farm % Colonization	
2	44.6	(4.9)a	39.2	(2.4)a
4	34.8	(4.8)a	36.2	(3.2)a
8	21.5	(5.3)a	26.6	(2.3)a
12	10.1	(1.8)a*	19.5	(2.4)b*

Week 10

	Burns				Veg Farm			
EC(t) (dS/m)	Colonization (%)		Extraradical hyphal length (m/g soil)		Colonization (%)		Extraradical hyphal length (m/g soil)	
2	43.0	(5.2)a	11.31	(1.83)a	34.8	(4.0)a	14.48	(2.03)a
4	32.7	(3.8)a	10.47	(1.12)a	32.6	(1.5)a	9.87	(0.93)a
8	29.8	(3.5)a	9.13	(0.66)a	27.8	(1.1)a	10.02	(0.55)a
12	26.2	(3.1)a	7.43	(1.49)a	29.9	(1.4)a	8.54	(1.06)a

EC(t)= electrical conductivity of treatment solutions

Figure 3.1 Fresh (A) and dry (B) mass of lettuce shoots inoculated before transplant with VAM fungal treatments: Burns (VAM from saline soil), Veg Farm (VAM from a nonsaline soil), or not inoculated (nonVAM); plants were grown in soil treated with four levels of NaCl (EC 2 control, EC 4, EC 8, and EC 12 dS/m). Means with the same letter are not different at $p \leq 0.05$ within the same salt level. Values are means of 10 replicate plants, and bars are (+/- SE).

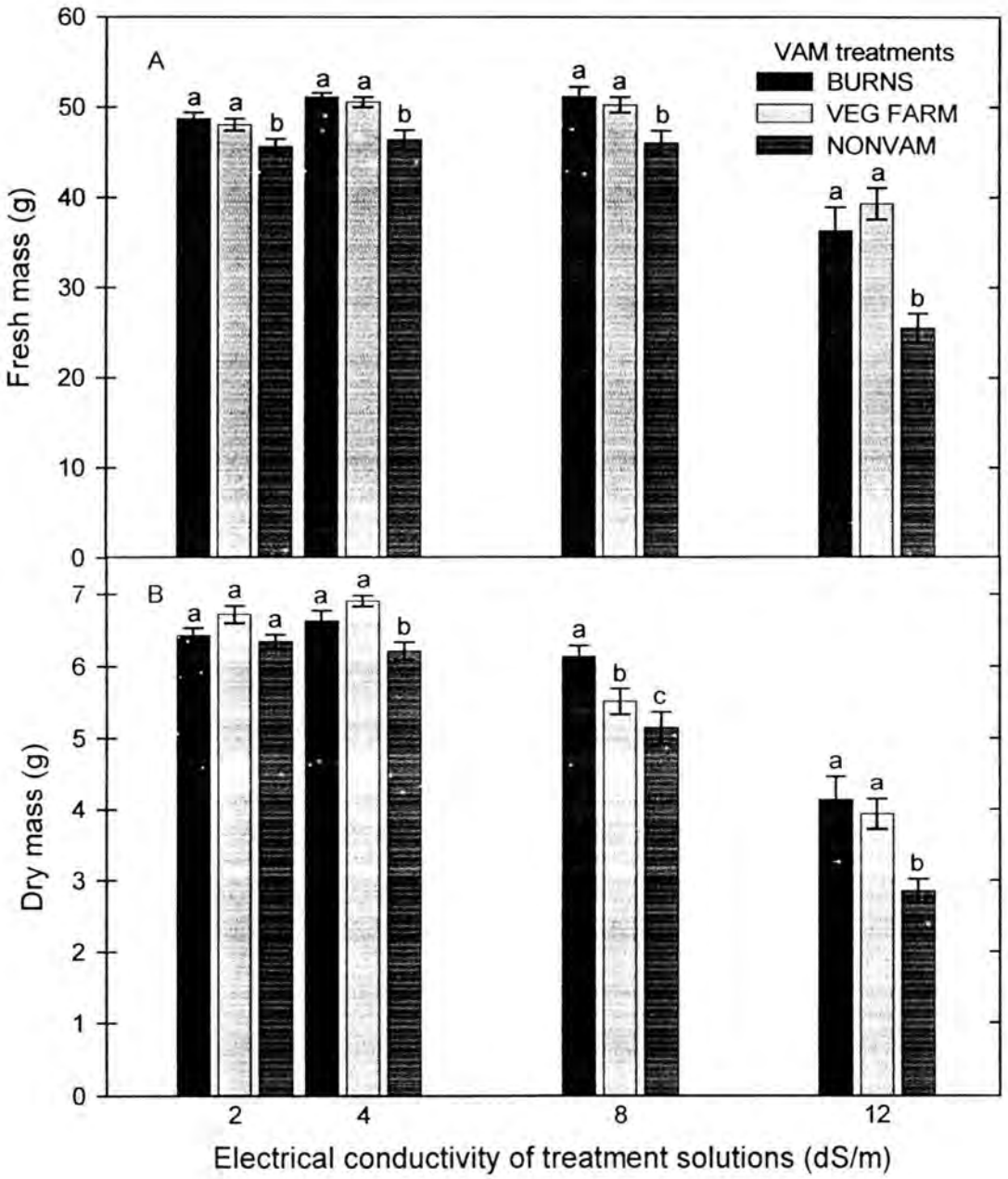
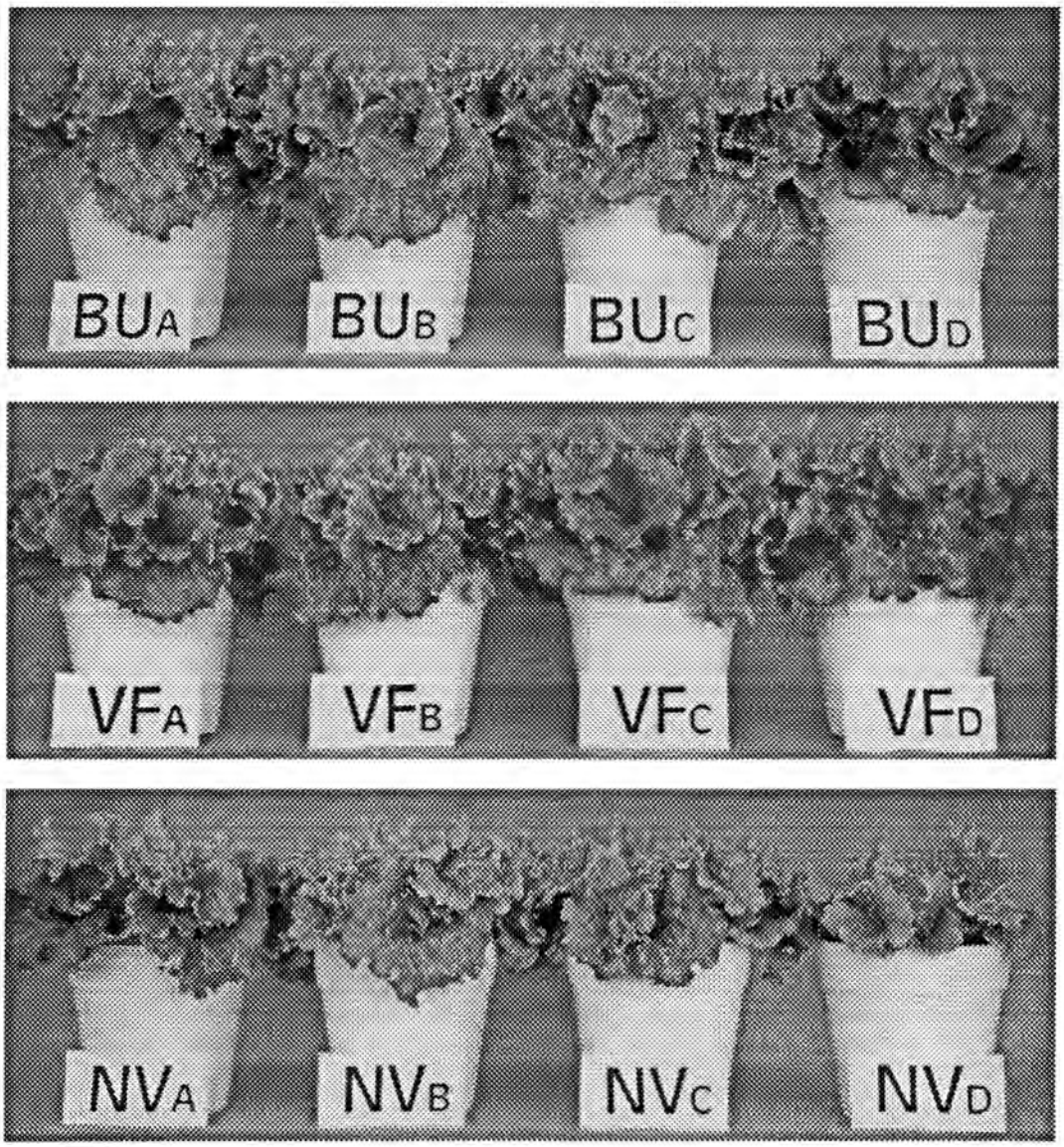


Figure 3.2 Photograph of representative lettuce plants grown in soil treated with four levels of NaCl solutions (A= EC 2 control, B= EC 4, C= EC 8, and D= EC 12 dS/m). Fungal treatments were: NV= nonVAM, VF= Veg Farm VAM fungi, or BU= Burns VAM fungi. At 12 dS/m, shoot dry weight of nonVAM lettuce plants was 29% less than that of the mean VAM plants combined from the Veg Farm and Burns inocula.



Farm inoculum treatments separately with the nonVAM treatment mean leaf fresh mass. The nonVAM fresh shoot mass mean was smaller than those of the Burns and Veg Farm inoculum plants by 6.3 and 4.9%, respectively at EC 2; by 9.2 and 8.2% at EC 4; by 10.1 and 8.4% at EC 8; and by 29.7 and 35.0% at EC 12. Significant differences on shoot fresh mass were not observed between the effects of inocula from the Burns and the Veg Farm sites.

Shoot dry masses (Figure 3.1B) of VAM plants were different from those of nonVAM lettuce at all salt levels (one-way ANOVA $p= 0.047$ at EC 2; $p= 0.001$ at EC 4; $p= 0.006$ at EC 8; $p= 0.001$ at EC 12). Mean dry mass of nonVAM plants was smaller than the mean of the VAM plants combined by 3.4% at EC 2; by 8.2% at EC 4; by 11.7% at EC 8; and by 29.3% at EC 12 (Figure 3.2). Mean dry mass of nonVAM plants was smaller than the mean of the Burns and Veg Farm inoculated plants by 1.3 and 5.5%, respectively, at EC 2; by 6.2 and 10.0% at EC 4; by 16.2 and 6.6% at EC 8; and by 31.0 and 27.6% at EC 12. No significant differences ($p\leq 0.05$) were observed in shoot dry mass between the lettuce inoculated with the either VAM inoculum.

Mortality of lettuce plants in experiment was low (total of 4) and was not treatment-related.

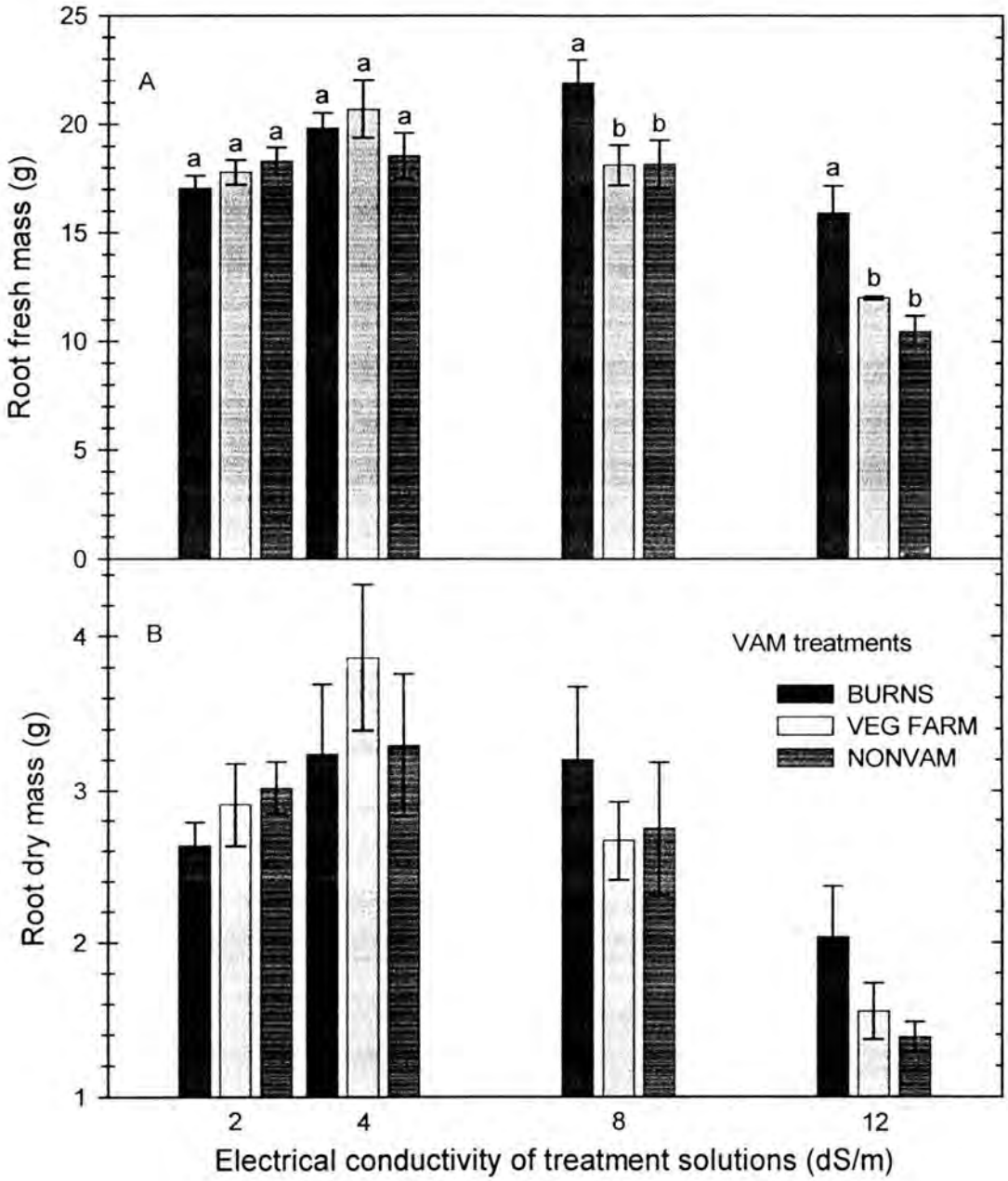
Root mass of lettuce

A statistical analysis of fresh root mass showed an interaction between VAM fungal source and salt (ANOVA, $p= 0.004$). Fresh mass of VAM lettuce roots was different from that of nonVAM lettuce roots ($p= 0.007$). Fresh root mass was significantly affected by salt (Figure 3.3A). Fresh mass of roots inoculated by Burns VAM fungi also was different from that of the roots inoculated by the Veg Farm VAM fungi ($p= 0.021$).

