REPORT

Title Impact of Saline Irrigation Water on Citrus Rootstocks in the Lower Rio Grande Valley

Prime Agreement Number: 06HQGR0130

Research Subcontract Number: 570651

Primary PI Catherine R. Simpson

Other PIs Shad D. Nelson, PhD

Abstract

The Texas citrus industry in the Rio Grande Valley experiences periodic droughts. During such times, water restrictions from the Amistad and Falcon Reservoirs can reduce water available for agriculture. Irrigation in this region is primarily surface waters from the Rio Grande where citrus orchards are flood irrigated. Irrigation practices that use less water are being explored and evaluated. Water used to irrigate crops usually contains between 800 and 900 mg L^{-1} of salt, the equivalent of adding between 2100 and 2400 lbs salt/acre foot. Limiting irrigation in agricultural areas may lead to salt accumulation in the crop rooting depth, especially where low water use systems like drip irrigation is utilized.

Currently, Citrus trees are grafted onto hardy rootstocks in order to ensure tree survival and production. These rootstocks are used to reduce pathogen impacts and enhance their tolerance to thermal, saline and other environmental stressors. It is vital to find saline tolerant citrus rootstocks for soil and environmental conditions in the Lower Rio Grande Valley (LRGV).

This study's objectives are to assess the salinity tolerance of citrus rootstocks using typical soils found in the Rio Grande Valley. We will evaluate irrigation water salinity tolerance levels for these rootstocks during greenhouse trials.

Problem and Research Objectives

Drought and water restrictions are an ongoing problem for farmers in the Rio Grande Valley. Finding citrus rootstocks that are able to tolerate increasing salinity while using less water is vital for the agricultural community and water conservation in South Texas.

Citrus (*Citrus spp.*) is an important economic crop in the LRGV, bringing in more than $50 million for growers annually (Sauls 2008). Citrus trees are traditionally grafted onto sour orange rootstock because of its ability to tolerate the calcareous, high pH soils and heavy soil conditions in south Texas (Sauls 2008; Louzada et al. 2008). However, the sour orange rootstock is susceptible to a variety of diseases and pathogens that were previously not a problem. Increasing concerns over Citrus tristeza virus transmitted by the new arrival, the Brown Citrus Aphid, and other diseases have initialized more research into finding alternative rootstocks. These pest and pathogen resistant rootstocks must be evaluated for the varying soil and water conditions found in the Rio Grande Valley.

The decreasing availability of irrigation waters from surface waters due to drought and restrictions have put limitations on irrigation practices in the LRGV. On average, typical irrigation practices consist of flooding fields with 0.5 acre-foot /acre between 4 and 6 times during the growing season. This could increase up to 9 times during the growing season in times of drought or water shortage. Given an average EC of 1.33 dS m^{-1} (850 mg L^{-1}), this means that in a growing season as much as 4624 to 10,404 lbs of salt/ acre are added annually to citrus orchards. While most salts will be leached away by excess water, some salts will continue to accumulate. This problem will be compounded if water restrictions limit the amount of water farmers will be able to apply to their land.

The intrusion of salt water from the Gulf of Mexico causes high salinity levels in groundwater throughout the LRGV. Groundwater in this region has not typically been used for irrigation due to high spatial variability in water quality and quantity (Chowdhury and Mace). Surface water limitations may force farmers to resort to saline or brackish groundwater in order to meet crop water demands. This has led to the need for further development and evaluation of saline tolerant rootstocks.

The objectives of this study were to evaluate and assess the salinity tolerance for several citrus rootstocks. There is also a need to evaluate irrigation water deficits to determine an optimal salinity tolerance level that will also meet the crop's water needs.

To determine these factors we will set up greenhouse trials using various citrus rootstocks. We used rootstock varieties adapted to soil conditions in the Rio Grande Valley and water with varying electrical conductivities and apply them at different increments in order to evaluate the optimal salinity tolerance in a water deficit situation.

This study's purpose is to obtain preliminary data in order to further research that may be conducted during field trials of the same rootstocks. This can potentially be valuable information for growers in times of drought or water restriction when they may have few options. Water quality and availability is a problem that is escalating as increased population growth in the LRGV as well as drought and water restrictions occur.

Materials/Methodology

Initially, rootstock seeds were evaluated for the potential to be used in this study by germinating seeds in a nutrient agar supplemented with salt solutions at different concentrations. This initial evaluation evaluated germination by observing seeds collected from rootstock parent varieties grown on Texas A&M University-Citrus Center property in Weslaco, TX. Four rootstock cultivars were evaluated based on their disease resistance, tolerance to calcareous clays, fruit quality and potential yield. These rootstock cultivars were Sour Orange, C-146, C-57 and C-22. One scion variety was also tested to evaluate salinity on the scion cultivar. This *in vitro* study was conducted to minimize contamination and reduce any additive effects of repeated saline water additions.

