PHYSIOLOGICAL EFFECTS OF SALINE WATER ON TWO ECONOMICALLY IMPORTANT HORTICULTURAL CROPS IN SOUTH TEXAS

A Dissertation

by

CATHERINE ROSS SIMPSON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Astrid Volder
Co-Chair of Committee,	Shad D. Nelson
Committee Members,	Juan Carlos Melgar
	Stephen King
	John Jifon
	Greta Schuster
Head of Department,	Daniel R. Lineberger

December 2013

Major Subject: Horticulture

ABSTRACT

Citrus and watermelons are valuable economic crops worldwide, contributing approximately $120 million combined each year in Texas alone. Both citrus and watermelons are sensitive to saline conditions, which can be problematic in the Lower Rio Grande Valley where they are commonly produced. As Texas increases the percentage of irrigated agriculture each year, and in turn the amount of land potentially exposed to salinization through this practice, grafting salt sensitive plants to tolerant rootstocks becomes a more feasible way to overcome this challenge. Grafting typically improves disease resistance, cold tolerance, yield, fruit quality and has been shown to improve salt tolerance as well. While citrus is commonly grafted to rootstocks that induce desirable qualities in the scion, watermelon grafting is only common in Asia and several European countries due to cost constraints. The main goal of this research was to assess selected rootstocks for salinity tolerance by evaluating plant growth and physiological parameters when subjected to several salinity levels.

In the first experiment, potential sour orange replacement rootstocks C22 and C146 were evaluated for salinity as ungrafted trees and grafted to the Olinda Valencia scion. These trees were then compared to the performance of grafted and ungrafted sour orange trees. The results suggest that C22 and C146 rootstocks are more tolerant to saline conditions than sour orange rootstocks at moderate salinity levels. However, grafting significantly decreased all measured growth and physiological parameters for all rootstocks implying that this scion-rootstock combination may not be ideal. In the

second experiment, TAMU mini watermelons were grafted to four rootstocks to determine if any of these would improve their performance when subjected to poor quality irrigation water. Of the four rootstocks and ungrafted TAMU mini watermelon, Strong Tosa showed the most growth when subjected to moderate salinity. Salinity treatments were found to increase fruit quality by increasing the percentage of sugar (brix) and fruit flesh firmness.

DEDICATION

I dedicate my dissertation to my beloved daughter, Makayla. You are my motivation and inspiration.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Volder, and co-chair Dr. Nelson, and my committee members Dr. Melgar, Dr. King, Dr. Jifon and Dr. Schuster, for their guidance, tolerance, encouragement, and support throughout the course of this research. I have been extremely lucky to have such a wonderful, supportive committee. My deepest appreciation goes out to all of you.

Thanks also go to my friends and colleagues and the department faculty and staff for making my time at Texas A&M University and Texas A&M University-Kingsville a great experience. I also want to extend my gratitude to the Texas Water Resources Institute and the USDA HSI funding agencies for providing support for my research, and to all of the organizations that provided me with scholarships during my graduate career. The following organizations and institutions provided me with funding that enabled me to complete my degree; my utmost gratitude goes to them. They are: Steidinger Scholarship, 2010; Belsterling Scholarship, 2011-2012; Margaret Plum Graduate Scholarship, 2012; William C. Welch Scholarship, 2013; Sugarland Garden Club Scholarship, 2013; Cisneros Asset Management Company Scholarship, 2013.

My sincerest appreciation goes to my mother and father for their encouragement and patience. Dad, thank you for calling me every day to motivate me to write and encouraging me every step of the way. To my daughter, thank you for putting up with all of my stress, continuous moving, and the days and nights spent at my work. And

finally, to my friends who have supported, laughed, and cried with me throughout this process, what's left of my sanity is due to you.

NOMENCLATURE

SO	Sour Orange
CTV	Citrus Tristeza Virus
C22	'Bitters' (Sunki mandarin x Swingle trifoliate rootstock cross)
C146	Sunki mandarin x Swingle trifoliate rootstock cross
EC	Electrical conductivity (dS m^{-1})
CEC	Cation exchange capacity
LRGV	Lower Rio Grande Valley
TAMU Mini	Watermelon variety being developed by Dr. S. King
RGR	Relative growth rate (μm mm^{-1} d^{-1})
SPAD	Soil Plant Analytical/Analysis Development
MANOVA	Multivariate analysis of variance
ANOVA	Analysis of variance
PSII	Photosystem II
ABA	Abscisic acid
ICP	Inductively coupled plasma
ATP	Adenosine tri-phosphate

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

1 INTRODUCTION

In recent years, the impact of climate change on rainfall distribution and the frequency of extremes has become more apparent as the occurrence of intensified precipitation events and prolonged periods of drought have increased (Easterling et al., 2000; Groisman et al., 1999). Incidents of drought and water scarcity are increasing, leading many agricultural producers to turn to alternative water sources as supplemental irrigation. In areas where irrigation is necessary for agricultural production, soil salinity can be exacerbated by the use of poor quality irrigation water from surface and ground water sources. Salinity has been estimated to impact one-third of arable lands, limiting crop production and reducing yields (Ghassemi et al., 1995). Glycophytic crops, which include most agricultural crop species, are negatively impacted by salt stress which results in yield and sometimes quality decreases (Bernstein, 1975; Maas, 1992). Important agronomic crops such as citrus and watermelons are classified as salt sensitive crops, and can tolerate up to 2 dS m^{-1} of salt before yields are reduced (Tanji, 1996). Since some species and genotypes are known to be resistant or tolerant to these soil-borne stress factors, they can be used as rootstocks to graft susceptible commercial varieties. Many fruit trees such as apples and citrus are commonly grafted to combat soil-borne diseases.

Grafting of salt sensitive scions to salt tolerant rootstocks may help increase yield and quality when plants experience salt stress (Cuartero et al., 2006; Edelstein et al., 2011; Estan et al., 2005; Huang et al., 2009; Lee, 1994; Rivero et al., 2003). While citrus

has classically been a grafted crop, watermelon grafting is less common. Sour Orange (SO) is a common rootstock used in the citrus industry, however, its susceptibility to Citrus Tristeza Virus (CTV) has led to a decline of its use in recent years. Several newer rootstock varieties are more CTV resistant; C22 and C146 rootstocks are among the most promising alternative rootstock source options for Texas regions, but have not been tested for their salinity tolerance. In contrast to citrus, watermelons are not typically grafted in the United States because of the labor and cost involved; however grafting of watermelon is practiced in other countries where labor is cheaper (Davis et al., 2008; Lee, 1994; Rivero et al., 2003). As grafting becomes more common and less cost-prohibitive, grafting watermelons is a more feasible option in areas afflicted with poor quality irrigation water or saline soil.

1.1 SALINITY

Salts are ionic compounds composed of cations and anions. The main ions that contribute to soil salinity are Na^+, Ca^{2+}, Mg^{2+}, K^+, NH_4^+, Cl^-, SO_4^{2-}, HCO_3^-, NO_3^-, and CO_3^{2-} (Gardiner and Miller, 2008). Soil salinity can be caused by many factors, such as low precipitation, salts in irrigation water, poor drainage, saline groundwater, evaporation, fertilizers, and salty marine parent material (Gardiner and Miller, 2008). Salinity is typically measured by electrical conductivity (EC) in dS m^{-1} (deci-Siemens per meter). Crop sensitivity to salt varies; however, salt sensitive crops can suffer dramatic reductions in yield at salinities less than 2 dS m^{-1}.

In arid and semiarid regions, runoff water and groundwater evaporate and dissolved salts accumulate in the soil. Water with dissolved salts also move upward

from saline groundwater sources and shallow water tables as water tables fluctuate. When the water evaporates, dissolved salts remain in the soil and can form saline, sodic or saline-sodic soils. These types of soil are predominantly located in semi-arid and arid regions, where rainfall is not sufficient for adequate leaching, usually areas that receive less than 51 cm yr^{-1} of precipitation. Approximately 10% of global land area is affected by salt, of these, about 20% of farm lands world-wide are saline and 35% are sodic (Ghassemi et al., 1995; Munns, 2002).

Sodium (Na^+) ions are particularly detrimental because of their toxic effects on plant physiology, growth and development, and other effects on soil structure (Essington, 2004). When a high percentage of the soil's cation exchange capacity (CEC) is occupied by Na^+, soil aggregates disperse, reducing aggregation and soil structure (Gardiner and Miller, 2008). These soils become impermeable to water, develop hard surface crusts, and reduce infiltration due to loss of structure. High salt concentrations in soil increase the amount of energy plants must use to extract water from the soil due to the soil osmotic potential which can prevent or reduce water movement into plant roots (Hasegawa et al., 2000).

Plants can be classified by their ability to tolerate soil salinity at various levels. For soil EC between 0-2 dS m^{-1}, the salinity level is classified as non-saline and results in negligible damage to glycophytic crops (Tanji, 1996). At salinities between 2-4 dS m^{-1}, soils are considered slightly saline and salinity restricts yield in salt sensitive crops. Soils with EC's between 4-8 dS m^{-1} are classified as moderately saline and these salinity levels result in yield decreases in many crops (Gardiner and Miller, 2008). At salinities

between 8-16 dS m^{-1} soils are considered to be severely saline and only a few tolerant crop species can produce satisfactory yield (Tanji, 1996). Soils over 16 dS m^{-1} are classified as very severely saline and only a few halophytic crops can grow in these conditions (Gardiner and Miller, 2008). Soil salinity limits plant growth by: 1) creating a water imbalance in the plant (physiological drought), 2) causing ion imbalances that result in increased energy consumption (carbohydrate respiration) to maintain metabolic processes, and 3) toxicity from high levels of Na^+ and Cl^- in plant tissues (Munns, 2002; Parida and Das, 2005). A high osmotic pressure in the soil solution causes a more negative soil water potential, reducing the ability of plants to extract water from soils (Munns, 2002). High salt content in soil causes osmotic stress in salt sensitive crops when plant cells are unable to adjust and lower their water potential in order to move water into the cells.

Ion toxicity is another issue that can be detrimental to plant growth and survival in saline conditions. In the cell cytoplasm, high concentrations of ions can cause protein denaturation, membrane destabilization through dehydration, and they can replace Ca^{2+} and K^+ within proteins embedded in cell membranes, which increases Na^+ influx into the cells, thus exacerbating toxic effects (Bernstein and Hayward, 1958; Flowers et al., 1977; Munns, 2002). Accumulation of Na^+, Cl^- and B in large quantities in plant tissues can lead to cell death and leaf abscission (Romero-Aranda et al., 1998). Accumulation of ions can also affect crop transpiration rates. For example, Moya et al.(2003) found that Cl^- uptake decreased transpiration rates in salt sensitive citrus varieties. They and other researchers also found that species which exhibited more salt tolerance excluded

larger quantities of Cl^- from the roots (Storey and Walker, 1999). Salt affected plants may exhibit stunted growth and have darker green leaf color. In woody species, salinity can also cause leaf burn and accumulation of Na^+ in leaves has been shown to cause necrosis of leaf tips and edges (Bernstein and Hayward, 1958; Boman et al., 2005). High Na^+ is toxic to plant roots, especially during drought conditions when the Na^+ concentration in soil solution increases. This makes the soil water potential more negative and enhances the risk for dehydration of root tissue.

In general, plant's ability to respond to salt stress depends on its inherent capability to modify its physiological and biochemical processes significantly. Plant physiological coping mechanisms and responses to stress vary amongst species and may include Na^+ transport to shoots, preferential accumulation of toxic ions in older leaves, high Cl^- uptake, lower K^+ uptake, lower biomass of shoots and roots, low P^+ and Zn^{2+} uptake, increase of non-toxic organic compatible solutes, increase in polyamine levels, increase of reactive oxygen species, and quick closing response of stomata when salt stress occurs (Flowers et al., 1977; Parida and Das, 2005). However, the predominant salt tolerance mechanisms in plants are 1) exclusion (i.e. restricting toxic ion uptake by roots), 2) osmoregulation, 3) excretion of salt (found in most halophytes), and 4) sequestration of the toxic ions in the vacuole or cell wall (Flowers et al., 1977; Munns, 2002; Parida and Das, 2005). Often salt tolerant plants exhibit a mixture of these responses.

1.2 GRAFTING

Grafting and budding are standard practices in production of many commercially important woody fruit and nut trees such as apples, pears, and citrus. Grafting is the unification of a root system (rootstock) and a shoot system (scion) with the intention of forming one fused plant that will result in desirable plant qualities (Hartmann et al., 2002). The main principle behind grafting is the joining of the rootstock and scion vascular tissues. A new vascular cambium is formed in successful grafts, leading to a connected vascular system and growth of the new shoot (Evans and Rasmussen, 1972; Soule, 1971; Troncoso et al., 1999; Yin et al., 2012). However, if plant species are incompatible or difficult to graft, there is a very low chance of successful unions between rootstock and scions. While the art of grafting remains one of the most expensive methods of propagation, it is necessary for the production of high quality plants with higher yield, increased disease resistance, vigor, fruit quality, size, maturity, stress tolerance, and overall tree performance (Hartmann et al., 2002).

There are many forms of grafting which have been developed for tree and vegetable crops. Budding is a form of grafting that utilizes the bud from scion budwood which is inserted into the bark of the rootstock (Hartmann et al., 2002; Mendel, 1936). The bud usually contains periderm, cortex, phloem, cambium and xylem tissues attached to the bud section. This bud is inserted next to the exposed xylem and cambium of the rootstock and the bud is sealed with grafting or budding tape to encourage cell proliferation and ensure a tight union (Bhagat et al., 2013; Patel et al., 2010). This bud eventually forms stems and branches that, once established, will replace the rootstocks'

original shoot system which is subsequently cut away or crippled. Crippling is a way of 'forcing' a bud to grow by bending or cutting through the rootstock above the grafted bud (Fann et al., 1983; Williamson et al., 1992; Williamson and Maust, 1995). This process allows apical dominance to be broken and for the scion bud to obtain more photosynthates (Bhagat et al., 2013; Cline, 1991; Samson and Bink, 1975). Budding is the primary form of grafting in citrus due to the rapid union development, lower cost, and high success rate (Bhagat et al., 2013; Mendel, 1936; Patel et al., 2010).

Vegetable grafting, such as used for watermelon, is not typically performed in the United States due to the labor intensity required and thus prohibitive cost of producing large amounts of transplants; however, Japan, Korea, and Europe have used these methods more and more over the past few decades because of automated grafting machinery and reduced land area available for farming (Davis et al., 2008; Lee, 1994). Vegetable grafting is performed using splice or cleft grafting, followed by an acclimatization period in a mist chamber with low lighting to ensure graft success (Denna, 1962; Lee, 1994). Vegetable grafting has shown promise for imparting salinity tolerance, vigor, and hardiness to herbaceous crops that previously could not grow in certain areas (Colla et al., 2010a; Cuartero et al., 2006; Edelstein et al., 2011; Huang et al., 2009; Martinez-Rodriguez et al., 2008).

The rootstock can have a profound effect on the developmental characteristics of the scion. Rootstocks have been known to control tree size in many species, from a dwarfing effect to vigorous growth (Brase and Way, 1959). Fruiting can also be affected by rootstock; rootstocks can improve fruit set, formation, maturity, yield and quality

(Preston, 1958; Strong and Miller-Azarenko, 1991). For example, citrus trees on sour orange rootstocks produce fruit with thin skins, more juice, and higher quality than those on sweet orange rootstocks (Bitters, 1961). Rootstocks have also been found to impart cold hardiness, increased nitrogen efficiency, disease resistance and tolerance of otherwise stressful environmental conditions to selected scions (Albrecht et al., 2012; Albrigo, 1977; Colla et al., 2010b; Hodgson, 1943; Louzada et al., 2008). Conversely, the scion can affect rootstock growth and development as well. Scions can occasionally stimulate growth of weak rootstocks, most likely due to increased photosynthate and phytohormone allocation and distribution (Hodgson, 1943; Jensen et al., 2003).

1.3 CITRUS

Citrus spp. are produced in tropical and subtropical regions and have many species and cultivars adapted to a wide range of climates and environmental conditions. Citrus can be grown in latitudes ranging from ~40° north to ~40° south of the equator, but most are grown between latitudes 20° and 40° (Spiegel-Roy and Goldschmidt, 1996). Citrus production in the United States centers around 3 main regions, Florida, California and Texas. Texas grapefruit production is ranked 2nd in the US behind Florida, and Texas is 3rd in orange production following Florida and California (United States Department of Agriculture, 2008). The citrus industry in Texas is located almost exclusively in the Lower Rio Grande Valley (LRGV) and is known for the production of deep red grapefruit varieties like the Rio Red. Citrus production in the LRGV has a statewide economic impact of approximately $50 million (United States Department of Agriculture, 2008) to $200 million (Sauls, 2008). The LRGV is the 3rd fastest growing

metropolitan area in the US. Currently, 90% of the water used from the Rio Grande River is used for agricultural purposes. However, population growth puts more pressure on water to be used for municipal purposes, leaving less water available for agriculture. The water from the Rio Grande River and the Amistad and Falcon Reservoirs serves the populations of both the US and Mexico. The proximity of the Gulf of Mexico leads to inclusions of salt water in groundwater along the LRGV (Michelsen, 2009), making it a less desirable water source than river water. However, agricultural, industrial and other human activity along the Rio Grande River increases salinity in Rio Grande surface waters. Thus, agricultural land that is irrigated with either groundwater or Rio Grande River water receives irrigation water with elevated concentrations of salt. Citrus production may be negatively impacted by the use of saline irrigation water. In fact, damage to agricultural yield and land from the use of saline irrigation water in the Rio Grande Basin is estimated at approximately $2.4 million (Michelsen, 2009). These problems may be exacerbated by projected future increased and intensified drought periods, as well as greater pollution pressure from increasing human activities. Ultimately the pressure of increasing population, unreliable rainfall and the decline of surface water availability will drive producers to use alternate water sources that may be of poor or questionable quality. Thus, it is imperative to find citrus rootstocks and management techniques that can tolerate irrigation with lower quality water than orchards typically receive at present.

Among the many types of grafting techniques currently practiced, the most relevant to this dissertation are budding and vegetable grafting using splice or cleft

grafting. Most citrus cultivars are budded onto rootstocks that can affect the yield, fruit quality and stress tolerance of the tree. New vegetation grows in 2-5 flushes depending on climatic conditions (Spiegel-Roy and Goldschmidt, 1996). In the spring both vegetative and reproductive shoots occur, and later flushes tend to be vegetative (Spiegel-Roy and Goldschmidt, 1996). Seven varieties that were tested in a previous study showed variable tolerance to salt, ranked from Sunki Benecke (good) > Sour Orange (moderate) > Swingle citrumelo (moderate) > Carrizo (moderate) > Valencia (moderate) > Troyer (poor) > *P. trifoliata* (poor) (Chapman, 1968; Zekri and Parsons, 1992).

Sour orange (*Citrus aurantium*) is a widely used rootstock with high adaptability and tolerance to diverse soil and environmental conditions; however trees grafted onto the sour orange rootstock tend to be susceptible to Citrus Tristeza Virus (CTV) (Grosser, 2004; Moreno et al., 2008). C-22, C-57, and C-146 are varieties developed from the cross of Sunki mandarin and Swingle trifoliate orange. These crosses were developed for their CTV resistance and are tolerant to the calcareous clays found in the LRGV (Louzada et al., 2008). Current interest in the citrus industry is focusing on CTV resistant rootstocks, and their replacement of the sour orange rootstock. Our goals to find a salt tolerant replacement for the sour orange rootstock that is CTV resistant have yielded promising results in the C22 and C146 rootstocks.

In arid and semi-arid regions where citrus is grown an increasingly unpredictable precipitation pattern causing periods of intensified drought during the growing season is one of the main concerns for future citrus production. Drought stress can have

devastating impacts on citrus physiology (CO_2 assimilation), growth, development, and yield (Garcia-Sanchez et al., 2007; Perez-Perez et al., 2007). Additionally, in these semi-arid and arid environments stressful conditions that negatively impact tree growth and yield are not restricted solely to drought. Other stress factors such as salinity, heat, excessive radiation, etc. often interact to reduce plant growth and yield (Hasegawa et al., 2000; Munns, 2002). High leaf temperatures increase leaf respiration, reduce rates of photosynthesis and increase the vapor pressure differences between leaves and air which drives the rate of transpiration. This increase in transpiration lowers efficiency of water usage by the plant and can reduce stomatal conductance and in turn reduce CO_2 assimilation (Farquar and Sharkey, 1982; Lawlor and Cornic, 2002). The rise in temperature in areas already subjected to heat and drought stress will exacerbate the impact of these stresses. Adequate water supplies can help reduce leaf temperature through evaporative cooling, if plants do not have enough water, stomata remain closed which leads to carbon starvation eventually resulting in leaf/tree death (Bañuls et al., 1997; Lloyd et al., 1990; Maas, 1992). To meet citrus transpirational demands many producers may have to start irrigating with saline ground water as easy access to high quality water decreases.

Saline irrigation water has many negative effects on plant growth. Salinity reduces water cell potential, dehydrates cells, leads to ion toxicity, reduces leaf expansion, cellular metabolism, stomatal conductance, photosynthesis, causes membrane destabilization, and cell death in salt sensitive plants like citrus (amongst other effects) (Al-Yassin, 2005; Balal et al., 2011; Bañuls et al., 1997; Behboudian et al., 1986;

Garcia-Sanchez et al., 2002b). Salinity mainly affects crops through osmotic effects that reduce water uptake and ion toxicities that cause reduced photosynthesis and leaf area (Ferguson and Grattan, 2005; Lauchli and Grattan, 2007). Accumulation of Na^+, Cl^- and B in large quantities in plant tissues can lead to cell death and leaf abscission (Romero-Aranda et al., 1998). Accumulation of ions can also be linked to water use efficiency of crops (Moya et al., 2003). They and others also found that more salt tolerant plants excluded more Cl^- from the roots (Storey and Walker, 1999). Chloride is often considered the most common cause of ion toxicity in citrus trees (Maas, 1992). The variability amongst citrus cultivars means that salt tolerance and ion accumulation is highly dependent upon individual plant mechanisms. However, a link between lower growth rate (reduced vigor) and salt tolerance has been reported (Moya et al., 2002). This reduced growth rate is related to the ion uptake and resulting toxic accumulation of ions in citrus trees (Brumos et al., 2009).

The overall objectives of this research were to characterize the physiological effects of saline irrigation water on several citrus rootstocks. The goal was to determine the salinity tolerance of two new rootstock varieties (C22 and C146) compared to the commonly used rootstock variety, sour orange. We also explored the effects that grafting had on salinity tolerance to test if scion-rootstock interactions had any beneficial impacts for citrus trees.

1.4 WATERMELON

Nationally, Texas is the third largest producer of watermelons (*Citrullus vulgaris*) with a statewide economic impact of approximately $50 million (United States

Department of Agriculture, 2008). In the LRGV, Hidalgo and Brooks counties account for 37% (2,889 hectares) of total Texas watermelon production (9,510 hectares). Knox, Gaines and Woods counties are the other main producers state-wide (United Nations Food and Agriculture Organization, 2012). These counties lie on several aquifers that have the potential for salinity problems if areas are heavily pumped (Scanlon et al., 2007).

Watermelons are a warm-season fruit that requires daytime temperatures of 26.6 - 35°C and night temperatures between 15.5 - 21°C. The total growing period ranges from 80-110 days; usually harvested in late April to early May in the Lower Rio Grande Valley. The key development stages of watermelons are the establishment period (10-15 days), the vegetative period (20-25 days), the blooming/flowering period (15- 20 days), fruit set/yield formation (20-30 days) and enlargement/ripening (15-20 days) (Roberts et al., 2007; Smith and Anciso, 2000). Watermelons are a moderately saline sensitive crop, generally affected at salinity levels over 2 dS m^{-1} (Tanji, 1996).

In southern Texas, the semi-arid climate, unpredictability of rainfall and low water holding capacity of soils used for watermelon production usually requires supplemental irrigation of crops. Watermelons require between 254-381 mm of water per season applied at critical periods, i.e. before seedling emergence, and early bloom before harvest (Roberts et al., 2007; Smith and Anciso, 2000). With increased irrigation, soils become more saline which can affect crop growth and yield. Soil salinity can reduce water infiltration, lower soil water potential, deteriorate the soil structure, limit the availability of plant nutrients, and reduce plant growth (Pitman and Lauchli, 2002;

Tanji, 1996). Salt stressed watermelon plants can have nutrient imbalances due to competition from Na^+ and Ca^{2+} which results in reduced crop yield and poor plant growth (Ruiz et al., 1997a). Salinity can cause phytotoxicity problems by the accumulation and partitioning of Na^+ and Cl^- in plant tissues (Uygur and Yetisir, 2009).

Grafting of higher quality crop scions onto tolerant rootstocks has been used to increase salinity tolerance and pathogen resistance in many plant species. Grafting is used to enhance plant growth, reduce pathogenic impacts and infections, and reduce the impact salt or high temperature may have on the plant (Rivero et al., 2003). The objective of the watermelon experiment was to determine if five selected rootstocks could impart salinity tolerance to watermelons. To determine if grafting had a significant effect on performance and salinity tolerance of watermelon, we compared growth and yield of grafted plants to ungrafted plants.

1.5 SUMMARY

It is increasingly important to find salt tolerant plants or techniques that enhance salt tolerance. The overall goal is to determine if selected rootstocks enhanced salinity tolerance for both citrus and watermelon. More specifically, for citrus we describe effects of salinity, rootstock and grafting on growth (section 2), and physiology (section 3), as well as the relationship between salinity, grafting, rootstock, and nutrient concentrations in citrus leaves (section 4). I hypothesized that grafting to more tolerant rootstocks would improve salinity tolerance and overall tree physiological performance.

For watermelon we were most interested in the effect of grafting of four rootstocks on salt tolerance of the TAMU mini cultivar. I hypothesized that at least one

of the five selected experimental rootstocks would improve plant growth and tolerance to salt. Yield and quality data of TAMU mini cultivar grafted to five rootstock cultivars and the ungrafted TAMU mini cultivar grown at three salt levels, are presented in section 5. The results of these studies are discussed in section 6, where all findings together are discussed in the context of the effects of grafting on salinity tolerance of citrus and watermelons.

2 GROWTH RESPONSE OF GRAFTED AND UNGRAFTED CITRUS TREES TO SALINE IRRIGATION

2.1 INTRODUCTION

Salinity leads to billions of dollars in crop losses worldwide (Ghassemi et al., 1995) and hundreds of thousands of hectares of cropland are taken out of production every year due to salinity problems (Ghassemi et al., 1995; Martinez Beltran and Licona Manzur, 2005). In coastal areas, groundwater may be exposed to inclusions of high salinity ocean water which lowers water quality and limits usability of groundwater for irrigation purposes. In arid and semi-arid lands salinity is also a major problem due to the accumulation of salts in the soil from naturally occurring salt minerals, salts dissolved in irrigation water, temporarily raised water tables that salinize soil through evapotranspiration, and applied fertilizers (Behboudian et al., 1986; Ben-Hayyim et al., 1985; García-Sánchez et al., 2005; Scanlon et al., 2007).

Salinity leads to a decline in crop production and reduces yields in glycophytic crops such as citrus (Maas, 1992; Tanji, 1996). Texas ranks second in the U.S. in grapefruit production and third for total citrus production (United States Department of Agriculture, 2012). Texas citrus are primarily grown in the Lower Rio Grande Valley (LRGV) due to its subtropical climate and mild winter temperatures. In the LRGV surface water is currently used for both domestic and agricultural purposes, but as population rises there will be a need to increase usage of lower quality ground water for agricultural purposes as available surface water declines (Gerber, 2011). One quarter of

the land in the LRGV is estimated as salt-affected due to groundwater intrusion and most groundwater is classified as a salinity hazard (Carter and Wiegand, 1965; McCoy, 1990). In 2009, over $2.4 million dollars' worth of agricultural crops were lost due to salinity along the Rio Grande River caused by poor quality return flows into the river, groundwater inflows, or wastewater effluent (Michelsen, 2009).

Most of the citrus grown in the LRGV is grafted onto sour orange (SO) rootstock, which has been a dependable rootstock for many decades since it is especially suitable to the LRGV's calcareous soils. However, SO is susceptible to several diseases that cause major losses in citrus orchards such as Citrus Tristeza Virus (CTV), which causes quick decline of citrus scions grafted on SO rootstocks (Grosser, 2004; Moreno et al., 2008). C22 and C146 trifoliate rootstock varieties are two promising rootstocks developed for their CTV resistance and high yield capabilities (Louzada et al., 2008). These varieties also have high tolerance to the high pH, calcareous clay soils of South Texas, which typically limit the use of rootstock varieties that may have been previously studied for salt tolerance.

Salt tolerance in plants can be achieved through exclusion of toxic ions, accumulation of ions within the vacuoles of the plant cells and osmotic adjustment through the production of osmotic substances in plant cells, which maintain osmotic pressure and consistent cell expansion (Grieve and Walker, 1983). Salt exclusion processes are dependent on selective uptake by root cells, preferential ion loading in the xylem and salt removal from the xylem (Munns, 2002). Among other mechanisms of salt tolerance is the accumulation of salts in older plant tissues followed by their subsequent

senescence. Lower water uptake and transpiration rates reduce the possibility of plant osmotic stress and can also result in less salt uptake into the plant. (Moya et al., 2002; Munns, 2002). One of the most important traits that determines salt tolerance in citrus species is their ability to restrict toxic ion accumulation in their tissues per volume of water absorbed via transpiration and prevent ion movement into leaves (Balal et al., 2012; Bañuls et al., 1997; García-Legaz et al., 1993; Lloyd et al., 1989; Moya et al., 2003; Moya et al., 2002). Most citrus are classified as salt sensitive with a sensitivity threshold of 1.7 dS m^{-1} soil salinity (Tanji, 1996). However, citrus rootstocks differ in their ability to exclude or accumulate Na^+ and Cl^- ions, so their tolerance to an excess of these ions depends on the variety (Bañuls and Primo-Millo, 1995; Garcia-Sanchez et al., 2006). Salt stress negatively affects physiological processes within plants, such as CO_2 assimilation/photosynthesis and stomatal conductance, as well as interfering with the uptake of other ions (Ca^{2+}, K^+) (Al-Yassin, 2005; Perez-Perez et al., 2007; Walker and Douglas, 1983; Zekri, 1991; Zekri and Parsons, 1990), that can negatively affect yield and plant growth.

Grafting of citrus is a common practice that imparts desirable qualities in the scion such as yield improvement, enhanced growth, disease resistance and salinity tolerance (Albrigo, 1977; Barry et al., 2004; Castle, 2010; Wutscher, 1979). Salinity tolerance is predominantly determined by rootstock tolerance but scions influence overall salinity tolerance of the plant as well (García-Legaz et al., 1993; Levy et al., 1999; Nieves et al., 1991; Vardi et al., 1988). Root hydraulic conductivity, transpiration rate and rootstock vigor play an important role in movement of ions throughout the

plant. Rootstock variety traits regarding these factors change with regards to each rootstock, some having higher root hydraulic conductivity, transpiration rates etc., and others having lower conductivities and transpiration rates, which will impact net photosynthesis and ion partitioning. Perception of salinity stress and subsequent signaling and gene expression throughout the plant in sensitive varieties are less responsive, failing to reduce the rate of photosynthesis and C assimilation, more tolerant citrus varieties respond rapidly and induce genetic expression of transporter genes while reducing metabolic processes within the plant (Brumos et al., 2009). Recent research also indicates that PIP1, an aquaporin subfamily of plasma membrane intrinsic protein subgroup which controls water movement throughout plant tissues, gene expression and H^+-ATPase activity is involved in Cl^- exclusion from leaves (Bañuls et al., 1995; Rodríguez-Gamir et al., 2012).