At EC 12, root fresh mass of lettuce plants inoculated with Veg Farm VAM fungi was 24.5% smaller ($p= 0.006$) than that of plants inoculated with Burns VAM fungi.

Uneven variances were observed in the root dry mass data (Figure 3.3B), and log transformation was used to correct for it. Only the salt main effect was significant (ANOVA p-value= 1.1×10^{-14}). Salt, at the highest level, reduced root dry mass of

Figure 3.3 Root fresh (A) and dry (B) mass of lettuce inoculated before transplant with VAM fungal mixtures from Burns (high salt), Veg Farm (low salt), or not inoculated (nonVAM) and grown in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). Means with the same letter are not different at $p \leq 0.05$ within the same salt level. Values are means of 10 replicate plants and bars are +/- SE.



lettuce by 42% with respect to the no-salt control. No significant differences between dry mass of the VAM and nonVAM roots (p= 0.225) or between the dry mass of plants inoculated with Burns or Veg Farm VAM fungi were observed (p= 0.628).

Percent VAM colonization of lettuce roots

Mean colonization percentages prior to transplant were estimated to be 18% for the lettuce roots inoculated with Veg Farm VAM fungal source and 13% for those inoculated with the Burns VAM fungal source (10 seedlings per treatment, one-way ANOVA p= 0.046). No VAM colonization was observed in control seedlings. VAM percent colonization by the two inocula significantly decreased with increased salinity at both sampling times (Table 3.1). Moreover, the percent colonization of both VAM inoculum sources was not significantly different at harvest (p-values> 0.05, Kruskal-Wallis nonparametric test). At week 7, the percent root colonization by the Burns inoculum at EC 12 was reduced by 77.4% with respect to the control (EC 2), while the percent root colonization by the Veg Farm inoculum was only reduced by 50.3%. At week 7, the VAM percent colonization by the two inocula was not significantly different (p-values> 0.05, Kruskal-Wallis nonparametric test) in the three lower salt levels (EC 2, EC 4, and EC 8), but the Veg Farm VAM fungi were stronger colonizers (two fold) at the highest salt treatment (EC 12) than the Burns VAM fungi. This difference was not significant by Kruskal-Wallis (p= 0.074), but it was significant by Tukey's (p= 0.006). Barlett's test of homogeneity of variance at EC 12 (p= 0.395) indicated that ANOVA tools (Tukey's) could be used at this salt level.

At week 10, percent colonization by the Burns inoculum was reduced by 39.1% and percent colonization of the Veg Farm inoculum was reduced only by 14.1% within the range of salt concentrations used. Percent colonization by the two VAM fungal inoculum sources was not significantly different at each salt level (p> 0.05, Kruskal-Wallis). Percent root colonization and colonized root length cannot be compared between the two

sampling times since the methods employed differed (i.e., coring vs. subsampling). No VAM colonization was observed in control pots.

Estimated root length of lettuce and its VAM colonized fraction

Root length of lettuce was significantly affected by salt (Table 3.2). At week 7, VAM lettuce roots inoculated with fungi from the Burns and the Veg Farm sites and treated with the highest salt level were shorter by 61.4% and 52.5%, respectively, compared with their corresponding nonVAM controls. At the maximum salt level, roots of plants inoculated with the Veg Farm VAM fungi were 18% longer (orthogonal contrast, $p= 0.322$) than those of plants inoculated with the Burns VAM fungi. At week 7, the root length colonized by the Veg Farm inoculum at the highest salt level was 58.6% (or 2.4 times) larger ($p= 0.074$, Kruskal-Wallis) than by the Burns inoculum; nevertheless, this difference was not significant at harvest. At week 10, lettuce inoculated with VAM fungi from the Veg Farm site and grown at EC 8 and EC 12 salt levels had an estimated root length significantly shorter (23.5%, $p= 0.0002$, and 27.6%, $p= 0.0003$ respectively) than plants inoculated with the Burns inoculum at the same salt levels. Colonized root length at EC 8 by Veg Farm VAM fungi appeared to be smaller (by 29.4%) than that of Burns VAM fungi ($p= 0.074$, Kruskal-Wallis). Nevertheless, significant differences were not observed in colonized root length at the highest salt level ($p= 0.371$).

Leaf length and number per lettuce plant

Differences in leaf length and number and plant height between VAM and nonVAM lettuce plants were not apparent until week 6 (only leaf length and number of leaves per plant presented). At that point, leaf length and leaf number per plant inoculated with Veg Farm VAM inoculum were greatest, and the nonVAM control the smallest (Figure 3.4). Mean leaf lengths of nonVAM lettuce plants were significantly different from those of the

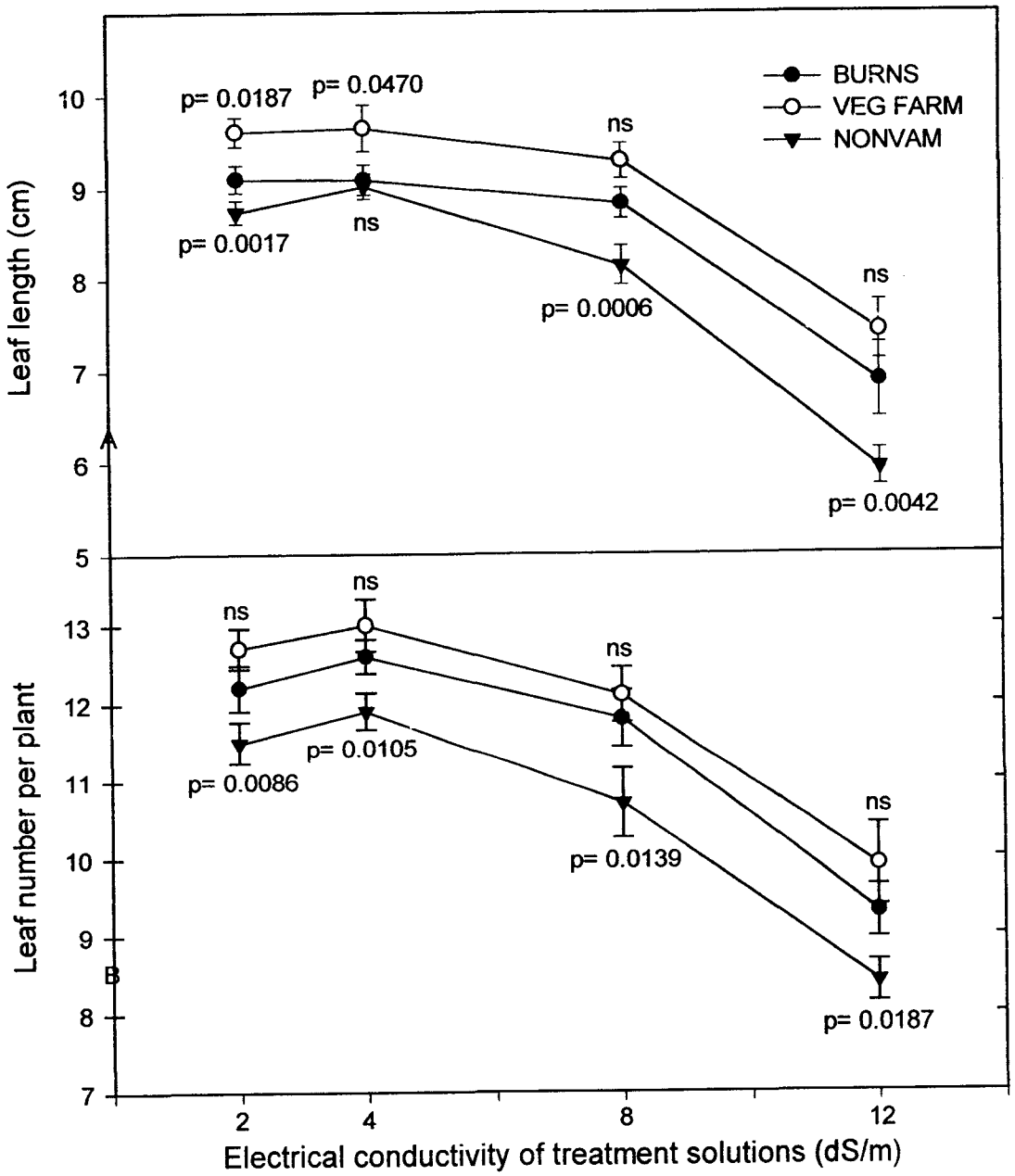
Table 3.2 Root length, colonized root length, and percent root colonization of lettuce plants preinoculated with two VAM fungal mixtures from Burns (high salt) or Veg Farm (low salt) sites and grown in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12). Values are means of 10 replicate plants and (+/- SE). Within each parameter and at each salt level, means of VAM fungal treatments followed by the same letter are not significantly different (at $p < 0.05$) as determined by Kruskal-Wallis nonparametric test (*Tukey's test, when appropriate).

Week 7	EC(t)	Root length (m/pot)				Colonized root length (m)				Colonization (%)			
	(dS/m)	Burns		Veg Farm		Burns		Veg Farm		Burns		Veg Farm	
	2	65	(3)a	64	(4)a	28	(3)a	25	(2)a	44.6	(4.9)a	39.2	(2.4)a
	4	66	(2)a	64	(4)a	23	(3)a	23	(2)a	34.8	(4.8)a	36.2	(3.2)a
	8	51	(5)a	52	(3)a	12	(4)a	14	(1)a	21.5	(5.3)a	26.6	(2.3)a
	12	25	(3)a	31	(5)a	3	(1)a	6	(2)b	10.1	(1.8)a*	19.5	(2.4)b*

Week 10	EC(t)	Root length (m/pot)				Colonized root length (m)				Colonization (%)			
	(dS/m)	Burns		Veg Farm		Burns		Veg Farm		Burns		Veg Farm	
	2	192	(6)a	209	(14)a	83	(11)a	73	(10)a	43.0	(5.2)a	34.8	(4.0)a
	4	214	(5)a	228	(15)a	69	(8)a	74	(5)a	32.7	(3.8)a	32.6	(1.5)a
	8	217	(7)a	166	(8)b	65	(7)a	46	(2)a	29.8	(3.5)a	27.8	(1.1)a
	12	146	(15)a	106	(2)b	39	(6)a	32	(2)a	26.2	(3.1)a	29.9	(1.4)a

EC(t)= electrical conductivity of treatment solutions

Figure 3.4 Leaf length (A) and leaf number (B) of lettuce plants preinoculated with VAM fungal mixtures from Burns (high salt site), Veg Farm (low salt site), or not inoculated (nonVAM) at six weeks after transplant into soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). The p-values above the curves correspond to orthogonal contrasts between VAM plants, and the p-values below the curves correspond to the orthogonal contrasts between the VAM and nonVAM plants. Nonsignificant differences in the contrasts ($p>0.05$) are indicated by ns. Values are means of 10 replicate lettuce plants and bars are +/- SE.



VAM lettuce plants combined (orthogonal contrast, $p< 0.05$). The mean leaf length of the lettuce plants inoculated with VAM fungi was 6.6% greater than that of the nonVAM at EC 2; 3.7% greater at EC 4; 10.0% greater at EC 8; and 17.1% greater at EC 12. The main effect of salt on leaf length was significant, and the highest salt concentration used in the experiment reduced leaf length by 26.1% compared to the control. Salt decreased leaf length in a linear way (orthogonal contrast, $p< 0.05$); nevertheless, plants in EC 4 had slightly greater leaf length (1.1% more, not statistically significant) than plants in EC 2 or EC 8. At week 6, leaf lengths of Veg Farm lettuce at the highest salt treatment were significantly greater (20.2%, $p= 0.007$) than those of nonVAM lettuce plants, while those of Burns lettuce were not (7.4%, $p= 0.109$). Salt reduced leaf length by 26%. Leaf-length data from prior to 6 weeks did not show significant differences between the fungal treatments.

As shown in Figure 3.4B, the mean number of leaves in the nonVAM lettuce was significantly different from the mean of the combined VAM plants (orthogonal contrast, $p< 0.05$). The mean number of leaves per plant of VAM lettuce was approximately one leaf greater than that of the nonVAM plants at each salt level. This difference was constant, irrespective of salt level. The salt effect was significant, and salt at the highest concentration reduced leaf number by 24.3% compared to the control.

Only at week 6 were the differences in mean leaf number per plant between Veg Farm-inoculated lettuce and nonVAM lettuce plants statistically significant ($p= 0.012$ at EC 2; 0.019 at EC 4; 0.043 at EC 8; and 0.030 at EC 12). No significant differences were observed between the mean leaf number of lettuce plants inoculated with the Burns VAM fungi and nonVAM lettuce plants at each salt level.

