Physiology of Salinity Stress in Almond: Influence of Rootstock, Scion and Supplemental Nutrition on Tree Growth, Ion Toxicity and Water Relations

Project No.: **15-HORT20-Brown/Grattan**

Project Leader: **Patrick H Brown**
Professor
Department of Plant Sciences
UC Davis
One Shields Ave.
Davis, CA 95616-8683
530.752.0929
phbrown@ucdavis.edu

Steve Grattan
Water Relations Specialist
Land, Air and Water Resources
UC Davis
One Shields Ave.
Davis, CA 95616-8683
530.752.4618
srgrattan@ucdavis.edu

Project Cooperators and Personnel:
Umit Baris Kutman, Postdoctoral Researcher, UC Davis
Francisco Valenzuela, PhD Student, UC Davis
Saiful Muhammad, Project Scientist, UC Davis

Objectives:

1) Investigate the growth and physiological responses of important almond rootstocks and cultivars to salinity stress
2) Elucidate the physiological mechanisms conferring different levels of salinity tolerance to different rootstocks and cultivars
3) Understand the interactions between toxic salt ions and essential minerals in almond and design nutritional salinity management strategies
4) Provide the physiological rationale and monitoring strategies needed to optimize almond selection and breeding programs for salinity tolerance

Interpretive Summary:

Salinity stress is a growing concern for California almond growers. Drought and increasing dependence on low-quality ground water for irrigation aggravates this problem. While it is recognized that almonds are a very salt-sensitive crop, not much was known about the physiology of salinity stress in almond, genotypic variations in salinity tolerance and tolerance mechanisms. On grafted almond trees grown in pots under field conditions, we studied the

effects of different levels of NaCl salinity and different supplemental salts on selected rootstocks and cultivars.

The first-year data obtained from the rootstock experiment showed that there was a great degree of variation in sensitivity to salinity among the tested rootstocks. Nonpareil trees grown on Nemaguard were most severely injured by salinity treatments. Hanson 536 conferred significantly greater salt tolerance than Nemaguard. Nonpareil trees grown on Empyrean-1 and Viking were virtually unaffected by salinity for the duration of the experiment. Tolerance levels correlated very well with the leaf Na and Cl concentrations throughout the season. In the first year of the cultivar experiment, Nonpareil, Mission, Monterey and Fritz did not exhibit marked differences in terms of salinity tolerance and Cl accumulation but important differences in their tissue Na distribution patterns were detected. Nonpareil, known to be Na-tolerant, was particularly efficient in storing Na in its woody tissues whereas Fritz and Mission were unable to do this and accumulated substantially more Na in their leaves. That the within-plant-distribution of Na can have a major impact on leaf Na levels was confirmed in a study on Nonpareil-Mission trees double-grafted on Nemaguard. Mission accumulated twice as much Na as Nonpareil in the leaves. In the rootstock-cultivar interaction experiment, rootstock appeared to be the major determinant of the salinity tolerance under our conditions although cultivars also played a role.

Both growth and carbon isotope discrimination data indicated that salinity-induced water stress did not play a significant role in our experiments. The observed effects were due to specific ion toxicities. Specifically, when the sole salinizing agent was NaCl, Cl accumulated much faster than Na in plant tissues and thus acted as the primary toxic ion. Counter ions affected the leaf accumulation rates of Na and Cl and KCl was more toxic than NaCl as it caused enhanced Cl uptake while Na_2SO_4 salinity had no negative impacts in the first season.

Materials and Methods:

Experiments in 2014:

In March 2014, the experiments for the first year of the project were started by planting young grafted almond trees in 7-gal pots filled with calcined clay (Turface®) as growth medium. This material has a high water-holding capacity and a high cation exchange capacity, drains very well and does not become compacted. The pot experiments were designed as factorial 4-replicate experiments and conducted under field conditions. Throughout the experiment, the trees were irrigated with complete nutrient solution containing different amounts and types of salinizing agents, depending on the treatment. Irrigation time and frequency were adjusted as needed to meet the demand of the trees and to provide some extra water for leaching. Each time the trees were irrigated, a leaching fraction of about 25% prevented the accumulation of nutrients and salts in the pots.