New rootstock varieties C22 and C146 have only recently been evaluated for their field performance (Louzada et al., 2008) and have not been extensively tested for salinity tolerance yet. However, other trifoliate rootstock varieties, such as *P. trifoliata* var. Rubidoux, Troyer citrange (*C. sinensis* x *P. trifoliata*), and Carrizo citrange (*C. sinensis* L., Osbeck x *P. trifoliata* L.) have varying tolerance to salinity (Balal et al., 2012; Bañuls and Primo-Millo, 1995; García-Sánchez et al., 2005). We hypothesize that C22 and C146, which were crossed from more salt tolerant lines, are more likely to exclude toxic ions and improve salt tolerance of Olinda Valencia grafted trees. The objectives of this study were to evaluate effects of saline irrigation water, grafting and

rootstock on plant mortality, height relative growth rate (RGR), leaf area development, and Na^+ and Cl^- accumulation in calcareous clay tolerant citrus varieties.

2.2 MATERIALS AND METHODS

2.2.1 Plant material and growing conditions

Citrus trees were obtained from the Texas A&M University-Kingsville Citrus Center in Weslaco, TX where they were originally propagated from seed. Sour Orange (SO), C22, and C146 rootstocks had either been grafted with Olinda Valencia budwood at approximately 1 year of age or were left ungrafted. C22 and C146 are cultivated from Sunki mandarin and Swingle trifoliate orange crosses developed in California (Louzada et al., 2008). Trees were grown in 10 cm x 10 cm x 36 cm tall pots in the Citrus Center greenhouse and transported to the greenhouse at Texas A&M University - Kingsville in January 2011. At the time of the experiment, the 75 grafted trees were 2 years old and the 75 ungrafted trees were 1 year old. Plants were grown in potting soil with Osmocote® slow release fertilizer (Indoor/Outdoor Smart-Release® Plant food, The Scotts Company, LLC, Columbus, OH) applied at 3 month intervals. Plants were irrigated twice per week with treatments of 0, 1, 3, 5 and 10 dS m^{-1} instant Ocean© sea salt solutions (United Pet Group, Blacksburg, VA) over six months until July 2011. Irrigation volume was designed to replenish transpirational water losses without excessive leaching from pots. Temperatures were regulated and maintained between a minimum temperature of 10 °C (January) and a maximum temperature of 36.5 °C (July) throughout the duration of the study.

2.2.2 Tree and leaf measurements

Plant height measurements were collected monthly. Height of grafted trees was measured from the graft to the top of the apical meristem and height of ungrafted trees was measured from a predetermined mark at the bottom of the trunk to the tip of the apical meristem. Leaf samples (most recent fully-expanded leaf) were sampled from the middle of the stem as described by Melgar et al. (2008). Leaf area was determined using a CI-202 Portable Laser Leaf Area Meter (CID Biosciences, Camas, WA). After six months, plants were harvested and total mortality, relative height growth rate (RGR), relative diameter growth rate were calculated (Ruiz et al., 1997a). Total mortality was determined at harvest, trees were considered dead if the trunk was dead. At harvest, trees were divided into leaves, stems, and roots, and washed, weighed, and dried at 60°C for 48 hours. Leaves were then weighed and ground to approximately 0.8 mm particle size. Leaves were subsequently analyzed for nutrient content and Na^+ and Cl^- analysis by Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory located in College Station, Texas.

2.2.3 Statistical analysis

The experimental design consisted of five replications for each rootstock per salinity treatment in a randomized complete block design. Data was analyzed using JMP®Pro 9.0.0 software (SAS Institute, Cary, NC). Treatment effects and interactions were analyzed using full factorial fit model and bivariate fit models when appropriate. Significant difference between treatments, rootstocks and grafting was tested through students T comparison of means.

2.3 RESULTS

2.3.1 Relative growth rate (RGR)

Height RGR (μm mm^{-1} d^{-1}) was greater for ungrafted plants at all salinity levels (Fig. 2-1). However, SO ungrafted rootstocks had a significantly lower RGR than C22 and C146 ungrafted rootstocks. There was no effect of rootstock on RGR of grafted trees. Additionally, grafted trees had a lower absolute height increase compared to ungrafted trees and SO grafted and ungrafted trees had a lower absolute height increase than C22 and C146 grafted and ungrafted trees (data not shown, $P_{\text{rootstock x graft x salinity}} = 0.044$).

Younger trees generally grow faster than older trees and ungrafted trees were younger than grafted trees. Thus, to eliminate RGR as a factor affecting salinity response, we calculated an RGR response ratio ($RGR_{\text{salinity treatment}}/RGR_{\text{without salinity}}$) within each grafting treatment. This response ratio shows the proportional effect of increasing salinity on growth (Fig. 2-2).

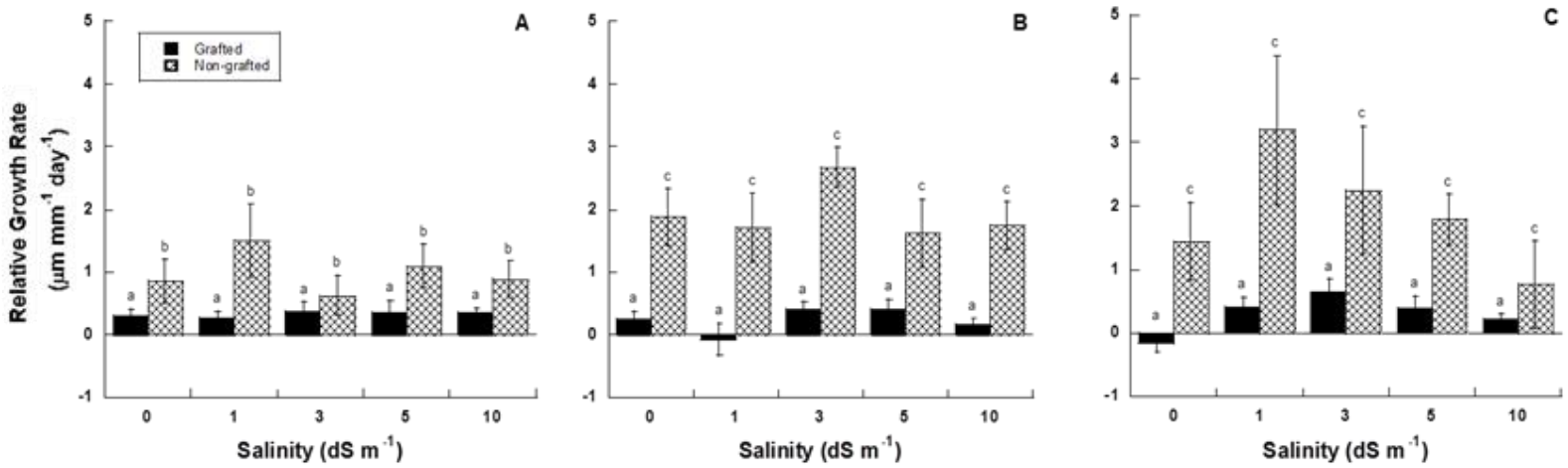


Figure 2-1. Plant height RGR of citrus rootstocks as affected by grafting and salinity. A) Sour orange (SO) rootstock, B) C22 rootstock, C) C146 rootstock. $P_{\text{rootstock x grafting}} = 0.016$. Error bars are ± 1 standard error. Different letters indicate statistically significant differences in RGR between treatment means ($P \leq 0.05$).

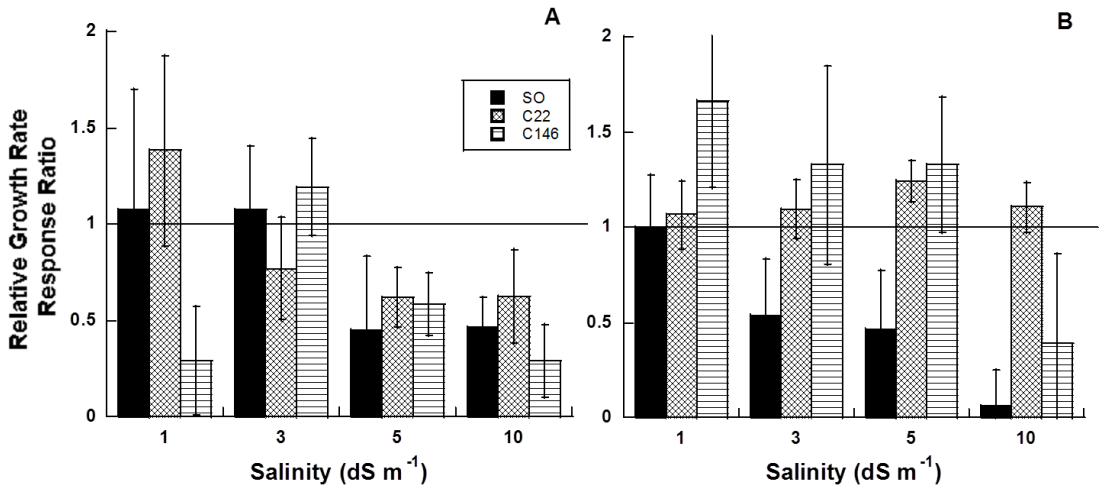


Figure 2-2. The ratio of change in tree RGR in response to salinity. A) grafted trees compared to the grafted control plant of each rootstock, B) ungrafted trees compared to the ungrafted control plant of each rootstock. A value of 1 indicates no effect of salinity, values <1 indicate reduced RGR in response to salinity and values >1 indicate increased RGR in response to salinity. ($P_{salinity}$ =0.001). Thin bars are ± 1 standard error of mean.

2.3.2 Standing leaf area

Sour orange (SO) ungrafted rootstocks increased their standing leaf area when irrigated with 1 dS m^{-1} when compared with control plants (1.295, Fig. 2-3) All other rootstock by grafting combinations reduced their standing leaf area in response to increasing salinity levels. At the end of the experiment, only ungrafted C22 and C146 rootstocks had any green leaf area at 10 dS m^{-1} (Fig. 2-3).

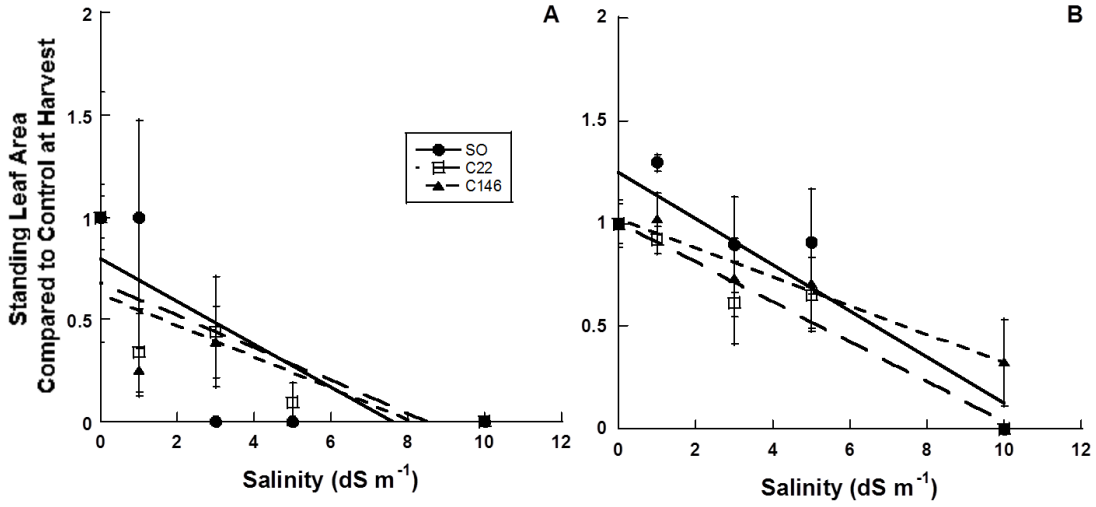


Figure 2-3. Effect of salinity on standing leaf area at harvest compared to control. (0 dS m^{-1} salinity) plants for three citrus rootstocks (SO, C22, C146), A) grafted plants B) ungrafted plants. Figures include data of all trees, including those with zero leaf area. $P_{grafted} < 0.001$, $P_{salinity} < 0.001$, $P_{grafting \times salinity} = 0.033$. Error bars are shown as ± 1 standard error. There was no effect of rootstock.

2.3.3 Tree mortality

Mortality increased with increasing salinity for grafted trees on all three rootstocks whereas ungrafted C22 and C146 rootstocks had lower mortality rates after 6 months (Fig. 2-4). SO and C22 grafted trees had 100% mortality at 10 dS m^{-1}, while C146 grafted trees had 80% mortality at 10 dS m^{-1}. Mortality increased for grafted trees when salinity increased. Ungrafted C22 and C146 rootstocks had lower mortality (0-20%) at all salinity levels while SO ungrafted rootstock mortality increased with salinity. Mortality of 60% in the SO grafted trees was attributed to an ant infestation within these three pots at the end of the study.

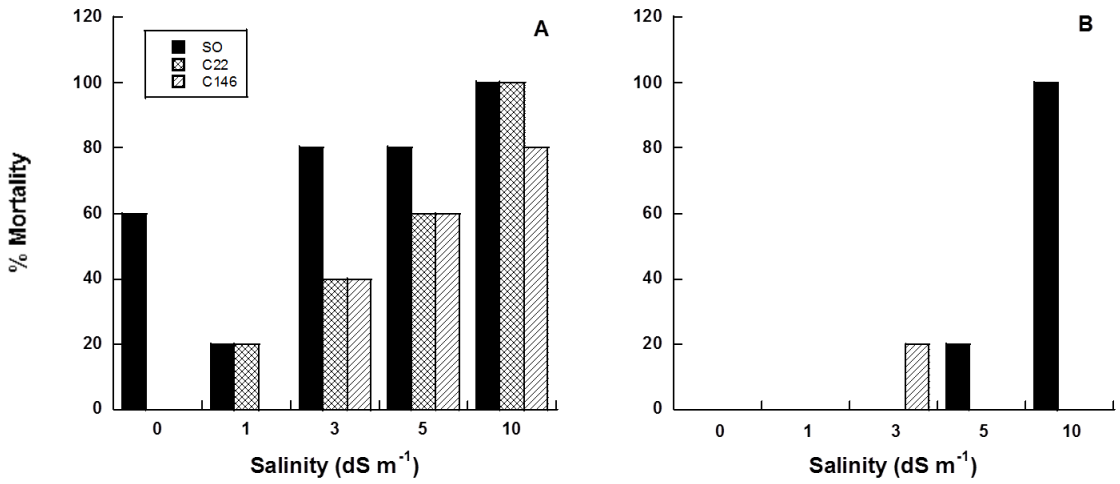


Figure 2-4. The effect of mortality on citrus rootstocks. A) Effect of salinity on mortality (%) of grafted rootstock varieties, B) Effect of salinity on mortality (%) of ungrafted rootstock varieties. P rootstock x graft x salinity ≤ 0.002.

2.3.4 Na^+ and Cl^- accumulation in leaves

Cl^- was taken up at higher levels than Na^+ in both grafted and ungrafted trees. Cl^- was accumulated mainly by SO ungrafted rootstocks at the 10 dS m^{-1} treatment level and C146 grafted trees at the 5 dS m^{-1} treatment level (Fig. 2-5). C146 grafted trees accumulated larger amounts of Cl^- ions in dry leaf matter than C22 grafted rootstocks at all salinity levels. There was a negative correlation between leaf area per plant and Na^+ and Cl^- accumulation in plant tissues (Fig. 2-6).

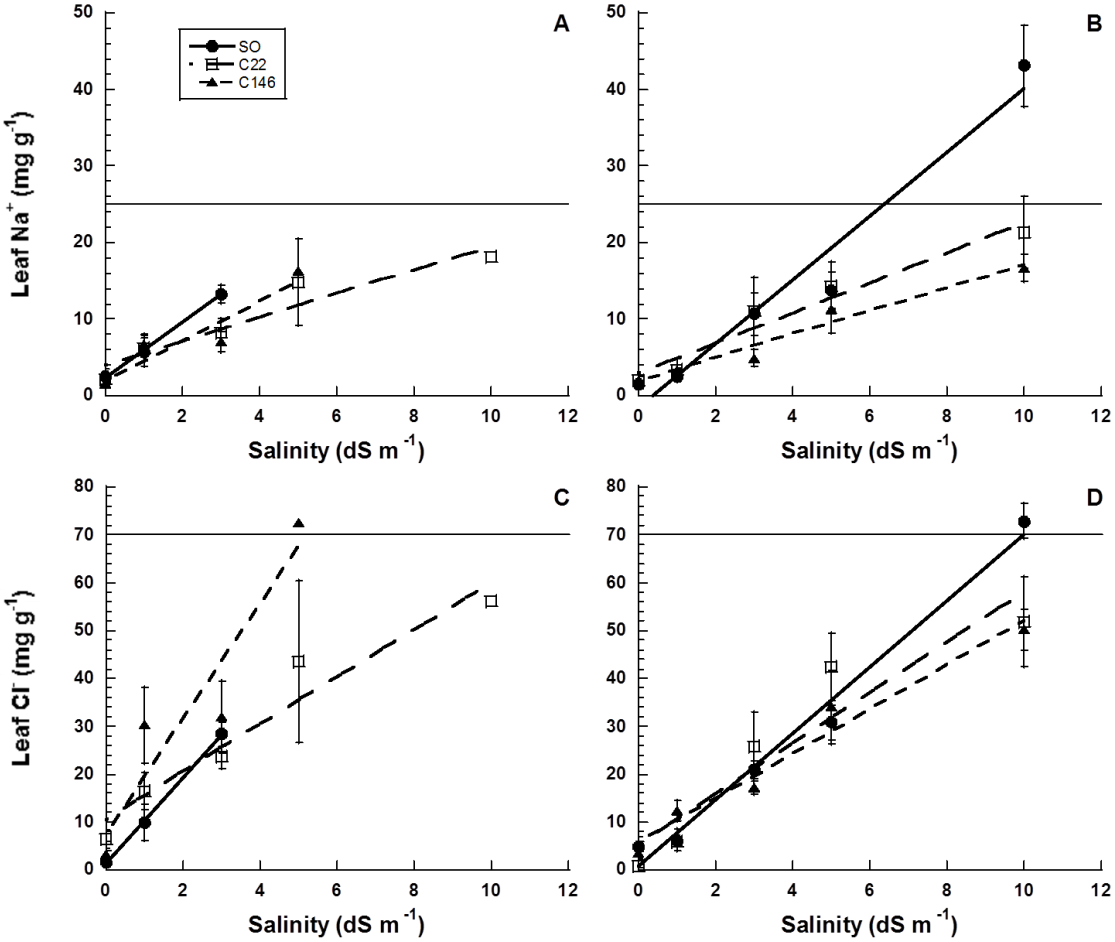


Figure 2-5. Accumulation of Na^+ and Cl^- in leaves at each salinity level. A) Leaf Na^+ concentration (mg g^{-1}) of grafted trees, B) leaf Na^+ concentration (mg g^{-1}) of ungrafted trees, C) leaf Cl^- (mg g^{-1}) of grafted trees, D) leaf Cl^- concentration (mg g^{-1}) of ungrafted trees. The typical toxicity threshold is approximated by a horizontal line (Chapman, 1949; Obreza et al., 1992; Sauls, 2008). Missing values are indicative of dead trees. Salinity, rootstock, and grafting interactions had a significant effect on Na^+ ($P \leq 0.001$) and Cl^- ($P \leq 0.01$) content in citrus leaves. Thin bars are ±1 standard error of the mean.

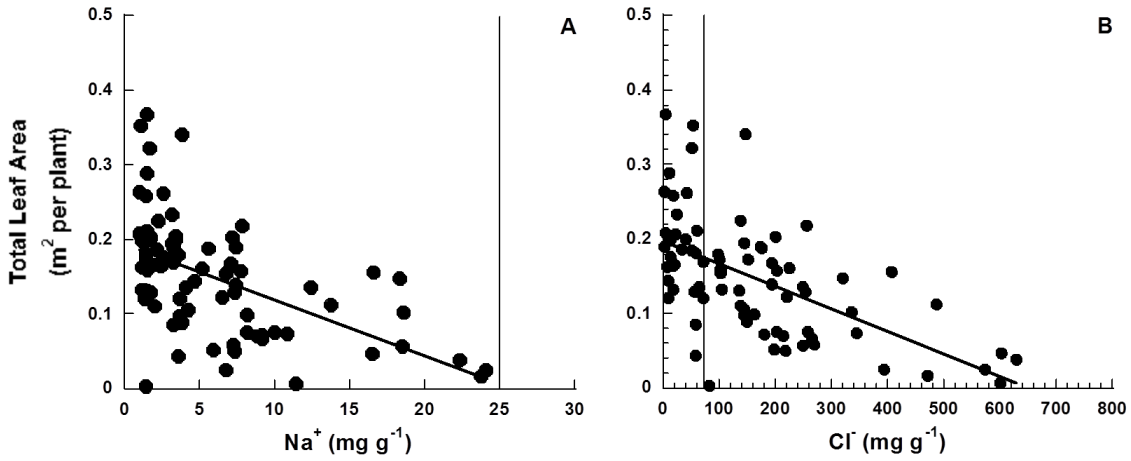


Figure 2-6. Concentration of Na^+ and Cl^- in leaves compared to total leaf area per plant. A) Leaf Na^+ concentration (mg g^{-1}) compared to the total leaf area per plant, B) Leaf Cl^- concentration (mg g^{-1}) compared to the total leaf area per plant. Vertical line approximates typical threshold levels for adult trees. (Chapman, 1949; Obreza et al., 1992; Sauls, 2008). ($P_{Na} \leq 0.001$, $P_{Cl} \leq 0.001$)

2.4 DISCUSSION

Sour orange has been the leading rootstock in Texas citrus production for many years; however, disease susceptibility has led to a decline in its use. In recent years C22 has been employed as a replacement for SO. C22 and C146 were top yielding and quality performers in recent rootstock trials for CTV resistance (Louzada et al., 2008), although they have not been tested for salinity tolerance. Our data show that C22 and C146 rootstocks were generally more salt tolerant than SO rootstocks in terms of mortality as well as relative growth rate, particularly for grafted rootstocks at low-

medium salinity levels. Mortality of ungrafted rootstocks was much lower for all rootstocks compared to grafted rootstocks, however, SO still showed 20 to 100% mortality at high salinity levels of 5 and 10 dS m^{-1}, respectively.

Reductions in growth due to salinity stress have been shown in citrus before (Bañuls and Primo-Millo, 1995; Maas, 1992; Munns, 2002; Perez-Tornero et al., 2009; Storey and Walker, 1999). However, salt tolerance is not uniform across rootstocks and scions. Zekri and Parsons (1989) found that Cleopatra mandarin and Sour Orange had greater salt tolerance than Swingle citrumelo, Carrizo, Milam lemon and trifoliate oranges, respectively. The ability of these rootstocks to restrict Cl^- and Na^+ accumulation in the scion can also vary (Maas, 1992). Our data showed that standing leaf area decreased with increasing salinity for both grafted and ungrafted trees. This reduced impact of salinity on standing leaf area and may indicate that grafting increases salinity sensitivity. Rootstock variety did not affect the response of grafted and ungrafted trees to salinity (Fig. 2-3). Greater standing leaf area of ungrafted plants most likely contributed to their increased survival rate because greater leaf area provides a greater photosynthetic capacity and thus reduced risk of carbon starvation (Munns, 2002). Additionally, ungrafted trees were smaller and 1 year younger than grafted trees, yet had higher standing leaf area.

It is well known that toxic accumulation of Na^+ and Cl^- in plant leaves can lead to early leaf death and senescence (Garcia-Sanchez et al., 2002a; Garcia-Sanchez et al., 2002b). The level of toxicity varies among ions, Na^+ toxicity data is inconsistent with one study reporting leaf injury starting at 0.1% leaf dry weight (Grattan, 2002) and

another reporting no injury until 0.5% dry weight (Sauls, 2008), however most experts use 0.25% as their toxicity threshold (Chapman, 1949, 1968; Obreza et al., 1992; Smith, 1962). Cl^- concentrations must be above approximately 0.7% leaf dry weight for Cl^- toxicity to occur in otherwise healthy, adult citrus trees (Ferguson and Grattan, 2005; Sauls, 2008). It should also be noted that toxicity ranges are typically for adult trees, spring flush leaves that are harvested at 3-7 months old, whereas the leaves analyzed in this experiment were not necessarily collected at this age due to defoliation. The majority of literature focuses on toxicity in adult trees and it is not widely known if seedlings are more or less sensitive to ion toxicity (Chapman, 1949; Cooper et al., 1952; Obreza et al., 1992). Our data indicates that leaf Cl^- concentrations exceeded these toxic threshold levels only at very high levels, but concentrations increased with increasing salinities. However, only the surviving green leaves were sampled for tissue analysis whereas those leaves that may have died as a result of toxic ion accumulation were not included. The inverse relationship between Na^+ and Cl^- concentrations and total leaf area suggests that toxic ion accumulation likely led to reduced leaf area, rate of growth and ultimately reduced survival rate (Fig. 2-6). However, the toxicity threshold may be higher than previously recommended. The relationship of both Na^+ and Cl^- and total leaf area was not affected by grafting ($P=0.6941$, $P=0.9521$, respectively). Na^+ in SO leaves accumulated at greater levels than in C22 and C146 leaves, potentially explaining the lower growth rate and increased mortality of SO rootstock. This is similar to results found by Cooper et al. (1952), who found that the salt tolerance of Valencia oranges on SO rootstock was less than for those grown on Cleopatra mandarin rootstocks. Both

Behboudian et al. (1986) and Cooper et al. (1952) found that accumulation of Cl^- was generally rootstock dependent. In contrast, Na^+ accumulation was scion dependent in various citrus varieties. Mortality was directly related to both Na+ and Cl- of leaves (P =0.0017 and P=0.0036, respectively). Our results suggest that ungrafted SO rootstocks are less effective Na^+ excluders at high salinity levels compared to C22 and C146 rootstocks. In general, C22 and C146 rootstocks had less Cl^- accumulation in the leaves. Grafting, rootstock and salinity treatments all played a significant role in the accumulation of Na^+ and Cl^- ions in leaves. Overall, these results also imply that C22 and particularly C146 rootstocks are more tolerant of saline conditions with regards to growth.

2.5 CONCLUSIONS

We found that while C22 and C146 out-performed SO in salinity tolerance as ungrafted trees, their salinity tolerance was reduced when the Olinda scion was grafted. This is similar to findings by Bañuls and Primo-Millo (1995) which showed that defoliation and growth reduction in response to salinity was higher on Navel orange trees grafted onto Troyer citrange rootstocks versus ungrafted rootstocks. Rootstock-scion compatibility can increase survival in saline conditions (Behboudian et al., 1986; García-Legaz et al., 1993) but our results suggest that the scion may decrease the survival of plants in the presence of high Na^+ and Cl^- conditions, although the accumulation of toxic ions in plant tissues depends on the ability of the rootstock to exclude them. Though C22 and C146 rootstocks provide improved salt tolerance to

Olinda Valencia scions, a more compatible option should be explored for areas with extreme salinity problems.

3 EFFECTS OF SALINITY ON PHYSIOLOGICAL PARAMETERS OF GRAFTED AND UNGRAFTED CITRUS TREES

3.1 INTRODUCTION

As salinity of arable lands becomes a major concern due to climate change and drought, crops sensitive to salt are more susceptible than ever. Identifying cultivars tolerant to these challenging conditions is vital for crop production in the future. Worldwide citrus is cultivated in sub-tropical to tropical climates, which are vulnerable to drought and subsequently salinity problems. Citrus is classified as sensitive to saline irrigation water with a sensitivity threshold of 1.7 dS m^{-1} (Maas, 1992; Spiegel-Roy and Goldschmidt, 1996). The ability of citrus rootstocks to restrict uptake and accumulation of toxic ions is one of the determining factors of their salinity tolerance. The ability to restrict these ions is dependent upon mechanisms that regulate sodium (Na^+) and chloride (Cl^-) absorption and transport throughout the plant. Toxic accumulation of Na^+ and Cl^- lead to reduced growth and can impair physiological and biochemical processes, depending the variety sensitivity (Garcia-Sanchez et al., 2006; Perez-Perez et al., 2007). Salinity is known to reduce CO_2 assimilation, photosynthesis, stomatal conductivity, and PSII efficiency as well as interfere with nutrient ion uptake and assimilation (Behboudian et al., 1986; García-Legaz et al., 1993). Salinity has also been known to increase cell membrane permeability leading to higher electrolyte leakage from affected cells (Ashraf and Harris, 2004; Lutts et al., 1996). These physiological factors indicate plant response to salinity stress and overall ability of a rootstock to tolerate saline

conditions. The objective of this study was to determine how grafted and ungrafted citrus rootstocks responded to 6 months of saline irrigation.

3.2 MATERIALS AND METHODS

3.2.1 Plant materials and growing conditions

As described in Simpson et al. 2013 (unpublished) plant materials used in this experiment were obtained from Texas A&M University-Kingsville Citrus Center in Weslaco, TX. From January to July 2011 citrus trees were grown in an experimental greenhouse located at Texas A&M University-Kingsville. Citrus rootstocks Sour Orange (SO), Bitters (C22) and C146 (C22 and C146 are both Sunki mandarin x Swingle trifoliate crosses) were used. Olinda Valencia scions were grafted onto half of the rootstocks whereas the other half remained ungrafted. Trees were grown in 10 cm x 10 cm x 36 cm tall pots filled with potting soil and treated with an Osmocote® fertilizer twice throughout the experiment. Plants were irrigated with salinity treatments of 0, 1, 3, 5, and 10 dS m^{-1} twice weekly throughout the study period. Saline solutions were prepared using Instant Ocean© sea salt (United Pet Group, Blacksburg, VA) and deionized water. Trees were set up in a random complete block design within each treatment. Greenhouse temperatures were regulated to remain between 10°C (January) and 36.5°C (July) to maintain sufficient survival and growing temperatures throughout the study.

3.2.2 Data collection

Physiological parameters were collected throughout the experiment. Leaf measurements were taken on a recent fully expanded mature leaf for each tree. Leaf chlorophyll fluorescence was measured using a chlorophyll fluorometer model OS1p (Opti-Sciences, Inc., Hudson, NH) using methodology described by Melgar et al. (2008). Stomatal conductance data was collected using a steady state diffusion leaf porometer (Model SC-1, Decagon Devices, Pullman, WA). Electrolyte leakage was determined using the method described by Lutts et al. (1996)Lutts et al. (1996). Two recently mature, fully expanded leaves were harvested, rinsed clean with deionized water and 6 discs (1 cm^2) sections per leaf were used in analysis. Leaf sections were added to test tubes with deionized water, then shaken overnight and then electrolyte leakage was measured using an electrical conductivity meter to determine the initial leakage. These containers were then autoclaved and electrolyte leakage was measured again after the solution cooled to approximately room temperature (~23ºC). Percent electrolyte leakage was calculated from the ratio of electrolyte leakage before and after plant cell membranes were burst during autoclaving. SPAD measurements were conducted on two leaves per tree per treatment using a chlorophyll meter (SPAD-502, Spectrum Technologies, Plainfield, IL).

3.2.3 Statistical analysis

Treatments were conducted using five replications of each rootstock per treatment and were set up in a complete randomized block design. Repeated measures MANOVA analysis was used to analyze time series data. Treatment interactions were analyzed

using full factorial and bivariate fit models and statistical significance (P <0.05) was determined using ANOVA with JMP® Pro 10.0.0 software (SAS Institute, Cary, NC).

3.3 RESULTS

3.3.1 Chlorophyll fluorescence

At higher salinities (5 and 10 dS m^{-1}), chlorophyll fluorescence ($F_vF_m^{-1}$) declined dramatically as trees were subjected to a longer time under salt stress ($P_{\text{duration of stress x salinity}} = 0.001$) (Fig. 3-1). Grafted trees showed a more pronounced decrease in fluorescence when irrigated with higher salinity solutions over the duration of the study ($P_{\text{grafting x salinity}} = 0.002$) than ungrafted trees. However, rootstocks showed significantly different reactions to salinity ($P_{\text{rootstock}} = 0.001$, $P_{\text{duration of stress x rootstock x salinity}} = 0.02$); C146 showed the most tolerance followed by C22 and SO. Marked differences in chlorophyll fluorescence were not obvious at the lower salinity levels, most remaining between 0.8 and 0.9 $F_vF_m^{-1}$, which generally indicates no negative effects on the functioning of PSII. We also found that the two highest salinity treatments resulted in a more rapid decrease in chlorophyll fluorescence over time for grafted plants than ungrafted plants ($P_{\text{duration of stress x grafting x salinity}} = 0.002$). Overall, non-grafted C146 and C22 rootstocks fared better than SO rootstocks when exposed to salinity.