Leaf color of lettuce

At week 7, leaf color of mature leaves was affected by salt and by VAM fungi (ANOVA p-values 0.0081 and 9.02×10^{-6}, respectively). Leaf color was negatively and linearly correlated with salt treatments (orthogonal contrast, $p= 0.002$). The color of mature

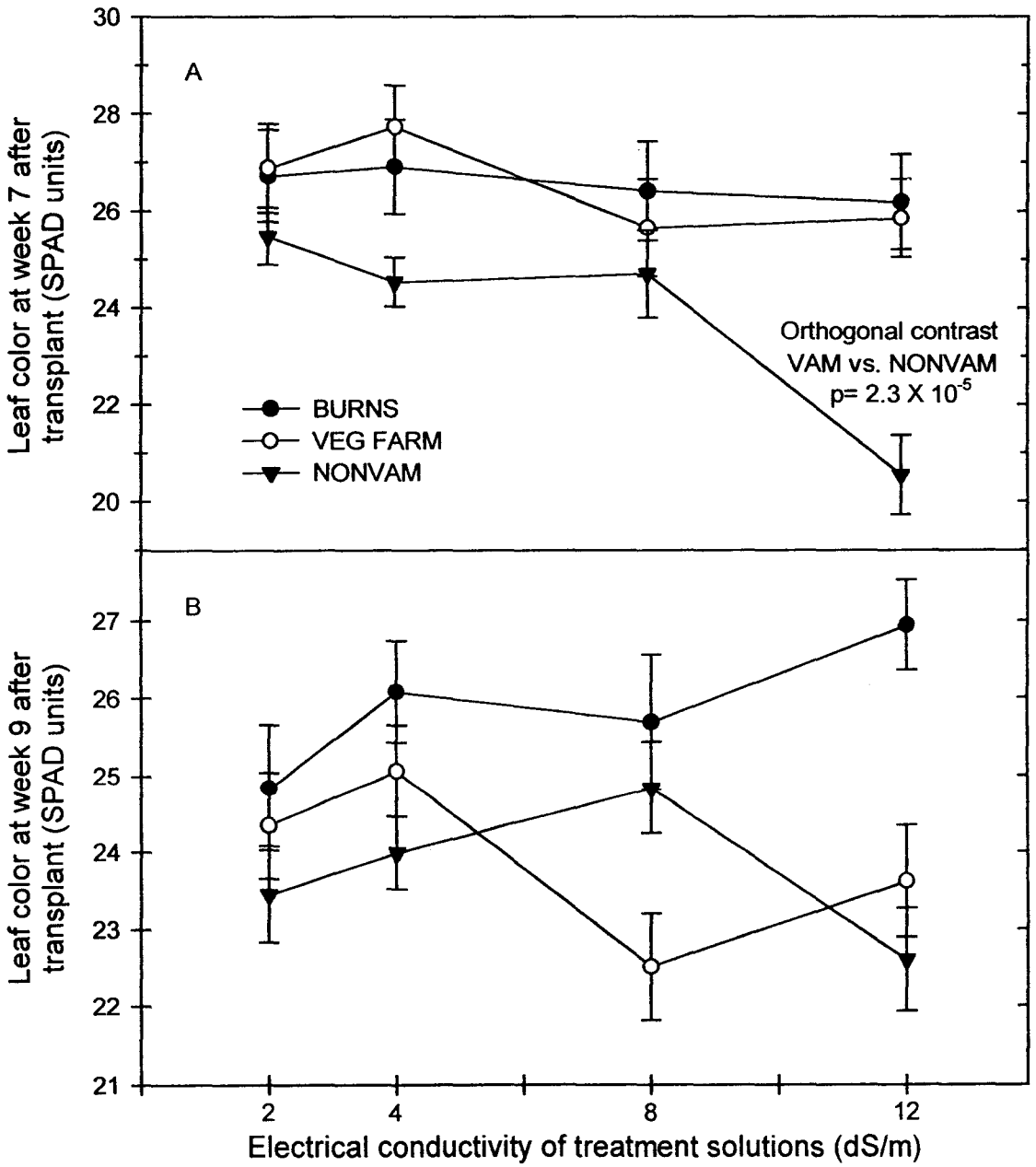
leaves (Figure 3.5A) of the VAM lettuce in the highest salt treatment was significantly different from that of the nonVAM plants (orthogonal contrast, $p < 0.001$). Leaf color means of the lettuce inoculated with Burns and Veg Farm VAM fungi at this salt level were compared separately to the mean of the nonVAM plants ($p = 0.0004$ and 0.0007, respectively). Greener leaves, as expressed by a higher number, occurred with the Burns inoculum treatments with a mean of 26.18 SPAD units (means were 25.85 and 20.55 for the lettuce plants inoculated with Veg Farm VAM fungi and the nonVAM lettuce, respectively). No significant differences were observed between the two VAM source treatments. No significant differences in leaf color were found in the new leaves at week 7. At week 9 (Figure 3.5B), the difference between leaf color in VAM and nonVAM mature lettuce leaves changed with salt (two-way ANOVA interaction, $p = 0.019$), but the main effect of salt was not significant. Leaf color of VAM lettuce plants was significantly different from that of the nonVAM lettuce at the highest salt level ($p < 0.001$), and the lettuce plants inoculated by Burns and Veg Farm VAM fungi were significantly different from each other at this salt level (orthogonal contrast, $p = 0.001$).

The leaf color mean for the lettuce inoculated with Burns VAM fungi was significantly different from that of the ones inoculated with Veg Farm VAM fungi and the nonVAM plants ($p = 0.0040$ and 0.0003, respectively); however, the color of the latter was not significantly different from the color of the lettuce inoculated with Veg Farm VAM fungi. Leaf color means for lettuce inoculated with Burns and Veg Farm VAM fungi, and nonVAM were 26.44, 23.62, and 22.60 SPAD units, respectively. No significant differences in leaf color were found in the new leaves at week 9.

Tissue elemental composition in lettuce

VAM lettuce plants had significantly different ($p < 0.05$) shoot Ca, P, Zn, B, Cu, Mg, and Mn contents than nonVAM plants (Appendix Table A1). Use of statistical tools and criteria of analysis are described in Materials and Methods. Lettuce root tissues were not analyzed for nutrients.

Figure 3.5 Leaf color of lettuce shoots of plants inoculated with VAM fungal mixtures from Burns (high salt site), Veg Farm (low salt site), or not inoculated (nonVAM) prior to transplant in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). Nondestructive measures were taken twice during the experiment. Values are means of repeated readings on 10 replicate plants and bars are +/- SE.



Calcium (Ca) in the nonVAM lettuce shoots did not increase with salt levels as much as it did in the VAM lettuce. Ca and sodium (Na) concentrations were highly correlated ($r= 0.908$, p-value< 0.001). Shoot Ca concentration of the combined VAM lettuce plants was significantly greater than that of the nonVAM plants ($p< 0.001$). As Table 3.3A shows, nonVAM lettuce in EC 2 (control) had 8% less Ca than the VAM lettuce. At the maximum salt level, this difference was of 34%. Shoot Ca concentration of the plants inoculated with Veg Farm VAM fungi was significantly greater than that of the plants inoculated with the Burns VAM fungi ($p< 0.001$). Shoot Ca concentrations of the lettuce inoculated with Burns VAM fungi were significantly less at EC 8 and EC 12 than those inoculated with the Veg Farm VAM fungi (36 and 38% less, respectively).

Phosphorus (P) concentrations in VAM plants were higher than those of nonVAM lettuce at all salt levels (Table 3.4). Significant differences between P concentration of lettuce plants inoculated with VAM fungi also were observed ($p= 0.0001$). The averaged P concentration of combined nonVAM lettuce across salt levels was 31% lower than that of the combined VAM lettuce plants. Lettuce plants inoculated with Burns VAM fungi had 9% less P than the lettuce plants inoculated with the Veg Farm VAM fungi.

Zinc (Zn) levels in VAM plants were significantly higher than those of the nonVAM plants (orthogonal contrast, $p< 0.001$) (Table 3.3A). Zn concentrations of the plants inoculated with VAM fungi from Burns and Veg Farm sites were significantly different (orthogonal contrast, $p= 0.001$). At EC 2 and EC 4, lettuce plants inoculated with Burns VAM fungi had larger mean concentrations of Zn than either the nonVAM lettuce or the lettuce plants inoculated with the Veg Farm inoculum. At EC 8 and EC 12, the mean Zn concentration of the Veg Farm-VAM plants was greater than either the nonVAM or the Burns-VAM lettuce. In VAM lettuce, Zn concentrations increased significantly with the addition of NaCl. Zn concentrations decreased between EC 8 and EC 12 in the nonVAM lettuce. Zn concentration was highly correlated with Mg and Cu concentrations ($r= 0.922$ and 0.952, respectively).

Table 3.3 Tissue analysis of lettuce shoots for elements in which two-way ANOVAs resulted in a significant VAM fungal source X salt interaction. Part A corresponds to the orthogonal contrasts between the element concentration of nonVAM shoots and that of VAM shoots. Part B corresponds to the orthogonal contrasts between the element concentration in plants inoculated with either VAM fungal source. See text for statistical significant differences.

A *

EC trmt.soln. (dS/m)	Ca% (a)	Mg%	C%	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Log Na **
2	*- 8%	- 14%	ns	ns	+ 41%	- 33%	- 18%	-56%
4	ns	- 8%	- 1%	ns	+ 48%	not error	- 20%	-30%
8	- 36%	- 38%	ns	ns	ns	ns	- 21%	-26%
12	- 34%	- 26%	ns	- 19%	ns	- 48%	- 45%	ns

* In part A: (-) = nonVAM < VAM; (+) = nonVAM > VAM; ns = nonsignificantly different.

Table 3.3 (Continued)

B ***

EC trmt.soln. (dS/m)	Ca%	Mg%	C%	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Log Na **
2	ns	ns	ns	+ 37%	ns	ns	+ 27%	-14%
4	ns	ns	ns	+ 32%	ns	not error	+ 21%	-27%
8	- 36%	- 42%	+ 2%	- 34%	- 48%	ns	- 14%	-33%
12	- 38%	- 49%	+ 2.5%	- 32%	- 67%	- 25%	- 36%	-36%

*** In part B: (-) = Burns < Veg Farm; (+) = Burns > Veg Farm; ns = nonsignificantly different.

Table 3.4 Tissue analysis of lettuce shoots for elements in which two-way ANOVAs resulted in a nonsignificant VAM fungal source X salt interaction.

	--Orthogonal contrast--	p-value	NV < VAM	BU < VF
P%	VAM vs nonVAM	<< 0.001	31%	9%
	Burns vs Veg Farm	0.0001		
B(ppm)	VAM vs nonVAM	0.0007	7%	ns
	Burns vs Veg Farm	0.0624		
			----------Medians----------	
			NV > VAM	BU > VF
Log K%	VAM vs nonVAM	0.0005	6%	11%
	Burns vs Veg Farm	<< 0.001		
Log N%	VAM vs nonVAM	0.0352	5%	9%
	Burns vs Veg Farm	0.0048		

NV= nonVAM; BU= Burns; VF= Veg Farm

Boron (B) concentration of VAM lettuce shoots was significantly different than that of nonVAM tissue ($p= 0.001$). The average difference in B contents of nonVAM lettuce plants across salt levels was 7% lower than for combined VAM lettuce plants (Table 3.4). Shoot B concentration of lettuce inoculated with Burns VAM fungi was not different from that of lettuce plants inoculated with the Veg Farm VAM fungi ($p= 0.062$).

Copper (Cu) concentration of VAM lettuce was significantly greater than that of the nonVAM lettuce (orthogonal contrast, $p= 1.13 \text{ X } 10^{-6}$). At the maximum salt level, VAM lettuce had significantly different Cu concentration (orthogonal contrast, $p< 0.001$; $n=9$) as compared with the nonVAM lettuce (Table 3.3A). Cu increased with increased salt in VAM lettuce, but it decreased in nonVAM plants between EC 8 and EC 12. At EC 12, lettuce inoculated with Burns VAM fungi had 25% less Cu than the lettuce plants inoculated with the Veg Farm VAM fungi (Table 3.3B). However, the contrast between Cu concentration of plants treated with Burns and Veg Farm VAM sources resulted in a p-value for which significance is arguable (orthogonal contrast, $p= 0.090$; full model $n= 36$).

Magnesium (Mg) concentration of VAM lettuce was significantly different than that of the nonVAM lettuce ($p< 0.001$). Significant differences between lettuce inoculated with Burns and Veg Farm VAM fungi were also observed ($p< 0.001$). Orthogonal contrasts in each salt level showed that the nonVAM lettuce plants had significantly lower concentrations of Mg in their tissues as compared with the VAM lettuce plants. Differences ranged from 8 to 38% but did not correlate with salt levels. Statistically significant differences in Mg concentrations were observed between the VAM lettuce plants in EC 8 and EC 12. Lettuce inoculated with Burns VAM fungi had 42% at EC 8 and 49% at EC 12 less Mg than lettuce plants inoculated with Veg Farm VAM fungi. Mg concentration was highly correlated with Ca ($r= 0.965$, $p< 0.001$).

Manganese (Mn) concentration of VAM lettuce was significantly different than that of the nonVAM lettuce ($p= 0.037$). Orthogonal contrasts in each salt level showed that only at EC 12 did nonVAM lettuce plants have significantly lower amounts of Mn in their tissues as compared with the VAM lettuce plants. Mn mean concentration of the lettuce inoculated with Veg Farm VAM fungi were lower than the lettuce inoculated with Burns-

VAM fungi by 37 and 32% in EC 2 and EC 4, respectively. In contrast at EC 8 and EC 12, lettuce inoculated with Burns VAM fungi had less (34 and 32%) Mn than those inoculated witth the Veg Farm VAM fungi. Shoot Mn concentration in the Burns-VAM lettuce decreased as salt increased. The salt-Mn relationship varied in other fungal treatments.

Chloride (Cl) concentrations were not statistically different between VAM and nonVAM lettuce plants ($p= 0.674$) or between plants inoculated with different VAM fungal sources ($p= 0.160$).

Potassium (K) concentrations in VAM lettuce were statistically significantly different from those in nonVAM lettuce (orthogonal contrast, $p= 0.0005$ on log transformed data). Lettuce inoculated with Burns VAM fungi statistically differed in their shoot K concentration from those inoculated with Veg Farm VAM fungi ($p< 0.001$). No analysis by salt level was warranted since the VAM X salt interaction was not significant. The median K concentration of nonVAM lettuce was 6% greater than that of VAM lettuce plants (Table 3.4). The median K concentration of the lettuce inoculated with Burns VAM fungi was 11% greater than that of the lettuce inoculated with the Veg Farm fungi. K concentration was highly correlated with Na and N concentrations ($r= 0.911$ and 0.972, respectively).

Carbon (C) concentrations were not statistically significantly different between VAM and nonVAM lettuce except at EC 4 (Table 3.3A). At the higher salt levels, lettuce inoculated with Burns VAM fungi had 2 to 2.5% ($p= 0.039$ and 0.027) more C than plants inoculated with the Veg Farm VAM fungi (Table 3.3B).

Median nitrogen (N) concentration of nonVAM lettuce was 5% greater than the median nitrogen concentration of the VAM lettuce (orthogonal contrast, $n=36$, $p= 0.035$). The median concentration of the lettuce inoculated with Burns VAM fungi was 9% greater than that of the lettuce inoculated with the Veg Farm VAM fungi (orthogonal contrast, $n= 36$, $p= 0.005$) (Table 3.4). N concentration was highly correlated with K concentration ($r= 0.972$, p-value< 0.001).

Sodium (Na) concentrations in shoot tissue in the three lower salt levels (EC 2, EC 4, and EC 8) were significantly higher in the VAM than in the nonVAM lettuce ($p< 0.001$).

