

SODIUM DISTRIBUTION AND PHYSIOLOGY IN PISTACHIO

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SUMMARY: When exposed to salinity, pistachio trees exclude a large percentage of any sodium in soil water completely, and extract much of the sodium that is not excluded along their transpiration streams, sequestering it into root, stem, and lower leaf tissues. Additionally, although exclusion is most effective at high concentrations of applied sodium, further dilution through dispersal offers no protection to photosynthesizing and growing leaves at concentrations so high that they overwhelm a tree's retrieval system.

INTRODUCTION: Pistachio growers, as managers of both a salt tolerant crop and a highly valued commodity, are able to maintain economically sound production despite expansion into marginally saline soils or the use of marginally saline irrigation water. Nonetheless, the physiological mechanisms that provide pistachio tolerance to salts remain largely unexplored. In a region where surface water availability and groundwater salinity are highly variable, improving our physiological understanding of this crop provides opportunities to meet environmental challenges with fresh perspective. The objectives of our research are to generate specific knowledge about the physiological limits of particular rootstocks, scions, and their combinations, as well as general knowledge about the mechanisms of salinity tolerance common to all pistachios. 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.

Defining Pistachio Salinity (NaCl) Tolerance: Species that have evolved under saline conditions have adapted mechanisms for salt management that allow them to maintain or even improve their growth rates under conditions of highly concentrated NaCl (Flowers and Colmer 2008). Though pistachio does not exhibit extreme halophytic tolerance mechanisms like excretion through salt glands or salt bladders, the commercially prevalent 'Kerman' scion does not begin to show reductions in growth until roughly 40-80mM NaCl or 4-8 dS/m EC_w (Behboudian *et al.* 1986, Ferguson *et al.* 2002, Pichionni 1990). This indicates some capacity for accommodation that exceeds that of most glycophytic crop plants (Maas and Hoffman 1997) and with the exception of dates, all other commercially viable tree crops (Hanson *et al.* 2006).

Components of Salinity (NaCl) Tolerance: The same gene, salt over sensitive gene (SOS1), is responsible for both reversing the passive channel flow of Na⁺ from soil into root cells and actively loading Na⁺ from root xylem parenchyma cells into the xylem (Adolf *et al.* 2013). Expression of this one gene thus both limits and, if the passive rate of uptake exceeds the active rate of exclusion, guarantees whole plant exposure to sodium. The positive implication of guaranteed whole plant exposure is the allowance of an energetically efficient (Raven, 1985, Munns 2002) osmotic gradient that favors water movement from root to transpiring tissues despite low soil water potentials. The negative implication for many plants is that

photosynthesizing leaf blades at the end of the transpiration stream tend to disproportionately accumulate inorganic osmolytes (Møller and Tester 2007).

Some degree of salinity tolerance may thus be derived from any process that reduces accumulation in mature leaf tissues while still allowing for low cost osmotic adjustment. For many plants, it seems that one key process is xylem retrieval. Increasing the expression of a Na⁺-specific xylem retrieval gene (HKT gene family) in both wheat (Munns *et al.* 2012) and arabidopsis (Sunarpi *et al.* 2005, Horie *et al.* 2009) has been shown to increase Na⁺ tolerance. In barley, Wolf demonstrated that significant quantities of Na⁺ are removed along the transpiration stream resulting in decreases in xylem sap [Na⁺] from 10mM at the plant base to 2mM at the leaf (Wolf *et al.* 1991). When the salt balance is assessed in lupine, Na⁺ has also been shown to decrease along the transpiration stream (Jeschke *et al.* 1992). If a similar xylem retrieval mechanism varies from one pistachio rootstock to another, the corresponding delivery of Na⁺ to transpiring leaves may help to explain variations in tolerance conferred to scions. Rootstock helps explain Na⁺ delivery to leaves in two more salt sensitive tree crops: avocado (Mickelbart *et al.* 2007) and citrus, though citrus shows more dramatic variations in leaf Na⁺ accumulation scion to scion (Lloyd *et al.* 1990).