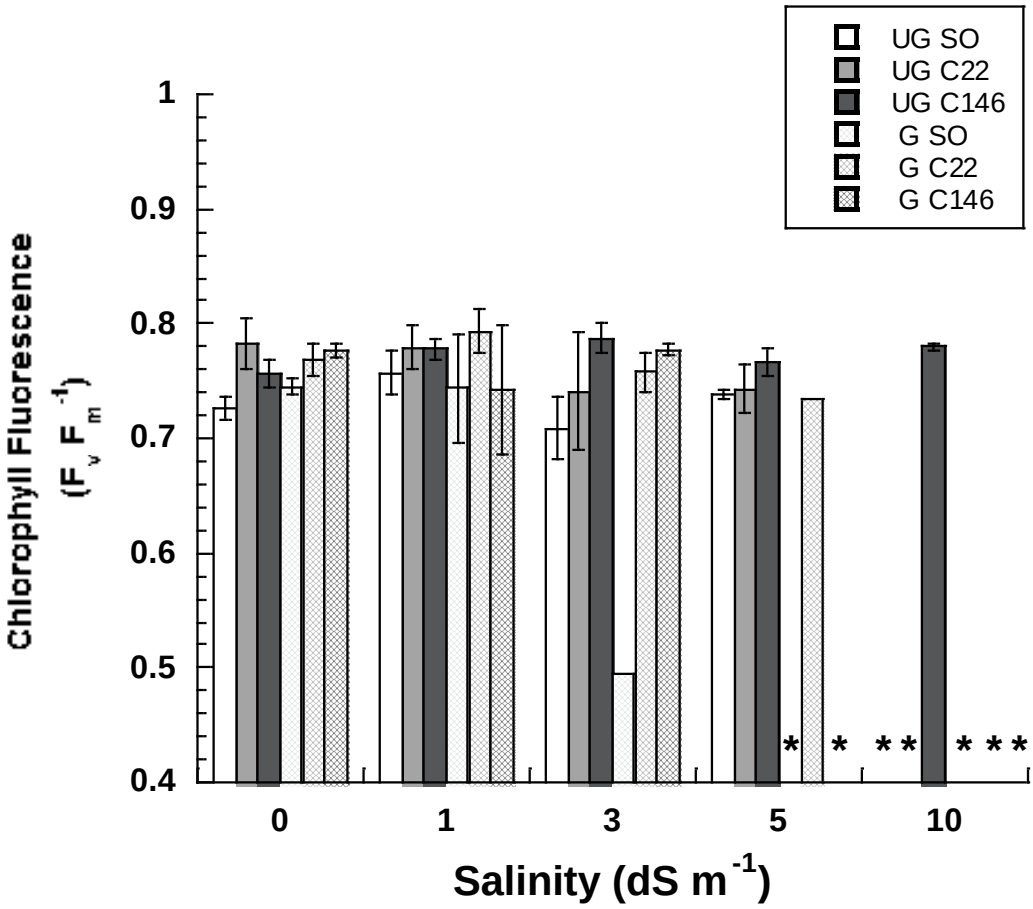


Figure 3-1 Chlorophyll fluorescence after 6 months of salinity treatment. Grafted trees are represented by solid shapes and solid lines, non-grafted trees are represented by open shapes and dashed lines. Error bars are shown as ±1 standard error of the mean.

Table 3-1. Chlorophyll fluorescence means at the end of the study. Significance was determined by full factorial fit model analysis, (*) denotes values with missing degrees of freedom and (na) denotes incalculable values.

Rootstock/Grafting Treatment	0 dS m^{-1}	1 dS m^{-1}	3 dS m^{-1}	5 dS m^{-1}	10 dS m^{-1}
UG SO	0.7263	0.7574	0.74375	0.738	
UG C22	0.783	0.779	0.741125	0.74325	
UG C146	0.757	0.778	0.786875	0.76625	0.77975
G SO	0.7445	0.743666667	0.494		
G C22	0.7688	0.793	0.75775	0.735	
G C146	0.7766	0.7425	0.7775		
	P values for treatments and interactions				
Time	0.031	0.290	0.008	0.530*	na
Rootstock	0.240	0.090	0.004	na	na
Grafting	0.024	0.055	0.071	0.157*	na
Time x Rootstock	0.570	0.710	0.004	na	0.004*
Time x Grafting	0.049	0.830	0.083	0.450*	na
Rootstock x Grafting	0.440	0.730	0.420	na	na
Time x Rootstock x Grafting	0.230	0.340	0.031	0.501*	0.026*

3.3.2 Stomatal conductance

Stomatal conductance was reduced more at higher salinities by the end of the study ($P_{\text{duration of stress x salinity}} < 0.001$). By the time of the last measurement, grafted trees had higher rates of stomatal conductance at 3 and 5 dS m^{-1} than ungrafted trees (Fig. 3-2). The highest stomatal conductance rates were observed at salinity levels between 1 and 3 dS $m^{-1}$for ungrafted trees, after which stomatal conductance declines with increasing salinity levels (Fig. 3-2). In contrast, C22 and C146 grafted rootstocks exhibited increased rates of stomatal conductance with increasing salinity levels until the point where trees were completely defoliated and no leaves were left to measure (Fig. 3-2).

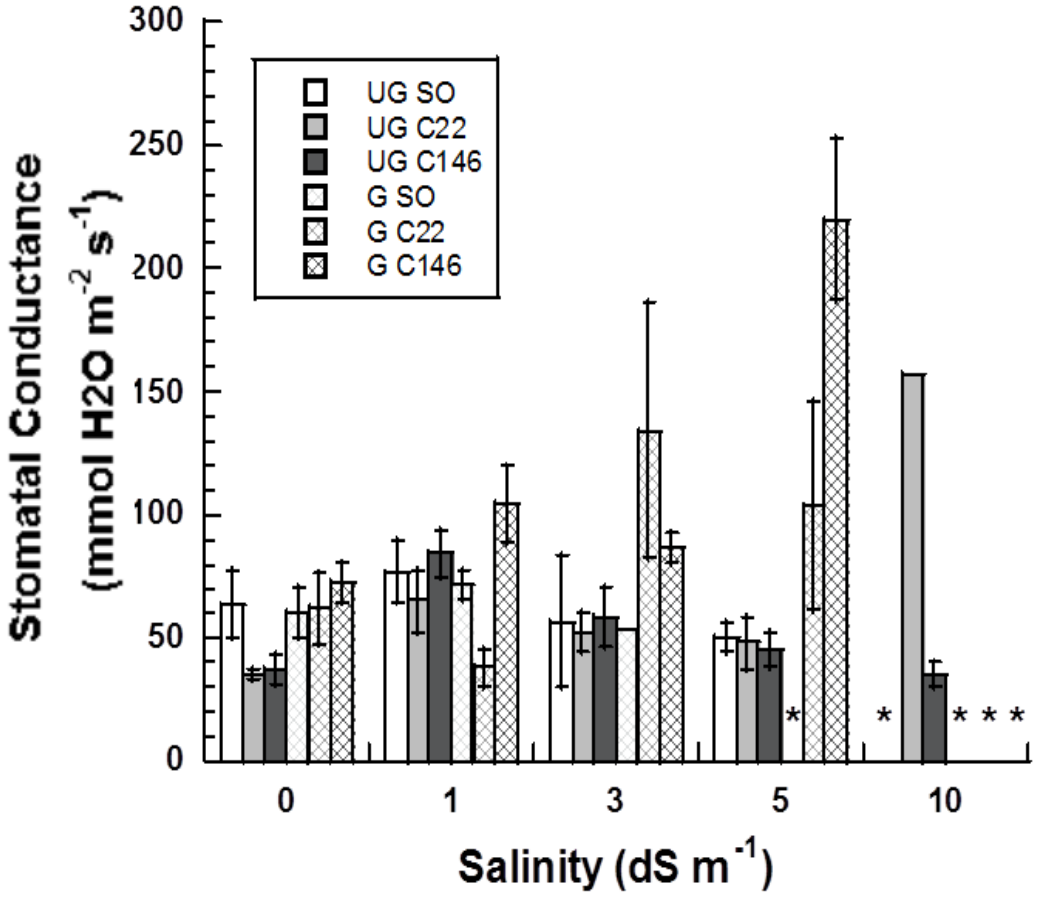


Figure 3-2. Stomatal conductance of trees at the end of the experiment as affected by salinity. Non-grafted (UG) trees are represented by solid bars, grafted (G) trees are represented by hatched bars. Error bars are shown as ±1 standard error of the mean. (*) denotes missing data as trees were dead or defoliated.

3.3.3 Electrolyte leakage

At the initiation of the study, trees exposed to all salinity levels had similar electrolyte leakage levels (approximately 15-20%). However, cell membrane stability

was reduced by high levels of salinity over the duration of the study ($P_{salinity} < 0.001$). At the middle point of the study only trees irrigated with the two highest salinity levels showed increases in electrolyte leakage. The largest increases in electrolyte leakage at day 55 (mid-way through the study) were in ungrafted and grafted SO at 10 dS m^{-1}, and grafted C146 at 5 and 10 dS m^{-1} (Fig. 3-3). C146 and C22 had lower % electrolyte leakage than SO ($P_{rootstock} = 0.006$, Fig. 3-3) and grafting increased electrolyte leakage ($P_{grafting} < 0.001$). Ungrafted C146 and C22 had the lowest electrolyte leakage percentages followed by ungrafted SO, and all grafted varieties (C22, C146 and SO) at most salinity levels (Fig. 3-3). At 10 dS m^{-1}, ungrafted C146 rootstocks had the lowest electrolyte leakage at 34.8% followed by ungrafted C22 at 95.3%, while ungrafted SO were completely defoliated. Grafted trees irrigated with 10 dS m^{-1} were completely defoliated by the end of the study, however, at 5 dS m^{-1} C22 grafted rootstocks had the lowest electrolyte leakage (36.0%) followed by grafted C146 (73.8%) and grafted SO (88.5%). Electrolyte leakage increased with the time trees were exposed to salinity stress, although at moderate salinities (3 dS m^{-1}) C146 ungrafted trees showed little change compared to C22 and SO grafted and ungrafted trees, whose electrolyte leakage almost doubled by the end of the experiment (Fig. 3-3).

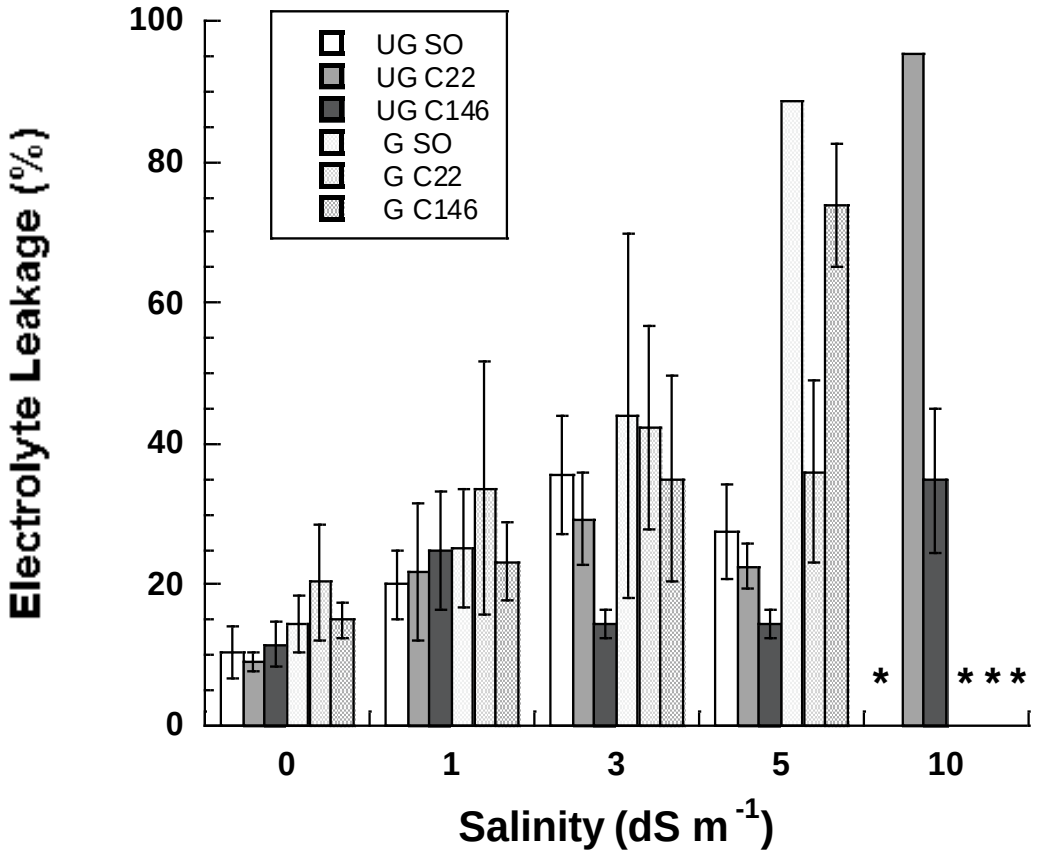


Figure 3-3. Electrolyte leakage of trees at the end of the experiment as affected by salinity, rootstock and grafting. Grafted trees are represented by unhatched bars, non-grafted trees are represented by hatched bars. ($P_{salinity} < 0.001$, $P_{rootstock \times salinity} = 0.008$, $P_{rootstock \times grafting \times salinity} = 0.005$). Error bars are shown as ±1 standard error of the mean. Defoliated trees denoted by (*).

3.3.4 SPAD

Duration of exposure to salinity, rootstock, grafting and salinity level all affected SPAD values, which is essentially a measure of leaf greenness (Grosser et al., 2012) ($P_{duration \times}$

rootstock x grafting x salinity = 0.002). SPAD values decreased as the time trees were exposed to salinity stress increased, indicating that the decline of SPAD can be related to salinity stress. Rouse et al. (1991) found that there was a high correlation between extracted chlorophyll content and SPAD readings ($r^2 = 0.96$). The degradation of chlorophyll or reduced synthesis of chlorophyll has been found to contribute to this occurrence (Santos, 2004). The decrease of SPAD values in ungrafted rootstocks ranged from 9-17% and ranged from 23 to 49% for grafted rootstocks at 5 dS m^{-1} when compared to 0 dS m^{-1} (Fig. 3-4), these findings were similar to the results found by Grosser et al. (2012) and (Hussain et al., 2012b). While all rootstocks showed a similar decline in SPAD values with increasing salinity levels, there was a significant rootstock effect ($P_{rootstock} = 0.009$), grafting effect ($P = 0.001$), and salinity effect ($P = 0.001$). Grafting and salinity treatments affect SPAD significantly, ungrafted SO, C22, and C146 SPAD readings declined in response to salinity increase ($r^2 = 0.56$, $r^2 = 0.67$, and $r^2 = 0.67$, respectively) as did the grafted trees ($r^2 = 0.81$, $r^2 = 0.98$, $r^2 = 0.74$, respectively). The decline was more dramatic for the grafted trees, slopes were -6.82, -3.17, and -5.47 SPAD dS^{-1} for grafted SO, C22 and C146, respectively (data not shown). SPAD readings of ungrafted trees declined less strongly with increasing salinity, -1.33, -4.31 and -3.65 for ungrafted SO, C22 and C146, respectively. There was also an interaction effect between rootstock, grafting, and salinity on SPAD measurements ($P_{rootstock \times grafting \times salinity} = 0.002$), where amongst grafted rootstocks SO had the strongest decline with increasing salinity (-6.82 SPAD dS^{-1}), while that for ungrafted rootstocks of SO was the least sensitive to salinity (-1.33 SPAD dS^{-1}).

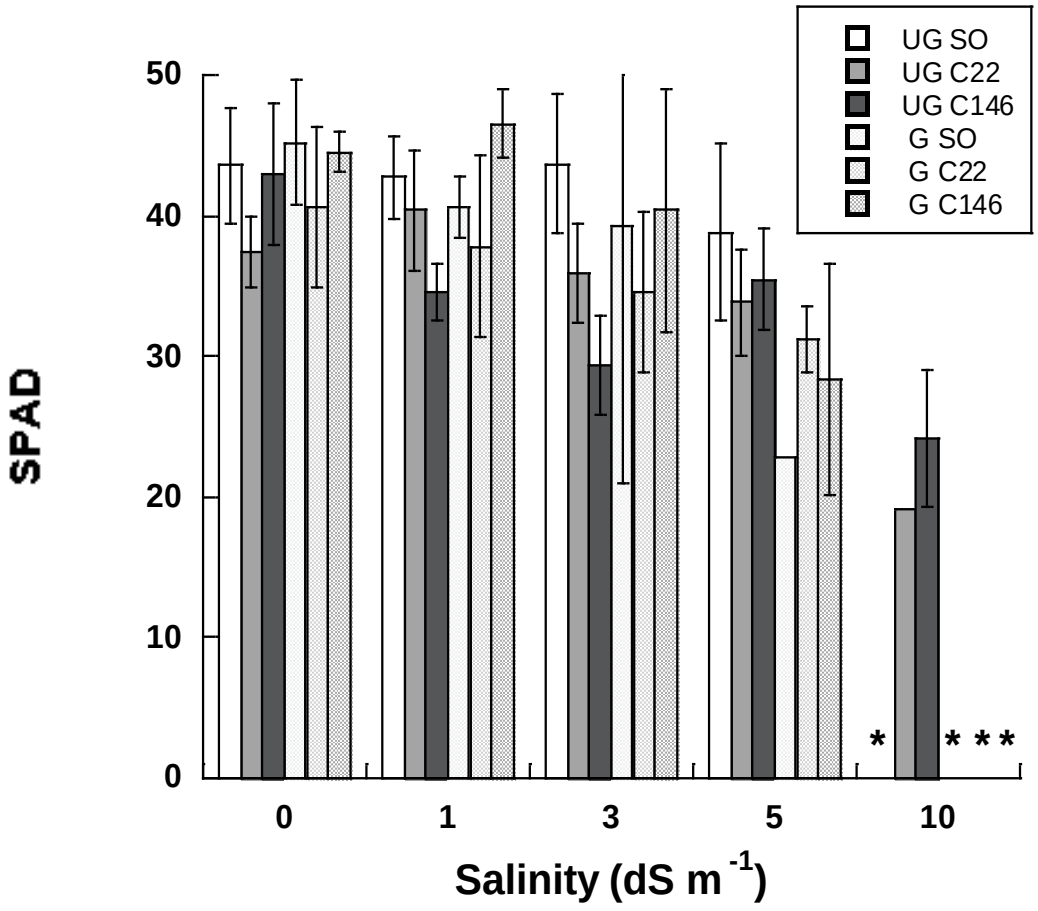


Figure 3-4. SPAD of grafted and ungrafted trees sorted by salinity. Grafted trees are represented by hatched bars, non-grafted trees are represented by unhatched bars. SPAD is an index of greenness which is correlated with visual greenness, values of 50-60 are typical for unstressed citrus (Grosser et al., 2012). Error bars are shown as ±1 standard error of the mean. Defoliated trees denoted by (*).

3.4 DISCUSSION

Salinity reduces chlorophyll fluorescence in plants by reducing energy transference to the reaction center of PSII (Santos, 2004). Often this reduction is due to toxic ion accumulation which can cause a decline in photochemical parameters such as reduced efficiency of electron transfer or can be due to osmotic effects from reduced water uptake (Levy and Syvertsen, 2004)(Levy and Syvertsen, 2004). In this study, rootstocks varied in their ability to withstand salinity stress. Because chlorophyll fluorescence is correlated with photosynthetic efficiency at the leaf level (Butler and Kitajima, 1975; Smethurst et al., 2005). Treatments that reduced $F_v F_m^{-1}$ in our experiment probably reduced photosynthetic efficiency in our trees, such that a combination of factors, grafting, salinity level and the length of time trees were exposed to salinity contributed to and likely reduced tree photosynthetic efficiency. Similar to these findings, we found that ungrafted SO rootstocks showed less chlorophyll fluorescence than C22 and C146 rootstocks. This contrasts with results found by Bleda et al. (2011)Bleda et al. (2011) which indicated that Cleopatra mandarin grafted onto Alemow rootstocks had higher levels of chlorophyll fluorescence when subjected to salinity stress. In the grafted plants, trees grafted to C146 had higher chlorophyll fluorescence values than trees grafted to C22 or SO rootstocks. Greater leaf area, higher PSII efficiency, ion assimilation and storage can indicate stress tolerance and may have attributed to these results (Lloyd et al., 1990; Munns, 2002; Storey and Walker, 1999).

Stomatal conductance values vary according to factors such as stress, abscisic acid (ABA), relative humidity, plant available radiation, internal CO_2 concentration and

leaf nitrogen content (Farquar and Sharkey, 1982; Lawlor and Cornic, 2002; Munns, 2002). Variations in these factors can cause increases or decreases in the stomatal conductance of measured plants. In this study, ungrafted trees showed little variation in stomatal conductance for all but the highest salinity treatments. While all grafted trees were defoliated in the 10 dS m^{-1} treatment by the end of the study, C22 and C146 displayed the highest rates of stomatal conductance for the 3 and 5 dS m^{-1} treatment levels (Fig. 3-3). This was most likely due to interference with osmotic regulation caused by high salinity in the irrigation water or due to damage in the leaf stomatal regulation from ion toxicity. The steady levels of stomatal conductance for the ungrafted rootstocks may signify that salinity stress affected stomatal closure at only the highest salinity treatment for these rootstocks. Due to these results, we believe that ungrafted rootstocks showed less stomatal indicated stress compared to grafted trees.

A lack of membrane stability under prolonged salinity stress has been correlated with spectral reflectance, antioxidant enzyme synthesis, membrane acyl lipid concentrations, water use efficiency, stomatal resistance and osmotic potential, all of which can be indicators of salinity stress (Ashraf and Harris, 2004; Bajji et al., 2001; Chen et al., 1999; Lutts et al., 1996). In living, healthy cells, membranes contain cell electrolytes and prevent them from leaking out into the apoplast. Electrolyte leakage reflects membrane damage as a result of lipid and protein degradation which may occur during stress (Rolny et al., 2011). Plant cells that remain undamaged maintain electrolytes within the plasma membrane, while electrolytes in damaged cells may leak out into the apoplast. Electrolyte leakages above 50% have been reported to be lethal to

citrus leaf cells, however this varies amongst varieties (Ebel et al., 2004; Levitt, 1956; Nesbitt et al., 2002). Increased leakage has also been associated with increased senescence and leaf injury (Bajji et al., 2001; Campos et al., 2003; Rolny et al., 2011; Whitlow et al., 1992). In our findings, chronic salinity stress increased electrolyte leakage in our experiment. We found that SO rootstocks had higher electrolyte leakage than C22 and C146, indicating that is more susceptible to salinity stress than the other two rootstocks. Grafting resulted in increased electrolyte leakage compared to non-grafted plants which is similar to findings by Ziogas et al. (2013).

SPAD (Soil Plant Analysis/Analytical Development) is a unit-less, objective measurement of chlorophyll indicated by leaf color. It has been used to detect nutrient deficiencies or other problems that result in color changes in leaves (Grosser et al., 2012; Hussain et al., 2012b; Jifon et al., 2005; Pestana et al., 2005). Jifon et al. (2005) found that the correlation between SPAD and chlorophyll content in leaves was greater than $r^2 = 0.92$ for all citrus varieties studied. In our experiment, SPAD indicated that increased salinity stress caused a decrease in leaf greenness (Fig. 3-4), suggesting a possible loss of chlorophyll (Jifon et al., 2005). Salinity has been found to cause nutrient deficiencies which result in lowered SPAD measurements and chlorotic leaf tissue (Hussain et al., 2012b; Pestana et al., 2005). While leaf nitrogen was not deficient in any of the rootstocks varieties evaluated, our nutrient data suggest that trees treated with 10 dS m^{-1} irrigation water had lower N concentrations than leaves of trees treated with lower salinity levels data not shown). This indicates that at lower salinities, nitrogen was not the cause of leaf chlorosis but it was more likely due to salinity stress.

3.5 CONCLUSIONS

These results suggest that increased salinity leads to reduced photosynthetic efficiency, stomatal conductance, leaf greenness, and increases membrane leakage. At salinities around 1 dS m^{-1} rootstocks were less affected by salinity, however, at higher salinities all SO rootstocks and grafted trees were more affected than other rootstocks and ungrafted trees. This indicates that when citrus trees are irrigated with low quality water, C22 or C146 rootstocks would be a better choice than SO rootstocks. While grafting reduced salinity tolerance of all trees, at moderate salinities C22 and C146 grafted trees may still be a better choice than SO rootstock.

4 THE RELATIONSHIP BETWEEN SALT STRESS AND NUTRITIONAL IMBALANCE IN GRAFTED AND UNGRAFTED CITRUS TREES

4.1 INTRODUCTION

Salinity stress has been known to affect the delicate balance of nutrients within plants, causing deficiencies and ineffective stress management within the plant system (Munns and Termaat, 1986)(Munns and Termaat, 1986). Macro and micronutrients are vital in plant physiological processes ranging from photosynthesis to water movement within the plant (Evans and Sorger, 1966). Many studies have linked tolerance of abiotic stress conditions to nutrient balance within the plant as reviewed by Cheeseman (1988) and Hayward and Bernstein (1958). For example, plant tolerance to stress is dependent upon the plant's ability to exclude toxic ions, acclimate to osmotic stress, and maintain growth and CO_2 assimilation when exposed to salt stress (Cheeseman, 1988; Munns and Termaat, 1986; Syvertsen et al., 1988). Low Ca^{2+} / Na^{+} and K^{+} / Na^{+} ratios can increase membrane permeability and therefore increase Na^{+} and Cl^{-} accumulation in tissues (Greenway and Munns, 1980; Syvertsen et al., 1988). This accumulation of toxic levels of ions can lead to cell and eventually tissue death in sensitive crops. Salinity negatively affects plant acquisition of nutrients, often leading to nutrient deficiencies in addition to toxic ion effects (Cramer, 1985; Ruiz et al., 1997a).

Citrus is a salt sensitive crop, showing declining yields when exposed to relatively low salinity levels (~1.7 dS m^{-1}) (Maas, 1992). Citrus is commonly grafted to acclimate

desirable citrus fruit varieties to conditions they would not be tolerant of and to improve plant yield and fruit quality (Spiegel-Roy and Goldschmidt, 1996). The search for citrus rootstocks that contribute to salt tolerance has spanned many decades, yielding only moderate results (Cooper et al., 1952; Grosser et al., 2012; Maas, 1992; Storey and Walker, 1999; Zekri and Al-Jaleel, 2003). Historically, sour orange rootstocks have been used extensively in citrus production in Texas, the third highest citrus producing state (Louzada et al., 2008; United States Department of Agriculture, 2009), however SO is not known to be salinity tolerant. We will discuss the ability of two new rootstocks and one commonly used rootstock, sour orange, to exclude toxic ions. Toxic ion exclusion (in particular Na^+ and Cl^- ions) is correlated with improved salt tolerance in citrus (Storey and Walker, 1999).

Na^+ and Cl^- toxicity levels are variable amongst studies in citrus. The threshold varies amongst variety and rootstock, and their interactions. Na^+ toxicity has been seen at levels as low as 10 µg g^{-1}, while others did not find toxicity until levels increased above 50 µg g^{-1} (Hayward and Bernstein, 1958; Sauls, 2008). The bulk of scientific studies use 25 µg g^{-1} as the threshold for Na^+ toxicity in citrus trees (Chapman, 1949; Ferguson and Grattan, 2005; Grattan and Grieve, 1999; Obreza et al., 1992). Cl^- toxicity has been shown at ranges from 10 µg g^{-1} to 70 µg g^{-1} (Chapman, 1949; Levy et al., 1999; Obreza et al., 1992). Research has been inconsistent in determining whether Na^+ or Cl^- is more toxic to citrus trees as some showed more sensitivity to Na^+ (Cooper et al., 1952; Lloyd et al., 1989) and others more sensitivity to Cl^- (Bañuls and Primo-Millo, 1995; Behboudian et al., 1986; Grieve and Walker, 1983; Maas, 1992).

The objectives of this study were to a) determine how salinity, grafting, and rootstock affect the nutritional status of citrus leaves, and b) determine how Na^+ and Cl^- impact tree mortality and physiological processes and whether this response is affected by grafting and rootstock.

4.2 MATERIALS AND METHODS

4.2.1 Plant materials and growing conditions

Citrus trees were propagated and grafted at the Texas A&M University-Kingsville Citrus Center in Weslaco, TX in 2009 and 2010. Olinda Valencia scions were budded onto Sour Orange (SO), C22, and C146 rootstocks in 2010. Ungrafted SO, C22 and C146 trees were propagated from seed and used in this experiment at approximately 1 year of age. Trees were grown in 10 cm x 10 cm x 36 cm tall pots in the Citrus Center greenhouse and moved north to Kingsville, Texas into the greenhouse located on campus at Texas A&M University-Kingsville in January 2011. C22 and C146 are cultivated from Sunki mandarin and Swingle trifoliate orange crosses that were developed at the University of California, Riverside (Louzada et al., 2008). Osmocote® slow release fertilizer (Indoor/Outdoor Smart-Release® Plant food, The Scotts Company, LLC, Columbus, OH) was applied to pots at 3 month intervals. A total of 11.4 g of N, 1.6 g P, and 6.0 g K^+ were added to each pot throughout the experiment. Plants were irrigated twice weekly with treatments of 0, 1, 3, 5 and 10 dS m^{-1} instant Ocean© sea salt solutions (United Pet Group, Blacksburg, VA) over six months until July 2011 (Table 4-1). The 0 dS m-1 treatments were irrigated with water filtered by reverse osmosis (RO).

Irrigation frequency and volume was designed to replenish transpirational water losses without excessive leaching from pots. Soil electrical conductivity (EC) was monitored throughout the study, and pots were leached with RO water if the EC went beyond the treatment level. Greenhouse temperatures were regulated and maintained between a minimum temperature of 10 °C (January) and a maximum temperature of 36.5 °C (July) throughout the duration of the study.

To address concerns regarding the concentrations of ions within the salt solutions, we calculated the approximate amount (g L^{-1}) of each element at 10 dS m^{-1} (Table 4-1). The concentrated solution is based on manufacturer guidelines and was calculated to be approximately 48.8 dS m^{-1} at full strength. At the highest salinity level (10 dS m^{-1}) 2.21 g of Na^{+} and 3.95 g of Cl^{-} were applied to trees with each liter of water applied. In addition to NaCl salts, SO_4^{-}, Mg^{2+}, K^{+}, and Ca^{2+} salts were also applied, increasing possible availability of these ions in solution (Table 4-1).

Table 4-1. Ions (g L^{-1}) in concentrated Instant Ocean® and at 10 dS m^{-1}. Instant Ocean® was calculated to be 48.8 dS m-1 at the recommended rate; all calculations are derived from these values. Adapted from a thesis by LeeRoy Rock, Texas A&M University – Kingsville, August, 2008.

Ion	g L^{-1} at 10 dS m^{-1}
Cl	3.95
Na	2.21
SO4	0.55
Mg	0.27
K	0.09
Ca	0.08
Carbonate/Bicarb	0.04
Br	0.00115
Strontium	0.00180
B	0.00115
Fluoride	0.00020
Li	0.00006
I	0.00005
Fe	0.00001
Mn	0.00001
Cu	0.00000
Zn	0.00000
NO3	0
PO4	0

4.2.2 Leaf tissue analysis

After six months, plants were harvested and separated into leaves, stems and roots. Leaves, stems and roots were washed, weighed and dried at 60°C for 48 hours. Leaves were weighed and ground to approximately 0.8 mm for N, P, K, Ca, Mg, Zn, Fe, Cu, Mn, S, B, Na and Cl analysis by Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory located in College Station, Texas. Total N was

determined by high temperature combustion as described by Nelson and Sommers (1973). B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn were determined through ICP analysis of nitric acid digestion (Havlin and Soltanpour, 1980). Cl was extracted using methods described by Liu (1998) and analyzed using a Cl ion selective electrode (Dr. Tony Provin, September 2013, personal communication).