Statistical differences in Na concentration between plants colonized with the two VAM fungal inoculum sources were observed at each salt level (p-values ranged from 0.01 to 5.95 X 10^{-5}). VAM lettuce plants absorbed greater amounts of Na in their tissues (Table 3.5), without deleterious visible effects, than nonVAM lettuce plants. Since VAM lettuce plants had more water in their tissues than the nonVAM lettuce plants, a dilution effect could to have occurred in the VAM lettuce plants. The differences in Na concentration due to differences in plant size were adjusted by obtaining estimates of the water content in each pooled sample and calculating the Na concentration (mmol Na L^{-1}) in that amount of water. Mean molar concentrations of the combined VAM lettuce plants also were greater than those of the nonVAM lettuce plants. Therefore, greater Na content of VAM lettuce was not due to the greater dilution in larger plants. Total content (concentration times mass) of elements is relevant since it helps integrating the size differences into the treatment comparisons. Total contents (in mg or in μg $plant^{-1}$) of Ca, P, Mg, Na, Cu, Zn, and B were greater in VAM than in nonVAM lettuce shoots (Table 3.5).

Final soil EC and pH

Table 3.6 shows that soil in which nonVAM lettuce was grown had a higher mean electrical conductivity (EC) at the end of the experiment than the soil in which the VAM lettuce (Burns and Veg Farm VAM fungal sources) was grown. The tissue analysis as well as the soil EC measured at the end of the experiment confirmed that greater quantities of Na were absorbed by the VAM lettuce than the nonVAM lettuce. Soil pH decreased with the addition of NaCl (salt main effect p= 0.000). There was also a VAM fungi main effect (p= 0.002). The pH of soils inoculated with the Veg Farm fungal source at higher salt levels were more acidic than either of the other two fungal treatments. Mean pH was ~6.3 at EC 2 and ~5.4 at EC 12.

Table 3.5 Total content per plant (dry matter times concentration) of Ca, P, K, Mg, Na, Cu, Zn, and B, and Na molar concentration in lettuce shoots as influenced by salinity when inoculated with VAM fungal mixtures or not inoculated. Fungal treatments were: Burns or Veg Farm (VAM fungi from a saline and a nonsaline soil, respectively), or not inoculated (nonVAM). Plants were grown in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). Values are means of three replicate tissue samples.

VAM inoculum source	EC trmt. soln. (dS/m)	mg/plant					mmolNa/L *	ug/plant			Water (ml)
		Ca	P	K	Mg	Na		Cu	Zn	B	
Burns	2	55	12	131	22	7	7.5	41	304	178	42.3
Veg Farm	2	53	13	116	22	9	9.2	38	233	172	41.3
NonVAM	2	48	8	128	19	5	5.5	25	214	152	39.3
Burns	4	52	11	144	22	19	18.9	40	314	177	44.5
Veg Farm	4	55	13	137	23	26	25.6	41	258	173	43.7
NonVAM	4	47	8	146	19	16	17.0	25	211	153	40.2
Burns	8	62	10	187	27	41	39.1	47	346	190	45.1
Veg Farm	8	88	11	154	42	49	47.2	48	362	160	44.8
NonVAM	8	49	7	162	19	31	33.2	38	249	149	40.9
Burns	12	54	7	163	20	43	58.6	34	232	124	32.2
Veg Farm	12	83	8	140	37	56	68.9	43	346	122	35.4
NonVAM	12	39	3	106	13	33	63.1	14	113	78	22.7

* concentration in relation to water in tissue sample

Table 3.6 Electrical conductivity (EC) of soil treated with NaCl solutions before transplanting of lettuce seedlings and measured at the end of the experiment. Fungal treatments were: Burns (VAM fungi from a saline soil), Veg Farm (VAM fungi inoculum from a nonsaline soil), or a noninoculated treatment (nonVAM). Values are means of 10 replicate soils (+/-SE).

	Burns		Veg Farm (dS/m)		NonVAM	
EC(t)*	EC(e)**		EC(e)		EC(e)	
2	0.18	(0.05)	0.15	(0.02)	0.17	(0.02)
4	0.32	(0.02)	0.28	(0.02)	0.45	(0.04)
8	2.71	(0.08)	2.47	(0.15)	3.06	(0.15)
12	6.66	(0.39)	6.47	(0.23)	8.32	(0.29)

*EC(t) electrical conductivity of treatment solutions applied to soil once prior to transplant.
**EC(e) electrical conductivity of soil extract at end of experiment.

Extraradical hyphal length in lettuce experiment

Salt had a significant effect on VAM fungal extraradical hyphal length (main effect p-value= 0.0132), but the interaction between VAM fungal source and salt was not significant (p-value= 0.5646). There were no significant differences between the VAM fungal inoculum source treatments (orthogonal contrast, p= 0.234). Hyphal lengths in the inoculated soils treated with four levels of salt measured at the end of the experiment are shown in Table 3.1.

At the maximum salt level, hyphal length of the Burns VAM fungi was reduced by 34.3% (p= 0.176) with respect to the control, while hyphal length of the Veg Farm VAM fungi was reduced by 41.0% (p= 0.060).

Differences in hyphal length between VAM fungal sources at each salt level were not statistically significant. More sensitive methods would be needed to detect differences between salt effects at the different levels on the fungal symbionts.

Experiment 2

Shoot fresh and dry mass of onion

In contrast with the lettuce experiment, colonization percent of onion roots differed between the two VAM fungal inoculum sources. Root colonization percent by the Burns VAM fungi was significantly higher than that by the Veg Farm VAM fungi at all salt treatments (Table 3.7). Therefore, the plant responses measured and discussed below may have been influenced by the different degrees of root colonization.

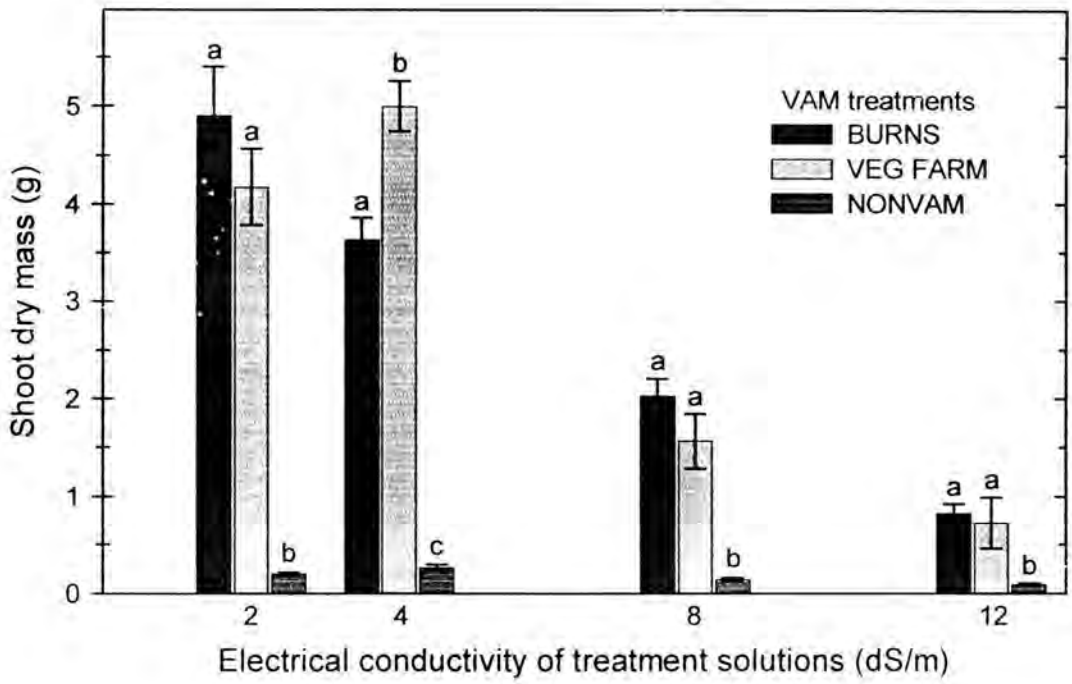
Increasing salt concentration progressively reduced shoot fresh and dry mass. Because trends in fresh and dry shoot mass were similar at each salt level, only dry shoot mass is shown (Figure 3.6). As the graph indicates, the mean shoot dry mass of the VAM-inoculated onions was six-to-eighteen fold greater than the nonVAM onions. At 12 dS/m,

Table 3.7 Percent colonization of onion roots by two VAM fungal mixtures from different sites and extraradical hyphal lengths at the end of the experiment. Plants were inoculated with VAM fungi from Burns (high salt soil) or Veg Farm (low salt soil) and grown in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). Values are percent root colonization and hyphal length mean of 10 replicate plants and (+/- SE). Within each parameter at each salt level, VAM fungal treatment means followed by the same letter are not significantly different (at $p < 0.05$) as determined by Kruskal-Wallis nonparametric test for percent root colonization and orthogonal contrasts for hyphal lengths.

	Burns				Veg Farm			
EC(t) (dS/m)	Colonization (%)		Extraradical hyphal length (m/g soil)		Colonization (%)		Extraradical hyphal length (m/g soil)	
2	61.7	(3.0)a	7.10	(1.01)a	28.0	(3.4)b	8.72	(0.75)a
4	59.4	(2.4)a	6.96	(0.86)a	28.8	(4.5)b	7.09	(0.75)a
8	49.0	(6.2)a	8.54	(0.98)a	20.3	(1.7)b	6.00	(1.76)a
12	38.8	(5.2)a	4.97	(0.75)a	18.3	(1.8)b	5.04	(0.79)a

EC(t)= electrical conductivity of treatment solutions

Figure 3.6 Dry mass of onion shoots inoculated before transplant with VAM fungal mixtures or not inoculated and grown in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). VAM fungal treatments were: Burns (from a saline soil), Veg Farm (from a nonsaline soil), or a noninoculated control (nonVAM). Means with the same letter are not different at $p \leq 0.05$ within the same salt level. Values are means of 10 replicate plants and bars are +/- SE.



shoot dry weight of nonVAM onion plants was 88% less than that of the mean VAM plants combined from the Veg Farm and Burns inocula (Figure 3.7).

In order to statistically analyze the data characterized by uneven variances of the fungal treatments, the Kruskal-Wallis nonparametric analysis of variance was used. When all salt levels were pooled, the test confirmed that at least one out of the three fungal treatments resulted in a different shoot dry weight (Table 3.8, overall values). The same test was repeated at each salt level including all three fungal treatments.

To keep consistency in the analysis and achieve the objective of comparing the effects of each one of the VAM fungal mixtures, another series of Kruskal-Wallis tests was performed leaving out the nonVAM treatment. As shown in Table 3.9, there were no statistical significant differences between shoot dry weights of onions inoculated by the Burns and Veg Farm VAM fungi except at EC 4.

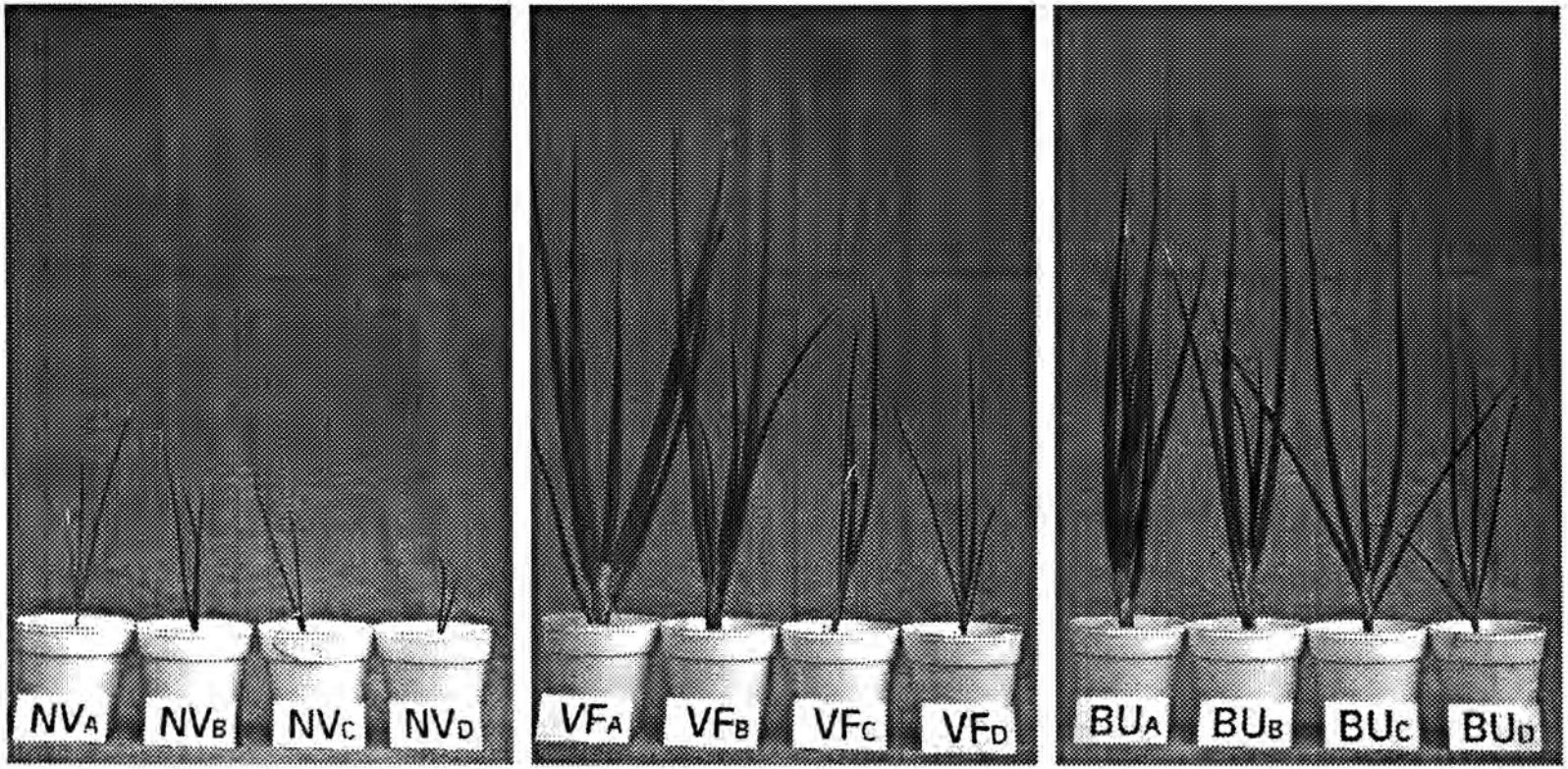
Table 3.8 Kruskal-Wallis analysis of variance by VAM fungal source (three treatments) of shoot dry weight of onion exposed to four different concentrations of NaCl.