This part of the study evaluated the *in vitro* germination and growth of citrus seeds in a nutrient agar supplemented with sea salt solution (Instant Ocean©, Spectrum Brands, Inc., Madison, WI) to have salt concentrations that correspond to approximately 0, 1, 3, 5, and 10 dS/m (+/- 1 dS/m) electrical conductivities. Each cultivar had 10 seeds per box and 2 boxes per treatment for a total of 100 seeds per cultivar and 20 seeds per treatment. The seeds were sanitized in a solution containing 10% bleach and 0.1% Tween 20 and stirring continuously for 2 hours, then rinsed with deionized water four times. In sterile conditions, the seed testa and cotyledons were cut (without damage to the micropylar end) in order to promote optimal germination and rule out seed coat factors in germination hindrance. Seeds were placed in a Magenta-7 vessel (Sigma-Aldrich) and containing Murashige and Skoog basal medium (Murashige and Skoog, 1962) supplemented with Gambourg's vitamins (Sigma-Aldrich, St. Louis, MO), and 0.4% Phytagel (Sigma-Aldrich, St. Louis, MO) along with the sea salt solution (Instant Ocean©). The seeds were kept in the dark at approximately 27°C for 2 weeks and then gradually introduced into natural light conditions. The germination was recorded daily until the 14 day point. The germinated seeds were measured for the following after 70 days total germination and growth.

- Germination rate and percentage
- Number of seedlings germinated per seed (polyembryony)
- Root length and width
- Shoot length and width
- Fresh weight and dry weight (average moisture content)

After the initial seed evaluation study, three rootstock varieties were chosen to determine the salinity tolerance of grafted and non grafted citrus trees. In this study the Sour Orange, C-22 and C-146 cultivars were evaluated. Grafted rootstocks had the scion variety 'Olinda' a Valencia sweet orange variety grafted onto the previously mentioned rootstocks, while the non-grafted varieties had no such treatment.

The trees were watered bi-weekly with a sea salt solution 0, 1, 3, 5 and 10 dS/m (+/- 1 dS/m). Each treatment contained three rootstock cultivars and 5 replications. The experimental setup as shown below was set up in a random complete block design.

Grafted Rootstocks

Grafted	**0 dS/m**	**1 dS/m**	**3 dS/m**	**5 dS/m**	**10 dS/m**
	C22-R1G- 0dS	SO -R1G- 1dS	C22-R1G- 3dS	C146- R1G- 5dS	SO -R1G- 10dS
	C146- R 1 G- 0dS	C22-R1G- 1dS	SO -R1G- 3dS	C22-R1G- 5dS	C146- R1G- 10dS
Rep 1	SO -R1G- 0dS	C146-R 1G- 1dS	C146- R1G- 3dS	SO -R1G- 5dS	C22-R1G- 10dS
	C146- R 2G -0dS	SO -R2G- 1dS	SO -R2G- 3dS	C146- R2G- 5dS	C22-R2G- 10dS
	SO -R2G- 0dS	C22-R2G- 1dS	C146- R2G- 3dS	C22-R2G- 5dS	SO -R2G- 10dS
Rep 2	C22-R2G- 0dS	C146- R2G- 1dS	C22-R2G- 3dS	SO -R2G- 5dS	C146- R2G- 10dS
	C22-R3G- 0dS	C146- R3G- 1dS	C146-R 3G- 3dS	C22-R3G- 5dS	SO -R3G- 10dS
	SO -R3G- 0dS	SO -R3G- 1dS	C22-R3G- 3dS	SO -R3G- 5dS	C146- R3G- 10dS
Rep 3	C146- R3G-0dS	C22-R3G- 1dS	SO -R3G- 3dS	C146- R3G- 5dS	C22-R3G- 10dS
	SO -R4G- 0dS	SO -R4G- 1dS	C146- R4G- 3dS	C22-R4G- 5dS	C22-R4G- 10dS
	C22-R4G- 0dS	C146- R4G- 1dS	C22-R4G- 3dS	SO -R4G- 5dS	C146- R4G- 10dS
Rep4	C146-R 4G-0dS	C22-R4G- 1dS	SO -R4G- 3dS	C146- R4G- 5dS	SO -R4G- 10dS
	C22-R5G- 0dS	C146-R5G-1dS	C22-R5G-3dS	SO-R5G-5dS	SO- R5G- 10dS
	C146- R5G- 0dS	C22-R5G-1dS	SO-R5G-3dS	C22-R5G- 5dS	C146 -R 5G- 10dS
Rep 5	SO-R 5G- 0dS	SO-R 5G- 1dS	C146-R5G-3dS	C146- R5G- 5dS	C22-R5G- 10dS