4.2.3 Statistical analysis

Data was analyzed using JMP®Pro 10.0.0 software (SAS Institute, Cary, NC). The experimental setup consisted of five replications for each rootstock per salinity treatment in a randomized complete block design. Treatment effects and interactions were analyzed using full factorial fit model and bivariate fit models when appropriate. P value was deemed significant between treatments, rootstocks and grafting was tested through students T comparison of the means.

4.3 RESULTS

4.3.1 Overall nutrient status by the end of the study

By the end of this experiment rootstocks showed similar nutrient concentrations for grafted and ungrafted trees (Fig. 4-1 and 4-2). None of the rootstocks, grafted or ungrafted, showed macronutrient deficiencies at any of the salinity treatment levels. However, Na^+ and Cl^- concentrations exceeded the reported minimum toxicity thresholds for all rootstocks in most or all of the salinity treatments (Fig. 4-3). When comparing N, P, and Ca^{2+} status between grafted and ungrafted rootstocks (Fig. 4-1.), grafted trees showed a steeper decline in nutrient concentrations with increasing salinity

levels than ungrafted trees. Na^+ and Cl^- ions reached toxic amounts at levels above 1 dS m^{-1} for grafted SO, C22, C146, and ungrafted C146 trees (Fig. 4-3). Ungrafted C22 and SO rootstocks reached toxic levels at 3 dS m^{-1} and above. The association of Na^+ and Cl^- toxicity is more evident in mortality, which increases with increasing Na^+ and Cl^- concentrations in the leaf tissue (Fig. 4-4.).

Concentrations of P, Mg^{2+} and S in citrus leaves did not reach deficient levels by the time of harvest and subsequent analysis. Moreover, concentrations of Mg^{2+} increased for at increasing salinity levels. Leaves of grafted trees contained lower concentrations of nutrients at corresponding salinity levels when compared to the ungrafted trees. However, only P and S showed significant difference between grafting treatments (Table 4-2).

Na^+ and Cl^- increased proportionally with increased salinity (Fig. 4-2.) Rootstock, salinity, and the interaction between rootstock and salinity influenced Na^+ and Cl^- significantly. Grafting treatment had a significant impact on Cl^- concentration in citrus leaves at the 3 dS m^{-1} level while Na^+ levels were significant only at the 1dS m^{-1} level (Table 4-2).

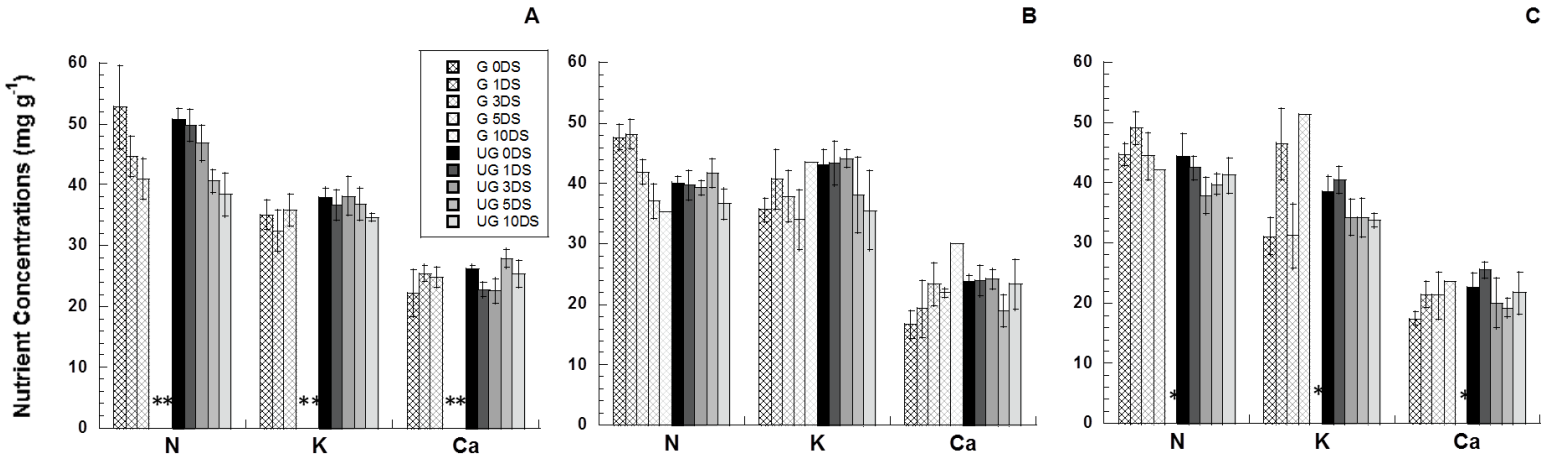


Figure 4-1. Concentrations of N, K, and Ca in leaves at 0, 1, 3, 5, and 10 dS m^{-1}. A) Concentrations of N, K, and Ca (mg g^{-1}) for grafted and ungrafted SO leaves, B), concentrations of N, K, and Ca (mg g^{-1}) for grafted and ungrafted C22 leaves, C) Concentrations of N, K, and Ca (mg g^{-1}) for grafted and ungrafted C146 leaves. Solid bars represent grafted trees and lined bars represent ungrafted trees. (*) represents defoliated or dead trees. Error bars are shown as ±1 standard error of the mean.

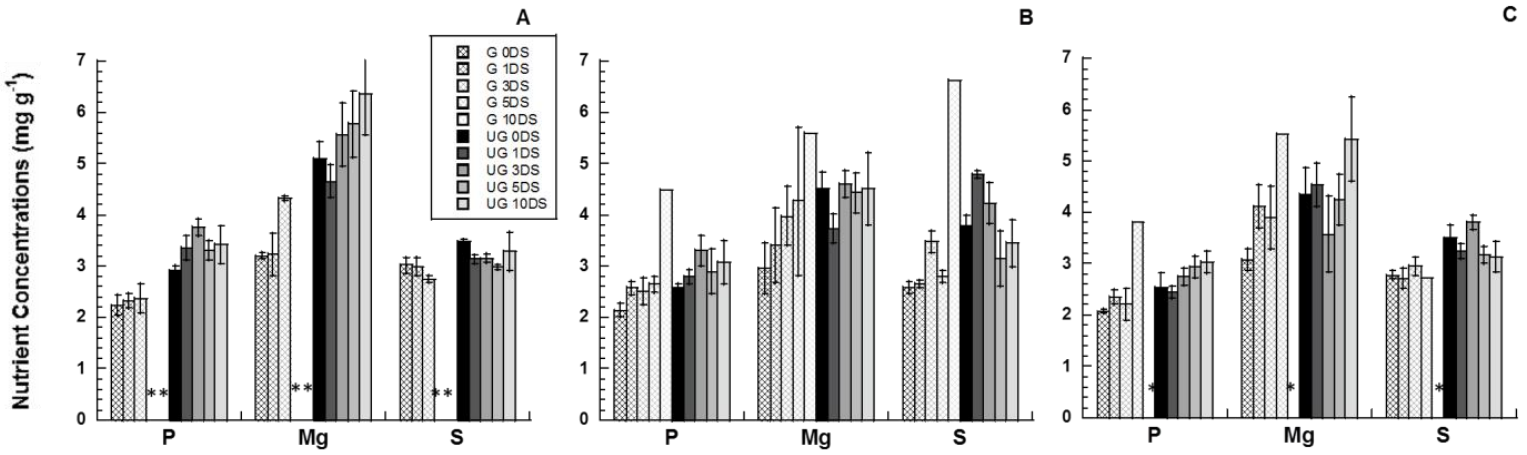


Figure 4-2. Concentrations of P, Mg, and S in leaves at 0, 1, 3, 5, and 10 dS m^{-1}. A) Concentrations of P, Mg, nd S (mg g^{-1}) for grafted and ungrafted SO leaves, B) Concentrations of P, Mg, and S (mg g^{-1}) for grafted and ungrafted C22 leaves, C) Concentrations of P, Mg, and S (mg g^{-1}) for grafted and ungrafted C146 leaves. Solid bars represent grafted trees and lined bars represent ungrafted trees. (*) represents defoliated or dead trees. Error bars are shown as ±1 standard error of the mean.

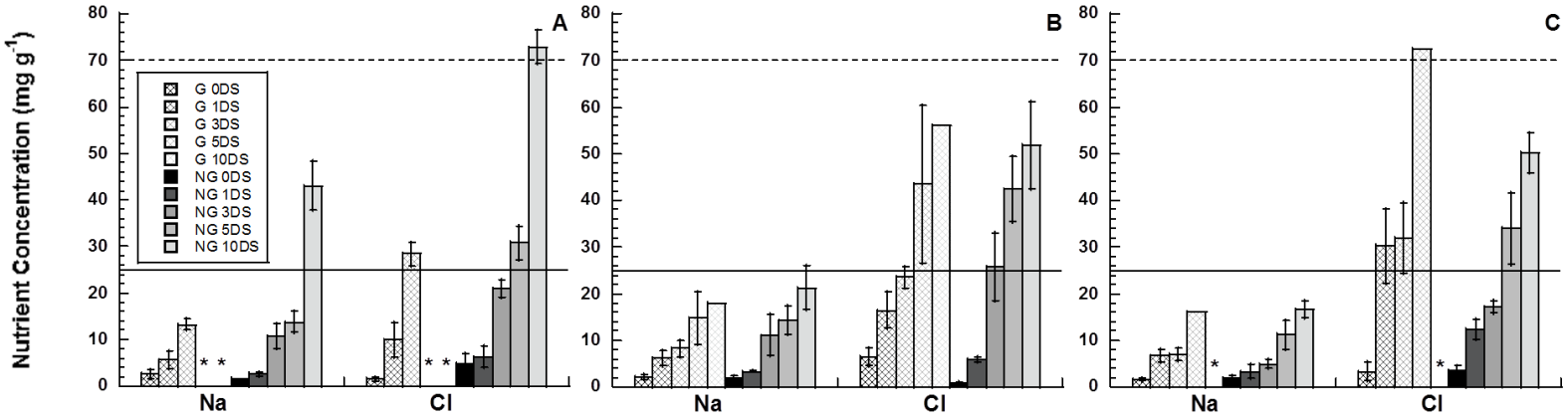


Figure 4-3. Concentrations of Na+ and Cl- in leaves at 0, 1, 3, 5, and 10 dS m^{-1}. A) Concentrations of Na^+ and Cl^- (mg g^{-1}) for grafted and ungrafted SO leaves, B) Concentrations of Na^+ and Cl^- (mg g^{-1}) for grafted and ungrafted C22 leaves, C) Concentrations of Na^+ and Cl^- (mg g^{-1}) for grafted and ungrafted C146 leaves. Solid bars represent grafted trees and lined bars represent ungrafted trees. (*) represents defoliated or dead trees. Error bars are shown as ±1 standard error of the mean. Solid horizontal lines indicate toxicity threshold ranges for Na+ and dashed horizontal lines indicate toxicity threshold ranges for Cl- (Obreza et al., 1992).

Salinity reduced N concentrations and increased P, Mg^{2+}, Na^{+}, Cl^{-} and Cu^{2+} at increasing salinities, while grafting reduced P, Zn, Fe, S, and B^{3+} concentrations (Table 4-2, Figs. 4-2 & 4-3). The only micronutrient found to be deficient was Cu^{2+}, and this deficiency was consistent across rootstocks and salinity treatments (Appendix A-10). Boron is toxic in levels above 200 µg g^{-1} (Obreza et al., 1992). Toxic levels of B^{3+} accumulated in all trees, grafted and ungrafted (Appendix A-10). However, there was no statistically significant link between B^{3+} and mortality for this experiment ($P = 0.1032$).

4.3.2 Relationship between ion concentration and mortality

Mortality of grafted and ungrafted trees was influenced by both Na^{+} ($P<0.001$) and Cl^{-} ($P= 0.001$) concentrations (Fig. 4-4). Our data indicate that lower concentrations of Na^{+} caused greater mortality in grafted and ungrafted trees than Cl^{-}. There was lower overall mortality in ungrafted trees and indicating that the threshold of toxicity may be lower for grafted trees compared to ungrafted trees.

Table 4-2. Statistical significance between nutrients, rootstock and grafting at each salinity level. Full factorial fit model analysis with nutrient as the response variable was run at each salinity level.

0 dS m^{-1}	**N**	**P**	**K**	**Ca**	**Mg**	**Zn**	**Na**	**Cl**	**Fe**	**Cu**	**Mn**	**S**	**B**
Rootstock	0.026	NS	NS	NS	NS	0.021	NS	NS	NS	NS	NS	NS	NS
Grafting	NS	0.001	0.008	0.002	0.001	0.001	NS	NS	NS	NS	0.005	0.001	NS
Rootstock x Grafting	NS	NS	NS	NS	NS	0.007	NS	NS	NS	NS	NS	NS	NS
1 dS m^{-1}	**N**	**P**	**K**	**Ca**	**Mg**	**Zn**	**Na**	**Cl**	**Fe**	**Cu**	**Mn**	**S**	**B**
Rootstock	NS	0.046	NS	NS	NS	0.001	NS	0.004	NS	NS	NS	0.001	NS
Grafting	NS	0.004	NS	NS	NS	0.001	0.018	0.014	NS	NS	NS	0.001	0.026
Rootstock x Grafting	0.041	0.028	NS	NS	NS	0.001	NS	NS	NS	NS	NS	0.001	NS
3 dS m^{-1}	**N**	**P**	**K**	**Ca**	**Mg**	**Zn**	**Na**	**Cl**	**Fe**	**Cu**	**Mn**	**S**	**B**
Rootstock	NS	NS	NS	NS	NS	NS	NS	0.013	NS	NS	NS	0.002	0.006
Grafting	NS	0.001	NS	NS	NS	NS	NS	NS	0.015	NS	NS	0.001	NS
Rootstock x Grafting	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5 dS m^{-1}	**N**	**P**	**K**	**Ca**	**Mg**	**Zn**	**Na**	**Cl**	**Fe**	**Cu**	**Mn**	**S**	**B**
Rootstock	NS*	NS*	NS*	NS*	NS*	NS*	~	~	NS*	NS*	NS*	NS*	NS*
Grafting	~	~	~	~	~	~	~	~	~	~	~	~	~
Rootstock x Grafting	NS*	NS*	NS*	NS*	NS*	NS*	~	~	NS*	NS*	NS*	NS*	NS*

NS indicates insignificant values. (~) denotes incalculable values and (*) denotes values missing degrees of freedom. 10 dS m-1 was not included in the statistical analysis because of too many missing values due to tree mortality or leaf drop.

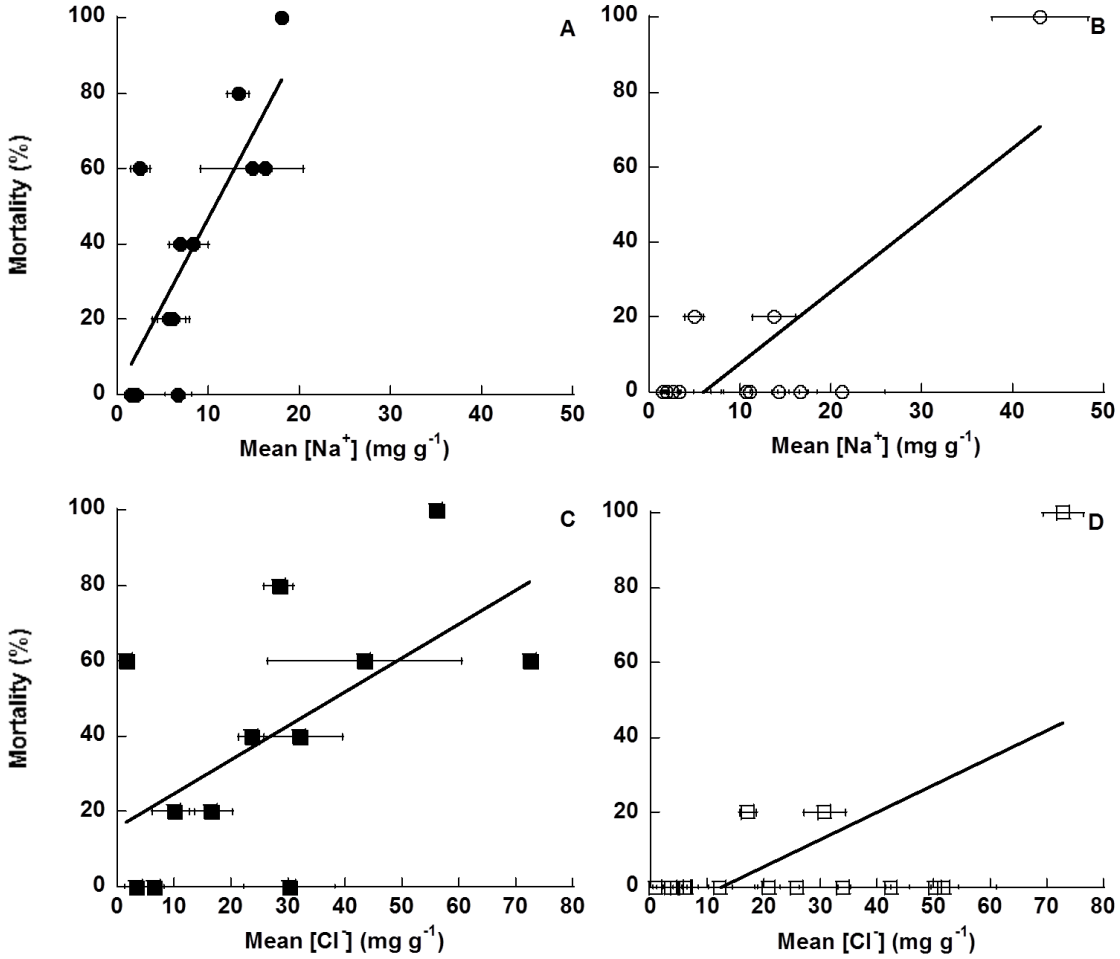


Figure 4-4. Concentrations of mean Na^+ (mg g^{-1}) and Cl^- (mg g^{-1}) compared to mortality in grafted and ungrafted citrus trees. A) Mean Na^+ correlated to mortality in grafted trees, B) Mean Na^+ correlated to mortality in ungrafted trees, C) Mean Cl^- correlated to mortality in grafted trees, D) Mean Cl^- correlated to mortality in ungrafted trees. Error bars are shown as ±1 standard error of the mean. ($P_{grafting \times Na^+} = 0.022$, $P_{grafting \times Cl^-} = 0.83$, $P_{grafting} < 0.001$, $P_{Cl^-} = 0.001$).

4.3.3 Nutrient to Na^+ ratio

K^+/Na^+ ratio decreased as salinity increases (Fig. 4-5A). Grafting decreased the K^+/Na^+ ratio significantly at 1 and 3 dS m^{-1} (P=0.001 and P=0.030, respectively). Significance for 5 and 10 dS m^{-1} could not be calculated due to lack of leaf data from defoliated trees. The Ca^{2+}/Na^+ ratio also declined with increasing salinity (Fig. 4-5B), with grafting significantly decreasing the ratio at 0 and 1 dS m^{-1} (P=0.025 and P=0.002, respectively). At 3 dS m^{-1} the impact of grafting was not significant, and at 5 and 10 dS m^{-1} significance could not be calculated due to defoliated trees. There were no rootstock effects, nor were there significant interactive effects between rootstock and grafting.

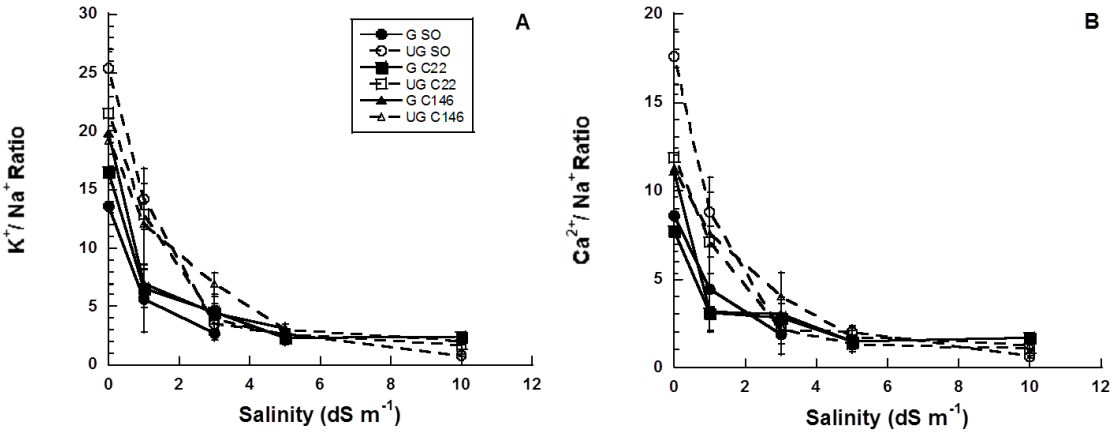


Figure 4-5. K^+/Na^+ and Ca^{2+}/Na^+ ratios in leaves for grafted and ungrafted trees at each salinity level. A) K^+/Na^+ ratio for grafted and ungrafted trees, B) Ca^{2+}/Na^+ ratio for grafted and ungrafted trees. Solid lines represent grafted trees, dashed lines represent ungrafted trees. Error bars are shown as ±1 standard error of the mean.

4.4 DISCUSSION

The relationship between plants exposed to salinity and their nutritional balance is complicated. Physiological processes such as stomatal regulation, photosynthesis, membrane permeability and CO_2 assimilation have all been linked to plant nutritional status and ion toxicity (Bañuls et al., 1991; Behboudian et al., 1986; García-Legaz et al., 1993; Lloyd et al., 1989, 1990).

Plant nutrients are vital for plant growth and reproduction. Nitrogen is taken up in the highest amounts for use in amino acids, proteins, nucleic acids, coenzymes etc. (Evans and Sorger, 1966). Phosphorus is a component of phosphates, nucleic acids, phospholipids, and ATP (Evans and Sorger, 1966). Potassium contributes to maintaining turgor pressure and solute transport within the xylem as well as protein synthesis, enzyme activation and maintaining optimal photosynthetic capacity during stress (Chow et al., 1990; Marschner, 1995). Sulfur plays an important role in structure and regulation of many plant processes, as well as e- transport and Fe-S clusters, enzymes and secondary metabolites (Evans and Sorger, 1966; Marschner, 1995). Sulfur and Zinc have also been shown to ameliorate some of the toxic effects of salt stress in many crops (Mehrotra et al., 1986; Shahriaripour et al., 2010). Furthermore, the interactions of Zn, SO_4^{2-}, and Ca^{2+} have been associated with reductions of B^{3+} and NaCl induced stress (Colla et al., 2012; Grattan and Grieve, 1999; Mehrotra et al., 1986; Ruiz et al., 1997a). Magnesium is the central element in the chlorophyll molecule and is essential in chloroplast function, but has to compete with Ca^{2+} for sites in the root plasma membrane (Evans and Sorger, 1966; Marschner, 1995). Calcium has been

known to preserve structural integrity of plant membranes by stabilizing cell wall structures and regulating ion transport and selectivity and has been known to reduce transport of Na^+ and Cl^- from roots to leaves in citrus, ultimately reducing negative effects of salinity (Bañuls et al., 1991; Bañuls et al., 1997; Maas, 1992; Zekri, 1993; Zekri and Parsons, 1990, 1992). Additionally, salinity has been found to reduce Mg^{2+} and Ca^{2+} concentrations in citrus (Ruiz et al., 1997a). During this experiment we also observed that as salinity increased N concentration within the leaves decreased for all rootstocks and treatments, but less dramatically for ungrafted C22 and C146. In contrast, P concentrations increased with increasing salinity. This could be caused by these nutrients being unaffected by reduced water uptake, fertilization, or the resulting reduced competition for uptake from a soil solution.

Salinity can cause an overall reduction in nutrient uptake which is a result of competition from the Na^+ and Cl^- ions for K^+ and NO_3^- ion channels (Cramer, 1985; Grattan and Grieve, 1999). There are many mechanisms involved with nutrient uptake under saline conditions; ion competition, reduced ion selectivity, genotypic sensitivity, and reduced water (and thus nutrient) uptake (Bernstein, 1980; Cramer, Lauchli, & Polito, 1985; Grattan & Grieve, 1999; Santa-Cruz, Martinez-Rodriguez, Perez-Alfocea, Romero-Aranda, & Bolarin, 2002). Ruiz et al. (1997a) found that salinity affected some citrus rootstocks more negatively than others. *Citrus macrophylla* had higher concentrations of K^+ as salinity increased, while other rootstocks had reduced K^+ in leaves and roots (Ruiz et al., 1997a) . However, most of these studies have been conducted with single salt (e.g. NaCl) solutions, which may not directly correlate with

irrigation waters composed of multiple salts. The application of an irrigation solution with Ca^{2+}, Mg^{2+} and S may reduce the negative effects seen in single salt irrigation studies. In our study, rootstock affected leaf N, Zn, P, Cl^-, S and B (Table 4-2) while grafting impacted concentrations of P, K^+, Ca^{2+}, Mg^{2+}, Zn, Mn, S, Fe, Na^+, Cl^- and B in leaves (Table 4-2). All treatments showed a decrease in N and an increase in P, Mg^{2+}, Na^+, and Cl^- at increasing salinities (Figs. 4-1, 4-2 and 4-3). The decline in N accumulation has been found to correspond with Cl^- antagonism of NO_3^- uptake or possibly the reduced ability of salinity affected plants to take up water (Ferguson and Grattan, 2005; Grattan and Grieve, 1992). The salt solution lacked additional N and P (Table 4-1), and thus availability of these nutrients in the nutrient solution did not increase with increasing salinity. Tissue P concentrations increased as salinity increased (Fig. 4-3) but was higher in ungrafted trees, suggesting that ungrafted trees were more efficient nutrient foragers. This effect was impacted by rootstock and grafting treatment at the 1 dS m^{-1} level as ungrafted SO trees had higher P tissue concentrations than grafted and ungrafted C22 and C146 trees. It may be that ungrafted SO were more effective at taking up P, however, as trees in the ungrafted treatment were generally smaller, and growth of SO in particular was very sensitive to salinity, the increase in leaf tissue P concentrations (which are expressed on a mass basis) may have also been caused by decrease in plant mass. Conversely, at 1dS m^{-1} tissue S concentrations were higher in ungrafted C22 and C146 trees than grafted trees and ungrafted SO. Sulfur decreased in grafted SO and ungrafted SO, C22, and C146 as salinities increased, but increased for the grafted C22 and C146 trees as salinity increased. Mg^{2+} increased with

increased salinities as well, though grafted trees at 0 dS m^{-1} had significantly higher concentrations of Mg^{2+} than ungrafted trees (P=0.001), implying that grafted plants were able to take up higher amounts of Mg^{2+} from the soil solution.

Mortality increased as tissue Na^+ and Cl^- concentrations increased, however, the relationship was not as strong for Cl^- concentrations; suggesting that Na^+ is more detrimental to these rootstocks than Cl^-. This is contrary to findings by Behboudian et al. (1986) and Cooper et al. (1952) who found Cl^- to be more toxic to citrus than Na^+ concentrations in several tissue studies.

K^+/Na^+ ratios have previously been implicated in improved salinity tolerance of glycophytic plants (Maathuis and Amtmann, 1999). Na^+ can interfere with plant uptake of K^+ because both Na^+ and K^+ compete for the same transport sites on plant root cells (Maathuis and Amtmann, 1999), disrupting root membrane selectivity in salt sensitive plants (Cachorro et al., 1993; Grattan and Grieve, 1999). Na^+ in cell cytoplasm can also compete with K^+ for binding sites, hindering metabolic processes which depend on K^+ (Maathuis and Amtmann, 1999). Plant tolerance to salt can be highly dependent upon their abilities to preferentially take up K^+ or to transport K^+ from tissues, while sequestering Na^+ (Walker, 1986), and their ability to maintain high K^+/Na^+ ratios (Cachorro et al., 1993; Maathuis and Amtmann, 1999). Ca^{2+} can partially mitigate Na^+ impacts. Results from several studies indicate that supplementary Ca^{2+} can decrease the negative effects of Na^+ by shifting channel selectivity from Na^+ to K^+ by decreasing membrane permeability (Bañuls et al., 1991; Cachorro and Cerdá, 1994). The results presented here indicate that grafted trees have less ability to maintain high K^+/Na^+ ratios

compared to ungrafted trees. However, results from Walker (1986) suggest that the plants have the ability to exchange K^+ for Na^+ in bark, stem, and root tissues, effectively immobilizing Na^+ in these tissues. They can then transport the mobile K^+ to leaves. These results imply that grafting may interfere with the translocation of K^+ and compartmentalization of Na^+ in woody tissues. The significant decline of Ca^{2+} in grafted trees at 0 and 1 dS m^{-1} may also signify an increase in membrane permeability and reduced K^+ selectivity in root cells.

4.5 CONCLUSIONS

In this experiment, we found that the complex ocean salt solution we used to irrigate our citrus trees did not reduce their nutritional status to deficiency thresholds. Although Na^+ and Cl^- concentrations increased significantly they only reached toxic thresholds previously used by others at the highest salinity treatment (Obreza et al., 1992). Leaf tissue concentrations of many of the elements still remained within adequate levels to maintain growth and reproductive functions, although several (e.g. N, K) decreased significantly with increasing salinity levels. The increasing presence of K^+, Ca^{2+}, and S concentrations in irrigation solutions of increasing salinity may have limited the reduction in uptake allowing plants to tolerate higher concentrations of Na^+ and Cl^-. In addition, most published toxic levels of Na^+ and Cl^- are from tests on mature trees, while the trees in our experiment were 1-2 year old seedlings. Our trees experienced high mortality, in spite of adequate tissue nutrients, this leads us to conclude that, either a) the Na^+ and Cl^- toxicity thresholds for seedlings are higher for ungrafted trees and/or b) the complex salt solution used to irrigate was not as detrimental to our ungrafted trees

compared to a single salt (i.e. NaCl) solution. The presence of sulfate and calcium salts and higher K^+/Na^+ ratios may have ameliorated some of the negative effects that NaCl has on plant physiological and nutritional status.

5 EFFECTS OF SALINITY ON GRAFTED WATERMELON PERFORMANCE

5.1 INTRODUCTION

Most crops are bred and evaluated for tolerance to abiotic stresses such as salinity, heat and cold tolerance, and disease resistance. One alternative method to induce stress tolerance is grafting of a productive scion on a stress tolerant rootstock. The development of techniques to graft watermelons began in Korea and Japan in the 1920's to allow for continuous cropping in areas prone to soilborne diseases (Davis et al., 2008). Grafting to vigorous rootstocks can enhance disease resistance, yield, nutrient acquisition, drought and cold tolerance, growth, fruit quality, and salt tolerance as found for different crops such as watermelon, tobacco and tomato (Colla et al., 2006; Edelstein et al., 2004; Ruiz et al., 2006; Santa-Cruz et al., 2002; Uygur and Yetisir, 2009). Grafted cucumber plants have higher photosynthesis rates and stomatal conductance in saline conditions than ungrafted plants (Yang et al., 2006). Several studies have shown that grafting led to increased yield and fruit quality (Alexopoulos et al., 2007; Huang et al., 2009; Lopez-Galarza et al., 2004; Salam et al., 2002). The greater yield and fruit quality of grafted plants can be due to increased water and nutrient uptake, increased hormone production, or enhanced scion vigor (Lee et al., 2010; Lee, 1994; Ruiz et al., 1997b), as well as potential other undocumented responses. Additional rootstock experimentation has indicated that there is a link between increased peroxidase activity and lower activity of superoxide dismutase in salt tolerant grafted watermelons (Liu, 2004). This leads

researchers to believe that stress response signaling from the rootstock to the scion is also involved in plant tolerance to abiotic stressors (Hasegawa et al., 2000; Huang et al., 2013; Jensen et al., 2003; Liu, 2006).