Starch and Salinity (and Frost): Once sodium is dispersed by xylem unloading or deposited in leaves, a species' or tissues' capacity to transport salts into cell vacuoles and there accommodate them provides a mechanism of salinity tolerance in and of itself. Vacuolar accumulation is a means of maintaining the homeostatic Na⁺ concentrations in the cytoplasm necessary for enzymatic and membrane function (Gorgham *et al.* 1990, Maathius and Amtmann 1999). The upregulation of Na⁺/H⁺ antiporters (NHX gene family) at the tonoplast has been shown to increase salt tolerance in several species including tomatoes (Zhang and Blumwald 2001), common buckwheat (Chen *et al.* 2008), and arabidopsis (Bassil *et al.* 2012). The drawback of vacuolar accumulation of inorganic osmolytes is that although they are energetically cheap themselves, they must be accompanied by an in-kind cytoplasmic accumulation of energetically expensive *organic* osmolytes (Yeo 1983, Raven 1985, Munns 2002), potentially expensive enough to significantly deplete carbohydrate reserves. Current work from our lab suggests that the common freeze thaw events characteristic of milder winters reduce the carbohydrate storage of Mediterranean species like pistachio between their transition into dormancy in the fall and leafout in the spring (Sperling *et al.* unpublished). If it is indeed over-drafted starch banks that are to blame for patchy events of poor leafout, what is being termed 'Winter Juvenile Tree Dieback' (Kallsen 2013), it may be that salt plays a crucial part in the story.

In apples, the relationship between drought-generated declining midday stem water potentials and increasing sorbitol: starch ratios in leaves (Naschitz *et al.* 2010) very clearly demonstrates that starch is being degraded so that solutes may be formed. The same paper also documents that starch concentration in branches is *greater* with a light crop because carbohydrates are not being mobilized for fruit production. A recent paper on drought stress and carbohydrate dynamics in forest tree species makes a related observation—drought stress consumes starch for osmotic adjustment but also conserves starch, comparatively, due to reductions in growth (Klein *et al.* 2014). The result is a balanced account.

It is reasonable to assume that, because xylem tension can be imposed by salinity stress as well as by drought stress, the two stresses may have similar consequences for carbohydrate storage. In both cases living plant cells are battling the soil for water and in both cases accumulating osmolytes helps the cells win (Wyn Jones and Storey 1978, Munns 2002). It is also reasonable to hypothesize that the most interesting moment in this carbohydrate data is the tipping point, the point at which investment in continued growth alongside osmotic adjustment limits long term viability or productivity more than investment in osmotic adjustment alone. This has particular relevance when carbohydrate storage is further tasked with responding to more frequent night freeze-day thaw events, as may be the case with ‘Winter Juvenile Tree Dieback’ in pistachios.

PRELIMINARY RESULTS: We found that xylem sap extracted from UCB1 stem segments cut just above the root crown exhibited sodium concentrations up to 85% lower than those of soil water (Figure 1). Exclusion averages were 63, 72, and 86 percent for the 10, 50, and 150mM treatments, respectively. These exclusion percentages corresponded with average xylem sap concentrations of 3.7, 13.83, and 20.73 mM for which standard errors were 1.17, 1.54, and 0.99 mM. Note that because of our sampling location at stem bases the term exclusion refers to any combination of active efflux and storage in roots.