SO=Sour Orange, R=Replication, G= Grafted

Non-Grafted Rootstocks

Non Grafted	0 dS/m	1 dS/m	3 dS/m	5 dS/m	10 dS/m
	C146- R1NG- 0dS	SO -R1NG- 1dS	SO -R1NG- 3dS	C146- R1NG- 5dS	C22-R 1NG- 10dS
	SO -R1NG- 0dS	C22-R1NG- 1dS	C146- R1NG- 3dS	C22-R1NG- 5dS	SO -R1NG- 10dS
Rep 1	C22-R1NG- 0dS	C146-R 1NG- 1dS	C22-R1NG- 3dS	SO -R1NG- 5dS	C146-R 1NG- 10dS
	SO -R2NG- 0dS	SO -R2NG- 1dS	C146- R2NG- 3dS	C22-R2NG- 5dS	C22-R 2NG- 10dS
	C22-R2NG- 0dS	C146-R 2NG- 1dS	C22-R2NG- 3dS	SO -R2NG- 5dS	C146-R 2NG- 10dS
Rep 2	C146- R2NG- 0dS	C22-R2NG- 1dS	SO -R2NG- 3dS	C146- R2NG- 5dS	SO -R2NG- 10dS
	C22-R3NG- 0dS	SO -R3NG- 1dS	C22-R3NG- 3dS	C146- R3NG- 5dS	SO -R3NG- 10dS
	C146-R 3NG- 0dS	C22-R3NG- 1dS	SO -R3NG- 3dS	C22-R3NG- 5dS	C146- R 3NG- 10dS
Rep 3	SO -R3NG- 0dS	C146-R 3NG- 1dS	C146- R3NG- 3dS	SO -R3NG- 5dS	C22-R 3NG- 10dS
	C22-R4NG- 0dS	C146- R4NG- 1dS	C146- R4NG- 3dS	C22-R4NG- 5dS	SO -R4NG- 10dS
	SO -R4NG- 0dS	SO -R4NG- 1dS	C22-R4NG- 3dS	SO -R4NG- 5dS	C146- R 4NG- 10dS
Rep 4	C146- R4NG- 0dS	C22-R4NG- 1dS	SO -R4NG- 3dS	C146- R4NG- 5dS	C22-R 4NG- 10dS
	C22-R5NG-0dS	C146-R5NG-1dS	SO- R5NG-3dS	C22-R5NG- 5dS	SO -R5NG- 10dS
	C146-R5NG-0dS	C22-R5NG-1dS	C22-R5NG-3dS	C146- R5NG- 5dS	C146- R 5NG- 10dS
Rep 5	SO-R5NG-0dS	SO-R5NG-1dS	C146-R5NG-3dS	SO -R5NG- 5dS	C22-R 5NG- 10dS

SO=Sour Orange, R= Replication, NG=Non-grafted

The trees are part of a continuing 6 month study, and the preliminary data will be presented in this report. Salt water solutions are applied bi-weekly at a volume determined by transpiration rate and soil moisture. The soil electrical conductivity (EC) is measured monthly and the soil will be periodically flushed with reverse osmosis water when soil EC is above the treatment levels.

Physiological effects are assessed on an incremental basis. The data presented in this report is incomplete, but salinity effects have been noted and will be discussed later. The evaluations presented in this report are for the following measurements.

- Height (monthly)
- Trunk diameter (pot level on non-grafted and 1.25 in above and below graft and at graft on grafted trees) (monthly)
- Stomatal conductance (monthly)
- Chlorophyll content (SPAD) (monthly)
- Bud growth (as needed)
- Track microclimate conditions in the greenhouse (temperature, humidity, air vapor pressure deficit) (continuously through datalogger)
- Visual observation of tree health and ranking (at 3 stages)
- Chlorophyll fluorescence (monthly)
- Electrolyte leakage (every 2-3 months)

- Leaf relative water content (monthly)

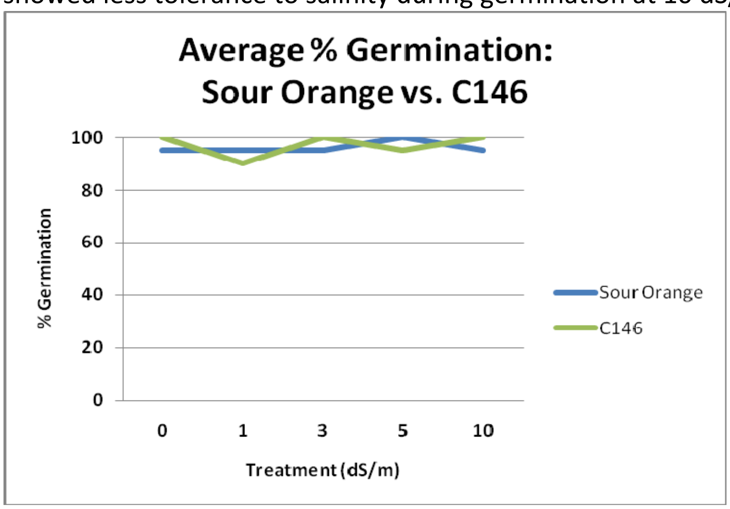
At the end of the project the trees will be harvested and follow up data on root area, length and plant dry weight will be assessed.

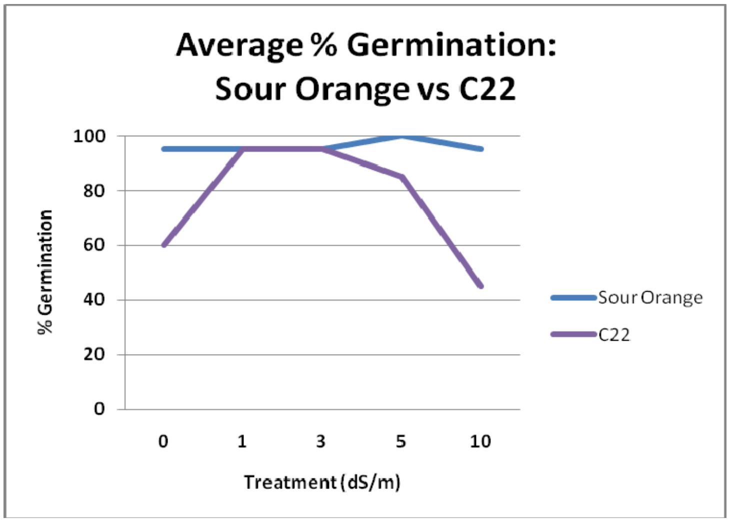
Grafted and non-grafted trees are compared on their visual, physiological and chemical parameters when subjected to salinity treatments. Rootstock varieties were also evaluated on their performance during the course of the treatment to assess which rootstock had better tolerance and overall tree health.