As of 2007, approximately half of the total farmland in the U.S. was irrigated (US Department of Commerce, 2007). Irrigated agriculture is predicted to increase in the next few decades as population and subsequent demand for food production increases (Ghassemi et al., 1995; Howell, 2001). As the irrigated area of land increases, so does soil salinization; especially in areas affected by drought and water scarcity (Ghassemi et al., 1995; Munns, 2002). Selecting crops for tolerance to salinity stress is vital for the future of agriculture in order to meet the future demands of consumers. Abiotic stress tolerance is of particular importance to producers in areas subjected to long periods of drought and low quality irrigation water. Salt stress impedes growth and yield of sensitive crops such as watermelon (Colla et al., 2010a; Tanji, 1996). The main mechanism that tolerant root systems use is decreasing the amount of salt taken up by the plant and accumulating salts in the rootstock tissues, preventing them from moving into the scion and causing toxic effects (Edelstein et al., 2011). Other mechanisms have been suggested, however, most evidence has indicated that exclusion and compartmentalization are the most common (Edelstein et al., 2011; Romero et al., 1997; Yetisir and Uygur, 2009). Production of watermelon requires large quantities of irrigation water. Production of watermelons in the United States exceeded 56,000 ha and $520 million in 2012 (USDA, 2012a, b), making it a valuable specialty crop. However, most watermelon production takes place in areas where saline water is already a problem

or may become a problem in the future where unreliable rainfall patterns and increasing population pressure reduce availability of high quality water. Thus it is important to identify methods to improve the salinity tolerance of watermelon. The objective of this experiment is to determine if selected rootstocks and grafting improve yield, salinity tolerance, or fruit quality of the experimental watermelon variety, TAMU mini.

5.2 MATERIALS AND METHODS

5.2.1 Plant materials and growing conditions

Four watermelon rootstocks were selected based on previous germination experiments (unpublished data, C. Simpson, 2009-2010) and the ungrafted TAMU Mini variety was used as a control. The rootstocks used were: Strong Tosa, Cold Tolerant PI red-seed (CTPI RS), NIZ 54-07, and smell melon. The scion variety, TAMU mini, is a smaller watermelon breeding line still being developed by Dr. Stephen King. Strong Tosa is a commercial interspecific hybrid (*Cucurbita maxima* x *C. moschata*) rootstock variety released by Syngenta® (Wilmington, DE). Cold Tolerant PI red-seeded (CTPI RS) is a watermelon accession variety being selected for cold tolerance. NIZ 54-07 were *Lagenaria spp*. samples from Nickerson-Zwaann experimental lines. The smell melon (*Cucumis odoratissimus*) seed was a breeding line developed from a wild accession collected from a field in Victoria Co., TX. A TAMU mini watermelon scion was grafted onto each rootstock, and then held in a mist chamber for 7-10 days. The watermelons were then slowly hardened off and transitioned to the greenhouse before experimentation took place. Once the grafted and control watermelon plants had acclimated to

greenhouse conditions they were transplanted to 5 gallon pots in a glass greenhouse. The plants were arranged in a randomized complete block design with 5 replications of each rootstock combination or control per treatment. Two salinity treatments (1.5 and 3 dS m^{-1}) were applied to watermelon plants throughout the study period, with reverse osmosis watered plants being the control (0 dS m^{-1}). The plants were watered through an automated drip irrigation system which was scheduled to water 4 times a day, 15 minutes per event, at a rate of 4 L per minute. Plants receiving salinity treatments were irrigated with a salt solution made up from Instant Ocean® salt (United Pet Group, Blacksburg, VA), which was injected via MicroDos® injectors (Hydro Systems Co., Cincinnati, OH) calibrated to inject the appropriate concentrations of the salt solutions, while the 0 dS m^{-1} treatment plants received only fertilizer with the irrigation water. Fertilizer 8-16-36 (Hydro-gardens, Colorado Springs, CO) was applied according to manufacturer guidelines and additional $MgSO_4$ and $CaNO_3$ fertilizers were added to the irrigation solutions according to recommendations for watermelons (Dr. Stephen King, September, 2011, personal communication). Micronutrient composition of applied fertilizer consisted of 0.05% B, 0.05% Cu, 0.2% chelated Fe, 0.1% Mn, 0.01% Mo, and 0.05% Zn. When the plants reached approximately 2 feet in length, they were attached to twine supports to allow for maximum space efficiency. Lateral branches were trimmed periodically to minimized inter-plant competition and interference. Each plant was self-pollinated to avoid outcrossing and the dates of pollination were recorded at each flower. Fruit were harvested at 45 days from pollination and processed within 1 day of harvest. Seeds were collected from each fruit, washed and dried for future experiments. Seed

germination percentage was approximately 60% on average after 14 days and was not affected by rootstock or salinity treatment.

5.2.2 Data collection

Data were collected on fruit brix, mass, firmness, number of seeds per fruit, and average mass per seed. Brix was determined using a digital pocket refractometer (Pocket Refractometer PAL-1, Spectrum Technologies, Inc., Aurora, IL) on two samples from each fruit. Fruit firmness was determined by taking the average of three peak compression measurements approximately 50 mm from the rind using a digital force gauge (Chatillon DFM 50, Ametek Test and Calibration Instruments, Largo, FL). Seeds from each fruit were collected, washed, sterilized and allowed to air dry for 1 day. After fruit was harvested, the plants were separated into the main central vine, and roots and any lateral branches were discarded. The length of the main central vine was recorded and the plants were placed in a drying oven to dry for approximately 5 days at approximately 40°C. After the plants were sufficiently dry, the dry vine biomass was weighed and recorded.

5.2.3 Statistical analysis

Data was analyzed using JMP® Pro 10.0.0 software (SAS Institute, Cary, NC). The experimental setup consisted of five replications for each rootstock per salinity treatment in a randomized complete block design. Treatment effects and interactions were analyzed using full factorial fit model and bivariate fit models when appropriate. P

value was deemed significant between treatments and rootstocks through students T comparison test. Standard errors were reported as ±1 error of the mean.

5.3 RESULTS

5.3.1 Watermelon growth and biomass response to salinity

Rootstock had a significant effect on the plant length at harvest (P=0.013) (Fig. 5-1A & Table 5-1). Vine length for smell melon was the shortest on average (4.18 m) and Strong Tosa was the longest on average (6.45 m). Salinity treatment did not affect length of plants grafted onto different rootstocks (P = 0.61). Rootstock also had a significant effect on dry biomass (P = 0.001) (Fig. 5-1B & Tables 5-1). Smell melon weighed the least on average (20.7 g) only under salinity treatments and Strong Tosa had the highest biomass (40.6 g). Other rootstocks and control plants had biomass statistically similar to that of smell melon. Salinity treatment was significant (P=0.054), with plants treated with 1.5 dS m^{-1} having the highest biomass and plants receiving no salinity treatment having the lowest biomass (Table 5-2). Rootstock x salinity treatment were found to be insignificant at the P=0.05 for length and mass.

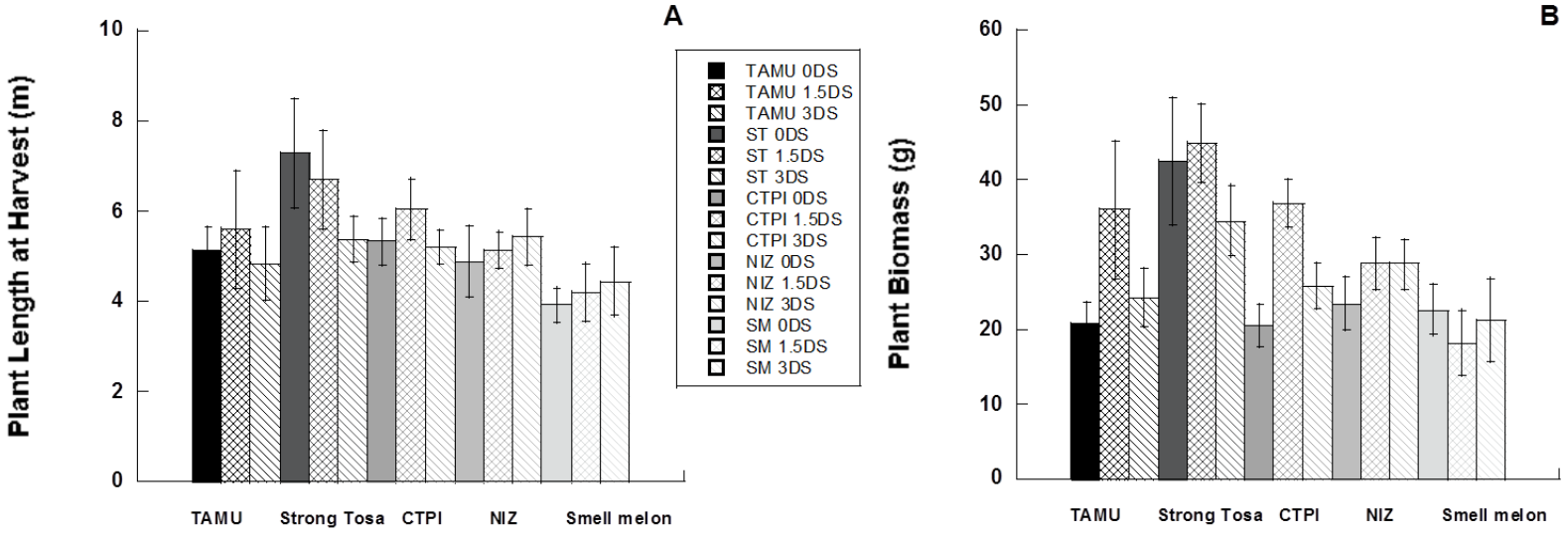


Figure 5-1. Watermelon growth and biomass response to salinity treatments. A) Average length of the watermelon main vines at the time of harvest for each salinity treatment, B) plant dry biomass of watermelon vines (g) for each salinity treatment. Error bars are shown as ±1 standard error of the mean.

Table 5-1. Rootstock effects on measured parameters.

Rootstock	Mean Plant Mass (g)	Mean Vine Length (m)	Mean Brix (%)	Mean Mass per Seed (g)
TAMU Mini	27.03b	5.19bc	6.09ab	0.028b
Strong Tosa	40.63a	6.45a	6.49ab	0.027b
CTPI RS	27.77b	5.52ab	7.55a	0.027b
NIZ 54-07	26.99b	5.15bc	6.47ab	0.026b
Smell melon	20.69b	4.18c	4.98b	0.035a

Significant differences between rootstocks are indicated by different letters as determined by students' T test. Mean plant mass (P=0.001), mean vine length (P=0.013), mean brix (P=0.11), mean mass per seed (P=0.065).

Table 5-2. Salinity treatment effects on brix and plant mass.

Treatment	Brix (%)	Mean Plant Mass (g)
0 dS m^{-1}	5.15b	25.97b
1.5 dS m^{-1}	6.58a	32.94a
3 dS m^{-1}	7.22a	26.92ab

Significant differences between rootstocks are indicated by different letters as determined by students' T test. Mean brix (P=0.003), mean plant mass (P=0.054).

5.3.2 Fruit quality measurements

Flower pollination and fruit set were variable amongst plants; however we limited production to only one fruit per plant. Watermelon grafted onto the Strong Tosa rootstocks produced the most fruit (13), followed by NIZ 54-07 (9), TAMU mini ungrafted (8), smell melon (8), and CTPI RS (7) (Fig 5-2). Fruit mass was affected by

rootstock and salinity level at P=0.082, with CTPI RS at 1.5dS m^{-1} having the highest masses and Strong Tosa, CTPI RS, and smell melon grafted watermelons irrigated with 3 dS m^{-1} and NIZ 54-07 irrigated with 1.5 dS m^{-1} fruit having the lowest masses. Fruit flesh firmness (Fig. 5-3 A & Table 5-3) was significantly affected by rootstock and treatment (P=0.049), with CTPI RS at 1.5 dS m^{-1} having the highest values and NIZ 54-07 and smell melon at 1.5 dS m^{-1} having the lowest values. Brix (%) was significantly affected by salinity treatment (P= 0.003); the 0 dS m^{-1} treatment had the lowest brix values while 1.5 and 3 dS m^{-1} had higher brix values (Fig 5-3 B, Tables 5-1 & 5-2). There was a slightly significant effect of rootstock on brix values (Table 5-1), although salinity did not affect brix values differently for fruit produced from each rootstock ($P_{\text{rootstock x treatment}} = 0.616$).

5.4 DISCUSSION

Grafted watermelon plants have shown promising results with regards to performance when exposed to salinity (Colla et al., 2006; Colla et al., 2012; Colla et al., 2010b; Edelstein et al., 2011; Martinez-Rodriguez et al., 2008). The enhanced ability of grafted plants to produce fruit may lead to greater yield. For example, Colla et al. (2006) found that grafted watermelon plants had 81% higher yield than ungrafted plants. While the results were not significant in our studies, fruit produced by smell melon and CTPI RS had greater mass at the 0 dS m^{-1} treatment than the fruit of other plants and fruit mass decreased with increased salinity. The fruit of TAMU mini, Strong Tosa, and NIZ 54-07 showed no significant effects of salinity on fruit mass. Studies by Mendlinger (1994) show that increased salinity decreased fruit mass and yield, mainly due to increased

concentrations of foliar Na^+, while Romero et al. (1997) found that grafting mitigated the negative effects of salinity on fruit yield. In our experiment, seeds produced per fruit were not affected by rootstock or salinity treatment. The rate of germination of these collected seeds and days to seedling emergence were not affected by salinity treatments either. We did find that fruit flesh firmness was significantly affected by rootstock and salinity treatment ($P = 0.05$). Fruit brix measurements increased significantly with increasing salinity ($P = 0.001$). This is similar to results published by Colla et al. (2006), who found that grafted watermelons irrigated with saline water produced smaller fruit with higher brix content due to osmotic potential reducing water uptake in salt stressed plants. These results are also supported by experiments conducted by Davis and Perkins-Veazie (2006) who found that some scion-rootstock combinations increased fruit quality and yield. However, in this study, plant biomass also increased at salinity levels of 1.5 dS m^{-1}, but was less for the 0 and 3 dS m^{-1} treatments. Rootstock variety influenced plant length and dry biomass significantly, with Strong Tosa ranking highest in both factors. Smell melon was the smallest rootstock of the four and may have had a slight dwarfing effect on the watermelon scion as evidenced by the lower dry mass and length at harvest.

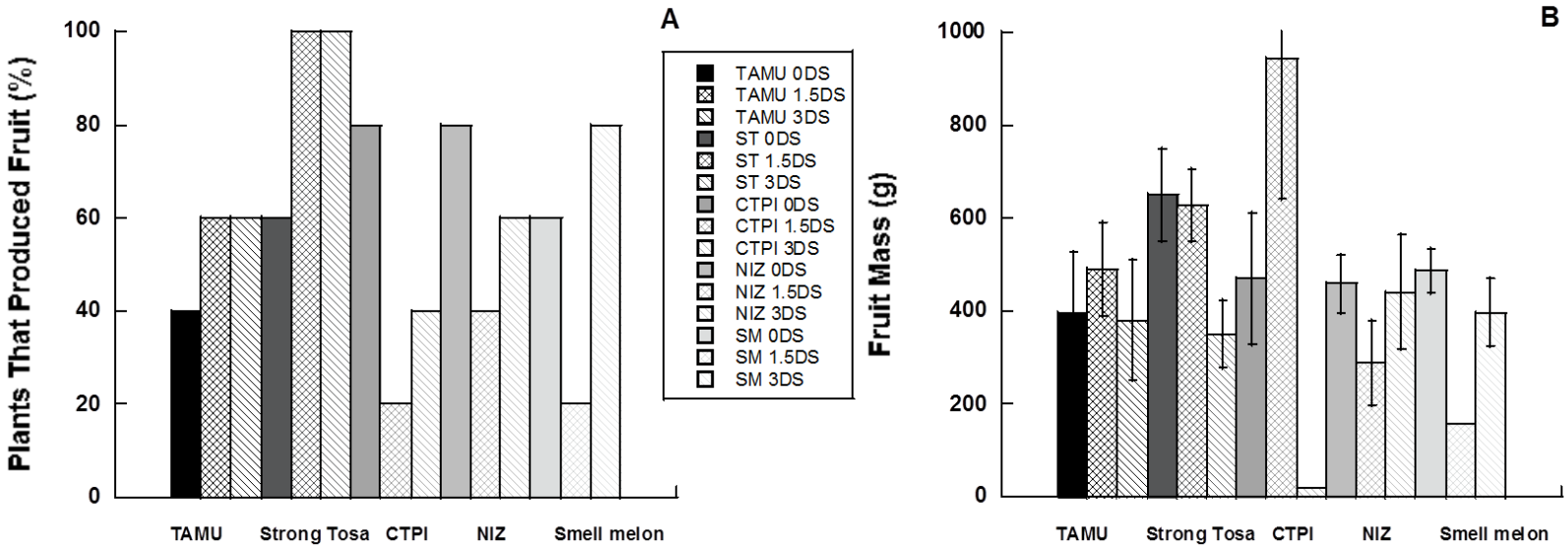


Figure 5-2. Fruit produced per plant and fruit mass. A) Percent of plants that produced fruit (out of 5), B) average fruit mass (g) for watermelons produced on each rootstock at each salinity level. A maximum of 1 fruit per plant was allowed to mature. Error bars are shown as ±1 standard error of the mean.

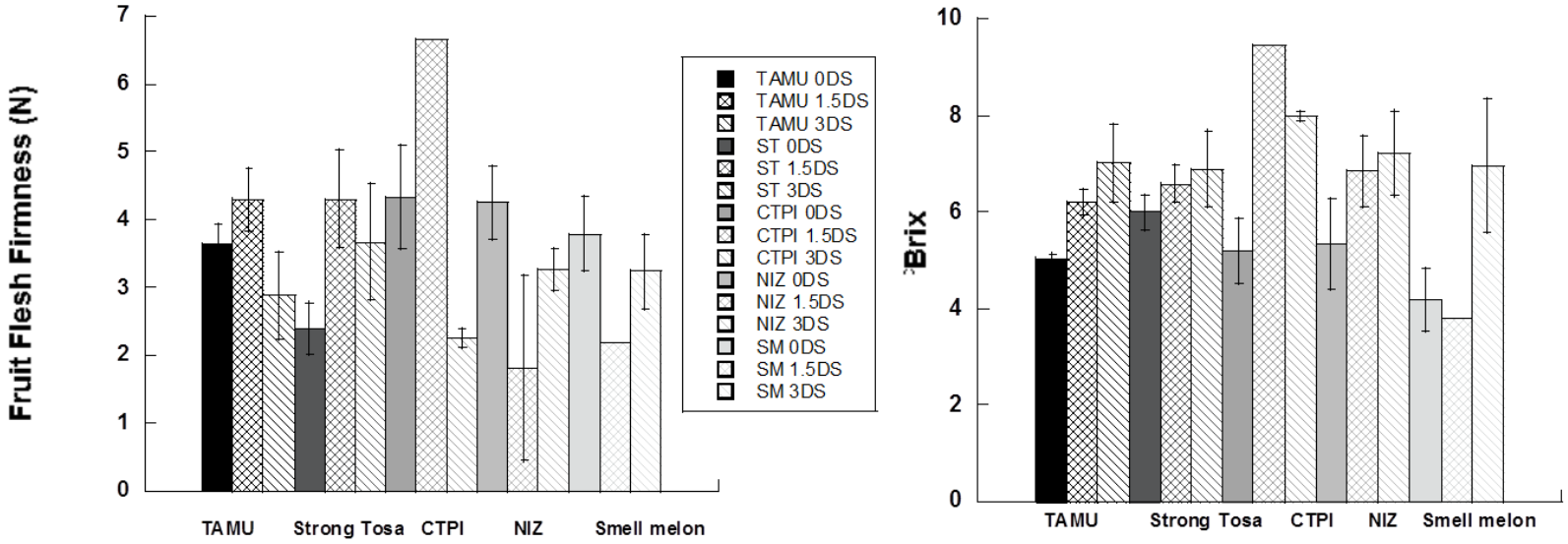


Figure 5-3. Fruit firmness and brix at each salinity level. A) Average fruit flesh firmness (peak compression in N) for each rootstock at each salinity level, B) average brix (%) for each rootstock at each salinity level. A maximum of 1 fruit per plant was allowed to mature. Error bars are shown as ±1 standard error of the mean.

Table 5-3. Treatment and rootstock effects on fruit mass and firmness.

Rootstock	Treatment	Mean Fruit Mass (g)	Mean Fruit Flesh Firmness (N)
TAMU	0 dS m^{-1}	394.63bc	3.63abcd
	1.5 dS m^{-1}	489.88bc	4.30abc
	3 dS m^{-1}	381.02bc	2.88bcd
Strong Tosa	0 dS m^{-1}	648.64ab	2.39cd
	1.5 dS m^{-1}	625.96ab	4.30ab
	3 dS m^{-1}	349.27c	3.68bcd
CTPI RS	0 dS m^{-1}	471.74bc	4.26abc
	1.5 dS m^{-1}	943.47a	1.81d
	3 dS m^{-1}	303.91c	3.27bcd
NIZ 54-07	0 dS m^{-1}	458.13bc	4.33abc
	1.5 dS m^{-1}	290.30c	6.65a
	3 dS m^{-1}	439.98bc	2.25bcd
Smell melon	0 dS m^{-1}	485.34bc	3.79abcd
	1.5 dS m^{-1}	158.76c	2.18bcd
	3 dS m^{-1}	399.16bc	3.23bcd

Significant differences between rootstocks are indicated by different letters as determined by students' T. Mean fruit mass (P=0.082), mean fruit flesh firmness (P=0.049).

5.5 CONCLUSIONS

Data from this experiment demonstrate that increasing salinity to 3 dS m^{-1} had very little effect on most watermelon growth parameters compared to irrigation with non-saline water. However, increased salinity did enhance fruit sugar content. At moderate salinities of 1.5 dS m^{-1}, growth, fruit flesh firmness, and brix were enhanced

significantly. Overall, the Strong Tosa rootstock outperformed the other rootstocks and the ungrafted control plants. We conclude that a combination of Strong Tosa rootstock with a TAMU mini scion is well-suited for moderately saline conditions.

6 DISCUSSION AND CONCLUSIONS

6.1 DISCUSSION

In this dissertation I have assessed the impacts that grafting has on salinity tolerance of selected citrus and watermelon rootstocks. Grafted and ungrafted citrus and watermelon rootstocks were subjected to irrigation with water of increasing salinity over a period of approximately 6 months and 4 months, respectively. Citrus findings are presented in sections 2, 3, and 4; and watermelon findings are presented in section 5.

It is well known that citrus are quite sensitive to salinity (Maas, 1992). While sour orange (SO) rootstocks have imparted relative salt tolerance compared to other rootstocks (Zekri and Parsons, 1989), they are being retired from the citrus industry due to their susceptibility to Citrus Tristeza Virus (CTV) (Moreno et al., 2008; Zekri, 1987, 1991). Most replacement rootstocks are not able to withstand the heavy, calcareous clay soils pervasive across southern Texas; the third largest citrus producing area in the U.S. (United States Department of Agriculture, 2009). In response, the Texas A&M University-Kingsville Citrus Center started screening rootstocks that showed resistance to CTV, tolerance to calcareous clay soils and good fruit quality (Louzada et al., 2008). The C22 and C146 varieties from California performed well in these screenings. In the citrus growing region of southern Texas soil conditions and water quality vary widely, often negatively affecting crop performance (Carter and Wiegand, 1965). Finding rootstocks that are salt tolerant is important due to the rapid decline in water availability and quality in southern Texas (Gerber, 2011; McCoy, 1990; Michelsen, 2009). Sour orange was of particular value because it was well adapted to calcareous soils and low

quality irrigation water and, until recently, the majority of citrus in the lower Rio Grande valley (LRGV) were grafted to this rootstock (Louzada et al., 2008). C22 and C146 have had excellent yield and fruit quality whilst thriving in the challenging soils of the LRGV (Louzada et al., 2008), however, they have not yet been evaluated for salinity tolerance.

Many factors may play a role in salinity tolerance; scion, age, length exposed to salt stress, and chemical composition of salts. Most studies that used citrus seedlings have been conducted using saline solutions of less than 5 dS m^{-1} NaCl over a variable amount of time (García-Legaz et al., 1993; Garcia-Sanchez et al., 2002a; Lloyd et al., 1990; Melgar et al., 2008; Zapata, 2004). The use of seedlings in experiments may not always yield results directly comparable to mature trees, however these studies can give indications of potential rootstocks than may impart salinity tolerance in long-term field trials (Garcia et al., 2002; Sykes, 1985). Grieve and Walker (1983) found that mature trees in a field setting excluded Cl^- better than seedlings grown in a greenhouse. Additionally, field trials of mature trees typically use yield as a performance indicator for trees subjected to salinity stress, however, the seedlings used in these experiments did not bear fruit, making this comparison impractical. Growth is a good indicator of stress in seedlings because both yield and growth decline proportionally when exposed to increasing salinity stress (Bielorai et al., 1978; Dasberg et al., 1991; Francois and Clark, 1980; Heller et al., 1973; Hoffman et al., 1984; Maas, 1992). However, in a long-term study conducted on an orchard of Valencia oranges, Grieve et al. (2007) initially found that fruit yield increased at moderate salinities in the first year of the study but declined in subsequent years. This was generally represented as a reduction in fruit

number per tree and fruit weight which declined with increased salinity, although fruit sugar and acid increased.

Another factor involved in salinity tolerance experimentation is the salt composition. In this experiment, we chose to use a complex salt solution to more accurately reflect the type of salinity trees may face when irrigated with brackish water. We hypothesized that the complex nature of these salts could possibly do less damage than a single salt solution because of the addition of multiple beneficial ions. We found that the additional K^+, Ca^{2+}, and Mg^{2+} supplied by the irrigation solution did not mitigate the negative effects salinity; any benefits may have been masked by the effects of osmotic stress induced by the salt solution. In a study conducted by Bañuls et al. (1991) the addition of Ca^{2+} increased growth and reduced defoliation in Navel orange scions exposed to salinity stress. Other studies have found that sulfate salts are less detrimental to citrus growth compared to chloride salts (Maas, 1992; Zekri and Parsons, 1990). In this experiment we found that S was associated with increased chlorophyll fluorescence ($P = 0.01$) and decreased electrolyte leakage ($P = 0.043$), indicating that salt composition may play a role in the ability of trees to tolerate saline conditions.

Chloride and sodium toxicity have contributed to poor yield in citrus in saline conditions (Bañuls et al., 1997; Lloyd et al., 1989; Zekri, 1987). Toxic accumulation of these ions can lead to defoliation and nutrient deficiency in affected trees (Ruiz, 1995; Storey and Walker, 1999). In general, exposure to saline conditions can reduce CO_2 assimilation, PSII efficiency, and reduce water uptake due to a negative soil osmotic potential (Munns, 2002). Sour orange rootstocks are Cl^- accumulators, generally taking

up more Cl^- into leaf tissues of the scion than Na^+ (Behboudian et al., 1986; Zekri, 1991). There is very little data on the C22 and C146 varieties, however, other crosses of Sunki mandarin have shown improved ability to prevent Na^+ and Cl^- ions from accumulation in leaf tissue (Hussain et al., 2012b; Lloyd et al., 1989, 1990; Maas, 1992). Bañuls et al. (1997) and Moya et al. (2003) have previously associated citrus salinity tolerance with the ability to exclude Cl^-, effectively limiting the amount of damage caused by its accumulation in leaf tissues. While rootstock ability to reduce toxic ion accumulation is vital in plant salt tolerance, scions may also affect the accumulation of Na^+ and Cl^- in leaves (Bañuls and Primo-Millo, 1995; García-Legaz et al., 1993; Nieves et al., 1991). The 'Valencia' orange scion is considered to be relatively tolerant to higher concentrations of foliar Cl^- and reduced Na^+ accumulation compared to other scions (Cooper et al., 1952; Lloyd et al., 1989). For example, Lloyd et al. (1989) found that 'Prior' Lemon trees budded onto two different rootstocks were more sensitive to salinity than 'Valencia' budded onto the same two rootstocks. Translocation rates of Na^+ along stems and its accumulation in basal stems may prevent reabsorption of Na^+ and movement into the xylem and eventually leaves of trees (Grieve and Walker, 1983). Interstock studies conducted by Cerda et al. (1990) found that 'Sanguina' orange interstocks restricted Cl^- movement into scions significantly, while studies by Zapata (2004) showed less improvement in salinity tolerance when using a 'Salustiano' orange interstock. There is some speculation on the exact mechanisms of restriction involved in both Cl^- and Na^+ movement within grafted trees, although Cl^- is thought to be mainly controlled by root membranes and Na^+ exclusion may be related to the ability of xylem

parenchyma cells to remove Na^+ from the stream and compartmentalize them in woody tissues (Walker, 1986; Walker and Douglas, 1983). It is still unknown if there are any mechanisms at the graft that restrict movement into the scion. Finding the optimal combination of Cl^- excluding rootstock and Cl^- tolerant scion will greatly improve the salinity tolerance of citrus. The goals of the citrus experiment described in this dissertation were to evaluate these C22 and C146 rootstocks for salinity tolerance compared to SO rootstocks, and determine what, if any, effect grafting has on salinity tolerance.

Chloride accumulated at higher concentrations than Na^+ in grafted and ungrafted rootstocks (Fig 2-5). I could not collect tissue Na^+ and Cl^- concentrations from completely defoliated trees and these were not represented in our results, however, there were linear increases in Na^+ and Cl^- concentrations as salinity levels in irrigation water increased. I found that both ions accumulated beyond levels considered to be toxic in citrus (Fig 2-6). These findings indicate that either a) leaves senesced at toxic concentrations and therefore were not available for analysis, or b) the thresholds for toxicity are higher for ungrafted rootstocks.

I found that the C22 and C146 rootstocks had lower mortality than SO rootstocks at increased salinity levels, however, grafting to the Olinda Valencia scion dramatically increased mortality for all rootstocks (Fig. 2-4). Mortality is rarely reported in the literature, the high rates recorded here may be due to higher salinity levels used or due to the length of time plants were irrigated with these solutions. Mortality was seen to be gradual, starting with defoliation followed by branch and trunk death. Both C22 and

C146 outperformed SO in height relative growth rate (RGR) when ungrafted. Grafting reduced height relative growth rate for all three rootstocks, while increasing salinity decreased the RGR response ratio (Fig. 2-1 & 2-2). Similarly, a study conducted by García-Legaz et al. (1993) found that reduced growth was a characteristic of more sensitive scions, concluding that ion toxicity and low osmotic potential reduced growth of lemon scions in their study. Bañuls and Primo-Millo (1995) also found that growth was more dependent upon the scion than the concentration of Na^+ or Cl^- in leaves, however, reductions in growth in response to salinization are likely due to negative effects on leaf water relations which reduced stomatal opening and photosynthesis. Although, reduced rates of growth can be beneficial as an increase in growth often coincides with increased water uptake and therefore increase salt uptake. Several authors have indicated the association between reduced growth and lower rates of Cl^- accumulation (Garcia et al., 2002; Levy and Syvertsen, 2004), however some fast growing rootstocks have shown comparable salinity tolerance (Levy, 2000). In this experiment, grafting and increasing salinity also reduced the standing leaf area of trees (Fig. 2-3). The higher standing leaf area of ungrafted plants likely led to the higher survival rate and growth of ungrafted plants. When I compared total leaf area per plant to the concentrations of Na^+ and Cl^- in leaf tissues I found that as concentrations of Na^+ and Cl^- increased, total leaf area per plant decreased (Fig. 2-6).