EC(t) (dS/m)	df	Chi-square	p-value
2	2	13.1	0.0014
4	2	16.8	0.0002
8	2	13.1	0.0014
12	2	16.8	0.0002
overall	2	60.2	0.0000

Table 3.9 Kruskal-Wallis analysis of variance by VAM fungal source (2 treatments) of shoot dry weight of onions exposed to four different concentrations of NaCl.

EC(t) (dS/m)	df	Chi-square	p-value
2	1	0.0	1.0000
4	1	7.2	0.0073
8	1	0.8	0.3711
12	1	3.2	0.0736
overall	1	0.2	0.6547

Figure 3.7 Photograph of representative onion plants grown in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). Fungal treatments were: NV= nonVAM, VF= Veg Farm VAM fungi, or BU= Burns VAM fungi. At 12 dS/m, shoot dry weight of nonVAM onion plants was 88% less than that of the mean VAM plants combined from the Veg Farm and Burns inocula.



Root mass of onion

Onion fresh root mass was analyzed using the Kruskal-Wallis nonparametric test. Results of analysis are not shown since the trends in this plant response were similar to those of root dry mass.

Root dry mass of VAM onions was significantly greater than that of nonVAM plants in all salt levels (Figure 3.8). Uneven variances were observed in the root dry mass of each fungal treatment within a salt level, and the Kruscal-Wallis Test was used to analyze the data. The test confirmed that at least one out of the three fungal treatments resulted in a different root dry weight when all salt levels were pooled (Table 3.10, overall values). The same test was repeated at each salt level including all three fungal treatments.

To keep consistency in the analysis and achieve the objective of comparing the effects of the Burns and the Veg Farm VAM inoculum sources, another series of Kruskal-Wallis tests was performed leaving out the nonVAM treatment within each salt level. As shown in Table 3.11, there were no statistically significant differences between root dry weights of onions inoculated by the Burns or Veg Farm VAM fungi. The mean root dry mass of combined VAM-inoculated onions was three-to-six fold greater than the mean root dry mass of the nonVAM onions.

Percent VAM colonization of onion root

At transplant, the initial colonization percents were estimated to be 30% for the onion roots inoculated with Veg Farm VAM fungi and 22% for those inoculated with the Burns VAM fungal source (10 replicate seedlings for each inoculum, one-way ANOVA p= 0.112). VAM percent colonization by the two inocula significantly decreased with increased salinity (Table 3.7). Moreover, the percent colonization of both VAM inoculum

Figure 3.8 Root dry mass of onion plants inoculated before transplant with VAM fungal mixtures or not inoculated and grown in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). VAM fungal treatments were: Burns (from a saline soil), Veg Farm (from a nonsaline soil), or a noninoculated control (nonVAM). Means with the same letter are not different at $p \leq 0.05$ within the same salt level. Values are means of 10 replicate onion plants and bars represent +/- SE.

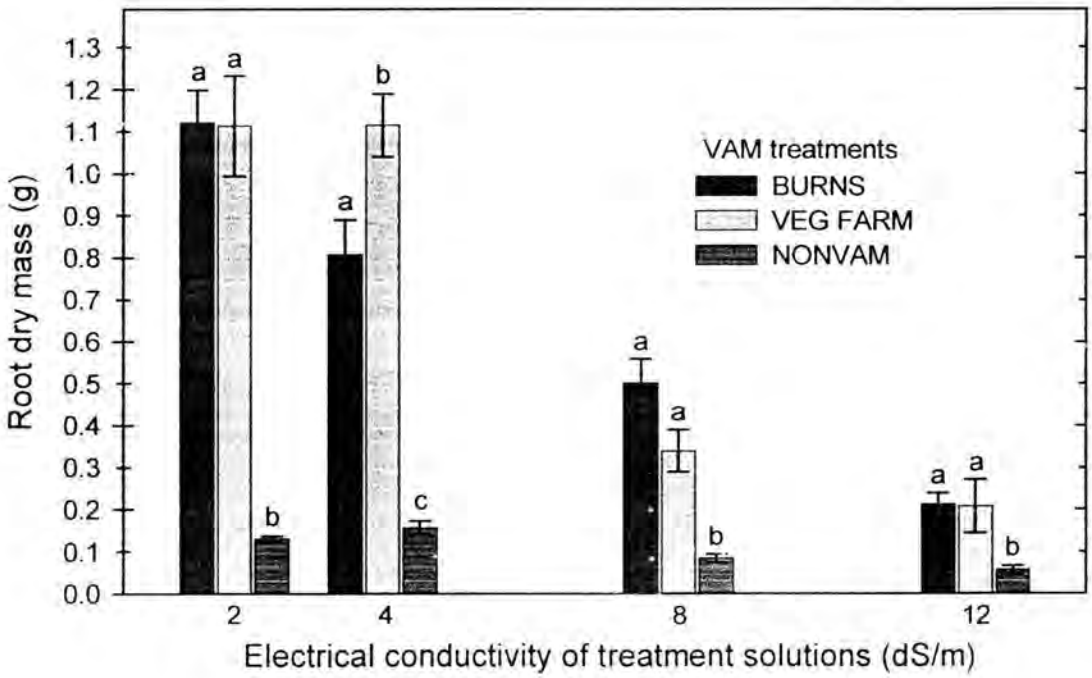


Table 3.10 Kruskal-Wallis analysis of variance by VAM fungal source (three treatments) of root dry weight of onions exposed to four different concentrations of NaCl.

EC(t) (dS/m)	df	Chi-square	p-value
2	2	15.2	0.0005
4	2	20.0	0.0000
8	2	16.8	0.0002
12	2	16.8	0.0002
overall	2	60.8	0.0000

Table 3.11 Kruskal-Wallis analysis of variance by VAM fungal source (2 treatments) of onion root dry weight. Plants were exposed to four different concentrations of NaCl.

EC(t)	df	Chi-square	p-value
2	1	0.8	0.3711
4	1	3.2	0.0736
8	1	0.0	1.0000
12	1	3.2	0.0736
overall	1	0.0	1.0000

sources within each salt level was different at harvest (p-values ranged from 0.0003 to 0.0073 among the four salt levels, Kruskal-Wallis nonparametric test). The root percent colonization of the onions inoculated with the Burns VAM fungi inoculum at EC 12 was reduced by 37.1% (p= 0.007) with respect to the control (EC 2), and the root percent colonization of the Veg Farm inoculum was reduced by 34.6% (p= 0.007). At the highest salt level (EC 12), the Burns VAM fungi were better colonizers (by two fold) than the VAM fungi from the Veg Farm. No VAM colonization was observed in control pots.

Estimated root length of onion and its colonized fraction

Root length of onion was significantly affected by salt (Table 3.12). At harvest, onion roots inoculated with VAM fungal inoculum from Burns and the Veg Farm sites and treated with the highest salt level were shorter by 84.6% (p= 0.0000) and 66.1% (p=

Table 3.12 Total final root length, VAM colonized root length, and percent VAM colonization of onion plants preinoculated with two VAM fungal mixtures from different sites Burns (high salt) or Veg Farm (low salt) and grown in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). Values are means of 10 replicate plants and (+/- SE). Within each parameter at each salt level, VAM fungal means followed by the same letter are not significantly different (at $p < 0.05$) as determined by Kruskal-Wallis nonparametric test.

EC(t)	Estimated root length/pot (m)				Colonized root length (m)				Colonization %			
(dS/m)	Burns		Veg Farm		Burns		Veg Farm		Burns		Veg Farm	
2	125	(11)a	26	(2)b	76	(7)a	7	(1)b	61.7	(3.0)a	28.0	(3.4)b
4	86	(9)a	29	(4)b	51	(6)a	8	(2)b	59.4	(2.4)a	28.8	(4.5)b
8	43	(6)a	20	(2)b	24	(6)a	4	(1)b	49.0	(6.2)a	20.3	(1.7)b
12	19	(3)a	9	(2)b	9	(2)a	2	(0.4)b	38.8	(5.2)a	18.3	(1.8)b

EC(t)= electrical conductivity of treatment solutions

0.0003), respectively, compared with their corresponding controls (EC 2). At each salt level, root length of onions inoculated with Burns VAM fungi was significantly greater than for plants inoculated with Veg Farm VAM fungi.

Root length colonized by both VAM inoculum sources within each salt level was statistically significantly different at harvest (p-value= 0.0000, 0.0003, 0.0073, and 0.0118 at EC 2, EC 4, EC 8, and EC 12, respectively. Kruskal-Wallis nonparametric test). At harvest, the root length colonized by the Burns VAM fungal inoculum at the highest salt level was 80.8% greater (or 4.2 times larger) than that colonized by the Veg Farm VAM fungal inoculum.

Shoot length, shoot number per plant, and shoot diameter

VAM onions had longer shoot lengths (Figure 3.9), more shoots per plant (Figure 3.10), and thicker shoot diameters at their bases (Figure 3.11) than did the nonVAM onions. For the most part, at all salt levels, changes started to be obvious by week 5. At times the low salt levels differences were apparent as early as week 3.

Tissue elemental composition in onion

Results of root and shoot analyses are shown in Table 3.13. Roots of VAM plants had greater P, Cu, and Na concentrations (Table 3.13A) and also greater Fe concentrations (except at the highest salt level) than those of nonVAM plants. In contrast, K and B concentrations of VAM onion roots were lower than those of nonVAM onion roots. N concentration of VAM roots was lower at EC 2 and EC 4 compared to that of nonVAM onion roots. The concentrations of other elements did not reflect a difference between VAM and nonVAM onion roots that remained consistent for all salt levels.

Figure 3.9 Shoot length of preinoculated onion plants with VAM fungi or not inoculated and grown for 10 weeks in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). VAM fungal treatments were: Burns (VAM fungi from a saline soil), Veg Farm (from a nonsaline soil), or a noninoculated control (nonVAM). Values are means of 10 replicate plants and bars are +/- SE.

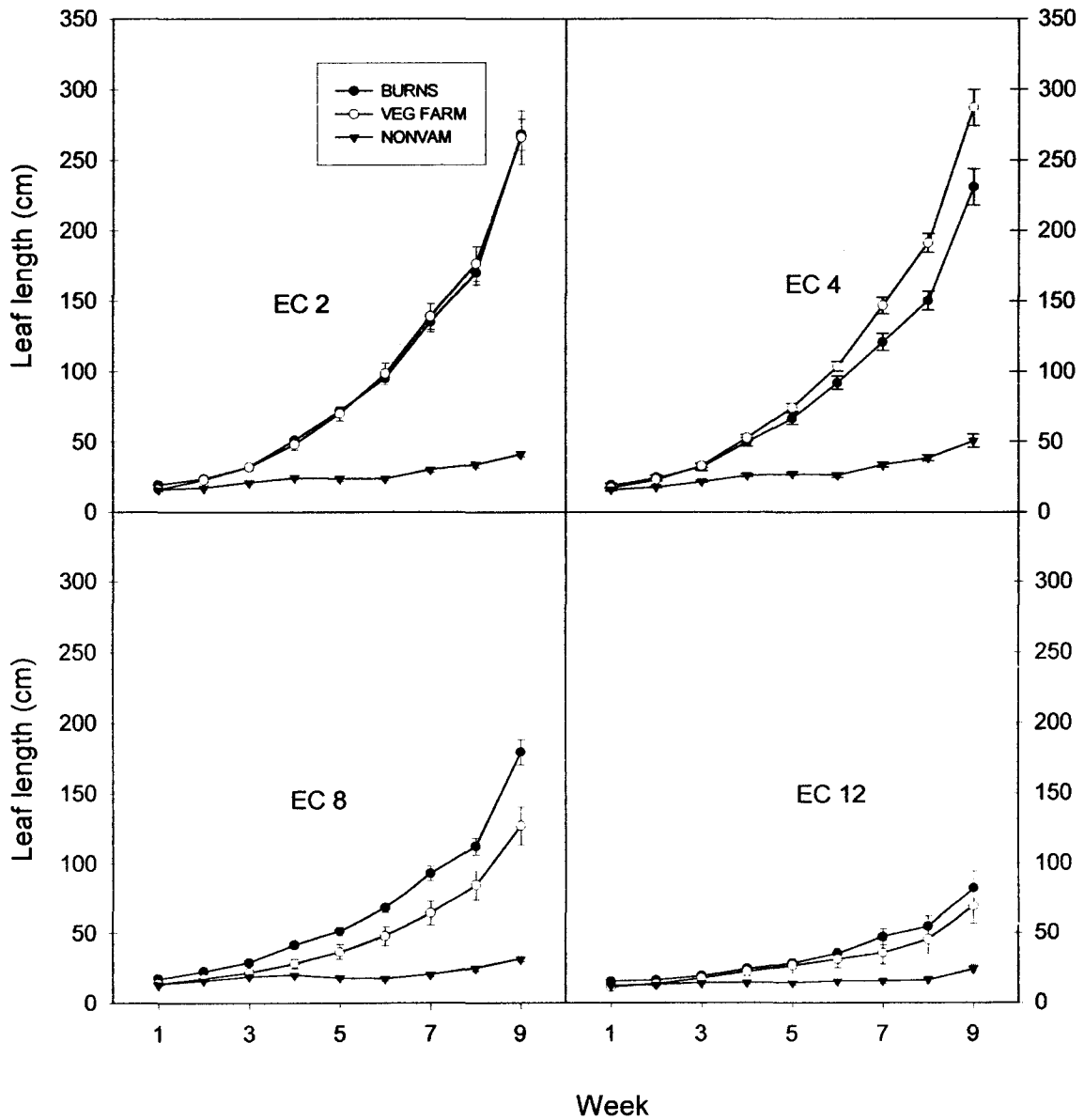


Figure 3.10 Number of leaves per onion plant preinoculated with VAM fungi or not inoculated and grown for ten weeks in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). VAM fungal treatments were: Burns (VAM from a saline soil), Veg Farm (from a nonsaline soil), or a noninoculated control (nonVAM). Values are means of 10 replicate plants and bars are +/- SE.