Principal Findings

As this study is still ongoing, findings at this time cannot be implied on a definite basis. However, preliminary findings will be discussed.

Germination
A preliminary study on the salinity tolerance of certain rootstock seeds was used to evaluate which rootstocks should be used for this study. According to this data, Sour Orange and C146 rootstocks tolerated salinity levels up to 10 dS/m without negative impacts on germination. C22 rootstock seeds showed less tolerance to salinity during germination at 10 dS/m levels as shown below.



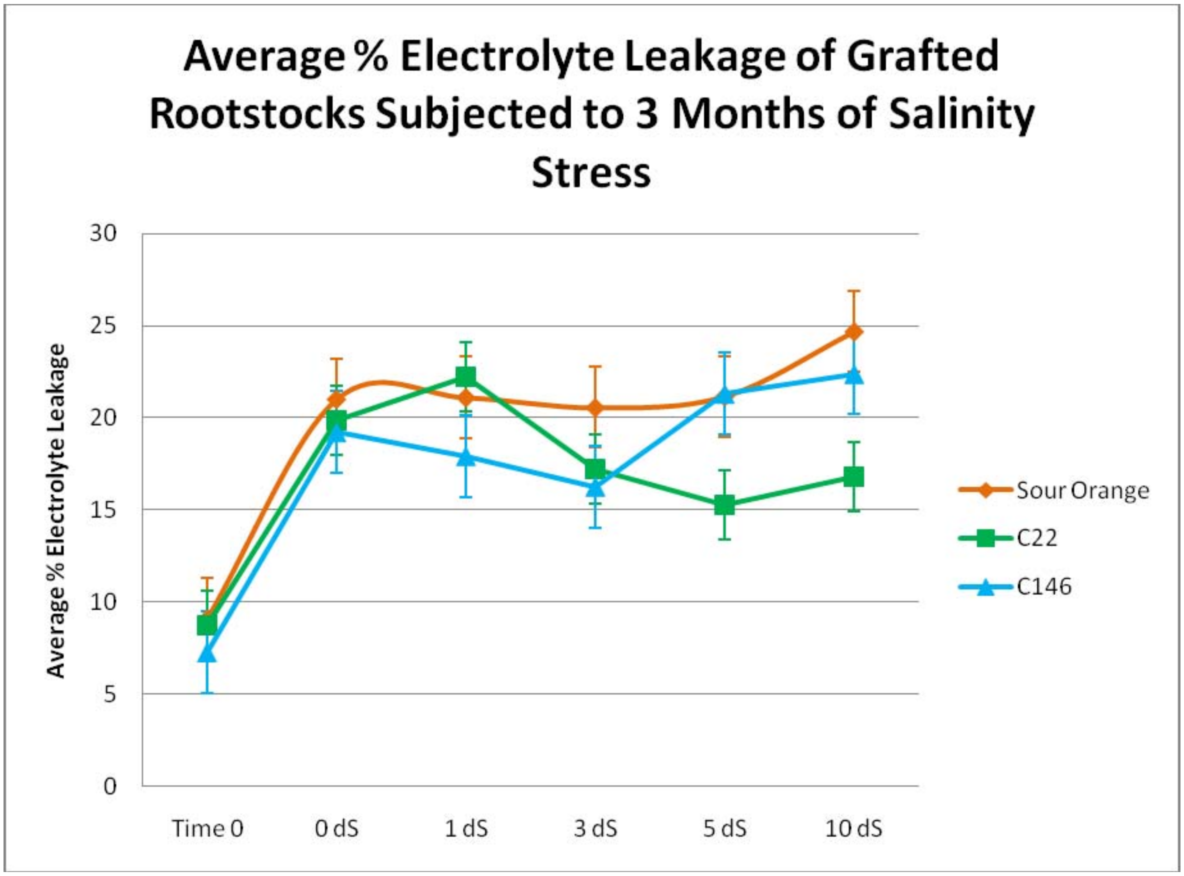


This data implies that germination of C22 seeds will be inhibited when exposed to saline conditions during initiation. From this, we decided to see how grafted and non-grafted plants of these varieties would behave when subjected to salinity in a greenhouse level study once the plants were established.