In order to further explore the effects of salinity and grafting on citrus physiology, I analyzed data on chlorophyll fluorescence, stomatal conductance, electrolyte leakage, and SPAD to determine possible stress caused by salinity, rootstock

or grafting treatments. As for mortality and growth, I found that grafted trees were more significantly affected by salinity than the ungrafted rootstocks. Chlorophyll fluorescence (Fv/Fm) is considered to be an excellent indicator of plant stress because it reflects the reduced efficiency of PSII based on reduced energy transference in the PSII reaction center which can be related to stress and photosynthetic efficiency of leaves, this is important because it gives a non-destructive measurement of plant stress at any given time (Downton and Millhouse, 1985; Gorbe and Calatayud, 2012; Santos, 2004). Grafted trees showed lower chlorophyll fluorescence at higher salinities than ungrafted rootstocks (Fig. 3-1). Ungrafted C22 and C146 rootstocks showed higher Fv/Fm values than SO, and trees grafted to the C146 rootstock showed similar Fv/Fm across all salinity treatments.

Stomatal conductance is also negatively affected by increasing soil salinity, usually due to a more negative osmotic potential in the soil which reduces water uptake and causes stomatal closure (Farquar and Sharkey, 1982; Lawlor and Cornic, 2002; Munns, 2002). Stomatal conductance of ungrafted C22 and C146 rootstocks were less affected by salinity than ungrafted SO. However, salinity had the most impact on stomatal conductance.

To analyze membrane stability of leaf cells I measured electrolyte leakage. Electrolytes are compartmentalized within a cell plasma membrane, when these membranes are subjected to stress the proteins and lipids imbedded in these membranes are degraded which can increase membrane permeability (Rolny et al., 2011). When plasma membrane integrity is damaged the electrolytes contained within them may leak

into the apoplast. By comparing the conductivity of the damaged and non-damaged tissues cell membrane injury may be estimated (Bajji et al., 2001). A correlation between increased electrolyte leakage and salinity stress has previously been found in citrus, making this a good indicator of the status of citrus tree salt tolerance (Ashraf and Harris, 2004; Chen et al., 1999; Ebel et al., 2004; Ziogas et al., 2013). However, it should be noted that electrolyte leakage is a measure of all charged solutes in the solution and does not indicate the exact ions which contribute to the measurement (Bajji et al., 2001). Electrolyte leakage was increased in response to increased salinity. Salinity, rootstock and grafting all had significant impacts on the degree of electrolyte leakage from cell membranes (Fig. 3-3). Again, ungrafted and grafted C22 and C146 rootstocks had lower electrolyte leakage than SO rootstocks. At the highest salinity level (10 dS m^{-1}) ungrafted C146 rootstocks had the lowest percentages of electrolyte leakage of all the rootstocks.

SPAD is a controversial measurement of plant nutritional status, typically used to indicate color changes in leaves caused by lower chlorophyll and thus reduced nutritional status (Grosser et al., 2012; Hussain et al., 2012a; Jifon et al., 2005; Pestana et al., 2005). I used this measurement to determine plant relative chlorosis in order to assess damage which may be attributed to salt toxicity. SPAD values decreased in response to salinity and values varied based on rootstock and grafting treatments (Fig. 3-4). Grafted trees had a more pronounced decline in SPAD values compared to the ungrafted trees, with trees grafted to C22 rootstocks having higher SPAD values than those grafted to SO and C146 rootstocks.

In summary, grafting decreased the ability of citrus rootstocks to maintain a healthy physiological status in response to salinity and the C22 and C146 rootstocks performed better than the SO rootstocks.

Salinity and nutritional status have been closely linked due to the complications that arise from salinity stress. Plant ability to take up nutrients responsible for growth and reproduction are limited when Na^+ ions compete with K^+ for entrance into the root (Cramer, 1985; Grattan and Grieve, 1999). This is further complicated by reduced plant water uptake in soils with low water potential (Bernstein, 1975; Cramer, 1985; Ruiz et al., 1997a). The plants' ability to maintain high K^+/ Na^+ ratios when exposed to salinity stress is an important factor in salinity tolerance (Cachorro et al., 1993; Maathuis and Amtmann, 1999). Other mineral elements such as Ca^{2+}, Mg^{2+} and S can also influence the uptake of toxic ions and plant overall salt tolerance (Bañuls et al., 1991; Colla et al., 2012; Grattan and Grieve, 1999; Mehrotra et al., 1986; Ruiz et al., 1997a). Discussion in section 4 addressed the plant nutrient status as affected by toxic accumulations of Na^+ and Cl^-. I found that salinity reduced concentrations of N, K^+, S and Ca^{2+} in leaf tissues but not to levels considered deficient in citrus (Figs. 4-1 and 4-2). Surprisingly, concentrations of P and Mg^{2+} increased with increased salinity treatments. While added Mg^{2+} was applied in the salt solution, P was added only in fertilizer and was expected to decline as was seen with N which was also added in the fertilizer. Because this occurrence was dependent upon grafting treatment, I speculate that this may be attributed to the ungrafted rootstock efficiency in nutrient foraging. Sulfur uptake was variable amongst salinity and grafting treatments, increasing in grafted C22 and C146

trees but decreasing for others. As expected, concentrations of Na^+ and Cl^- increased with increasing salinity treatments (Fig 4-3). Grafted trees were seen to have higher concentrations of Na^+ and Cl^- than ungrafted trees at comparable salinity levels. Chloride concentrations were dependent upon rootstocks at the 1 dS m^{-1} treatment level, indicating higher concentrations in ungrafted C146 rootstocks than the other two. To determine whether Na^+ or Cl^- played a more significant role in toxicity and mortality in our citrus trees I examined the relationship between mean Na^+ and Cl^- to mortality (Fig. 4-4). I found that lower concentrations of Na^+ resulted in higher mortalities for grafted and ungrafted trees. This finding contrasts with results found by Bañuls et al. (1997) and Moya et al. (2002) who found Cl^- to be the predominant factor resulting in citrus toxicity. Because this might have been due to the ratios of K^+/Na^+ in plant tissues I explored this factor as well (Fig 4-5) and found that K^+/Na^+ ratio decreased as salinity increased. A similar trend was discovered in Ca^{2+}/ Na^+ ratios which have previously been implicated in salinity tolerance. Rootstock did not affect the ratios of K^+ or Ca^{2+} to Na^+, but grafting decreased these ratios significantly. This supports our findings throughout this study that grafting negatively impacted salt tolerance in these trees.

Overall, increased salinity levels negatively affect citrus, however C22 and C146 rootstock imparted greater salinity tolerance than the SO rootstock. These results imply that grafting onto the C22 rootstock in particular, can improve salinity tolerance at moderate salinity levels, but a more tolerant scion may be needed at higher salinities.

In section 5 the effects of salinity on grafted and ungrafted watermelons were investigated to determine if selected rootstock varieties were able to contribute to

watermelon salinity tolerance. Greenhouse watermelon production is faced with many limitations. Our experiment involves several factors that may have influenced the fruit and biomass production. Growing watermelons in greenhouses requires that vines be trellised up supporting twine and any lateral branches must be trimmed to save space and minimize interference between plants, this can influence fruit production in several ways. Often, lateral branches are removed to save space and focus carbohydrate resources on main vine development and fruit production (Choi et al., 2012; Kato et al., 1984). However, in a study conducted by Choi et al. (2012) found that removing only half of lateral branches increased fruit sucrose compared to plants with all lateral branches removed. From this, they concluded that these lateral branches may delay ripening but later become a source of C for maturing fruit. The trimming of lateral branches in our experiment may have led to lower brix values of fruit or may have delayed ripening. Our fruit were harvested 45 days after pollination, and while our results are comparable within our experiment, this may not be representative of brix values that may be seen in plants with no lateral branches removed. Ramirez et al. (1988) also found that defoliation reduced total plant weight and fruit weight in cucumbers. Defoliation of our plants may have led to reduced plant and fruit mass, however, we cannot compare greenhouse cultivated watermelons with field grown because of the different growing circumstances. These results are only indicative of greenhouse production and should be taken into account for future field experimentation.

While grafting vegetable crops is not yet considered cost effective in North America, other countries such as Japan, Korea and European countries have used

grafting to reduce the deleterious effects of monocropping systems (Cohen et al., 2007; Davis et al., 2008). Vegetable grafting has been found to increase disease resistance, cold tolerance, and in certain cases yield and fruit quality (Davis et al., 2008; Liu, 2004; Rouphael and Colla, 2005). Results from previous research indicate that salinity tolerance can be enhanced by certain scion-rootstock combinations in watermelon and other cucurbit crops (Colla et al., 2006; Colla et al., 2010a). My findings suggest that grafting can enhance watermelon plant performance with regards to growth and biomass, as the Strong Tosa rootstock resulted in the plants with greatest biomass and length (Fig 5-1). Percent brix and fruit flesh firmness was increased by increasing the salinity of the irrigation solution (Fig. 5-3), a finding that can be used to improve fruit quality. These results are similar to experiments conducted using deficit irrigation to induce stress in grafted watermelon, in these studies they found that production and plant and fruit quality improved (Proietti et al., 2008; Rouphael and Colla, 2005). This could be due to osmotic stress which results in reduced water uptake in plants and leads to the accumulation of osmolytes in fruit (Lauchli and Grattan, 2007).

Of the trial rootstocks selected, smell melon scored lowest on most of the factors assessed here. Salinity increased plant biomass significantly at the 1.5 dS m^{-1} treatment level for all rootstocks which contrasts with the results of several studies (Colla et al., 2006; Edelstein et al., 2011; Uygur and Yetisir, 2009). Number of seeds, fruit weight, and germination were not affected by any of the tested factors. While Strong Tosa rootstocks showed the most positive results in this experiment, future research should be conducted to more fully evaluate the effects of salinity on grafted watermelons.

6.2 CONCLUSIONS

The citrus industry in Texas and elsewhere faces many environmental challenges that will have to be addressed in order to meet future consumer demands. One of these problems is salinity which is often associated with drought. Data from the current study indicates that two new rootstocks, C22 and C146 may have moderate salinity tolerance and could be useful in commercial citrus production to mitigate moderate salinity problems; however, more research should be conducted to evaluate scions which may be more compatible when subjected to higher salinities. Contrary to previous studies, the current data also suggests that Na^+ may be more problematic for these rootstock-scion combinations than Cl^-. Among the rootstocks evaluated for watermelon, Strong Tosa had the best performance in terms of growth and biomass. Salinity treatments increased fruit quality by increasing brix and firmness values of fruit. This coincides with results by Colla et al. (2006), but should be evaluated further when multiple fruit can be grown and harvested from each plant. We have found that grafting can be a useful tool for improving salt tolerance in salt sensitive plants; however, finding the most compatible scion-rootstock combination may be challenging.

REFERENCES

Al-Yassin, A. (2005). Adverse effects of salinity on citrus. *International Journal of Agriculture and Biology* **7**, 668-680.

Albrecht, U., McCollum, G., and Bowman, K. D. (2012). Influence of rootstock variety on Huanglongbing disease development in field-grown sweet orange (*Citrus sinensis* [L.] Osbeck) trees. *Scientia Horticulturae* **138**, 210-220.

Albrigo, L. G. (1977). Rootstocks affect 'Valencia' orange fruit quality and water balance. *Proceedings, International Society of Citriculture*, 62-65.

Alexopoulos, A., Kondylis, A., and Passam, H. (2007). Fruit yield and quality of watermelon in relation to grafting. *Journal of Food, Agriculture & Environment:* **5**, 178-179.

Ashraf, M., and Harris, P. J. C. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Science* **166**, 3-16.

Bajji, M., Kinet, J. M., and Lutts, S. (2001). The use of the electrolyte leakage method for asessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regulation*, 1-10.

Balal, R. M., Ashraf, M., Kahn, M. M., Jaskani, M. J., and Ashfaq, M. (2011). Influence of salt stress on growth and biochemical parameters of citrus rootstocks. *Pakistan Journal of Botany* **43**, 2135-2141.

Balal, R. M., Khan, M. M., Shahid, M. A., Mattson, N. S., Tahira, A., Muhammad, A., Garcia-Sanchez, F., Usman, G., Gimeno, V., and Zafar, I. (2012). Comparative studies on the physiobiochemical, enzymatic, and ionic modifications in salt-

tolerant and salt-sensitive citrus rootstocks under NaCl stress. *Journal of the American Society for Horticultural Science; 2012* **137**, 86-95.

Bañuls, J., Legaz, F., and Primo-Millo, E. (1991). Salinity-calcium interactions on growth and ionic concentration of Citrus plants. *Plant and Soil* **133**, 39-46.

Bañuls, J., and Primo-Millo, E. (1995). Effects of salinity on some citrus scion-rootstock combinations. *Annals of Botany* **76**, 97-102.

Bañuls, J., Ratajczak, R., and Luttge, U. (1995). NaCI stress enhances proteolytic turnover of the tonoplast H^+-ATPase of Citrus sinensis: Appearance of a 35 kDa fragment of subunit A still exhibiting ATP-hydrolysis activity. *Plant, Cell & Environment* **18**, 1341-1344.

Bañuls, J., Serna, M. D., Legaz, F., Talon, M., and Primo-Millo, E. (1997). Growth and gas exchange parameters of Citrus plants stressed with different salts. *Journal of Plant Physiology* **150**, 194-199.

Barry, G. H., Castle, W. S., and Davies, F. S. (2004). Rootstocks and plant water relations affect sugar accumulation of 'Valencia' sweet orange via osmotic adjustment. *Acta Horticulturae; 2004*, 159-165.

Behboudian, M. H., Torokfalvy, E., and Walker, R. R. (1986). Effects of salinity on ionic content, water relations and gas exchange parameters in some citrus scion-root stock combinations. *Scientia Horticulturae* **28**, 105-116.

Ben-Hayyim, G., Spiegel-Roy, P., and Neumann, H. (1985). Relation between ion accumulation of salt-sensitive and isolated stable salt tolerant cell lines of *Citrus aurantium*. *Plant Physiology* **78**, 144-148.

Bernstein, L. (1975). Effects of salinity and sodicity on plant-growth. *Annual Review of Phytopathology* **13**, 295-312.

Bernstein, L., and Hayward, H. (1958). Physiology of salt tolerance. *Annual Review of Plant Physiology* **9**, 25-46.

Bhagat, S., Thakur, A., and Dhaliwal, H. S. (2013). Organic amendments influence growth, buddability and budding success in rough lemon (*Citrus jambhiri* Lush.). *Biological Agriculture & Horticulture* **29**, 46-57.

Bielorai, H., Shalhevet, J., and Levy, Y. (1978). Grapefruit response to variable salinity in irrigation water and soil. *Irrigation Science* **1**, 61-70.

Bitters, W. (1961). Physical characters and chemical composition as affected by scions and rootstocks. *In* "The orange: Its biochemistry and physiology" (W. Sinclair, ed.). University of California Division of Agricultural Sciences, Berkeley, California.

Bleda, J. F., Madrid, R., Garcia-Torres, A. L., Garcia-Lidon, A., and Porras, I. (2011). Chlorophyll fluorescence and mineral nutrition in citrus leaves under salinity stress. *Journal of Plant Nutrition* **34**, 1579-1592.

Boman, B. J., Zekri, M., and Stover, E. (2005). Managing salinity in citrus. *HortTechnology* **15**, 108-113.

Brase, K., and Way, R. (1959). "Rootstocks and methods used for dwarfing fruit trees." New York State Agricultural Experiment Station Bulletin, Geneva, New York.

Brumos, J., Colmenero-Flores, J. M., Conesa, A., Izquierdo, P., Sanchez, G., Iglesias, D. J., Lopez-Climent, M. F., Gomez-Cadenas, A., and Talon, M. (2009). Membrane

transporters and carbon metabolism implicated in chloride homeostatsis differentiate salt stress responses in tolerant and sensitive Citrus rootstocks. *Functional and Integrative Genomics* **9**, 293-309.

Butler, W. L., and Kitajima, M. (1975). Energy transfer between Photosystem II and Photosystem I in chloroplasts. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **396**, 72-85.

Cachorro, P., and Cerdá, A. (1994). Implications of calcium nutrition on the response of *Phaseolus vulgaris* L. to salinity. *Plant and Soil* **159**, 205-212.

Cachorro, P., Ortiz, A., and Cerdá, A. (1993). Growth, water relations and solute composition of *Phaseolus vulgaris* L. under saline conditions. *Plant Science* **95**, 23-29.

Campos, P. S., Quartin, V., Ramalho, J., and Nunes, M. (2003). Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea* sp. plants. *Journal of Plant Physiology* **160**, 283-292.

Carter, D. L., and Wiegand, C. L. (1965). Interspersed salt-affected and unaffected dryland soils of the Lower Rio Grande Valley: I. Chemical, physical, and mineralogical characteristics. *Soil Science* **99**, 256-260.

Castle, W. S. (2010). A career perspective on citrus rootstocks, their development, and commercialization. *HortScience* **45**, 11-15.

Cerda, A., Nieves, M., and Guillen, M. G. (1990). Salt tolerance of lemon trees as affected by rootstock. *Irrigation Science* **11**, 245-249.

Chapman, H. (1949). Citrus leaf analysis. *California Agriculture* **3**, 10-14.

Chapman, H. (1968). The mineral nutrition of citrus. *In* "The Citrus Industry II" (W. Reuther, L. D. Batchelor and H. J. Webber, eds.), pp. 243-266. University of California, Oakland, California.

Cheeseman, J. M. (1988). Mechanisms of salinity tolerance in plants. *Plant Physiology* **87**, 547-440.

Chen, Q., Zhang, W.-H., and Liu, Y.-L. (1999). Effect of NaCl, glutathione and ascorbic acid on function of tonoplast vesicles isolated from barley leaves. *Journal of Plant Physiology* **155**, 685-690.

Choi, E.-Y., Cho, I.-H., Moon, J.-H., and Woo, Y.-H. (2012). Impact of secondary-lateral branch removal during watermelon production. *Horticulture, Environment, and Biotechnology* **53**, 24-31.

Chow, W., Ball, M., and Anderson, J. (1990). Growth and photosynthetic responses of spinach to salinity: Implications of K^+ nutrition for salt tolerance. *Functional Plant Biology* **17**, 563-578.

Cline, M. G. (1991). Apical Dominance. *Botanical Review* **57**, 318-358.

Cohen, R., Burger, Y., Horev, C., Koren, A., and Edelstein, M. (2007). Introducing grafted cucurbits to modern agriculture: The Israeli experience. *Plant Disease* **91**, 916-923.

Colla, G., Roupahel, Y., Cardarelli, M., and Rea, E. (2006). Effect of salinity on yield, fruit quality, leaf gas exchange, and mineral composition of grafted watermelon plants. *HortScience* **41**, 622-627.

Colla, G., Rouphael, Y., Leonardi, C., and Bie, Z. (2010a). Role of grafting in vegetable crops grown under saline conditions. *Scientia Horticulturae* **127**, 147-155.

Colla, G., Rouphael, Y., Rea, E., and Cardarelli, M. (2012). Grafting cucumber plants enhance tolerance to sodium chloride and sulfate salinization. *Scientia Horticulturae* **135**, 177-185.

Colla, G., Suãrez, C. M. C., Cardarelli, M., and Rouphael, Y. (2010b). Improving nitrogen use efficiency in melon by grafting. *HortScience* **45**, 559-565.

Cooper, W. C., Gorton, B. S., and Olson, E. O. (1952). Ionic accumulation in citrus as influenced by rootstock and scion concentrations of salts and boron in the substrate. *Plant Physiology* **27**, 191-203.

Cramer, G. R. (1985). Displacement of Ca by Na from the plasmalemma of root cells : A primary response to salt stress? *Plant Physiology* **79**, 207-11.

Cuartero, J., Bolarin, M. C., Asins, M. J., and Moreno, V. (2006). Increasing salt tolerance in the tomato. *Journal of Experimental Botany* **57**, 1045-58.

Dasberg, S., Bielorai, H., Haimowitz, A., and Erner, Y. (1991). The effect of saline irrigation water on “Shamouti” orange trees. *Irrigation Science* **12**, 205-211.

Davis, A. R., and Perkins-Veazie, P. (2006). "Rootstock effects on plant vigor and watermelon fruit quality." *Cucurbit Genetics Cooperative Report* **28**, 39-42.

Davis, A. R., Perkins-Veazie, P., Sakata, Y., López-Galarza, S., Maroto, J. V., Lee, S.-G., Huh, Y.-C., Sun, Z., Miguel, A., King, S. R., Cohen, R., and Lee, J.-M. (2008). Cucurbit Grafting. *Critical Reviews in Plant Sciences* **27**, 50-74.

Denna, D. (1962). A simple grafting technique for cucurbits. *In* "Proceedings of the American Society for Horticultural Sciences", pp. 369-370.

Downton, W. J. S., and Millhouse, J. (1985). Chlorophyll fluorescence and water relations of salt stressed plants. *Plant Science Letters* **37**, 205-212.

Easterling, D. R., Meehl, G. A., Parmesan, C., Changnon, S. A., Karl, T. R., and Mearns, L. O. (2000). Climate extremes: Observations, modeling, and impacts. *Science* **289**, 2068-2074.

Ebel, R. C., Carter, P. A., Dozier, W. A., Findley, D. A., Nesbitt, M. L., Hockema, B. R., and Sibley, J. L. (2004). Pattern of exotherm and electrolyte leakage measured at high frequency of Satsuma mandarin exposed to subfreezing temperatures. *HortScience* **39**, 1614-1616.

Edelstein, M., Ben-Hur, M., Cohen, R., Burger, Y., and Ravina, I. (2004). Boron and salinity effects on grafted and non-grafted melon plants. *Plant & Soil* **269**, 273-284.

Edelstein, M., Plaut, Z., and Ben-Hur, M. (2011). Sodium and chloride exclusion and retention by non-grafted and grafted melon and Cucurbita plants. *Journal of Experimental Botany* **62**, 177-184.

Essington, M. E. (2004). "Soil and water chemistry: An integrative approach," CRC Press, Boca Raton, Florida.

Estan, M. T., Martinez-Rodriguez, M. M., Perez-Alfocea, F., Flowers, T. J., and Bolarin, M. C. (2005). Grafting raises the salt tolerance of tomato through limiting the

transport of sodium and chloride to the shoot. *Journal of Experimental Botany* **56**, 703-12.

Evans, G., and Rasmussen, H. (1972). Anatomical changes in developing graft unions of Juniperus. *Journal of the American Society for Horticultural Science* **97**, 228-232.

Evans, H. J., and Sorger, G. (1966). Role of mineral elements with emphasis on the univalent cations. *Annual Review of Plant Physiology* **17**, 47-76.

Fann, Y., Davies Jr., F., and Paterson, D. (1983). Correlative effects of bench chip budded 'Mirandy' roses. *Journal of the American Society for Horticultural Science* **108**, 180-183.

Farquar, G., and Sharkey, T. (1982). Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **33**, 317-345.

Ferguson, L., and Grattan, S. R. (2005). How salinity damages citrus: Osmotic effects and specific ion toxicities. *HortTechnology* **15**, 95-99.

Flowers, T. J., Troke, P. F., and Yeo, A. R. (1977). Mechanism of salt tolerance in halophytes. *Annual Review of Plant Physiology and Plant Molecular Biology* **28**, 89-121.

Francois, L. E., and Clark, R. A. (1980). Salinity effects on yield and fruit quality of 'Valencia' orange. *Journal of the American Society for Horticultural Science* **105**, 199-202.

García-Legaz, M. F., Ortiz, J. M., Garcí-Lidón, A., and Cerdá, A. (1993). Effect of salinity on growth, ion content and CO_2 assimilation rate in lemon varieties on different rootstocks. *Physiologia Plantarum* **89**, 427-432.

García-Sánchez, F., Botia, P., Fernández-Ballester, G., Cerdá, A., and Lopez, V. M. (2005). Uptake, transport, and concentration of chloride and sodium in three citrus rootstock seedlings. *Journal of Plant Nutrition* **28**, 1933-1945.

Garcia-Sanchez, F., Jifon, J. L., Carajal, M., and Syvertsen, J. P. (2002a). Gas exchange chlorophyll and nutrient contents in relation to Na^+ and Cl^- accumulation in 'Sunburst' mandarin grafted on different rootstocks. *Plant Science* **162**, 705-712.

Garcia-Sanchez, F., Martinez, V., Jifon, J. L., Syvertsen, J. P., and Grosser, J. W. (2002b). Salinity reduces growth, gas exchange, chlorophyll and nutrient concentrations in diploid sour orange and related allotetraploid somatic hybrids. *Journal of Horticultural Science and Biotechnology* **77**, 379-386.

Garcia-Sanchez, F., Perez-Perez, J. G., Botia, P., and Martinez, V. (2006). The response of young mandarin trees grown under saline conditions depends on the rootstock. *European Journal of Agronomy* **24**, 129-139.

Garcia-Sanchez, F., Syvertsen, J. P., Gimeno, V., Botia, P., and Perez-Perez, J. G. (2007). Responses to flooding and drought stress by two citrus rootstock seedlings with different water-use efficiency. *Physiologia Plantarum* **130**, 532-542.

Garcia, M. R., Bernet, G. P., and Puchades, J. (2002). Reliable and easy screening technique for salt tolerance of citrus rootstocks under controlled environments. *Australian Journal of Agricultural Research* **53**, 653-662.

Gardiner, D. T., and Miller, R. W. (2008). "Soils In Our Environment," 11th/Ed. Pearson Education, Inc., Upper Saddle River, New Jersey.

Gerber, J. (2011). Fact Sheet: Lower Rio Grande Basin Study. *In* "Reclamation: Managing water in the west". U.S. Bureau of Reclamation, http://www.lrgvdc.org/water.html.

Ghassemi, F., Jakeman, A. J., and Nix, H. A. (1995). " Salinisation of land and water resources: human causes, extent, management and case studies," CAB International. Wallingford, Oxon, United Kingdom.

Gorbe, E., and Calatayud, A. (2012). Applications of chlorophyll fluorescence imaging technique in horticultural research: A review. *Scientia Horticulturae* **138**, 24-35.

Grattan, S. R. (2002). Irrigation water salinity and crop production. (Div. of Agriculture and Natural Resources, ed.). UC DANR electronic publications, University of California, Oakland, California.

Grattan, S. R., and Grieve, A. M. (1999). Salinity-mineral nutrient relations in horticultural crops. *Scientia Horticulturae* **78**, 127-157.

Grattan, S. R., and Grieve, C. M. (1992). Mineral element acquisition and growth response of plants grown in saline environments. *Agriculture, Ecosystems & Environment* **38**, 275-300.

Greenway, H., and Munns, R. (1980). Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology* **31**, 149-190.

Grieve, A. M., Prior, L. D., and Bevington, K. B. (2007). Long-term effects of saline irrigation water on growth, yield, and fruit quality of 'Valencia' orange trees. *Australian Journal of Agricultural Research* **58**, 342-348.

Grieve, A. M., and Walker, R. R. (1983). Uptake and distribution of chloride, sodium and potassium ions in salt-treated citrus plants. *Australian Journal of Agricultural Resources* **34**, 133-43.

Groisman, P. Y., Karl, T. R., Easterling, D. R., Knight, R. W., Jamason, P. F., Hennessy, K. J., Suppiah, R., Page, C. M., Wibig, J., Fortuniak, K., Razuvaev, V. N., Douglas, A., Førland, E., and Zhai, P.-M. (1999). Changes in the probability of heavy precipitation: Important indicators of climatic change. *Climatic Change* **42**, 243-283.

Grosser, J. W. (2004). Building a replacement Sour Orange rootstock: Somatic hybridization of selected Mandarin + Pummelo combinations. *Journal of the American Society for Horticultural Science* **129**, 530-534.

Grosser, J. W., Omar, A. A., Gmitter, J. A., and Syvertsen, J. P. (2012). Salinity tolerance of 'Valencia' orange trees on allotetraploid rootstocks. *Proceedings of the Florida State Horticultural Society* **125**, 50-55.

Hartmann, H., Kester, D., Davies, F., and Geneve, R. (2002). "Plant propagation principles and practices," 7th/Ed. Prentice Hall, Upper Saddle River, New Jersey.

Hasegawa, P. M., Bressan, R. A., Zhu, J.-K., and Bohnert, H. J. (2000). Plant cellular and molecular responses to high salinity. *Annual Review of Plant Biology* **51**, 463-499.

Havlin, J. L., and Soltanpour, P. N. (1980). A nitric acid plant tissue digest method for use with inductively coupled plasma spectrometry 1. *Communications in Soil Science and Plant Analysis* **11**, 969-980.

Hayward, H., and Bernstein, L. (1958). Plant-growth relationships on salt-affected soils. *The Botanical Review* **24**, 584-635.

Heller, J., Shalhevet, J., and Goell, A. (1973). Response of a citrus orchard to soil moisture and soil salinity. *In* "Physical Aspects of Soil Water and Salts in Ecosystems" (A. Hadas, D. Swartzendruber, P. E. Rijtema, M. Fuchs and B. Yaron, eds.), Vol. 4, pp. 409-419. Springer, Berlin, Heidelberg, Germany.

Hodgson, R. (1943). Some instances of scion domination in citrus. *In* "Proceedings of the American Society of Horticultural Sciences", Vol. 43, pp. 131-138.

Hoffman, G. J., Oster, J. D., Maas, E. V., Rhoades, J. D., and Van Schilfgaarde, J. (1984). Minimizing salt in drain water by irrigation management — Arizona field studies with citrus. *Agricultural Water Management* **9**, 61-78.

Howell, T. A. (2001). Enhancing water use efficiency in irrigated agriculture contrib. from the USDA-ARS, Southern Plains Area, Conservation and Production Resources Lab., Bushland, TX 79012. *Agronomy Journal* **93**, 281-289.

Huang, Y., Bie, Z., Liu, P., Niu, M., Zhen, A., Liu, Z., Lei, B., Gu, D., Lu, C., and Wang, B. (2013). Reciprocal grafting between cucumber and pumpkin

demonstrates the roles of the rootstock in the determination of cucumber salt tolerance and sodium accumulation. *Scientia Horticulturae* **149**, 47-54.

Huang, Y., Tang, R., Cao, Q., and Bie, Z. (2009). Improving the fruit yield and quality of cucumber by grafting onto the salt tolerant rootstock under NaCl stress. *Scientia Horticulturae* **122**, 26-31.

Hussain, S., Curk, F., Dhuique-Mayer, C., Urban, L., Ollitrault, P., Luro, F., and Morillon, R. (2012a). Autotetraploid trifoliate orange (*Poncirus trifoliata*) rootstocks do not impact clementine quality but reduce fruit yields and highly modify rootstock/scion physiology. *Scientia Horticulturae* **134**, 100-107.

Hussain, S., Luro, F., Costantino, G., Ollitrault, P., and Morillon, R. (2012b). Physiological analysis of salt stress behaviour of citrus species and genera: Low chloride accumulation as an indicator of salt tolerance. *South African Journal of Botany* **81**, 103-112.

Jensen, P. J., Rytter, J., Detwiler, E. A., Travis, J. W., and McNellis, T. W. (2003). Rootstock effects on gene expression patterns in apple tree scions. *Plant Molecular Biology* **493**, 394-511.

Jifon, J. L., Syvertsen, J. P., and Whaley, E. L. (2005). Growth environment and leaf anatomy affect nondestructive estimates of chlorophyll and nitrogen in *Citrus* sp. leaves. *Journal of the American Society of Horticultural Sciences* **130**, 152-158.

Kato, T., Fukumoto, Y., and Kinoshita, S. (1984). Effect of training, pinching and defoliation on the development and quality of fruit in watermelon [*Citrullus lanatus*]. *Research Reports of the Kochi University. Agricultural Science* **33**.

Lauchli, A., and Grattan, S. R. (2007). Plant growth and development under salinity stress. *In* "Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops" (M. A. Jenks, ed.), pp. 1-32. Springer, New York.

Lawlor, D. W., and Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant , Cell and Environment* **25**, 275-294.

Lee, J.-M., Kubota, C., Tsao, S. J., Bie, Z., Echevarria, P. H., Morra, L., and Oda, M. (2010). Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Scientia Horticulturae* **127**, 93-105.

Lee, J. (1994). Cultivation of grafted vegetables. I. Current status, grafting methods, and benefits. *HortScience* **29**, 235-239.