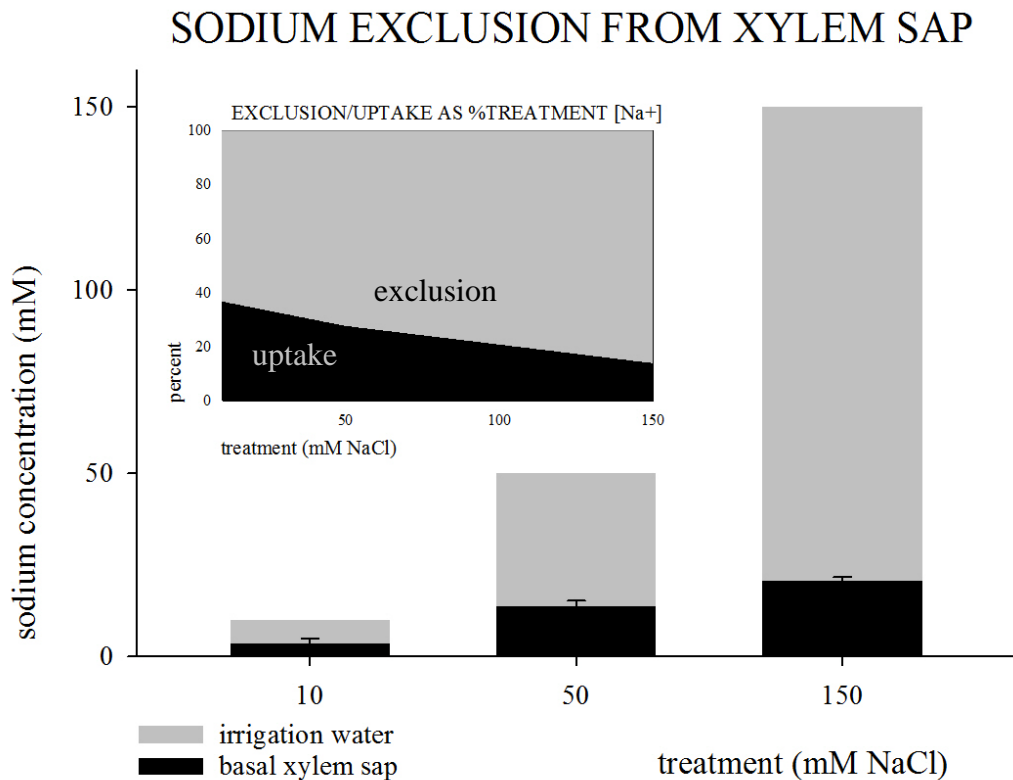


Figure 1 Sodium exclusion expressed as the difference between concentration applied in irrigation water and concentration in stem xylem sap. The smaller figure presents the same data as percent uptake v. percent exclusion.

Preliminary findings in the UCB1 rootstock also indicate that Na⁺ not excluded is retrieved along the transpiration stream and deposited in adjacent tissue. The setup was a completely randomized design of 32 trees in a soil medium (UC Mix C) irrigated to drainage at 1600h each day with one of four NaCl solutions: 0mM, 10mM, 50mM, and 150 mM (roughly 0, 1, 5, and 15

dS/m). We selected these concentrations to approximate the conditions leading to 100% (0 mM and 10mM), 90% (50mM), and 50% relative productivity observed in the 2002 Ferguson study. The 10mM treatment was included to confirm that there are no beneficial effects of very low NaCl application. 35 days after the start of treatments, measurements of basal and apical vacuum-extracted (Secchi and Zwieniecki 2012) xylem sap $[Na^+]$ were significantly different for the 50 and 150 mM treatments with two-tailed p-values of 0.014 and 0.001, respectively. For a visual comparison, the 1:1 line diverges up from the 95% confidence interval of the regression correlating basal and apical xylem sap at higher applied NaCl concentrations, when apical sap is the dependent variable (Figure 2).