Electrolyte leakage in established plants

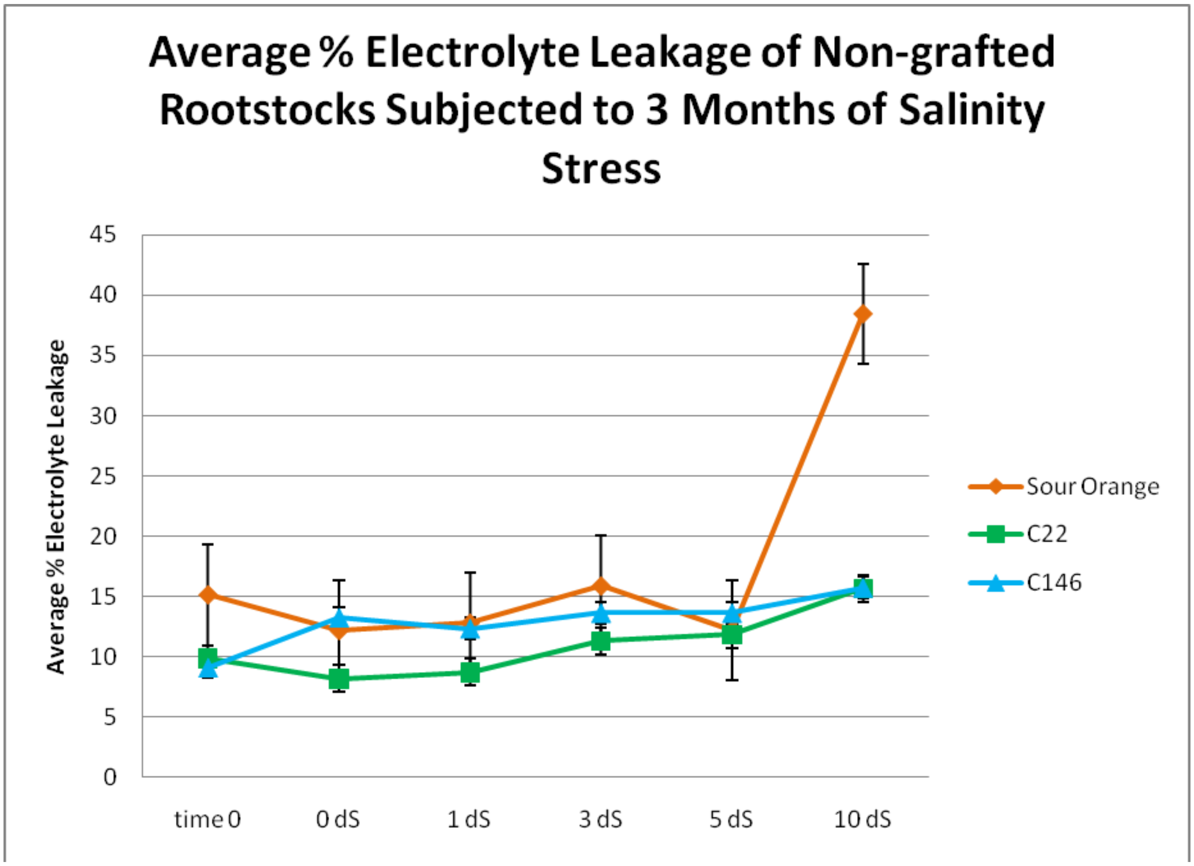
Electrolyte leakage gives a measure of the stability of cell membranes within plant tissues. Stressors cause electrolytes to leak into adjacent tissues of the plant.

Grafted plants show some differences between treatments and rootstocks with Sour Orange having more electrolyte leakage from cells, followed by C146 and C22.



Plants at Time 0 were not subjected to any stressors such as heat or salinity, the measurements were taken at the time the experiment started. These results also indicate that temperatures within the greenhouse may cause stress within the plants as well.

Non-grafted plant data showed some similarities among plants and treatments with the only real negative impact occuring to Sour Orange rootstock at 10 dS/m.



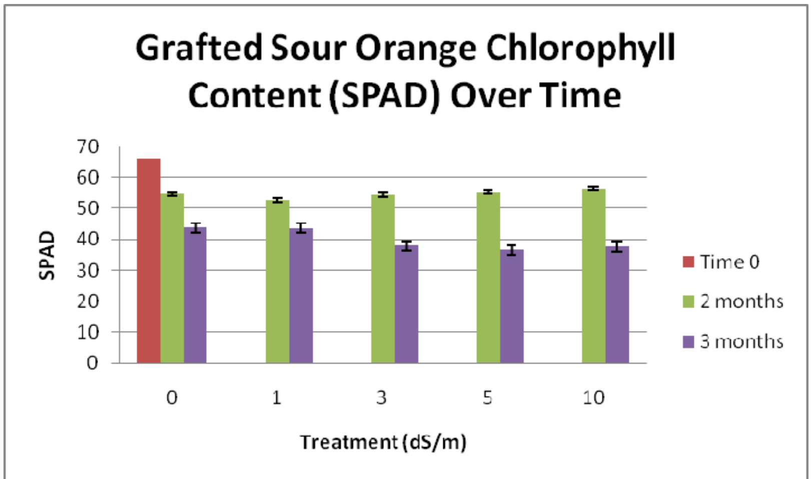
This would indicate that non grafted C22 and C146 rootstocks may be less affected by higher salinity levels while Sour Orange is negatively impacted only at the 10 dS/m level.

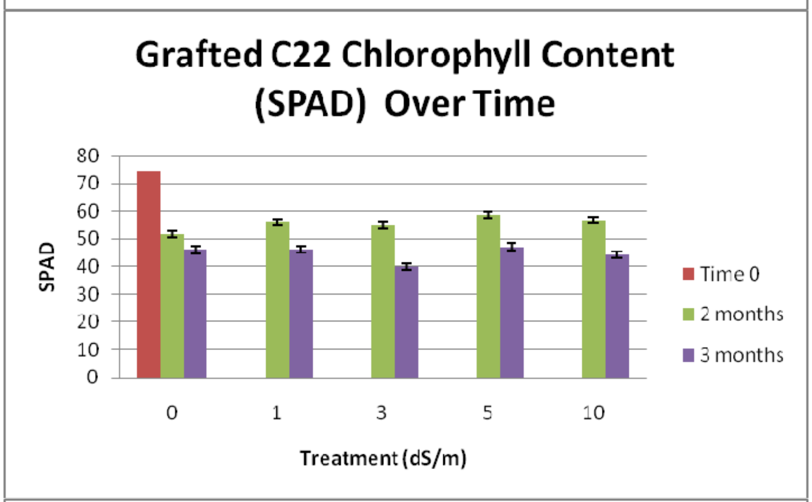
Further research must be conducted to see if new and continued growth is impacted similarly by salinity stress.

