2015 REPORT FOR THE CALIFORNIA PISTACHIO RESEARCH BOARD
Sodium Distribution and Physiology in Pistachio

Jessie Godfrey, PhD Candidate
Department of Plant Sciences
University of California, Davis
jmgodfrey@ucdavis.edu
(510) 847-7664

Collaborators
Maciej Zwieniecki*, Steve Grattan**, Louise Ferguson*
**Department of Plant Sciences*
***Department of Land Air and Water Resources*
University of California, Davis

Summary: Of the three rootstocks (PGII, PGI, and UCB1) we observed in this study, UCB1 excludes the most sodium, potentially by way of preferentially selecting for potassium along a shared uptake pathway. PGII and UCB1 exclude chloride equally and more effectively than PGI. All three rootstocks extract much of the sodium that enters transpiration streams, sequestering it into root and stem living parenchyma tissues observed as a decreasing concentration of sodium in xylem sap. The same extraction strategy does not to apply to chloride, as chloride concentration is sap remains equal up the stem. Of the three rootstocks that we used in this experiment, PGII performs best under the conditions we imposed based on its potential for salt exclusion as well as its maintenance of growth.

Introduction: Pistachio is a high value crop with relatively high salt tolerance. Thus, pistachio growers are often able to maintain economically sound production despite orchard expansion onto land with marginally saline soils or land dependent on marginally saline irrigation water. The physiological mechanisms that provide pistachio tolerance to salts remain largely unexplored. In an agricultural region where surface water availability and groundwater salinity are highly variable, improving our physiological understanding of this crop provides opportunities to meet the environmental challenges that salinity imposes with fresh perspective. The objectives of our research are to generate specific knowledge about the mechanisms of salinity tolerance common to all pistachios and to determine if variations in these mechanisms can explain salinity tolerance differences among rootstocks, scions, and their combinations. Our overall goal is to strengthen the industry by identifying potential breeding strategies and developing management tools that will help to sustain yields under saline conditions. This year's report is an update on our work in 2015 emphasizing findings about the relative performance of PGI, PGII, and UCB1 when treated with sodium chloride.

Methods: The data here presented were generated by an experiment conducted during the summer of 2014 using nine rootstock-scion combinations: self-budded UCB1 seedlings, PGI seedlings, and PGII clones, unbudded UCB1 seedlings, PGI seedlings, and PGII clones, and Kerman-budded UCB1 seedlings, PGI seedlings, and PGII clones. Budding was completed in the fall of 2013. If a bud did not take in the fall the rootstock was rebudded in early April 2014. Trees were provided with twice weekly irrigation throughout the winter, then daily irrigation with nutrient solution from early February until the start of treatment in early July 2014. All rootstocks were cut to between 40 and 50cm (just above the scion) once the scion had taken. The experiment was conducted in pots filled with coarse sand. Applied salinity treatments were 0, 50, and 100 mM NaCl. 25 and 50mM CaCl were added to the 50 and 100mM NaCl treatments, respectively, to avoid severe calcium deficiency symptoms. Irrigations of 3 gallons per pot were applied daily at 1600 h to maintain drainage ECs below 1dS/m in our 0mM treatment, between 6 and 7 dS/m in our 50mM treatment, and between 12 and 14 dS/m in our 100mM treatment. There were in total nine rootstock/scion combinations, three levels of salt treatments and five replications for a total of 135 trees.

We used three different growth metrics to follow performance of the 9 rootstock-scion combinations—rootstock diameter, scion diameter, and extension growth. Rootstock and scion diameter were measured at marked locations on the trunk every two weeks. For the rootstock the marked location was approximately 20cm above the root crown and for the scion the marked location was approximately 5 cm above the graft union. To establish extension growth, we tagged all branches on the most apical node present at the start of treatment then measured branch length beyond the tag every two weeks. The values used to calculate the presented averages are the sums of all branch lengths for a particular tree. Physiological measurements including stomatal conductance and water potential were conducted every two weeks (not presented—under analysis).