END POINT XYLEM SAP $[Na^+]$

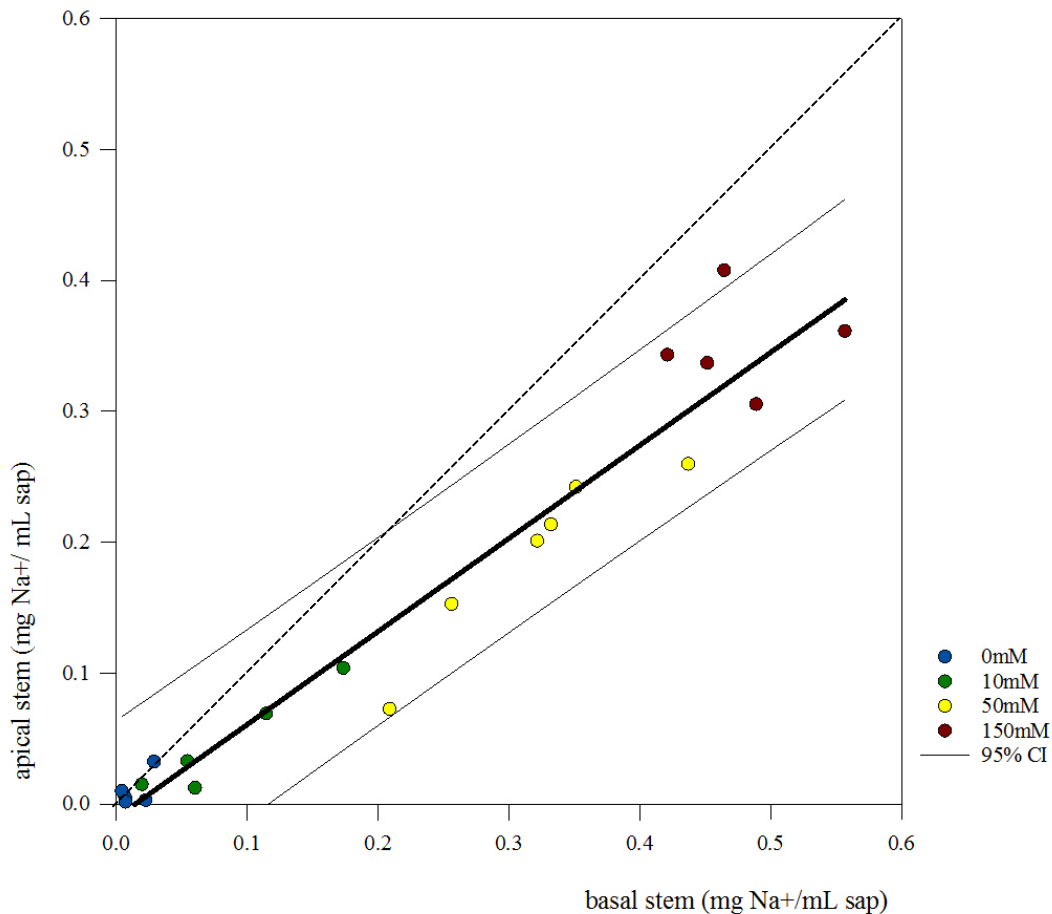


Figure 2 The bold solid line is the regression correlating basal and apical stem xylem sap $[Na^+]$ after 35 days of irrigation with 0, 10, 50, and 150 mM NaCl (approximately 0, 1, 5, and 15 dS/m). The dashed line is the 1:1 line. The softer solid lines border the 95% confidence interval.

The observation of declining xylem sap $[Na^+]$ up the stem is complemented by observations of declining tissue $[Na^+]$ along the transpiration stream in each tissue type—roots, stems, and leaves—for

all but the 150mM treatment (Figure 3). With the exception of roots in the 50mM treatment, categorically ranking location confirms significantly negative slopes up the transpiration stream for all tissue types in the 0, 10, and 50mM treatments (data not shown). The slope does not differ significantly from zero in the 150mM treatment. This lack of deviation suggests that at 150mM

NaCl, retrieval and sequestration are no longer adequately diluting delivery of Na⁺ to the apical tissues where photosynthesis and growth are most concentrated.