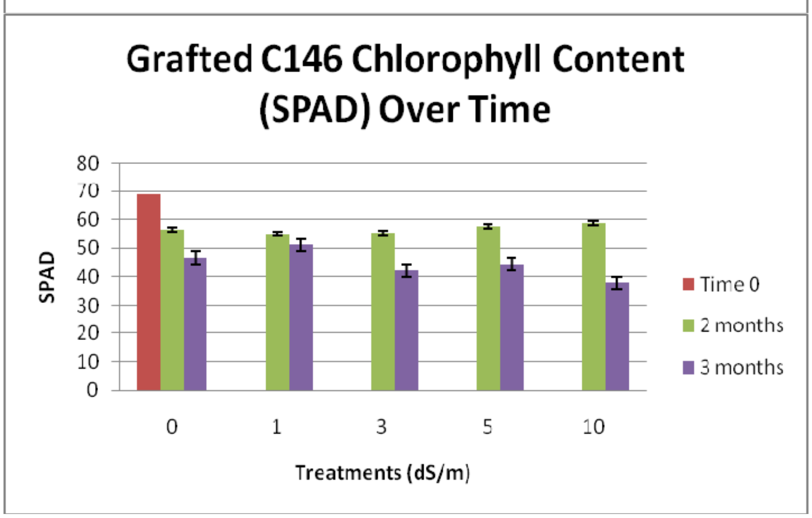
Chlorophyll content in established trees over time

Chlorophyll content measurement by SPAD is an indicator of plant health. As plant health declines, chlorophyll content decreases, usually causing yellowing and necrosis.

Chlorophyll content in grafted trees was measured over 3 months to show the health status of the plant as the study progressed. The following graphs illustrate the chlorophyll decline over time.

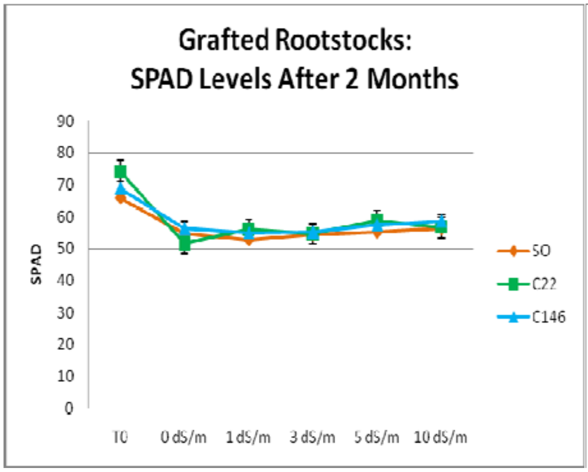


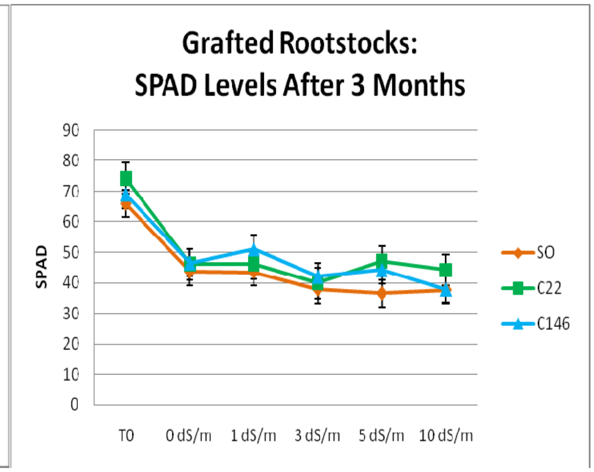




These graphs show that that there seems to be a decline in chlorophyll content in each rootstock variety over time with increased salinity treatments. It also shows that there is a slight decrease in chlorophyll content for all rootstocks over time with no salt treatment.

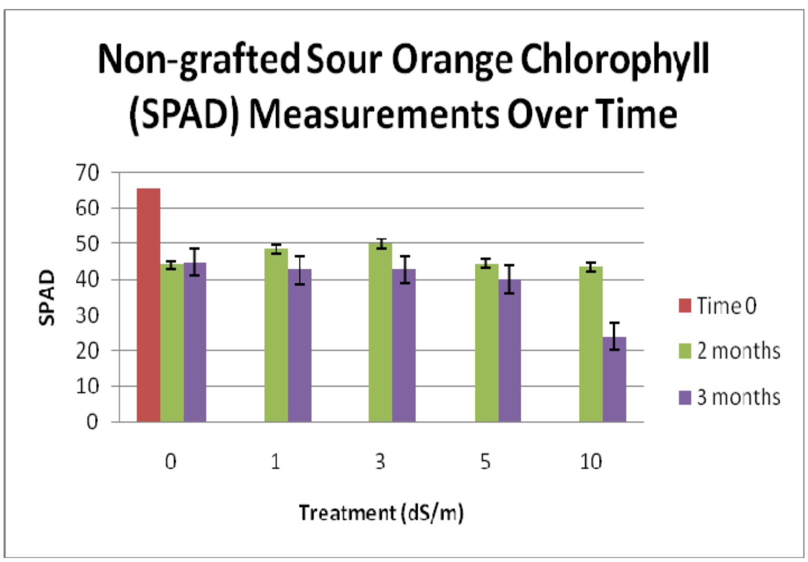
However, when the rootstock varieties are compared to one another, they show a similar trend.