At the conclusion of the experiment multiple tissue samples were collected (including roots, stem and branches, leaves – 11 sampling points) and xylem sap (four points). Concentrations of sodium, potassium, magnesium, calcium, and chloride were measured in xylem sap as well as in all tissues. Xylem sap was extracted from 20cm long segments by removing bark, cutting segments into approximately 1cm long pieces to break xylem vessels, and centrifuging. Xylem sap was then pipetted from centrifuge tubes, diluted with 3% nitric acid and analyzed using inductively coupled mass spectrometry (ICPMS). Tissues were first digested using a combination of nitric acid and hydrogen peroxide, then diluted and also analyzed using ICPMS. We have already analyzed more than a thousand tissue samples (wood, leaf, and root), but several hundred remain to be analyzed. Xylem sap samples were all analyzed and these data appear in this year's report.

Results: Xylem sap samples extracted from approximately 20cm stem sections beginning just above the root crown confirmed that UCB1 rootstocks exclude significantly more sodium from the transpiration stream than PGI or PGII. UCB1 appears to achieve sodium exclusion by way of selecting for potassium instead. This makes sense as they are comparably sized and charged cations that may share the same uptake pathway (although it is worthwhile to mention that while some membrane transporters do not distinguish between ions, there are also specific sodium and potassium transporters and rootstocks might differ in respect to their presence or activity; ongoing efforts of the research board to quantify the genome of the species may help future efforts to understand these behaviors at the expression level). Note that potassium levels are maintained at all treatment levels in UCB1 whereas it declines from control levels for both PGII and PGI. With regard to chloride, PGII and UCB1 appear to share some moderate exclusion mechanism (Figure 1).