Levitt, J. (1956). "The Hardiness of Plants," Academic Press, New York.

Levy, Y., Lifshitz, J., De Malach, Y., and David, Y. (1999). The response of several citrus genotypes to high salinity irrigation water. *HortScience* **34**, 878-881.

Levy, Y., and Syvertsen, J. P. (2004). Irrigation water quality and salinity effects in citrus trees. *Horticultural Reviews* **30**, 37-83.

Levy, Y. and Lifshitz, J. (2000). Field screening of citrus rootstocks using trickle linear salinity gradient. *Acta Horticulturae* **511**, 197-203.

Liu, H. (2004). Effect of low temperature stress on chilling tolerance and protective system against active oxygen of grafted watermelon. *The Journal of Applied Ecology* **15**, 659-662.

Liu, H. Y. (2006). Characteristics of the sugar metabolism in leaves and fruits of grafted watermelon during fruit development. *Plant Physiology Communications* **42**, 835-840.

Liu, L. (1998). Determination of chloride in plant tissue. *In* "Handbook of Reference Methods for Plant Analysis" (Y. P. Kalra, ed.), pp. 57-61. CRC Press, Boca Raton, Florida.

Lloyd, J., Kriedemann, P. E., and Aspinall, D. (1989). Comparative sensitivity of 'Prior Lisbon' lemon and 'Valencia' orange trees to foliar sodium and chloride concentrations. *Plant, Cell & Environment* **12**, 529-540.

Lloyd, J., Kriedemann, P. E., and Aspinall, D. (1990). Contrasts between Citrus species in response to salinisation: An analysis of photosynthesis and water relations for different rootstock-scion combinations. *Physiologia Plantarum* **78**, 236-246.

Lopez-Galarza, S., San Bautista, A., Perez, D., Miguel, A., Baixauli, C., Pascual, B., Maroto, J., and Guardiola, J. (2004). Effects of grafting and cytokinin-induced fruit setting on colour and sugar-content traits in glasshouse-grown triploid watermelon. *Journal of Horticultural Science & Biotechnology* **79**, 971-976.

Louzada, E. S., Del Rio, H. S., Setamou, M., Watson, J. W., and Swietlik, D. M. (2008). Evaluation of citrus rootstocks for the high pH, calcareous soils of South Texas. *Euphytica* **164**, 13-18.

Lutts, S., Kinet, J. M., and Bouharmont, J. (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Annals of Botany* **78**, 389-398.

Maas, E. V. (1992). Salinity and citriculture. *Tree Physiology* **12**, 195-216.

Maathuis, F. M., and Amtmann, A. (1999). K^+ nutrition and Na^+ toxicity: The basis of cellular K^+/Na^+ratios. *Annals of Botany* **84**, 123-133.

Marschner, H. (1995). "Mineral nutrition of higher plants," Academic Press, London.

Martinez-Rodriguez, M. M., Estañ, M. T., Moyano, E., Garcia-Abellan, J. O., Flores, F. B., Campos, J. F., Al-Azzawi, M. J., Flowers, T. J., and Bolarín, M. C. (2008). The effectiveness of grafting to improve salt tolerance in tomato when an 'excluder' genotype is used as scion. *Environmental and Experimental Botany* **63**, 392-401.

Martinez Beltran, J., and Licona Manzur, C. (2005). Overview of salinity problems in the world and FAO strategies to address the problem. *Proceedings of the international salinity forum - managing saline soils and water: science, technology and social issues*. Riverside, California.

McCoy, T. W. (1990). "Evaluation of ground-water resources in the lower Rio Grande Valley, Texas." Texas Water Development Board, Austin, Texas.

Mehrotra, N. K., Khanna, V. K., and Agarwala, S. C. (1986). Soil-sodicity-induced zinc deficiency in maize. *Plant and Soil* **92**, 63-71.

Melgar, J. C., Syvertsen, J. P., Martinez, V., and Garcia-Sanchez, F. (2008). Leaf gas exchange, water relations, nutrient content and growth in citrus and olive seedlings under salinity. *Biologia Plantarum* **52**, 385-390.

Mendel, K. (1936). The anatomy and histology of the bud-union in citrus. *Palestinian Journal of Botany* **1**, 13-46.

Mendlinger, S. (1994). Effect of increasing plant density and salinity on yield and fruit quality in muskmelon. *Scientia Horticulturae* **57**, 41-49.

Michelsen, A. M. M., T.; Sheng, Z.; Lacewell, R.; Creel, B. (2009). "Rio Grande Salinity Preliminary Economic Assessment Report." Texas AgriLife Research, New Mexico State University, Texas Water Resources Institute, New Mexico Water Resources Research Institute.

Moreno, P., Ambros, S., Albiach-Marti, M. R., Guerri, J., and Pena, L. (2008). Citrus tristeza virus: a pathogen that changed the course of the citrus industry. *Molecular Plant Pathology* **9**, 251-268.

Moya, J. L., Gomez-Cadenas, A., Primo-Millo, E., and Talon, M. (2003). Chloride absorption in salt-sensitive Carrizo citrange and salt-tolerant Cleopatra mandarin citrus rootstocks is linked to water use. *Journal of Experimental Botany* **54**, 625-633.

Moya, J. L., Tadeo, F. R., Gomez-Cadenas, A., Primo-Millo, E., and Talon, M. (2002). Transmissible salt tolerance traits identified through reciprocal grafts between sensitive carrizo and tolerant cleopatra citrus genotypes. *Journal of Plant Physiology* **159**, 991-998.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell and Environment* **25**, 239-250.

Munns, R., and Termaat, A. (1986). Whole-plant responses to salinity. *Australian Journal of Plant Physiology* **13**, 143-160.

Nelson, D. W., and Sommers, L. E. (1973). Determination of total nitrogen in plant material. *Agronomy Journal* **65**, 109-112.

Nesbitt, M. L., Ebel, R. C., Findley, D. A., Wilkins, B., Woods, F., and Hemelrick, D. (2002). Assays to assess freeze damage in Satsuma mandarin. *HortScience* **37**, 871-877.

Nieves, M., Cerda, A., and Botella, M. (1991). Salt tolerance of two lemon scions measured by leaf chloride and sodium accumulation. *Journal of Plant Nutrition* **14**, 623-636.

Obreza, T. A., Alva, A., Hanlon, E., and Rouse, R. (1992). "Citrus grove leaf tissue and soil testing: Sampling, analysis, and interpretation," Cooperative Extension Service, University of Florida, Institute of Food and Agricultural Sciences.

Parida, A. K., and Das, A. B. (2005). Salt tolerance and salinity effects on plants: A review. *Ecotoxicology of Environmental Safety* **60**, 324-49.

Patel, R. K., Babu, K. D., Singh, A., Yadav, D. S., and De, L. C. (2010). Soft wood grafting in Mandarin (*C. reticulata* Blanco): A novel vegetative propagation technique. *International Journal of Fruit Science* **10**, 54-64.

Perez-Perez, J. G., Syvertsen, J. P., Botia, P., and Garcia-Sanchez, F. (2007). Leaf water relations and net gas exchange responses of salinized Carrizo citrange seedlings during drought stress and recovery. *Annals of Botany* **100**, 335-45.

Perez-Tornero, O., Tallon, C. I., Porras, I., and Navarro, J. M. (2009). Physiological and growth changes in micropropagated *Citrus macrophylla* explants due to salinity. *Journal of Plant Physiology* **166**, 1923-33.

Pestana, M., Varennes, A. d., Abadía, J., and Faria, E. A. (2005). Differential tolerance to iron deficiency of citrus rootstocks grown in nutrient solution. *Scientia Horticulturae* **104**, 25-36.

Pitman, M., and Lauchli, A. (2002). Global impact of salinity and agricultural ecosystems. *In* "Salinity: Environment-Plants-Molecules." (A. Lauchli and U. Luttge, eds.), pp. 1-51, Kluwer Academic Publisher.

Preston, A. (1958). Apple rootstock studies: Fifteen years' results with some M.IX crosses. *Journal of Horticultural Science* **42**, 41-50.

Proietti, S., Rouphael, Y., Colla, G., Cardarelli, M., De Agazio, M., Zacchini, M., Rea, E., Moscatello, S., and Battistelli, A. (2008). Fruit quality of mini-watermelon as affected by grafting and irrigation regimes. *Journal of the Science of Food and Agriculture* **88**, 1107-1114.

Ramirez, D. R., Wehner, T., and Miller, C. H. (1988). Source limitation by defoliation and its effect on dry matter production and yield of cucumber. *HortScience* **23**, 704-706.

Rivero, R. M., Ruiz, J. M., and Romero, L. (2003). Role of grafting in horticultural plants under stress conditions. *Food, Agriculture & Environment* **1**, 70-74.

Roberts, W., Damicone, J., Edelson, J., Motes, J., and Duthie, J. (2007). "Watermelon Production." Oklahoma Cooperative Extension Service.

Rock, L. (2008). Salinity tolerance of Texas native and introduced grasses and forbs, Texas A&M University-Kingsville, Kingsville, Texas.

Rodríguez-Gamir, J., Ancillo, G., Legaz, F., Primo-Millo, E., and Forner-Giner, M. A. (2012). Influence of salinity on pip gene expression in citrus roots and its relationship with root hydraulic conductance, transpiration and chloride exclusion from leaves. *Environmental and Experimental Botany* **78**, 163-166.

Rolny, N., Costa, L., Carrion, C., and Guiamet, J. J. (2011). Is the electrolyte leakage assay an unequivocal test of membrane deterioration during leaf senescence? *Plant Physiology and Biochemistry* **49**, 1220-7.

Romero-Aranda, R., Moya, J. L., Tadeo, F. R., Legaz, F., Primo-Millo, E., and Talon, M. (1998). Physiological and anatomical disturbances induced by chloride salts in sensitive and tolerant citrus: benficial and detrimental effects of cations. *Plant , Cell and Environment* **21**, 1243-1253.

Romero, L., Belakbir, A., Ragala, L., and Ruiz, J. M. (1997). Response of plant yield and leaf pigments to saline conditions: Effectiveness of different rootstocks in melon plants (*Cucumis melo* L.). *Soil Science and Plant Nutrition* **43**, 855-862.

Rouphael, Y., and Colla, G. (2005). Growth, yield, fruit quality and nutrient uptake of hydroponically cultivated zucchini squash as affected by irrigation systems and growing seasons. *Scientia Horticulturae* **105**, 177-195.

Ruiz, D., Martínez, V., and Cerdá, A. (1997a). Citrus response to salinity: Growth and nutrient uptake. *Tree Physiology* **17**, 141-150.

Ruiz, D. M., V.; and Cerda, A. (1995). Citrus response to salinity growth and nutrient uptake. *Tree Physiology* **17**, 141-150.

Ruiz, J. M., Belakbir, A., López-Cantarero, I., and Romero, L. (1997b). Leaf-macronutrient content and yield in grafted, melon plants. A model to evaluate the influence of rootstock genotype. *Scientia Horticulturae* **71**, 227-234.

Ruiz, J. M., Ríos, J. J., Rosales, M. A., Rivero, R. M., and Romero, L. (2006). Grafting between tobacco plants to enhance salinity tolerance. *Journal of Plant Physiology* **163**, 1229-1237.

Salam, M., Masum, A., Chowdhury, S., Dhar, M., Saddeque, M., and Islam, M. (2002). Growth and yield of watermelon as influenced by grafting. *Journal of Biological Sciences* **2**, 298-299.

Samson, J., and Bink, J. (1975). Citrus budding in the tropics: Towards explanation of the favorable results of lopping. *In* "Conference of the International Organization of Citrus Virologists". **7**, 213-231.

Santa-Cruz, A., Martinez-Rodriguez, M. M., Perez-Alfocea, F., Romero-Aranda, R., and Bolarin, M. C. (2002). The rootstock effect on the tomato salinity response depends on the shoot genotype. *Plant Science* **162**, 825-831.

Santos, C. V. (2004). Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Scientia Horticulturae* **103**, 93-99.

Sauls, J. P. (2008). Texas Citrus and Subtropical Fruits. *In* "Citrus Nursery Production", Vol. 2012. Texas AgriLife Extension, http://aggie-horticulture.tamu.edu/citrus/nutrition/L2288.htm.

Scanlon, B. R., Jolly, I., Sophocleous, M., and Zhang, L. (2007). Global impacts of conversions from natural to agricultural ecosystems on water resources: Quantity versus quality. *Water Resources Research* **43**, 1-18.

Shahriaripour, R., Pour, A. T., Mozaffari, V., Dashti, H., and Adhami, E. (2010). Effects of salinity and soil zinc application on growth and chemical composition of pistachio seedlings. *Journal of Plant Nutrition* **33**, 1166-1179.

Smethurst, C., Garnett, T., and Shabala, S. (2005). Nutritional and chlorophyll fluorescence responses of lucerne (*Medicago sativa*) to waterlogging and subsequent recovery. *Plant and Soil* **270**, 31-45.

Smith, D., and Anciso, J. (2000). "Watermelons in Texas: Brief on production, pests and pesticides." Texas A&M University System, College Station.

Smith, P. F. (1962). A case of sodium toxicity in citrus. *In* " Proceedings of the Florida State Horticultural Society". **75**, 120-124.

Soule, J. (1971). Anatomy of the bud union in mango (*Mangifera indica* L.). *Journal of the American Society for Horticultural Science* **96**, 380-383.

Spiegel-Roy, P., and Goldschmidt, E. E. (1996). "Biology of Citrus," Cambridge University Press, Cambridge.

Storey, R., and Walker, R. R. (1999). Citrus and salinity. *Scientia Horticulturae* **78**, 39-81.

Strong, D., and Miller-Azarenko, A. (1991). Dry matter partitioning in 'Starkspur supreme delicious' on nine rootstocks. *Fruit Variety Journal* **45**, 238-241.

Sykes, S. (1985). Effects of seedling age and size on chloride accumulation by juvenile citrus seedlings treated with sodium chloride under glasshouse conditions. *Australian Journal of Experimental Agriculture* **25**, 943-953.

Syvertsen, J., Lloyd, J., and Kriedemann, P. (1988). Salinity and drought stress effects on foliar ion concentration, water relations, and photosynthetic characteristics of orchard citrus. *Australian Journal of Agricultural Research* **39**, 619-627.

Tanji, K., Ed. (1996). "Agricultural Salinity Assessment and Management," American Society of Civil Engineers, New York, New York.

Troncoso, A., Linan, J., Cantos, M., Acebedo, M., and Rapoport, H. (1999). Feasibility and anatomical development of an in vitro olive cleft-graft. *Journal of Horticultural Science and Biotechnology* **74**, 584-587.

United Nations Food and Agriculture Organization (2012). "Coping with water scarcity: An action framework for agriculture and food security." Food and Agriculture Organization of the United Nations, Rome, Italy.

United States Department of Agriculture, National Agricultural Statistics Service, Texas Field Office (2008). "Texas Agricultural Statistics 2008." Texas Department of Agriculture, Austin, TX.

United States Department of Agriculture, National Agricultural Statistics Service, Texas Field Office (2009). "Texas Agricultural Statistics 2009." Texas Department of Agriculture, Austin, TX.

United States Department of Agriculture, National Agricultural Statistics Service, Texas Field Office (2012). Citrus fruits 2012 summary. United States Department of

Agriculture, National Agricultural Statistic Service, Texas Field Office, Austin, Texas.

US Department of Commerce, USDA and National Agricultural Statistic Service (2007). U.S. Watermelon Industry: Census harvested & irrigated watermelon area by State, 1982-2007. United States Department of Agriculture, Economic Research Service. Ithaca, New York.

USDA, (2012a). U.S. watermelon industry: U.S. watermelon area, yield, production, price & value, 1950-2012. United States Department of Agriculture, Economic Research Service. Ithaca, New York.

USDA, Vegetables Annual Summary and Texas Agricultural Statistics Service (2012b). U.S. Watermelon Industry: Texas acreage, yield, prodn, price & value, 1950-2012. United States Department of Agriculture, Economic Research Service. Ithaca, New York.

Uygur, V., and Yetisir, H. (2009). Effects of rootstocks on some growth parameters, phosphorous and nitrogen uptake watermelon under salt stress. *Journal of Plant Nutrition* **32**, 629-643.

Vardi, A., Spiegel-Roy, P., Ben-Hayyim, G., Neumann, H., and Shalhevet, J. (1988). Response of Shamouti orange and Minneola tangelo on six rootstocks to salt stress. *Citriculture.* Balaban Publishers, Rehovot, Israel.

Walker, C. W. (1986). Sodium exclusion and potassium-sodium selectivity in salt-treated trifoliate orange (*Poncirus trifoliata*) and Cleopatra mandarin (*Citrus reticulata*) plants. *Australian Journal of Plant Physiology* **13**, 293-303.

Walker, R. R., and Douglas, T. J. (1983). Effect of salinity level on uptake and distribution of chloride, sodium and potassium ions in citrus plants. *Australian Journal of Agricultural Resources* **34**, 145-153.

Whitlow, T. H., Bassuk, N. L., Ranney, T. G., and Reichert, D. L. (1992). An improved method for using electrolyte leakage to assess membrane competence in plant tissues. *Plant Physiology* **98**, 198-205.

Williamson, J., Castle, W. S., and Koch, K. (1992). Growth and ^{14}C-photosynthate allocation in citrus nursery trees subjected to one of three bud forcing methods. *Journal of the American Society for Horticultural Science* **117**, 37-40.

Williamson, J., and Maust, B. (1995). Growth of budded, containerized, citrus nursery plants when photosynthesis of rootstock shoots is limited. *HortScience* **30**, 1363-1365.

Wutscher, H. K. (1979). Citrus rootstocks. *Horticultural Reviews; 1979* **1**.

Yang, L. F., Zhu, Y. L., Hu, C. M., Liu, Z. L., and Zhang, G. W. (2006). Effects of NaCl stress on the contents of the substances regulating membrane lipid oxidation and osmosis and photosynthetic characteristics of grafted cucumber. *Acta Botanica Boreali-Occidentalia Sinica* **26**, 1195-1200.

Yetisir, H., and Uygur, V. (2009). Plant growth and mineral element content of different gourd species under salinity stress. *Turkish Journal of Agricultural Forestry* **33**, 65-77.

Yin, H., Yan, B., Sun, J., Jia, P., Zhang, Z., Yan, X., Chai, J., Ren, Z., Zheng, G., and Liu, H. (2012). Graft-union development: a delicate process that involves cell–

cell communication between scion and stock for local auxin accumulation. *Journal of Experimental Botany* **63**, 4219-4232.

Zapata, J. M. C. (2004). Interstock-induced mechanism of increased growth and salt resistance of orange (*Citrus sinensis*) trees. *Tree Physiology* **24**, 1109-1117.

Zekri, M. (1987). Effects of NaCl and polyethylene glycol on the water relations growth and morphology of citrus rootstock seedlings. Dissertation, University of Florida.

Zekri, M. (1991). Effects of NaCl on growth and physiology of sour orange and Cleopatra mandarin seedlings. *Scientia Horticulturae* **47**, 305-315.

Zekri, M. (1993). Salinity and calcium effects on emergence, growth and mineral composition of seedlings of eight citrus rootstocks. *Journal of Horticultural Science* **68**, 53-62.

Zekri, M., and Al-Jaleel, A. (2003). Citrus rootstock evaluation for sweet oranges. *Proceedings of the Interamerican Society of Tropical Horticulture* **47**, 242-247.

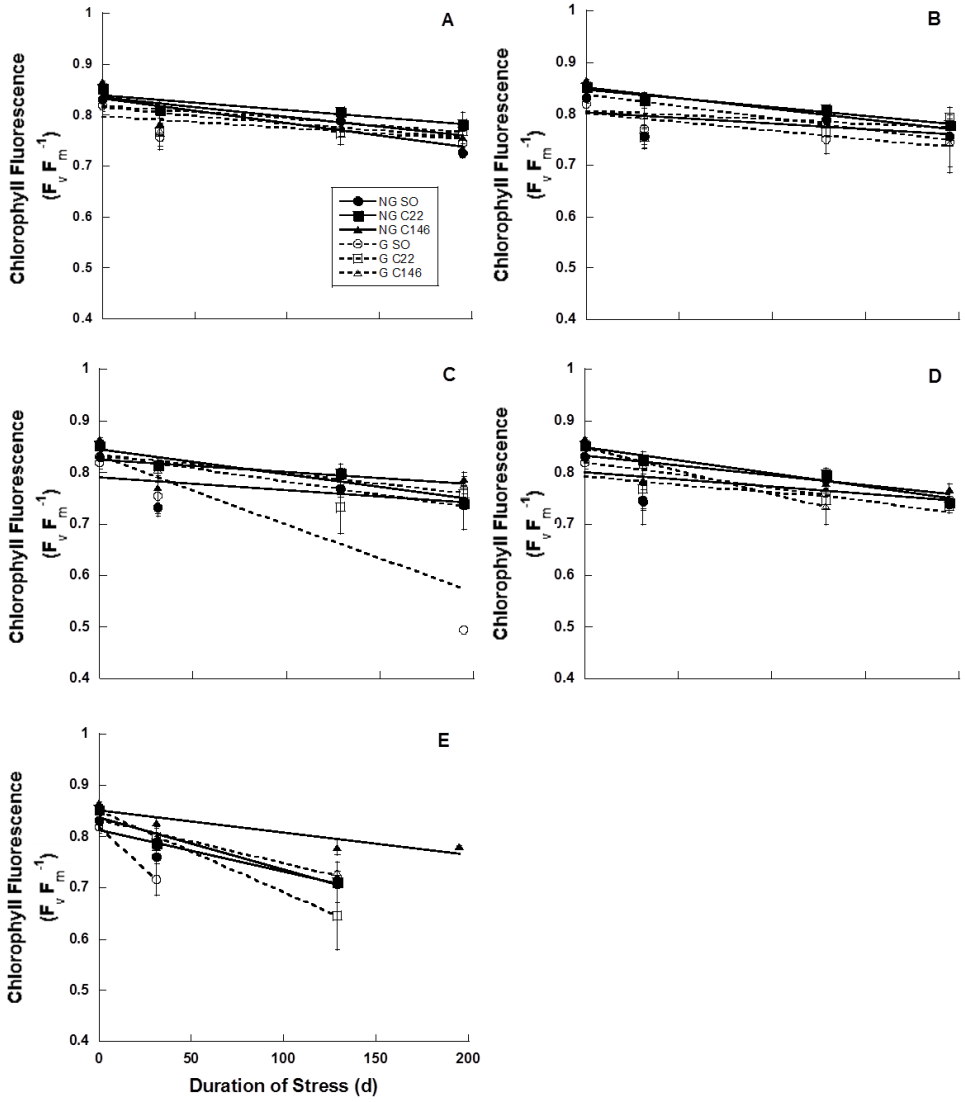
Zekri, M., and Parsons, L. R. (1989). Growth and root hydraulic conductivity of several citrus rootstocks under salt and polyethylene glycol stresses. *Physiologia Plantarum* **77**, 99-106.

Zekri, M., and Parsons, L. R. (1990). Calcium influences growth and leaf mineral concentrations of citrus under saline conditions. *HortScience* **25**, 784-786.

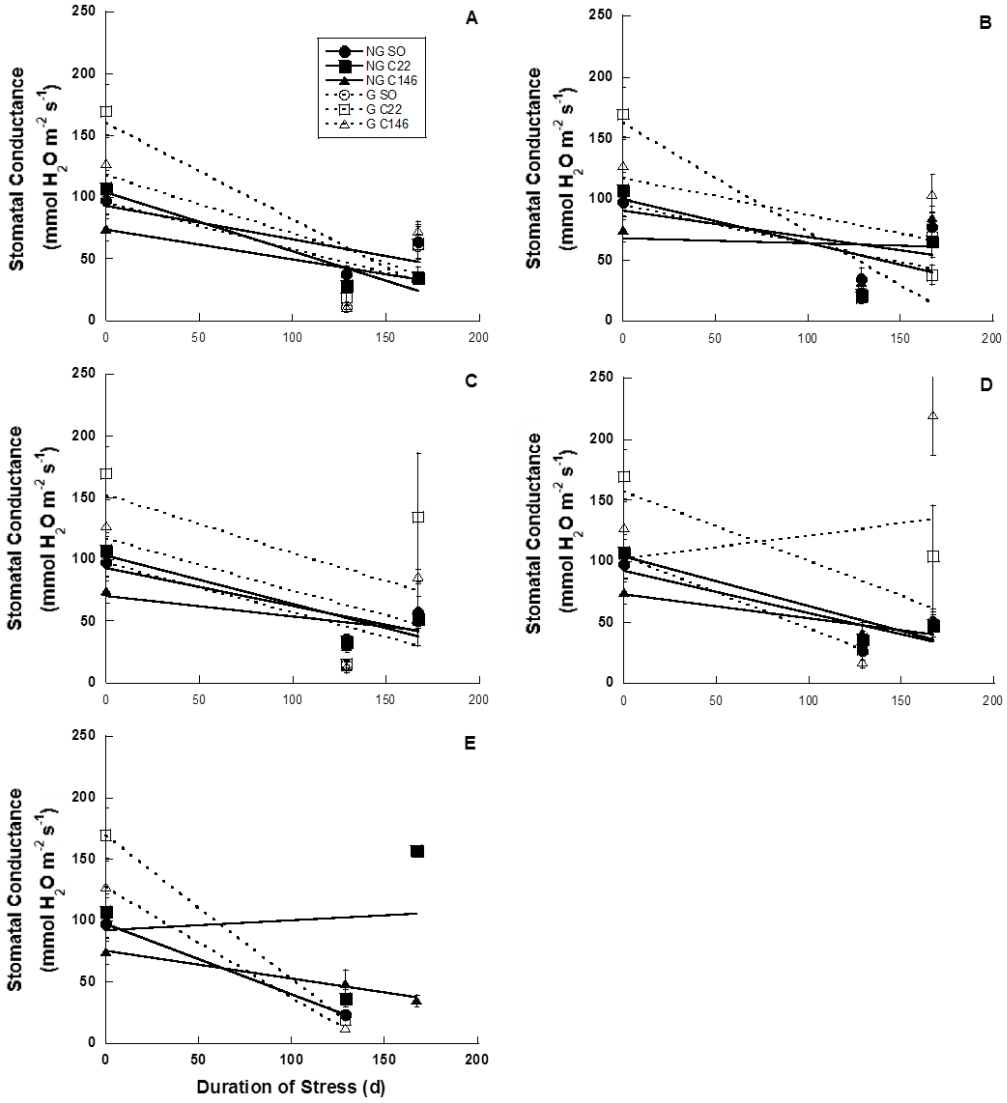
Zekri, M., and Parsons, L. R. (1992). Salinity tolerance of citrus rootstocks: Effects of salt on root and leaf mineral concentrations. *Plant and Soil* **147**, 171-181.

Ziogas, V., Tanou, G., Filippou, P., Diamantidis, G., Vasilakakis, M., Fotopoulos, V., and Molassiotis, A. (2013). Nitrosative responses in citrus plants exposed to six abiotic stress conditions. *Plant Physiology and Biochemistry* **68**, 118-26.

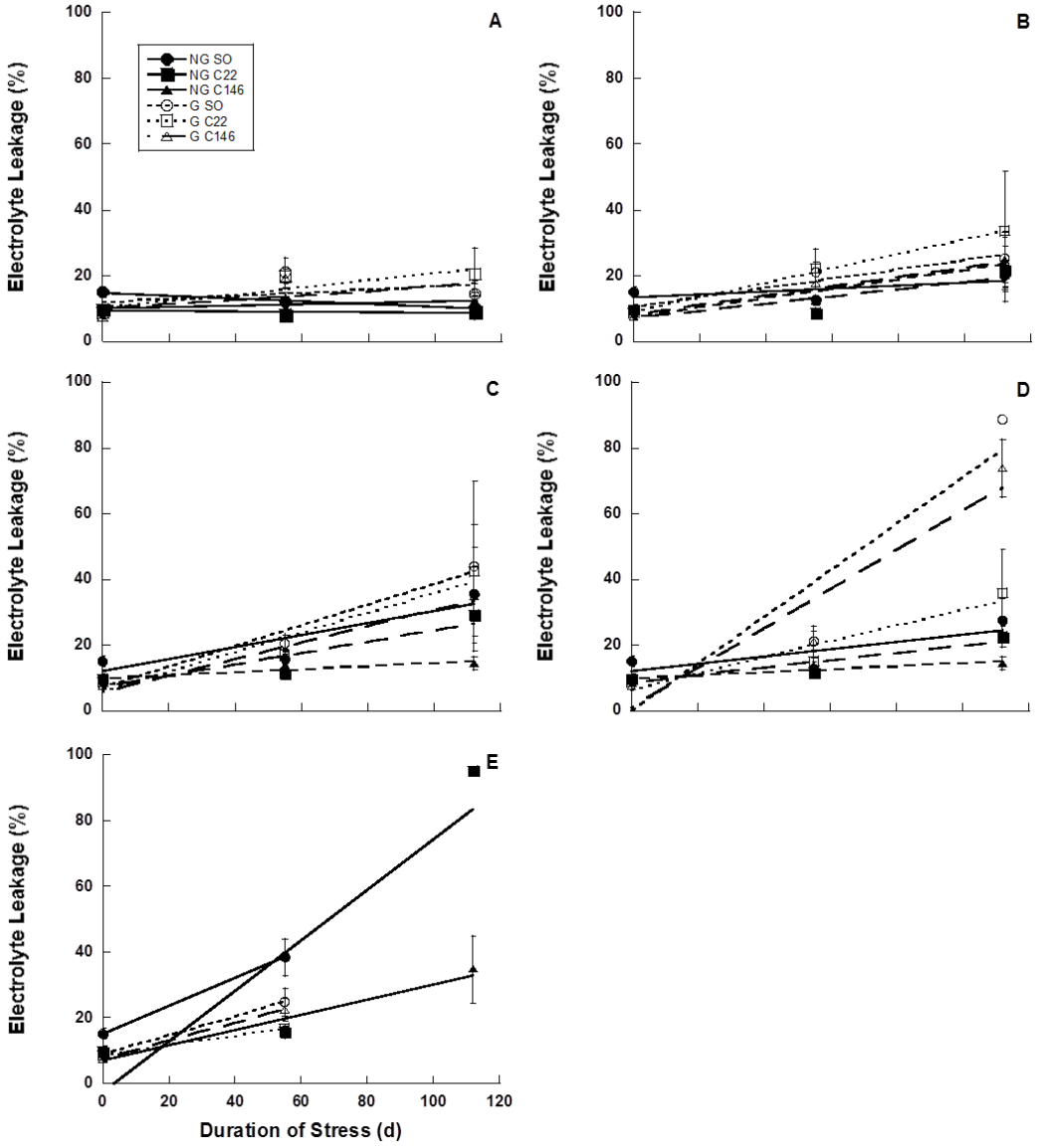
APPENDIX A



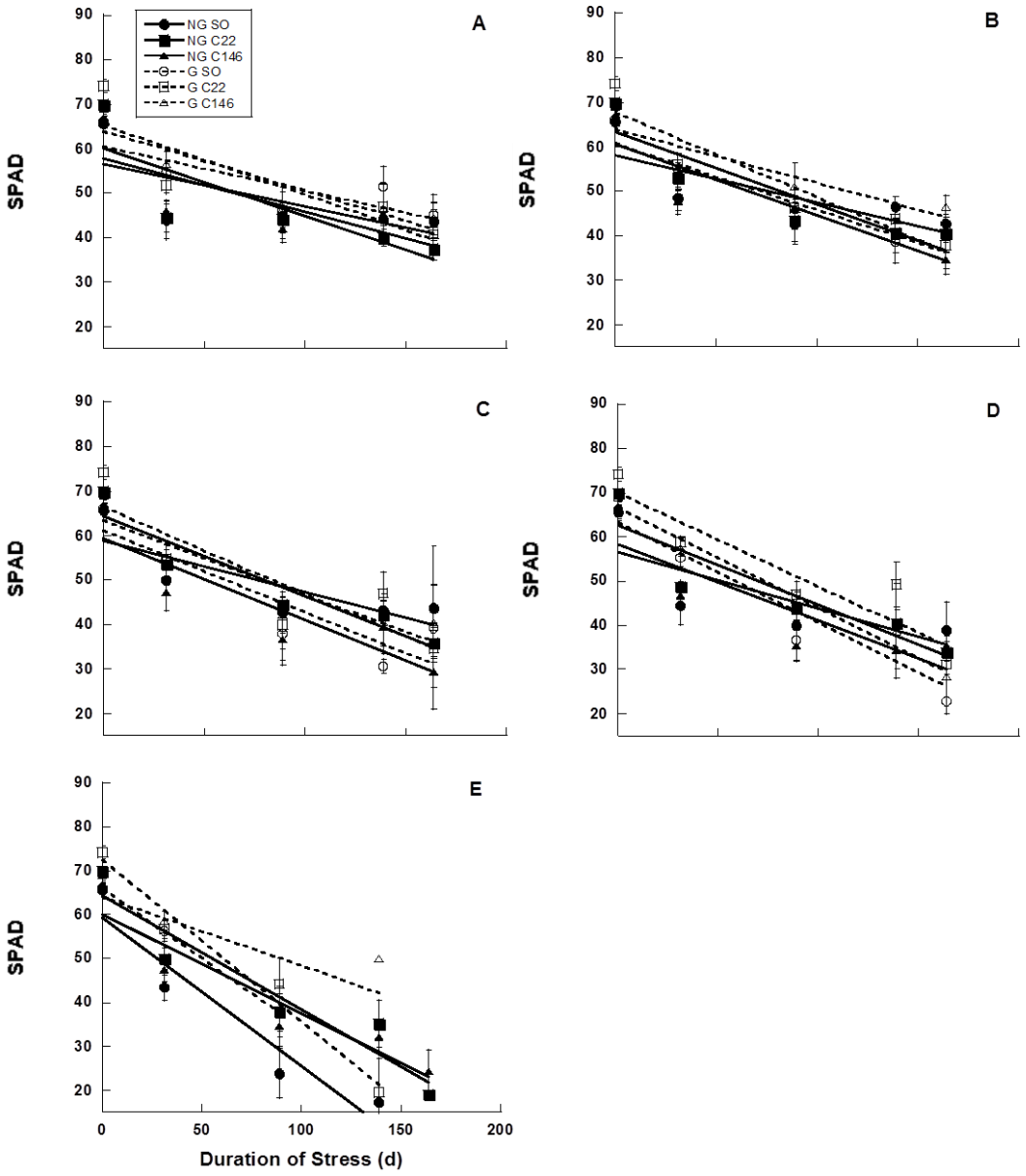
Appendix A-1. **Chlorophyll fluorescence over time as affected by rootstock, grafting, and salinity.** Grafted trees are represented by solid shapes and solid lines, non-grafted trees are represented by open shapes and dashed lines. A) Chlorophyll fluorescence ($F_v F_m^{-1}$) over time for control treatment (0 dS m^{-1}), B) Chlorophyll fluorescence over time for trees irrigated with 1 dS m^{-1} water, C) Chlorophyll fluorescence over time for trees irrigated with 3 dS m^{-1} water, D) Chlorophyll fluorescence over time for trees irrigated with 5 dS m^{-1} water, E) Chlorophyll fluorescence over time for trees irrigated with 10 dS m^{-1} water. Error bars are shown as ±1 standard error of the mean.