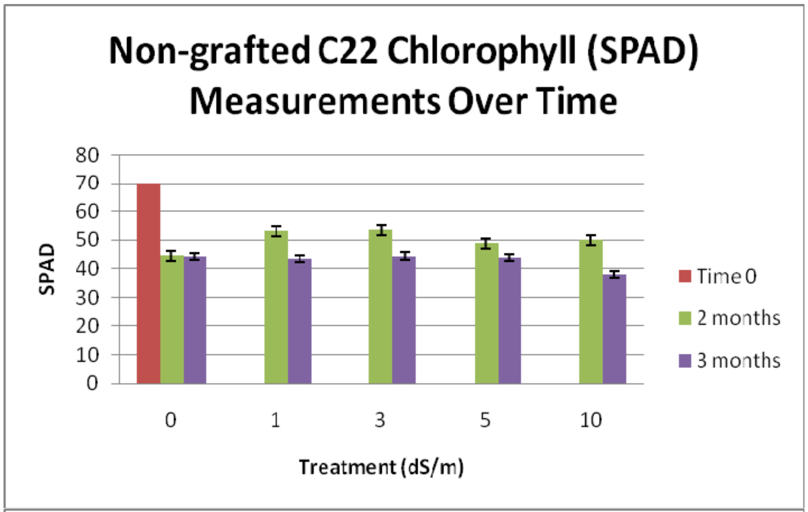


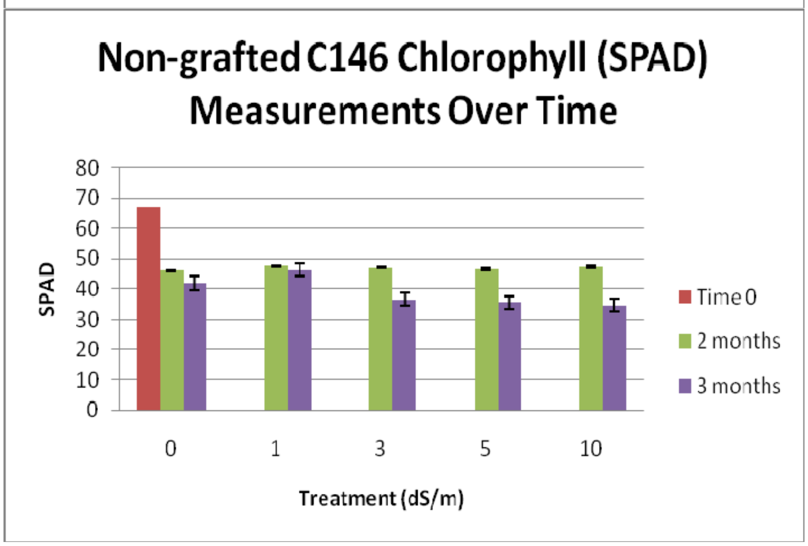


After three months, the chlorophyll content of each grafted roostock variety and each treatment look very similar. However, the total chlorophyll content has declined in each variety over three months. This may change by the culmination of the study. This may indicate that short term salinity applications may have little effect on the chlorophyll content of all of the rootstock varieties shown here.

Chlorophyll content in non-grafted trees was measured over 3 months to show the health status of the plant as the study progressed. The following graphs illustrate the chlorophyll decline over time.

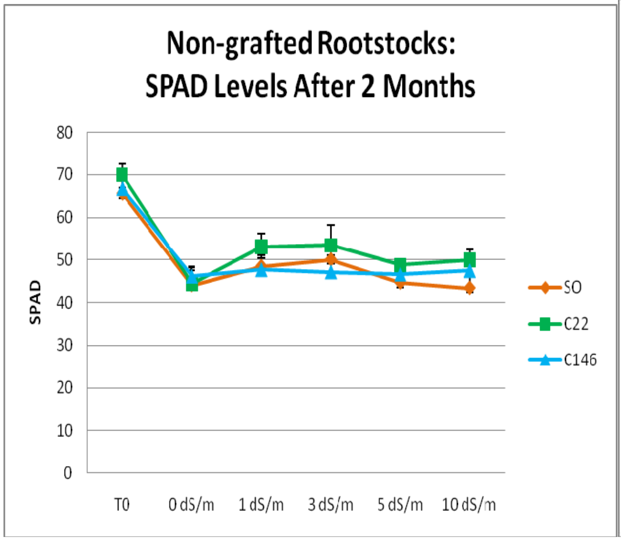


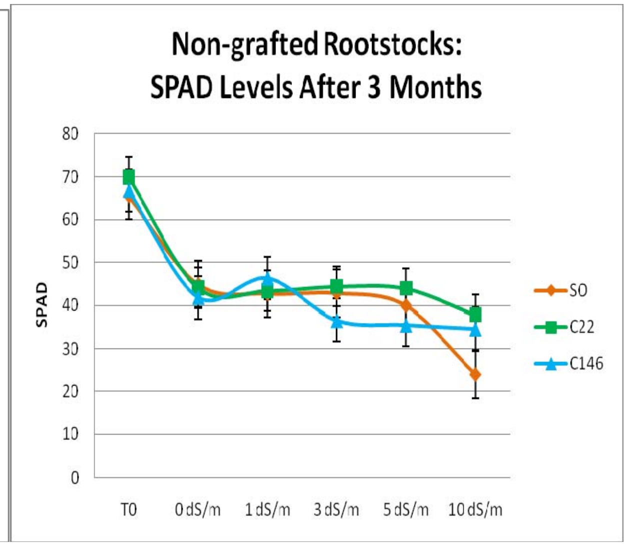




These graphs show that non-grafted Sour Orange rootstocks had the most dramatic decrease in chlorophyll content over time for the 10 dS/m treatment. C22 and C146 rootstocks showed some decline over time and per treatment.