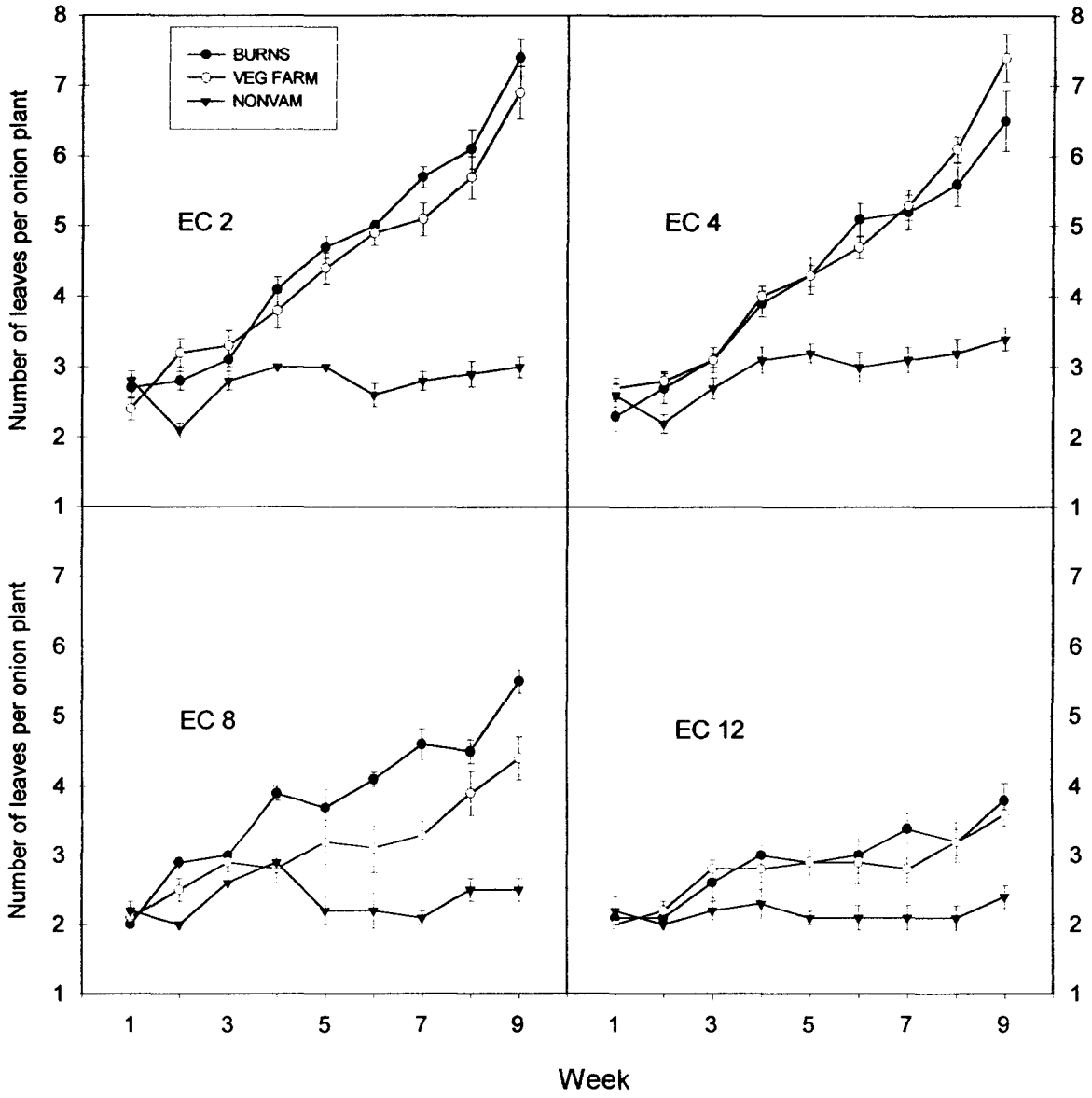


Figure 3.11 Diameter of shoot bases of onion plants preinoculated with VAM fungi or not inoculated and grown for ten weeks in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). VAM fungal treatments were: Burns (VAM from a saline soil), Veg Farm (from a nonsaline soil), or a noninoculated control (nonVAM). Values are means of 10 replicate plants and bars are +/- SE.

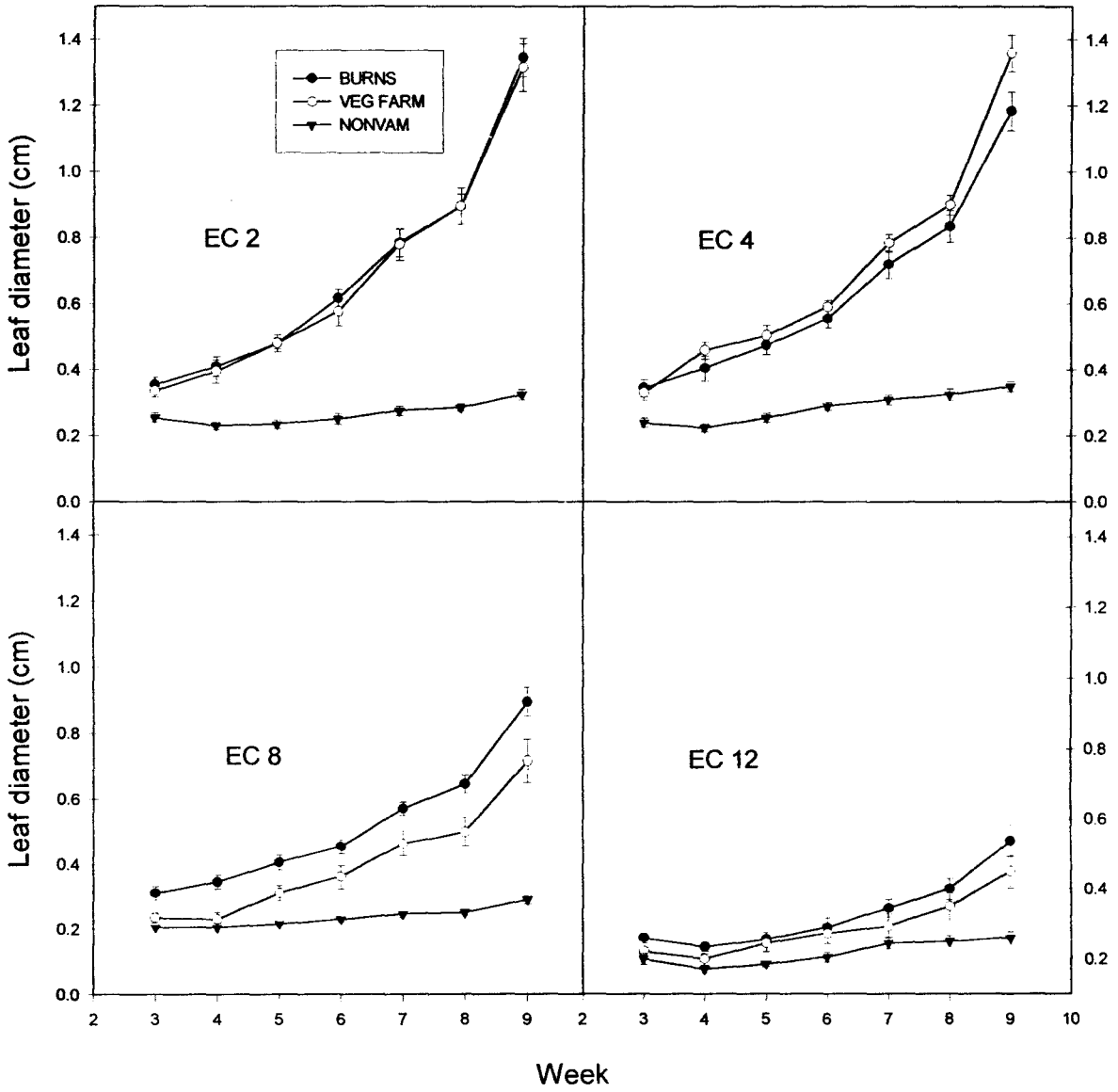


Table 3.13 Concentrations of minerals in onion roots (A) and shoots (B) as influenced by preinoculation with VAM fungal mixtures compared with no inoculation and the additions to the soil of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). Fungal treatments were: Burns (high-salt site), Veg Farm (low-salt site), or not inoculated (nonVAM). Samples were composed of the 10 plants in each treatment.

A

VAM fungi source	EC(t) (dS/m)	P%	K %	Ca %	Mg %	Mn ppm	Fe ppm	Cu ppm	B ppm	Zn ppm	Na ppm	Cl ppm	C%	N%
BU	2	0.31	1.60	0.8	0.57	233	3057	71	42	82	6206	1896	42	2.66
VF	2	0.30	1.44	0.59	0.46	164	2523	50	31	34	5575	1514	42.7	2.32
NV	2	0.11	3.34	1.05	0.69	178	2306	14	55	26	1204	6341	40.5	3.82
BU	4	0.32	1.79	0.82	0.65	183	2467	56	34	89	12707	15147	40.7	3.43
VF	4	0.27	1.62	0.67	0.50	185	3032	45	33	45	11901	17155	40.8	2.38
NV	4	0.11	3.46	0.99	0.57	146	2004	13	51	31	2449	12412	40.8	4.00
BU	8	0.33	2.66	0.67	0.63	173	2304	47	45	95	16093	25430	40.0	3.89
VF	8	0.33	2.93	0.62	0.58	202	2511	62	50	50	15834	24164	39.1	3.94
NV	8	0.10	3.05	0.87	0.62	155	2012	11	70	33	6752	16288	41.1	3.78
BU	12	0.31	3.12	0.58	0.61	181	1913	40	38	86	13895	28525	40.7	4.13
VF	12	0.29	2.88	0.61	0.57	196	2771	47	43	48	16296	24360	38.9	3.78
NV	12	0.12	2.70	0.77	0.55	203	2590	12	91	35	10058	-------	40.5	3.78

BU= Burns; VF= Veg Farm; NV= nonVAM.
EC(t)= electrical conductivity of treatment solutions

Table 3.13 (continued)

B

VAM fungi source	EC(t) (dS/m)	P%	K %	Ca %	Mg %	Mn ppm	Fe ppm	Cu ppm	B ppm	Zn ppm	Na ppm	Cl ppm	C%	N%
BU	2	0.23	1.82	1.59	0.33	133	147	6	23	40	145	1612	44.0	2.85
VF	2	0.22	1.44	1.53	0.36	119	124	6	23	24	109	1535	44.6	2.45
NV	2	0.10	2.75	1.34	0.27	198	349	4	28	42	196	3942	43.3	4.59
BU	4	0.21	1.95	1.60	0.32	114	119	7	20	41	240	11122	43.5	2.74
VF	4	0.22	1.55	1.18	0.26	97	104	6	19	23	247	8721	44.4	2.28
NV	4	0.09	2.75	1.37	0.27	182	300	4	27	46	282	7210	43.2	4.52
BU	8	0.20	2.50	1.49	0.30	125	153	7	21	43	747	20376	42.8	3.38
VF	8	0.25	2.64	1.76	0.35	147	153	9	21	35	818	19736	43.2	3.40
NV	8	0.08	2.20	1.76	0.31	145	433	4	29	43	1390	17389	42.5	4.31
BU	12	0.19	2.74	1.72	0.31	136	227	7	22	45	1651	27063	42.2	3.72
VF	12	0.20	2.58	1.94	0.36	210	181	8	22	35	1205	24280	42.8	3.44
NV	12	0.08	2.12	2.00	0.38	275	640	5	30	40	3153	29369	41.1	4.34

BU= Burns; VF= Veg Farm; NV= nonVAM.
EC(t)= electrical conductivity of treatment solutions

Shoots of VAM plants also had greater concentrations of P and Cu, but not Na. Na concentration (mg Na per g of dry tissue) in VAM onion shoots was lower than that of nonVAM onion shoots (Table 3.13B). At the highest salt level, Na concentration of nonVAM onions was more than twice that of the VAM onion shoots. More will be said about root and shoot Na concentrations below.

Table 3.14 shows that the total contents of all elements analyzed (concentration times dry mass) of VAM onions (roots and shoots) were greater than those of the nonVAM onions. As in the lettuce experiment, VAM onion plants had more Na in their tissues than nonVAM plants (Tables 3.13 and 3.14). Because VAM onion plants had more water in their tissues than the nonVAM onion plants, a dilution effect could have occurred in the VAM onion plants that might have influenced concentration. The differences in Na concentration due to differences in plant size were adjusted by obtaining estimates of the water content in each pooled sample and calculating the Na concentration (mmol Na L^{-1}) in that amount of water. Molar concentrations of the combined VAM onion roots were higher than those of the nonVAM onion roots. Mean Na molar concentrations of the combined VAM onion roots were more than five fold that of nonVAM roots at EC 2 level, four fold at EC 4, two fold at EC 8, and nearly equal at EC 12 (Table 3.15). At the highest salt level, onion roots inoculated by the Veg Farm VAM fungal mixture had a larger molar Na concentration than the onion roots inoculated with the Burns VAM fungal mixture. Na molar concentrations of onion shoots inoculated with Burns and Veg Farm VAM fungi were similar except at the highest salt level where the shoots inoculated with Burns VAM fungi had a greater Na molar concentration. The lower the ratio between root and shoot Na molar concentration, the more these concentrations are alike. Ratios between Na molar concentration of roots and shoots were considerably lower in nonVAM onion plants compared to those ratios of the VAM onion plants. This suggests that VAM fungi may have influenced the mechanisms by which the onion plants were able to compartmentalize Na in the roots while excluding it from the shoots.

Table 3.14 Total content per plant (concentration times dry weight) of minerals in onion roots (A) and shoots (B) as influenced by preinoculation with VAM fungal mixtures compared with no inoculation and the addition to the soil of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). VAM fungal treatments were: Burns (from high salt site), Veg Farm (from low salt site), or not inoculated (nonVAM). Samples were composed of the 10 plants in each treatment.