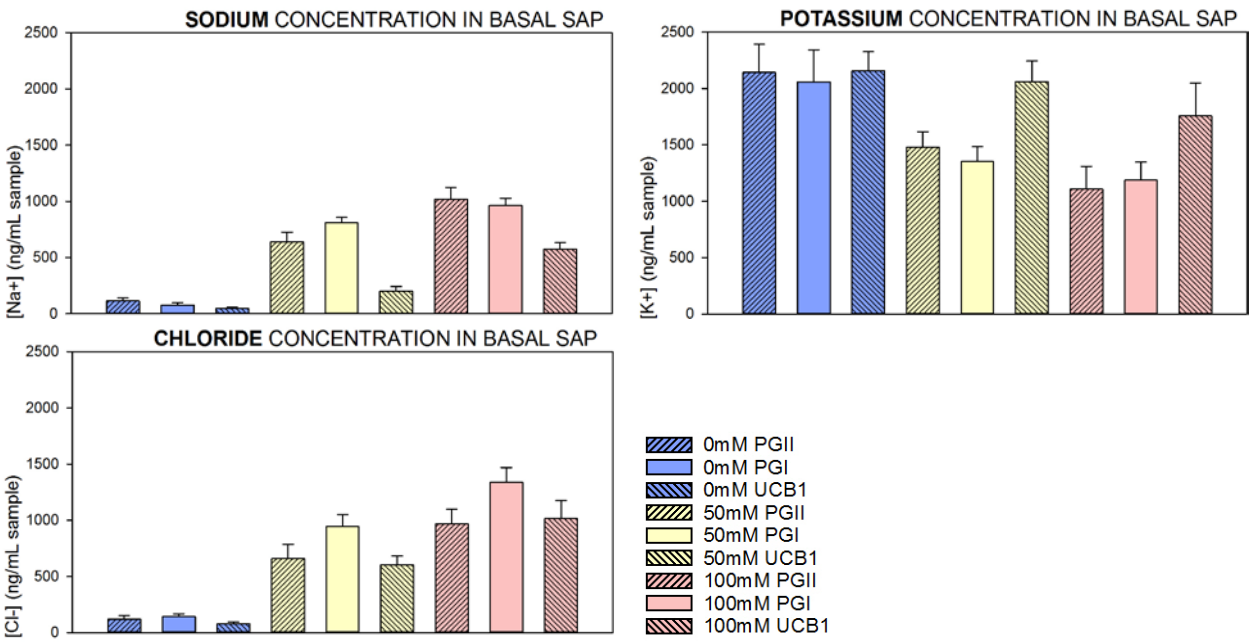

Figure1. Concentrations of sodium, potassium, and chloride separated in color by low (0mM, blue), mid (50mM, yellow), and high (100mM, red) sodium chloride treatments. Shading slanting downwards to the left indicates PGII rootstocks, no shading indicates PGI rootstocks, and shading slanting downwards to the right indicates UCB1 rootstocks. Sap concentrations are presented in nanograms per milliliter of sap (ng/mL sample). Error bars represent standard error.

This year's sap data also built on last year's observation that sodium not excluded by pistachios is extracted along the transpiration stream for storage in living stem parenchyma cells. To better understand the physiological response of pistachio trees (rather than of a particular rootstock) we pooled xylem sap information from all trees used in the study and analyzed for chloride, sodium, and potassium. Our findings show that while sodium in sap is effectively diluted on its way to leaves (suggesting sequestration along the path), chloride does not show any decline in concentrations (Figure 2). Interestingly, potassium appears to be lower in rootstocks but maintains the level of the no salt control above the graft union. This suggests that the loss of sodium from the transpiration stream might be related to sodium sequestration and release of potassium from living cells. Once we complete tissue analysis, we will be able to complement sap analysis and resolve stem storage capacity.