END POINT [Na⁺] IN ALL TISSUES

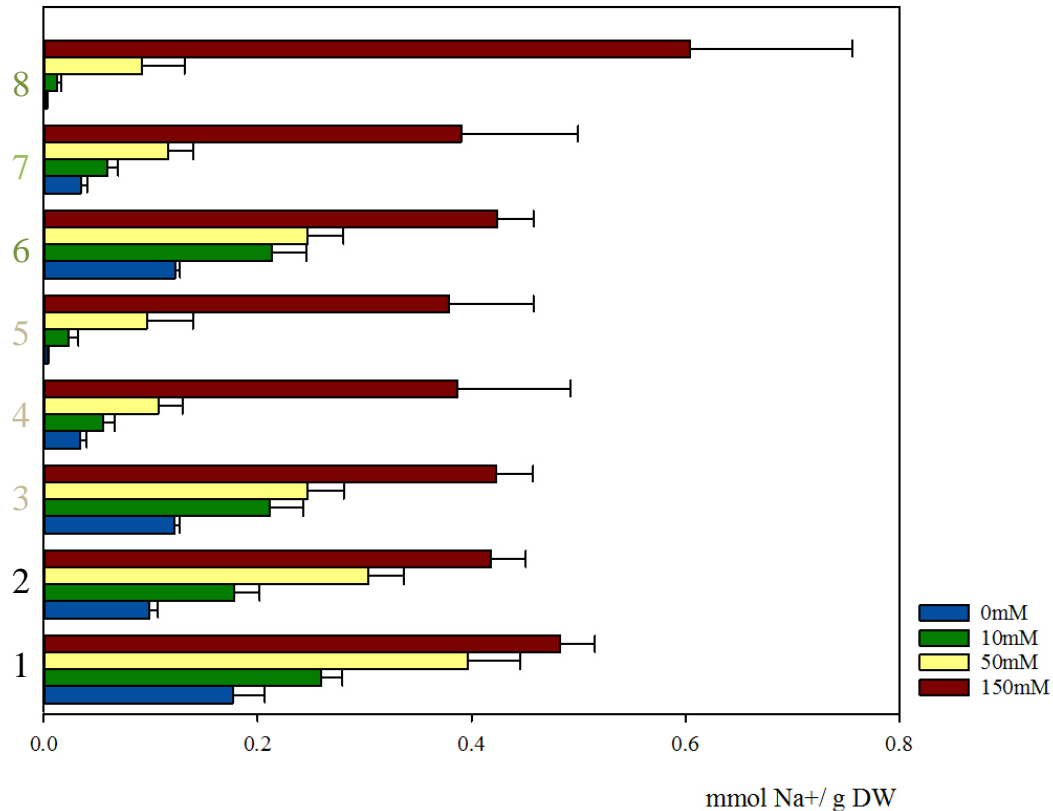


Figure 3 Tissue [Na⁺] up the stem of UCB1 rootstocks after 35 days of irrigation with 0, 10, 50, and 150 mM NaCl. Error bars reflect standard error. Along the y-axis, numbers correspond with tissues as follows: 1 = fine roots, 2 = support roots, 3 = root crown, 4 = midstem, 5 = apical meristem, 6 = old leaves, 7 = mature leaves, 8 = immature leaves. Note that in all but the 150mM treatment, tissue-type [Na⁺] declines up the transpiration stream. Root tissues are labeled on the y-axis in black, stem tissues in brown, leaf tissues in green.

CONCLUSIONS AND FUTURE DIRECTIONS: Xylem sap and tissue ion analyses offer a definitive focus for experiments comparing rootstocks. The maximum exclusion values and extraction rates for not only sodium, but also other harmful inorganic solutes like chloride, are potential screening targets for breeders. More data is forthcoming.

Measurements of sodium concentration in stem tissues collected at multiple heights also offer a potential new tool for assessing salinity's infringement upon a tree's safety margin. The retrieval systems of trees that continue to exhibit tissue concentration declines up the stem are operational and offering some degree of protection to photosynthesizing leaves. This should be true regardless of rootstock or scion. Whether or not operational systems of xylem retrieval are useful for predicting yield reductions must of course be validated in the field. However, samples collected over the summer of 2014 from a tank study we conducted here at UC Davis and in the fall of 2014 from an 11-year field trial established and managed by UC crop extension specialist Blake Sanden will offer more insight in the near future.

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