A

VAM fungi source	EC(t) (dS/m)	P	K	Ca	Mg	C	N	Mn	Fe	Cu	B	Zn	Na	Cl
		mg/plant						ug/plant						
BU	2	3.5	17.9	9	6.4	471	29.8	261	3428	80	47	92	6960	2126
VF	2	3.3	16	6.6	5.1	475	25.8	183	2809	56	35	38	6207	1686
NV	2	0.1	4.4	1.4	0.9	53	5	23	301	2	7	3	157	827
BU	4	2.6	14.5	6.6	5.3	329	27.7	148	1993	45	27	72	10263	12234
VF	4	3	18.1	7.5	5.6	455	26.5	206	3379	50	37	50	13261	19116
NV	4	0.2	5.4	1.6	0.9	64	6.3	23	315	2	8	5	385	1950
BU	8	1.7	13.4	3.4	3.2	201	19.5	87	1157	24	23	48	8079	12766
VF	8	1.1	10	2.1	2	133	13.4	69	854	21	17	17	5384	8216
NV	8	0.1	2.6	0.7	0.5	35	3.2	13	169	1	6	3	567	1368
BU	12	0.7	6.7	1.2	1.3	87	8.8	39	409	9	8	18	2968	6093
VF	12	0.6	6	1.3	1.2	81	7.9	41	577	10	9	10	3393	5072
NV	12	0.1	1.5	0.4	0.3	23	2.2	12	148	1	5	2	573	----

BU= Burns; VF= Veg Farm; NV= nonVAM.
EC(t)= electrical conductivity of treatment solutions

Table 3.14 (continued)

VAM fungi source	EC(t) (dS/m)	P	K	Ca	Mg	C	N	Mn	Fe	Cu	B	Zn	Na	Cl
		mg/plant						ug/plant						
BU	2	11.3	89.3	78	16.2	2159	139.8	653	721	29	113	196	712	7910
VF	2	9.2	60.1	63.9	15	1862	102.3	497	518	25	96	100	455	6408
NV	2	0.2	5.5	2.7	0.5	87	9.2	40	70	1	6	8	39	792
BU	4	7.6	70.8	58.1	11.6	1579	99.5	414	432	25	73	149	871	40384
VF	4	11	77.5	59	13	2220	114	485	520	30	95	115	1235	43596
NV	4	0.2	7.3	3.7	0.7	115	12.1	49	80	1	7	12	75	1925
BU	8	4.1	50.8	30.2	6.1	869	68.6	254	311	14	43	87	1516	41363
VF	8	3.9	41.3	27.6	5.5	676	53.2	230	240	14	33	55	1281	30897
NV	8	0.1	3.2	2.5	0.4	61	6.2	21	62	1	4	6	200	2504
BU	12	1.6	22.5	14.1	2.5	346	30.5	112	186	6	18	37	1354	22192
VF	12	1.5	18.7	14.1	2.6	311	25	153	132	6	16	25	876	17644
NV	12	0.1	1.9	1.8	0.3	37	3.9	25	58	0.46	3	4	287	2673

BU= Burns; VF= Veg Farm; NV= nonVAM.
EC(t)= electrical conductivity of treatment solutions

Table 3.15 Total content per plant (dry matter times concentration) of Na in onion roots and shoots as influenced by preinoculation with VAM fungal mixtures compared with no inoculation and the addition to the soil of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). VAM fungal treatments were: Burns (high salt site), Veg Farm (low salt site), or not inoculated (nonVAM). Samples were composed of the 10 plants in each treatment.

		Roots			Shoots			Ratio of
VAM fungi source	EC(t) (dS/m)	mg Na/plant	mmolNa/L (1)	Water (mL)	mg Na/plant	mmolNa/L *	Water (mL)	molar conc. root/shoot
BU	2	7.0	29.7	10.2	0.71	0.91	34.2	33
VF	2	6.2	27.5	9.8	0.45	0.60	33.2	46
NV	2	0.2	5.7	1.2	0.04	1.55	1.1	4
BU	4	10.3	54.4	8.2	0.87	1.30	29.2	42
VF	4	13.3	55.2	10.5	1.23	1.46	36.7	38
NV	4	0.4	11.3	1.5	0.08	2.30	1.4	5
BU	8	8.1	68.7	5.1	1.52	4.00	16.5	17
VF	8	5.4	58.3	4.0	1.28	4.67	11.9	12
NV	8	0.6	30.2	0.8	0.20	12.21	0.7	2
BU	12	3.0	56.5	2.3	1.35	10.46	5.6	5
VF	12	3.4	73.7	2.0	0.88	7.66	5.0	10
NV	12	0.6	42.8	0.6	0.29	25.63	0.5	2

BU= Burns; VF= Veg Farm; NV= nonVAM.

* concentration in relation to water in tissue sample

EC(t)= electrical conductivity of treatment solutions

Final soil EC and pH

As Table 3.16 shows, the electrical conductivity (EC) of soil not inoculated with VAM fungal mixtures was significantly higher than that of the VAM-inoculated soils (p-values <0.01, Kruskal-Wallis nonparametric test) at the end of the experiment. The test confirmed that at least one out of the three fungal treatments resulted in a different soil electrical conductivity as each salt level was analyzed. Final electrical conductivity of the noninoculated soil was about 18, 6, 3 and 1 times as large as the electrical conductivity of the combined VAM-inoculated soils as they were treated once with NaCl solutions of 2, 4, 8, and 12 dS/m, respectively. VAM fungi may have indirectly reduced the salt contents in the soil by facilitating greater absorption of salt (minerals) into onion roots.

The same test was repeated at each salt level comparing only the two VAM inoculum sources. Electrical conductivity of soils treated with two VAM fungal sources mixtures were not different (p-values> 0.05, Kruskal-Wallis nonparametric test).

Soil pH decreased with the addition of NaCl at EC 8 and EC 12 (5.7), irrespective of fungal treatment, but this effect of salt was somewhat offset by VAM at EC 4 (VAM= 6.3, nonVAM= 5.7). Soil inoculated with the Burns VAM fungi had a mean pH of 6.5 at EC 2 and 6.34 at EC 4. Similarly, soil that received the Veg Farm VAM inoculum had a mean pH of 6.4 at EC 2 and 6.2 at EC 4. Noninoculated soil had a mean pH of 5.8 at EC 2 and 5.7 at EC 4.

Table 3.16 Electrical conductivity of soil treated with NaCl solutions before transplant of onion seedlings and measured at the end of the experiment. Values are means of 10 replicate soils.

	Burns		Veg Farm		NonVAM	
EC(t)*	EC(e)**	SE	EC(e)	SE	EC(e)	SE
(dS/m)			(dS/m)		(dS/m)	
2	0.12	0.02	0.24	0.05	3.25	0.10
4	0.99	0.16	0.51	0.09	4.63	0.11
8	5.90	0.33	7.44	0.68	8.60	0.17
12	11.49	0.18	10.86	0.68	12.95	0.12

*EC(t) electrical conductivity of treatment solutions applied to soil once prior to transplant.
**EC(e) electrical conductivity of soil extract at end of experiment.

Extraradical hyphal length in onion experiment

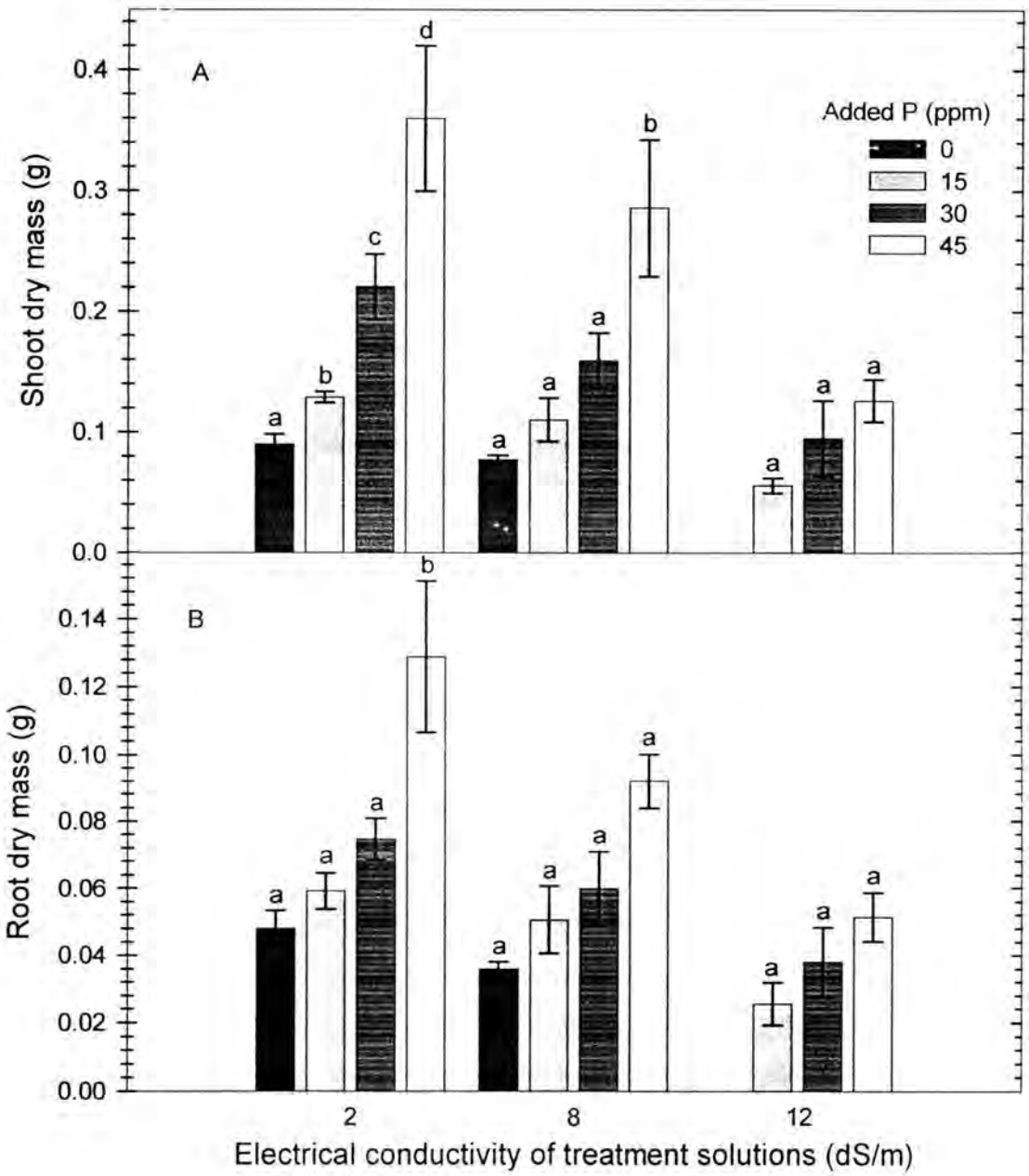
VAM fungi and salt main effects on VAM fungal hyphal length were not significant ($p= 0.296$ and 0.062). The interaction between VAM fungi and salt was not significant (p-value= 0.272). There were no significant differences in extraradical hyphal length between the VAM fungal source treatments (orthogonal contrast, $p= 0.810$). VAM fungal hyphal lengths in the inoculated soils treated with four levels of salt are shown in Table 3.7. At the highest salt level, hyphal length of the Burns VAM fungi was reduced by 30.0% ($p= 0.200$) with respect to the control (EC 2), while hyphal length of the Veg Farm VAM fungi was reduced by 42.2% ($p= 0.027$). Differences in hyphal length between VAM fungal source at each salt level were not significant ($p> 0.05$). More sensitive methods would be needed to detect differences between salt effects at the different salt levels on the fungal symbionts.

Experiment 3

Dry shoot and root mass of not inoculated onions. Increasing salt concentration progressively reduced onion shoot fresh and dry mass. Since trends in fresh and dry shoot mass were similar at each salt level, only dry shoot mass is shown (Figure 3.12A).

Significant differences in shoot dry mass were observed at each P level in the absence of NaCl (EC 2). This indicated that in the absence of salt onion shoots responded to each level of P fertilization. At EC 8, only onion shoots treated with the highest P level (45 ppm P) were significantly larger than the rest. As the graphs indicate (Figure 3.12), the mean shoot and root dry mass of onions treated with 45 ppm P were two-fold greater than those of the onions treated with 15 ppm P at the highest salt level (EC 12). At this salt level, all onions without additional P died. At the maximum salt level no significant differences in shoot and root weights were observed independent of the P level. This suggested that 45 ppm P was not sufficient to overcome the growth reduction caused by high salt levels

Figure 3.12 Dry shoot (A) and root (B) of onion plants not inoculated with VAM fungi, grown at four levels of P (0,15, 30, and 45 ppm P) and in soil treated with three levels of NaCl solutions (EC 2 control, EC 8, and EC 12 dS/m). Means with the same letter are not different at $p \leq 0.05$ within the same salt level. Values are means of 10 replicate onion plants and bars represent +/- SE.



(EC 12). It was only at the intermediate salt level (EC 8) that 45 ppm P added to the experimental soil offset salt effects in onion shoots. The only significant difference observed in root dry mass was at EC 2 and under high P treatment (Figure 3.12B).

CHAPTER FOUR.
DISCUSSION AND CONCLUSION

The finding that lettuce and onion plants preinoculated with VAM fungi and grown in saline soil had greater fresh and dry shoot mass than noninoculated plants (Figures 3.1, 3.2, .3.6, and 3.7) supports our first hypothesis that preinoculated VAM plants yield better than nonVAM plants under saline conditions. Mortality of lettuce and onions was low under these experimental conditions and it was not treatment-related.

Inoculation of transplants prior to salt exposure bypasses the inhibitory effects that salt could have on VAM fungal spore germination and the ability of VAM hyphae to locate and colonize plant roots in a saline soil environment. These inhibitory effects have been reported previously (McMillen et al., 1998; Koske et al., 1996; Juniper and Abbott, 1993). The establishment of the mutualistic symbiosis prior to salt exposure gives the plants physiological advantages that could represent increased yields under saline conditions. In the past, researchers have studied VAM effects on plants under saline conditions, but only few adjusted the inocula used in order to eliminate significant differences in VAM root colonization, and all studies have progressively added saline solutions to soil after some time allowed for establishment. Under field conditions, salt is rarely an adjustable variable. Techniques developed in this study can be of practical importance in the cultivation of many horticultural crops as well as in reclamation projects. These techniques can be implemented even within a low-budget system since VAM fungi are ubiquitous in most soils. If the preinoculation technique were utilized in agriculture or restoration projects, it would be necessary to monitor root colonization through time under field conditions as results evolve.

Several studies have shown that VAM fungi mitigate the growth reduction caused by increased levels of salt in the soil (Hirrel and Gerdemann, 1980; Ojala et al., 1983; Pond et al., 1984; Poss et al., 1985; Pfeiffer and Bloss, 1988; Gupta and Krishnamurthy, 1996; Tsang and Maun, 1999). Only few of these studies offered an explanation of the mechanism involved. Poss (1985) concluded that the salt-tolerance mechanism in VAM

onion is primarily related to P nutrition. Similarly, Pfeiffer and Bloss (1988) indicated that "the major effect of the mycorrhiza on sodium uptake is through mediation of phosphorus accumulation." Other mechanisms that improve salt-tolerance and that may be altered by VAM fungi could include cell membrane altered permeability, which facilitates cell effective compartmentation and prevents root cytoplasmic leakage, balanced nutrition, selective ion intake, and induction of osmotica such as proline.