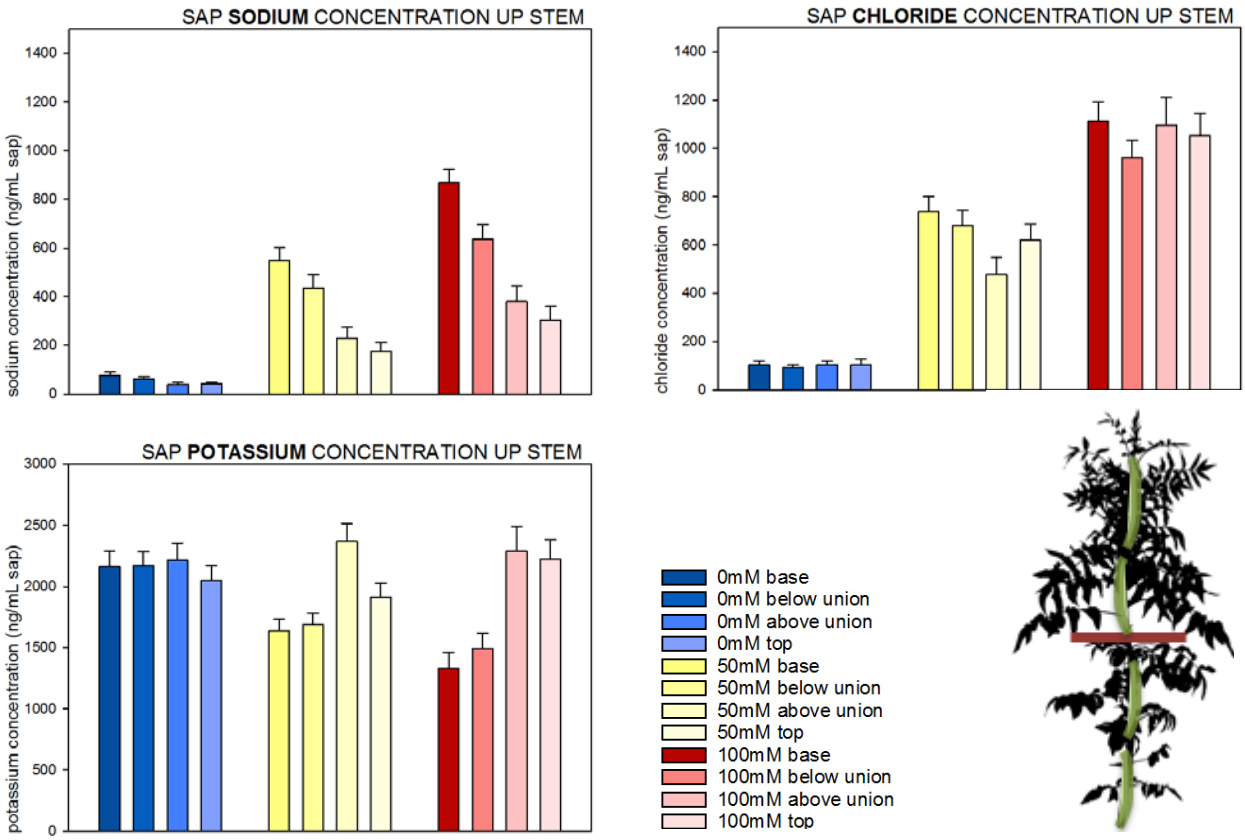

Figure2. Four sap samples in total were taken for each tree, two below the graft union and two above. One sample was extracted from a stem section extending 20 cm up from just above the root crown (base), one from a stem section extending 20 cm down from just below the graft union (below union), one sample from a stem section extending 20 cm up from just above the graft union (above union), and one sample from a stem section extending 20 cm up from 2-5cm higher along the scion from the above union section (top). All rootstock-scion combinations are pooled together within a particular salt treatment. Salt treatments are indicated by blues (0mM), yellows (50mM), and reds (100mM). Error bars represent standard error.

The last set of observations that we will discuss in this year's report relate to growth performance. The presented data narrow in on Kerman-budded rootstocks to make comparisons most relevant to growers (Figure 3). By both metrics of scion performance—diameter and extension growth—the 100mM treatment impeded growth equally regardless of rootstock. Under the 50mM treatment, PGII appears to be the best performer of the three rootstocks we used in this study by the metric of scion diameter, followed by UCB1 and then PGI. By the metric of extension growth, PGII and UCB1 perform comparably, followed by PGI.