When comparing each rootstock variety, the difference is more clearly illustrated.





After two months of treatment each roostock has a similar chlorophyll content, regardless of treatment. After three months of treatment chlorophyll content decrease is most dramatic in the Sour Orange variety at the 10 dS/m treatment. This indicates that the Sour Orange rootstock may be less suited to highly saline conditions when compared to the C22 and C146 varieties. It also shows that at salinity levels of 3 and 5 dS/m the C22 and Sour Orange varieties may be less affected by salinity stress than the C146 variety. This also may imply that short term salinity application may have little effect on chlorophyll content of each variety of rootstock.

This research will continue to study the impact of salinity on chlorophyll content for rootstocks for a total of 6 months.

These data are not complete and more information will be added by the culmination of the study. Cell membrane stability shows more consistency in non-grafted plants than grafted plants. Grafted plants showed less cell membrane stability (more electrolyte leakage) over the treatment time regardless of salinity application. However this could be due to a variety of reasons, more research must be done to clarify the data. The only non-grafted rootstock that showed less tolerance to high levels of salinity was the Sour Orange variety. From these data we find that by the 3 month stage of the experiment chlorophyll content on non-grafted and grafted rootstocks has been reduced by close to 50% in some treatments. The chlorophyll content did not vary much by treatment with the exception of the Sour Orange rootstock treated with 10 dS/m saline solution. More data must be collected to see if there is a significant difference in chlorophyll content between treatment and rootstock.

Significance

The citrus industry in Texas and throughout the US is changing. The influx of new diseases and insect pests along with increased water demands in a rapidly rising population lead to many new pressures on citrus producers. The future of citrus lies in developing virus free rootstocks, disease resistant varieties, and abiotic stress tolerance.

This research will give data on salinity tolerance of disease resistant rootstock varieties. Preliminary results show that germination of C22 seeds may be inhibited by higher salinity levels in a germination medium, while C146 and Sour Orange are less affected. However, once established, C22 rootstock varieties seem to be more tolerant of highly saline conditions when compared to the widely used Sour Orange rootstock variety. The C22 rootstock variety has been shown to produce superior yield and disease tolerance in a study done by Louzada et al. 2008. If it is found to have a high salinity tolerance it may be a better option when faced with low quality irrigation water or in areas with saline soil. This would expand the areas and conditions in which citrus can be grown in Texas and possibly globally.

References Cited (if needed)

Chowdhury, AH and RE Mace. Geochemical evolution of the groundwater in the gulf coast aquifer of south texas. Texas Water Development Board Report. http://www.igeograf.unam.mx/aih/pdf/T2/T2-19.pdf Last retrieved: December 17, 2009.

Louzada, ES; HS del Rio; M Setamou; JW Watson and DM Swietlik (2008) Evaluation of citrus rootstocks for the high pH, calcareous soils of South Texas. Euphytica 164, 13-18.

Sauls JW (2008) The Texas Citrus Industry. *Agri*Life Extension Publication. http://aggie-horticulture.tamu.edu/citrus/l2286.htm Last retrieved: December 17, 2009.

Sauls JW (2008) Rootstock and Scion Varieties. *Agri*Life Extension Publication. http://aggie-horticulture.tamu.edu/Citrus/cultivars/L2304.htm Last retrieved: December 17, 2009.

PUBLICATION

List all reports, dissertations, publications, etc., published during the reporting period as a result of the project supported by the USGS grant and required matching funds, including base grants. Please follow the following provides guidelines on how to list and format the publications based on publication type.

Simpson, C.R.; S.D. Nelson; S. Cornell; G. Schuster; and M. Setamou. 2011. Evaluation of Salinity on Citrus and Watermelon Rootstock Seed Germination. Oral Presentation. Southern Region of American Society of Horticultural Science meetings. February 5-8, 2011

NOTABLE AWARDS AND ACHIEVEMENTS

Provide a brief description of any especially notable achievements and awards resulting from work supported by section 104 and required matching funds and by supplemental grants during the reporting period.

Simpson, C.R.; S.D. Nelson; S. Cornell; G. Schuster; and M. Setamou. Evaluation of Salinity on Citrus and Watermelon Rootstock Seed Germination. Oral Presentation. Southern Region of American Society of Horticultural Science meetings. February 5-8, 2011 *2nd place Oral PhD competition.*

Received the Gerald O. Mott Meritorious Graduate Student Award in Crop Science, 2011. Awarded May 2011.

Received the 2011Outstanding PhD Student Award for the Department of Agriculture, Agribusiness and Environmental Sciences at Texas A&M University-Kingsville. Awarded April 2011.