Summarizing the effects of NaCl on root cells helps explain why P nutrition is relevant in plant salt-tolerance. Multiple changes occur in structures and functions of epidermal and cortical cells of ion-excluding plants under NaCl salinity (Koyro, 1997). These include an increase in (1) deposition of polysaccharides in cell wall, (2) build-up of transfer cells, and (3) number of mitochondria and vesicles. These changes suggest an increased supply of energy for osmotic adjustment and for selective uptake and transport processes. Active transport processes, which maintain ion compartmentation and fuel protein synthesis, are two of the most costly energy sinks in a plant (Penning De Vries, 1975). As the concentration of Na^+ increases in the apoplast (by simple diffusion), and the symplast (by facilitated diffusion), cells dehydrate to reach equilibrium with the ionic concentration of the environment (osmotic adjustment), thus loosing turgor pressure. The uptake of essential nutrient ions is disturbed and ionic imbalance occurs. According to Koyro (1997), the densities of cytoplasm, mitochondrial and nucleoplasm matrices decrease under NaCl salinity. Perhaps this is due to leaking membranes. Permeability of root membranes has been measured by K^+ efflux (Ratnayake et al., 1978) and has been shown to be highly dependent on P nutrition (Graham et al., 1981). Ratnayake et al. (1978) showed that larger quantities of K^+ were lost from roots grown in soil with low P. Graham et al. (1981) found that root exudates (as measured by the amounts of reducing sugars and amino acids present) increased with low or zero P added to the soil. Their data support the hypothesis that improved P nutrition decreases membrane permeability as shown by a decline in root exudation.

P contents of VAM onion and lettuce plants in our experiments were higher than those of nonVAM plants (Tables 3.4, 3.5, 3.13, and 3.14). We do not know if the P levels in VAM roots and shoots were optimal for these plant species (given that they were grown

in soil with low available P). Nevertheless, increased growth compared to nonVAM plants is evidence of the beneficial effects of VAM fungi on plants growing in salinized soil. The ability to protect the plants from salt stress was correlated with the growth promoting effect of VAM fungi. Plants under salt stress can remain alive, although not actively growing, as long as they are able to selectively absorb the nutrients that are most limiting. Selective ion intake may be the reason why onion plants in Experiment 3 grew only after more P was available. Growth results from adequate water absorption and balanced nutrition, and VAM fungi facilitated both (Tables 3.5, 3.14, and 3.15). Plants with sufficient P in their tissues are more efficient in maintaining cell structures and functions and growing in spite of salt stress. Salt ions taken into the cell are nonuniformly compartmented. Most ions are stored in the vacuole, thus relatively lowering the ion concentration in the cytoplasm. Consequently, the cytoplasmic enzyme systems are directly exposed to a lower concentration of salt. Ion pumps in the plasma membrane and tonoplast bring about and maintain salt compartmentation (Larcher, 1980). Reducing cell membrane permeability and increasing energy levels by providing P via VAM or inorganic fertilizer to plant cells (particularly root cells) enhances cell structural organization. As cells are able to maintain membrane integrity under saline conditions, it is possible to prevent interference of excessive ions with metabolic processes (e.g., photosynthesis). Most VAM lettuce plants had greener leaves than nonVAM plants, especially at high salt levels (Figure 3.5). This suggested that their chlorophyll synthesis (and thus photosynthesis) had not been as affected as that of nonVAM plants. Increased photosynthetic rates by VAM fungi have been reported previously (Ruiz-Lozano et al., 1996; Tsang and Maun, 1999).

We propose that the negative effects of Na^+ and Cl^- in onion and lettuce plants were reduced by increased P nutrition via VAM fungi, which resulted in maintained vacuolar membrane integrity and prevented these ions from interfering in growth metabolic pathways. In maintaining membrane integrity (Marshner, 1995), VAM fungi also participated by increasing root absorption of Zn (Tables A1, 3.5, 3.13, and 3.14). Contrary to this proposal, the evidence that the effects of VAM on tolerance to salt cannot be attributed to P nutritional differences (Ruiz-Lozano et al., 1996) was achieved by

producing control (nonVAM) plants that were not P limited. The mechanisms by which VAM fungi alleviated salt stress were involved with physiological processes (e.g., carbon dioxide exchange, transpiration, stomatal conductance, and water use efficiency) enhanced by the symbiosis. Similar conclusion (that salt alleviation by VAM is not related to improved P nutrition) was presented by Jindal et al. (1993) as they found that total P concentrations in VAM and nonVAM plants were the same.

Water absorption was greater in VAM than in nonVAM plants as expressed by the differences between fresh and dry weight (Tables 3.5 and 3.15). This effect has been previously reported (Rosendahl and Rosendahl, 1991; Rozema et al., 1986; Ruiz-Lozano et al., 1996). The increased water content of VAM plants was not as striking in lettuce as in onion plants. The final soil ECs (Tables 3.6 and 3.16) were lower than the initial level; therefore, plants absorbed the salt since it was not lost by leaching. VAM plants absorbed greater amounts of Na in their tissues (Tables A1, 3.5, 3.13, 3.14, and 3.15) than nonVAM plants. Tissue elemental composition as well as final EC confirmed that VAM plants absorbed more Na than nonVAM plants. Onion root Na concentrations were higher than shoot concentrations (Table 3.13). This suggested that Na ions were actively excluded from onion shoot tissue. A similar relationship occurred with Fe and Cu concentrations in roots and shoots. Na (content per g of dry tissue, total amount per plant, and molar) concentrations were higher in VAM onion and lettuce plants than in nonVAM plants (Tables A1, 3.5, 3.13A, 3.14A, and 3.15). Higher Na concentrations in VAM plant tissues as compared to nonVAM tissues had been reported previously (Allen and Cunningham, 1983). Pfeiffer and Bloss (1988) showed that the addition of NaCl resulted in reduced concentrations of P, Cu, and Zn. In contrast, our results showed that VAM onion and lettuce had increased P, Cu, and Zn concentrations at high levels of NaCl treatments.

According to Kuiper (1984), uptake and translocation of nutrient ions such as K^+ and Ca^{2+} are greatly reduced by salinity stress. This was true for the total K^+ and Ca^{2+} contents in onion plants (Table 3.14), but it did not occur in lettuce plants (Tables A1 and 3.5).

We observed a greater inhibitory salt effect on hyphal growth of VAM fungi isolated from a nonsaline soil than on VAM fungi from a saline soil as expected. Reduction of soil

hyphal length in the salt range used in Experiments 1 and 2 was greater for the soil hyphal length of the Veg Farm VAM fungi than for that of the Burns VAM fungi.

Percent root colonization of lettuce was not statistically different between the two VAM fungal sources at harvest (Table 3.1) irrespective of salt level, but there were statistical differences in percent root colonization of onion (Table 3.7) even after equalizing the number of VAM propagules in the fungal mixtures. These results could be due to root architecture differences between the two plant species. Nevertheless, the increase in shoot mass brought about by each VAM fungal source was statistically significant for both plant species (Figure 3.1A, 3.2, 3.6, and 3.7) compared to the nonVAM plants. This suggests that the beneficial effects of VAM fungi on plant growth were, to some degree, independent of percent root colonization and of adaptation to salt by the fungal symbionts. VAM effects on plant growth as measured by leaf length and number, fresh and dry shoot mass, and leaf color (only in lettuce) for the most part did not differ between the two VAM fungal sources. This contradicted our second hypothesis, which stated that, under saline conditions, growth of plants colonized by VAM fungi from a saline soil would be greater than that of plants colonized by VAM fungi from a nonsaline soil. As far as we know, only Pond et al. (1984) and Copeman et al. (1996) investigated crop plant responses brought about by VAM fungi from saline soils as compared to the responses to VAM fungi from a nonsaline soil. They collected VAM fungal sources from sites with elevated ECs in order to select those that improved the salt tolerance of tomato. Results of Pond et al. (1984) were inconclusive since the same VAM fungus species (obtained from different sites) would either increase or decrease shoot dry weight (in comparison to a noninoculated and salinized or not controls) of tomato plants grown in salinized soil. Conversely, results of Copeman et al. (1996) showed that tomato shoot growth was enhanced by inoculation with VAM fungi from a nonsaline soil and was inhibited by inoculation with VAM fungi from a saline soil. Based on their potential for colonization, results on plant growth responses to VAM fungi from different edaphic environments conflict. Data from Pond et al. (1984) showed a positive correlation between shoot growth and percent root colonization. In contrast, Dickson et al. (1999) found that the more aggressive colonizers prevented growth increases or depressed

growth. It is also possible that the host preference between plant and VAM fungi at the species level and individual plant species metabolic processes induced by the symbiosis are involved in the differential responses.

Our results confirm that salt reduces VAM root colonization as reported previously by Chambers et al. (1980) and Tsang and Maun (1999). Percent root colonization in onion by both VAM fungal sources decreased similarly with increasing salt (31.7% vs. 34.6% reduction within the salt range used). However, the percent root colonization by Burns VAM fungi was two fold greater than that by the Veg Farm VAM fungi at each salt level (Table 3.7). This indicated that Burns VAM fungi were more aggressive colonizers of onion roots under saline conditions, but at the same time, the enhanced growth was comparable to that of the Veg Farm VAM fungi which colonized considerably less. In lettuce, the reduction in percent root colonization was greater for Burns VAM fungi (39.1% vs. 14.1% within the range of salt used). It is possible that salt effect was somewhat offset by the host preference or specificity in the symbiosis between Veg Farm VAM fungal species and lettuce. The saline soil experimental conditions could have posed more difficulties for the physiological development of Burns VAM fungi than for that of the Veg Farm VAM fungi. Dickson et al. (1999) reported that different VAM species have preferences in pH, which affect the level of colonization. Similarly, Hoefnagels et al. (1983) showed different percentage of root length colonized in two soils with different properties (pH and mineral concentrations). In our experiments, changes in pH alone between the original site and that of the Newberg series (experimental) soil at EC 12 (8.8 vs. 5.4) were larger than those to which the Veg Farm VAM fungi were subjected (6.3 vs.5.4). These may be some reasons why the Burns VAM fungi, albeit more aggressive colonizers, had considerably greater reductions in percent colonization of lettuce roots in saline soils.

Additions of inorganic P are known to relieve salt stress in plants (Champagnol, 1979; Awad et al., 1990). The same effect of additional inorganic P has been observed in saline soil in the absence of VAM fungi (Hirrel and Gerdemann, 1978; Poss et al., 1985; Ruiz-Lozano et al., 1996). Based on the results in Experiment 3, we conclude that additions of inorganic P were not available for plant uptake. Growing nonVAM onions to a size that

compares with that of VAM onions in a loam, nonsaline soil from the Willamette River banks required 44 ppm P added weekly (Linderman, personal communication). Nevertheless, in a soil with a high P-fixing capacity, as the experimental (Newberg series) soil, 45 ppm P added at the beginning of the experiment and given weekly for the last 9 weeks was insufficient to completely overcome this fixing capacity and yield normal-size onion plants. It is conceivable that the addition of P fertilizer necessary to overcome salt effects on plant growth in a soil with a high P-fixing capacity would exceed economical thresholds in crop production. Grattan and Grieve (1999) indicated that P availability is reduced in saline soil not only because of ionic strength effects that reduce the activity of P, but also because P concentrations in soil solution are tightly controlled by sorption processes and by the low-solubility of Ca-P precipitates. Therefore, P fertilization may be successful in alleviating plant salt stress if the rates applied overcome the soil's P-fixing capacity before becoming toxic to plants.

In conclusion, preinoculation of transplants with either of the two VAM fungal sources protected onions and lettuce plants against the detrimental effect of salt in the growth medium. Different edaphic origins of these VAM fungi (saline and not saline) did not cause one fungal source to be more efficient than the other in protecting plants against salt stress. Additional research is needed to determine the cell structural changes of plant tissues treated with NaCl and how VAM fungi ameliorate plant growth reduction imposed by salt. It is important to determine the levels of soil P fertilization that will allow sufficient available P for plant uptake, considering the fixing capacity of the soil and possible precipitation, and still produce nonVAM (control) plants that are not P limited in the experimental soil used. In future experiments aimed to determine the mechanisms by which VAM fungi alleviate salt stress, it would be desirable to have nonVAM-P-fertilized and VAM-without-P-fertilizer plants of similar size. Recommended physiological responses to be measured are carbon dioxide exchange as a parameter for photosynthetic activity, transpiration, stomatal conductance, the responses measured in this study, and water use efficiency in addition to total sugars and proline accumulations.

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APPENDIX

A1. Tissue analysis of lettuce shoots from plants inoculated with VAM fungi or not inoculated and grown in soil treated with four levels of NaCl solutions (EC 2 control, EC 4, EC 8, and EC 12 dS/m). Fungal treatments were: Burns (VAM inoculum from a saline soil), Veg Farm (VAM inoculum from a nonsaline soil), and a noninoculated treatment (nonVAM). Values are means of three replicate plants.

VAM inoc. source	EC(t) (dS/m)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Fe (ppm)	Cu (ppm)	B (ppm)	Zn (ppm)	Na (ppm)	Cl (ppm)	C (%)	N (%)
Burns	2	0.19	2.03	0.86	0.35	305	213	6.33	27.67	47.33	1135	261	43	1.60
Veg Farm	2	0.20	1.73	0.79	0.33	191	298	5.67	25.67	34.67	1297	95	43	1.50
NonVAM	2	0.13	2.01	0.76	0.29	273	434	4.00	24.00	33.67	779	277	43	1.75
Burns	4	0.17	2.17	0.78	0.33	233	258	6.00	26.67	47.33	2924	9608	42	1.66
Veg Farm	4	0.18	1.99	0.79	0.34	159	239	6.00	25.00	37.33	3726	4221	43	1.48
NonVAM	4	0.12	2.35	0.76	0.30	199	474	4.00	24.67	34.00	2529	1804	42	1.67
Burns	8	0.17	3.05	1.02	0.43	210	345	7.67	31.00	56.33	6609	17449	42	2.12
Veg Farm	8	0.19	2.79	1.60	0.75	318	666	8.67	29.00	65.67	8826	8643	41	1.90
NonVAM	8	0.14	3.14	0.95	0.37	198	467	7.33	29.00	48.33	6074	6766	41	2.03
Burns	12	0.17	3.94	1.31	0.47	199	244	8.33	30.00	56.33	10524	3720	40	2.65
Veg Farm	12	0.20	3.57	2.11	0.93	294	747	11.00	31.00	88.00	14264	23243	39	2.51
NonVAM	12	0.12	3.72	1.36	0.44	200	416	5.00	27.33	39.67	11575	26084	40	2.63