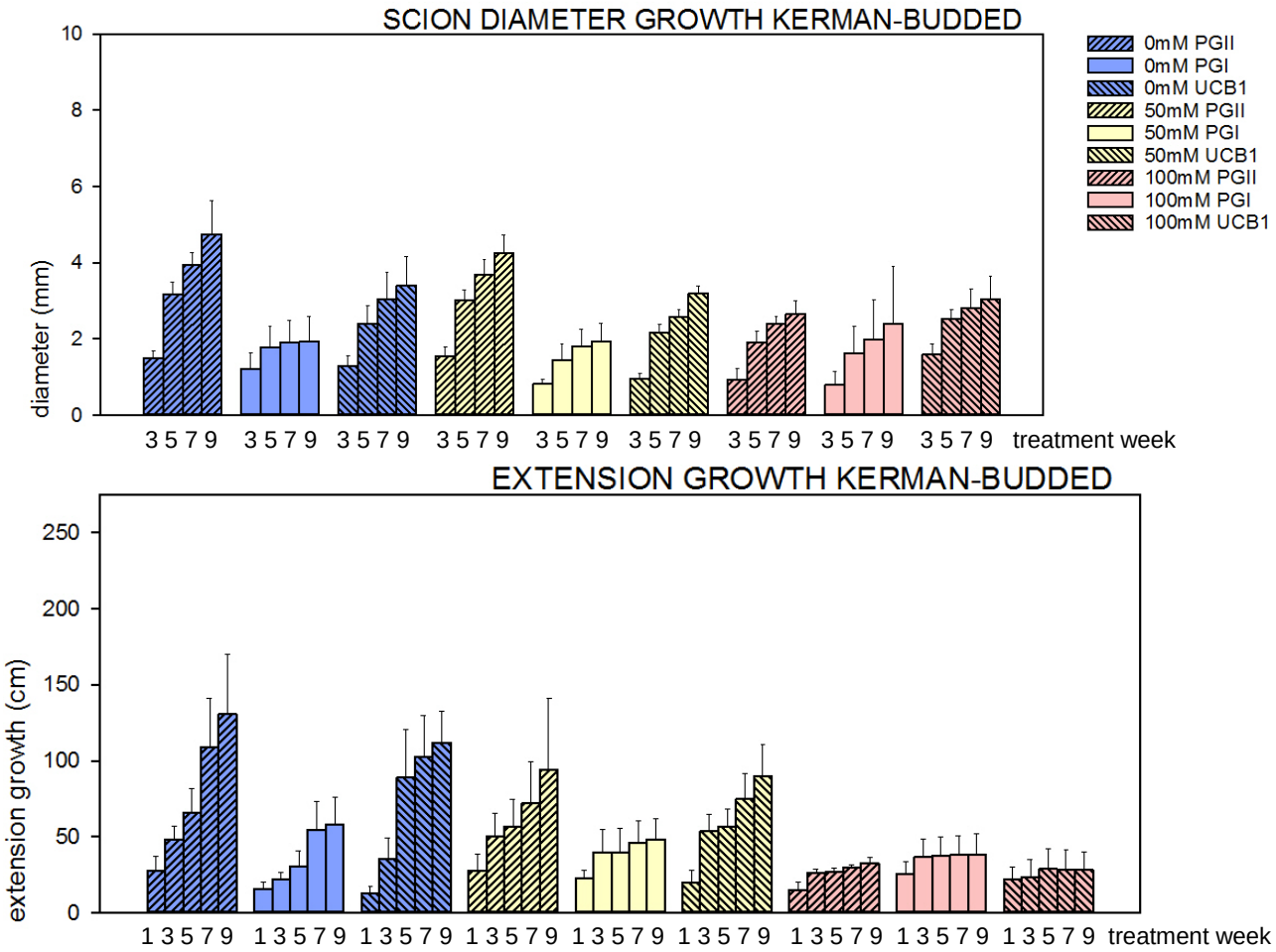

Figure3. Scion diameter (above) and extension growth (below) of Kerman-budded rootstocks, separated in color by low (0mM, blue), mid (50mM, yellow), and high (100mM, red) sodium chloride treatments. Shading slanting downwards to the left indicates PGII rootstocks, no shading indicates PGI rootstocks, and shading slanting downwards to the right indicates UCB1 rootstocks. For scion diameter, values in mm along the y-axis present the difference between a second measurement and the measurement at week 1; treatment week along the x-axis indicates the treatment week of the second measurement. For extension growth, values in cm along the y-axis indicate the actual branch length measured beyond a tag placed at the start of treatment; treatment week along the x-axis indicates the week of the measurement.

Conclusions and Future Directions: Of the three commercially available rootstocks that we used in this experiment—PGII, PGI, and UCB1—UCB1 appears to have two clear mechanistic advantages when it comes to salinity tolerance. The first advantage is its selectivity for potassium over sodium at the site of uptake, regardless of external sodium conditions. As potassium and sodium are similarly sized and charged cations that have been observed to share an uptake pathway, and as potassium is a noted essential macronutrient, a high potassium: sodium ratio is often used as a measure of salt tolerance. The second advantage, which UCB1 shares with PGII, is moderate exclusion of chloride. The fact that PGII outperforms UCB1 in terms of growth despite sharing only one of these apparent advantages suggests that the other, potassium selectivity/ sodium exclusion, may in fact be of little benefit. These findings have implications for rootstock choice as well as future breeding programs.

Although it requires submission of our samples to a separate laboratory for analysis we would like to know whether or not reductions in chloride uptake are correlated with increased nitrate uptake. Chloride and nitrate are both negatively charged and, like potassium and sodium, may share pathways. We hope to present this data in our final report as well as a complete tissue analysis to complement our sap analysis and the findings from this year's study which is following carbohydrate balance under saline conditions.