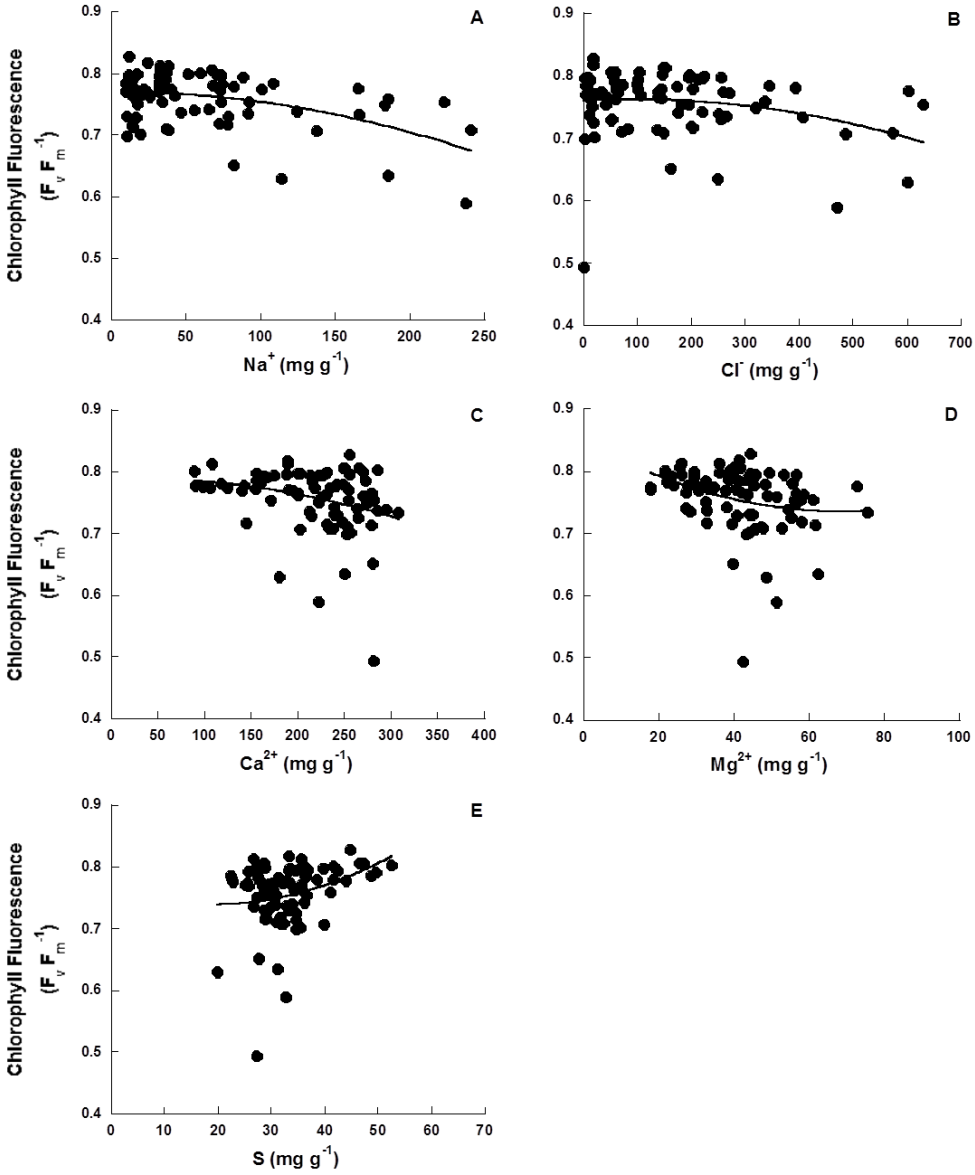
Appendix A-2.Stomatal conductance over time as affected by rootstock, grafting, and salinity. Grafted trees are represented by solid shapes and solid lines, non-grafted trees are represented by open shapes and dashed lines. A) Stomatal conductance (mmol H_2O m^{-2} s^{-1}) over time for control treatment (0 dS m^{-1}), B) Stomatal conductance over time for trees irrigated with 1 dS m^{-1} water, C) Stomatal conductance over time for trees irrigated with 3 dS m^{-1} water, D) Stomatal conductance over time for trees irrigated with 5 dS m^{-1} water, E) Stomatal conductance over time for trees irrigated with 10 dS m^{-1} water. Error bars are shown as ±1 standard error of the mean.



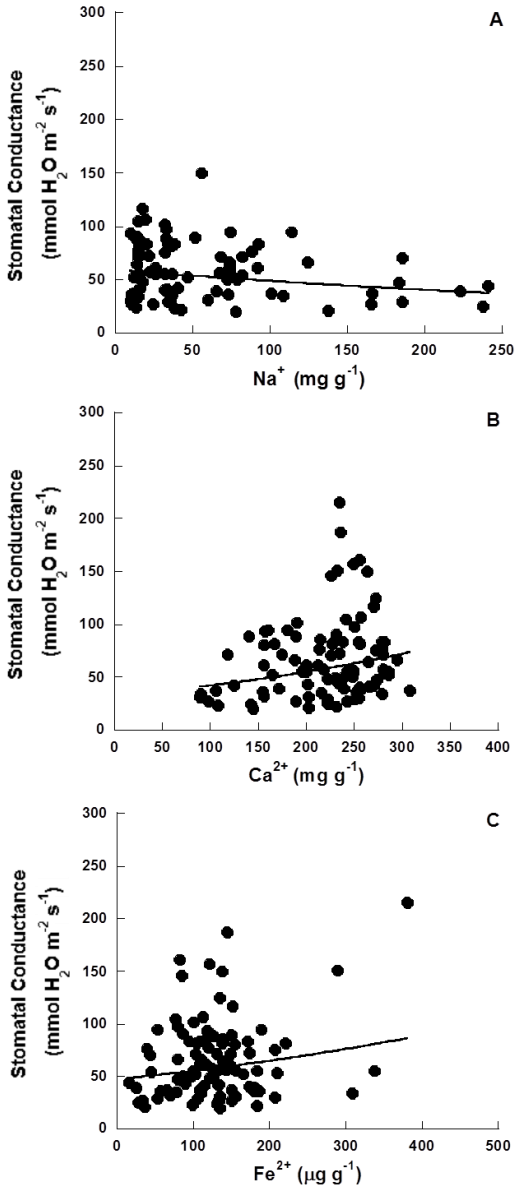
Appendix A-3. Electrolyte leakage over time as affected by rootstock, grafting, and salinity. Grafted trees are represented by solid shapes and solid lines, non-grafted trees are represented by open shapes and dashed lines. A) Electrolyte leakage (%) over time for control treatment (0 dS m^{-1}), B) Electrolyte leakage over time for trees irrigated with 1 dS m^{-1} water, C) Electrolyte leakage over time for trees irrigated with 3 dS m^{-1} water, D) Electrolyte leakage over time for trees irrigated with 5 dS m^{-1} water, E) Electrolyte leakage over time for trees irrigated with 10 dS m^{-1} water. Error bars are shown as ±1 standard error of the mean.



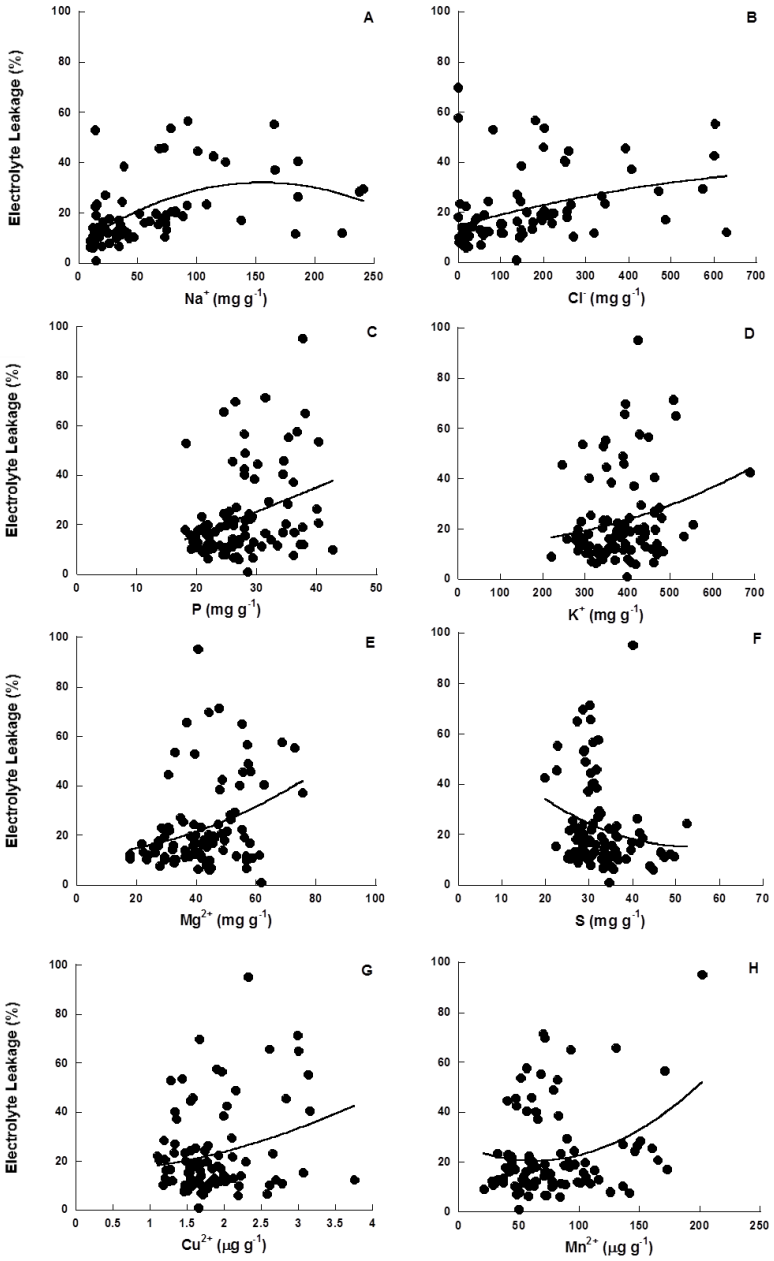
Appendix A-4. SPAD over time as affected by rootstock, grafting and salinity.
Grafted trees are represented by solid shapes and solid lines, non-grafted trees are represented by open shapes and dashed lines. A) SPAD over time for control treatment (0 dS m^{-1}), B) SPAD over time for trees irrigated with 1 dS m^{-1} water, C) SPAD over time for trees irrigated with 3 dS m^{-1} water, D) SPAD over time for trees irrigated with 5 dS m^{-1} water, E) SPAD over time for trees irrigated with 10 dS m^{-1} water. Error bars are shown as ±1 standard error of the mean.



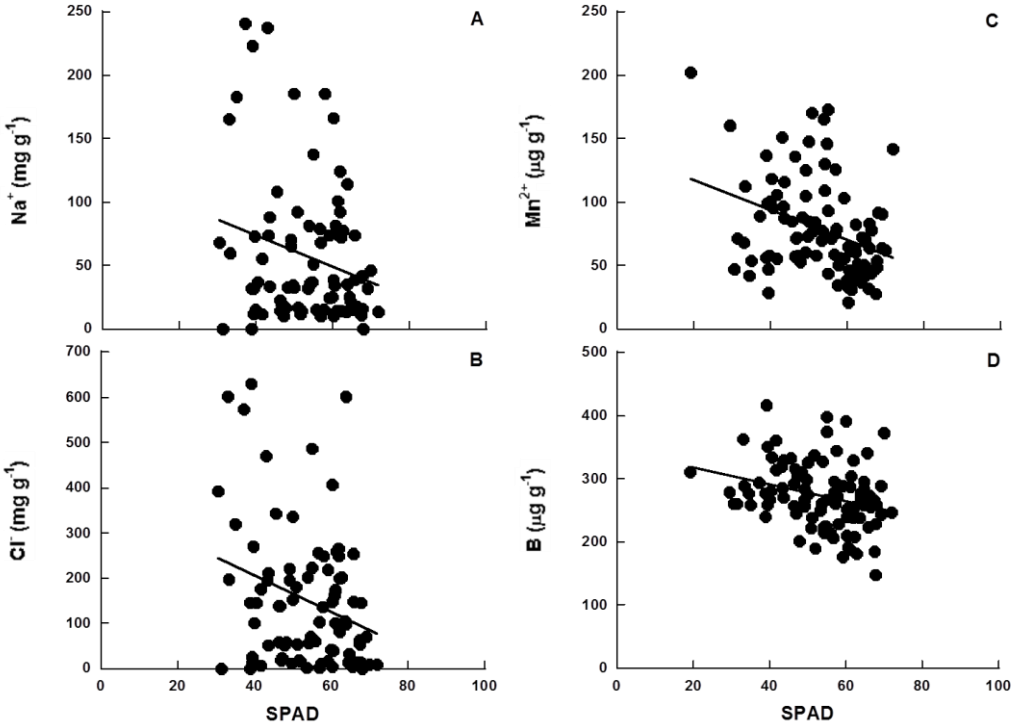
Appendix A- 5. Chlorophyll fluorescence response to nutrients. Nutrient correlation with chlorophyll fluorescence. A) Na^+ ion accumulation associated with chlorophyll fluorescence decline (P=0.007), B) Cl^- ion accumulation associated with chlorophyll fluorescence decline (P=0.031), C) Ca^{2+} ion accumulation associated with chlorophyll fluorescence decline (P=0.009), D) Mg^{2+} ion accumulation associated with chlorophyll fluorescence decline (P=0.011), and E) S ion association with higher chlorophyll fluorescence values (P=0.010).



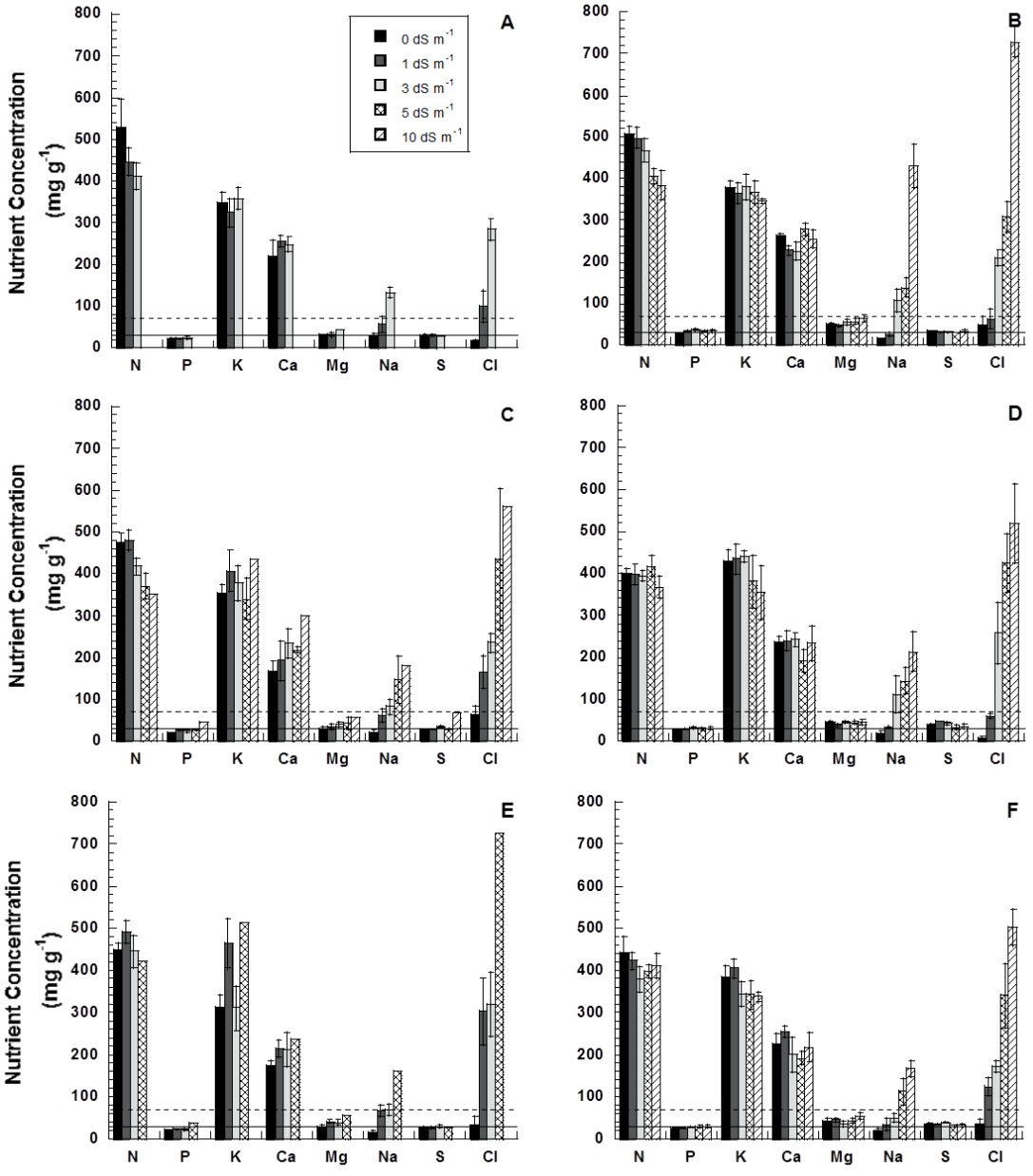
Appendix A- 6. Nutrient correlation with stomatal conductance. A) Na^+ ion accumulation associated with decline stomatal conductance (P=0.024), B) Ca^{2+} ion accumulation associated with higher stomatal conductance decline (P=0.039), C) Fe^{2+} ion association with higher stomatal conductance values (P=0.022).



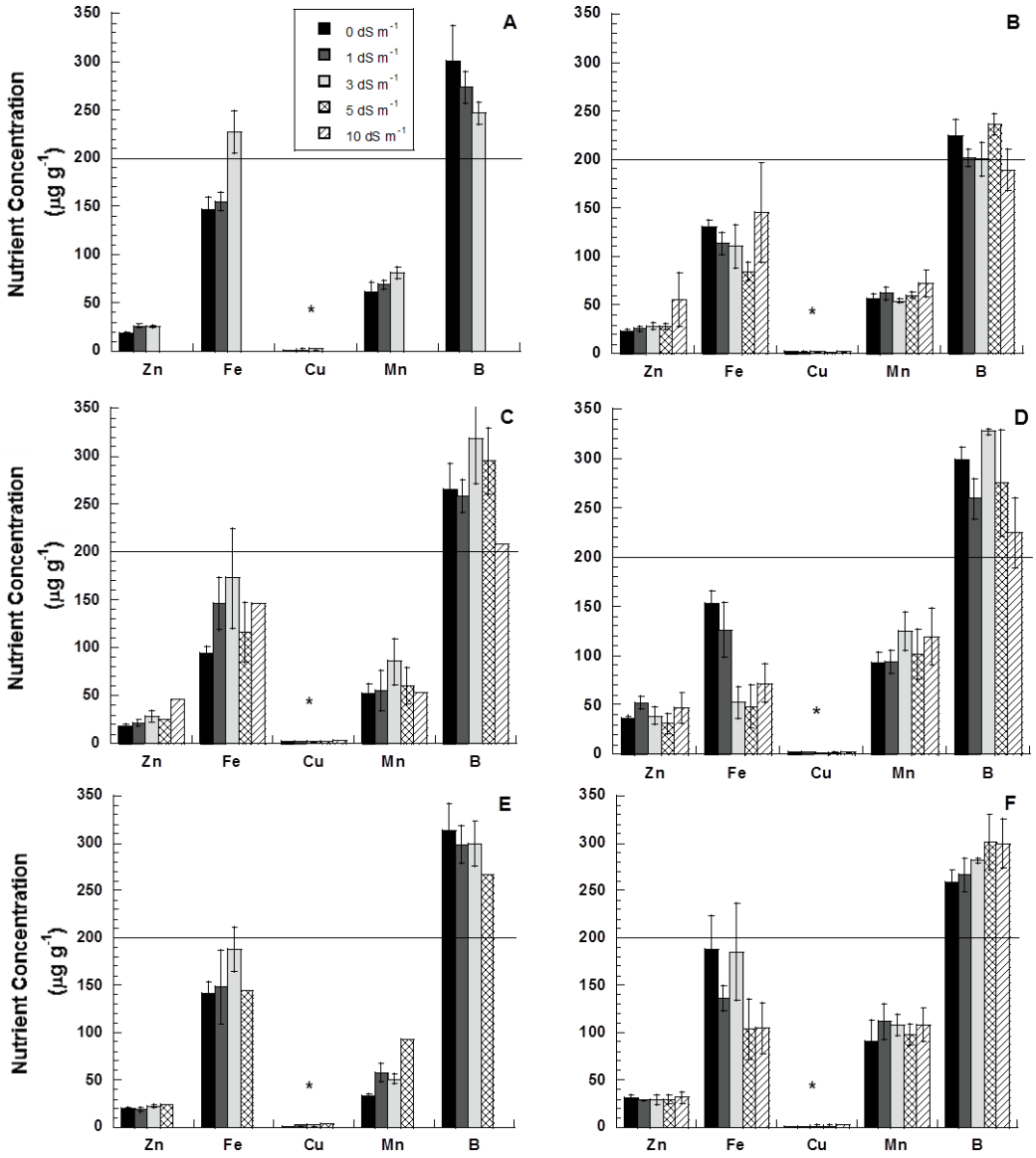
Appendix A-7. Nutrient correlation with electrolyte leakage. A) Na^+ accumulation associated with increased electrolyte leakage (P=0.004), B) Cl^- accumulation associated with increased electrolyte leakage (P=0.001), C) P accumulation associated with increased electrolyte leakage (P=0.002), D) K^+ accumulation associated with increased electrolyte leakage (P=0.032), E) Mg^{2+} accumulation associated with increased electrolyte leakage (P=0.002), F) S accumulation associated with decreased electrolyte leakage (P=0.043), G) Cu^{2+} accumulation associated with decreased electrolyte leakage (P=0.016), and H) Mn^{2+} accumulation associated with increased electrolyte leakage (P=0.019).



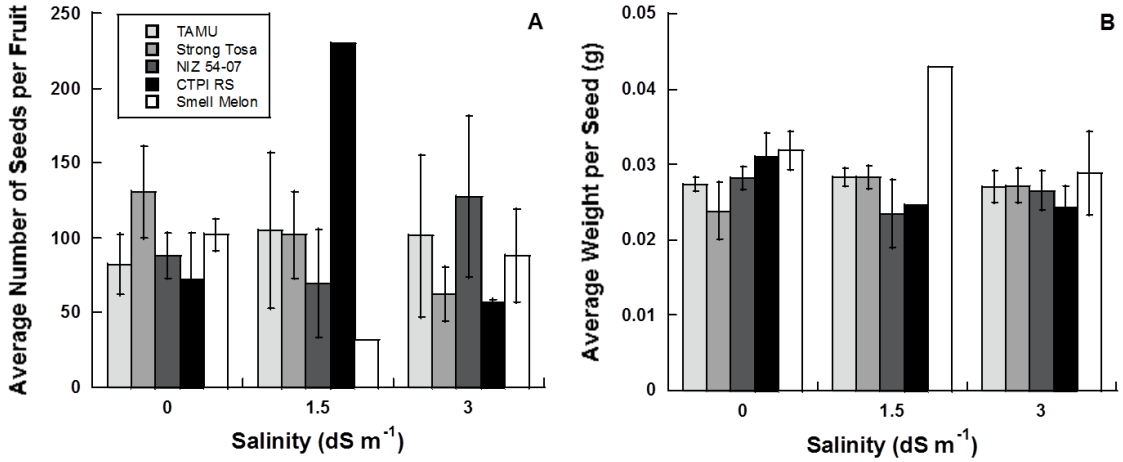
Appendix A-8. Nutrient correlation to SPAD measurements. A) SPAD decline associated with higher Na^+ ion concentrations (P=0.037), B) SPAD decline associated with higher Cl^- ion concentrations (P=0.011), C) SPAD decline associated with higher concentrations of Mn^{2+} (P=0.001), D) SPAD decline associated with higher concentrations of B ions (P=0.004).



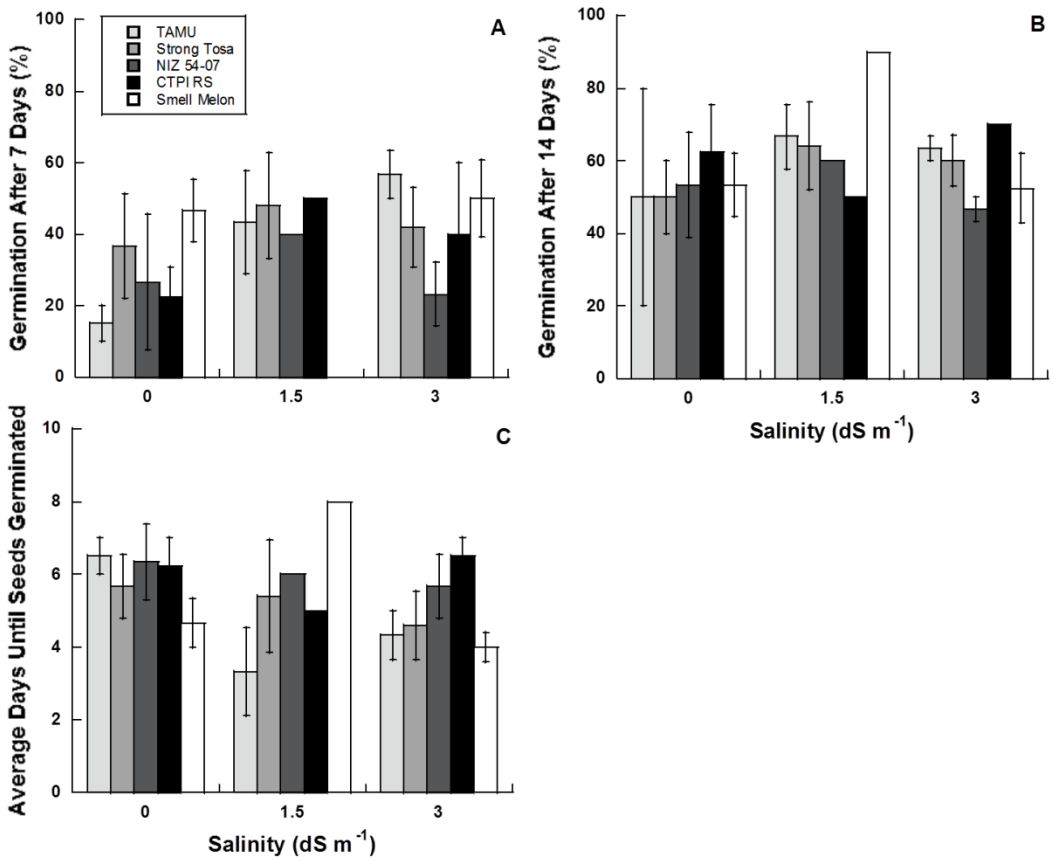
Appendix A-9. Rootstock macronutrient status of trees at 0, 1, 3, 5, and 10 dS m^{-1}. A) Macronutrient concentrations (mg g^{-1}) for grafted SO trees, B) macronutrient concentrations for SO ungrafted trees, C) macronutrient concentrations for grafted C22 trees, D) macronutrient concentrations for C22 ungrafted trees, E) macronutrient concentrations for grafted C146 trees, F) macronutrient concentrations for C146 ungrafted trees. Error bars are shown as ±1 standard error of the mean. Solid horizontal lines indicate toxicity threshold ranges for Na^+ and dashed horizontal lines indicate toxicity threshold ranges for Cl^-.



Appendix A-10. Rootstock micronutrient status of trees at 0, 1, 3, 5, and 10 dS m^{-1}.
Rootstock micronutrient status of trees at 0, 1, 3, 5, and 10 dS m^{-1}. A) Micronutrient concentrations (µg g^{-1}) for grafted SO trees, B) micronutrient concentrations for SO ungrafted trees, C) micronutrient concentrations for grafted C22 trees, D) micronutrient concentrations for C22 ungrafted trees, E) micronutrient concentrations for grafted C146 trees, F) micronutrient concentrations for C146 ungrafted trees. Error bars are shown as ±1 standard error of the mean. (*) indicates deficiencies according to recommended plant nutrient concentrations. Horizontal lines indicate toxicity threshold ranges for B^{3+}.



Appendix A-11. Seeds produced and seed weights by plants on each rootstock for each salinity treatment. A) Average number of seeds produced per plant, B) average weight (g) per seed. Error bars are shown as ±1 standard error of the mean.



Appendix A-12. Germination after 7 and 14 days and days til emergence for each rootstock at each salinity level. A) % germination after 7 days, B) % germination after 14 days, and C) average number of days until seeds germinated. Error bars are shown as ±1 standard error of the mean.