1. Rootstock experiment:

Nonpareil almonds grafted on the following 4 *Prunus* rootstocks were tested for their salt tolerance in this experiment:

- Nemaguard
- Hansen536
- Empyrean-1
- Viking

The experimental trees were grown at 3 salinity levels:

- Control (EC = ~0.8 dS/m),
- Low salt (EC = ~2.8 dS/m),
- High salt (EC = ~4.8 dS/m).

The control solution contained only the complete set of mineral nutrients. Sodium chloride (NaCl) was used as the single salinizing agent for the salt treatments. For the low and high salt treatments, 20 mM and 40 mM NaCl were added to the irrigation water, respectively.

2. Cultivar experiment:

The following 4 almond cultivars grafted on the rootstock Nemaguard were tested for their performance at different salinity levels:

- Nonpareil
- Mission
- Monterey
- Fritz

As in the rootstock experiment, the trees were grown at 3 salinity levels:

- Control (EC = ~0.8 dS/m),
- Low salt (EC = ~2.8 dS/m),
- High salt (EC = ~4.8 dS/m).

While the control solution contained only the complete set of mineral nutrients, 20 mM and 40 mM NaCl were added to the irrigation water for the low and high salt treatments, respectively.

3. Rootstock-cultivar interaction experiment:

To study the rootstock-cultivar interaction in almond trees in terms of salinity responses, the cultivars Nonpareil and Fritz were combined with the rootstocks Nemaguard and Hansen536 in a factorial design:

- Nonpareil on Nemaguard
- Nonpareil on Hansen536
- Fritz on Nemaguard
- Fritz on Hansen536

The salt treatments were the same as in the first two experiments:

- Control (EC = ~0.8 dS/m),
- Low salt (EC = ~2.8 dS/m),
- High salt (EC = ~4.8 dS/m).

For the low and high salt treatments, 20 mM and 40 mM NaCl were added to the control solution containing all the essential nutrients, respectively.

4. Double-graft experiment:

For this experiment, Nemaguard rootstocks were double-grafted with two scions:

- Nonpareil
- Mission

These two cultivars were chosen based on preliminary data and orchard observations indicating a marked contrast between them in terms of salinity tolerance.

The objective of this experiment was to investigate the salt accumulation characteristics and salt tolerance levels of two cultivars growing on the same rootstock.

As in the other experiments described above, the trees were subjected to 3 salinity levels:

- Control (EC = ~0.8 dS/m),
- Low salt (EC = ~2.8 dS/m),
- High salt (EC = ~4.8 dS/m).

For the low and high salt treatments, 20 mM and 40 mM NaCl were added to the control solution containing all the essential nutrients, respectively.

5. Salt type experiment:

In order to elucidate the individual toxic effects of Na and Cl on almond trees, Nonpareil almonds grafted on Nemaguard were treated with the following 3 salts in this experiment:

- Sodium chloride (NaCl)
- Potassium chloride (KCl)
- Sodium sulfate (Na_2SO_4)

While KCl enabled us to focus on the individual effects of Cl without the interference of Na, Na_2SO_4 made it possible to study Na toxicity without the interference of Cl. The counter ions potassium (K) and sulfate (SO_4) are not expected to cause any direct toxicity problems.

The trees were grown at 3 salinity levels as in the other experiments:

- Control (No salinizing agent),
- Low salt (20 mM Na and/or Cl in any form),
- High salt (40 mM Na and/or Cl in any form).

In all these experiments, the high salt treatment was continued for 100 days and then terminated not to kill the severely stressed trees and study their response to a recovery treatment. During the recovery period, which started in the middle of August and continued until the end of the 2014 growing season, the former high-salt trees were irrigated with the control solution. Thus, all trees survived the 2014 growing season and could be treated with high salt again in 2015 to collect second-year data.

Measurements and analyses:
To monitor tree growth, all experimental trees were photographed once a month in front of a large, white background, and the images were analyzed by using the software ImageJ to estimate the canopy size. These high-resolution images were also used to document the development of leaf symptoms caused by ionic toxicities. Complementary growth data were obtained from periodic trunk diameter measurements taken 15 cm above the graft union.

Once a month during the growing season, 20 mature leaf samples per tree were collected from the older halves of non-lignified branches for mineral analysis. They were dried, ground and analyzed for the concentrations of Na, Cl and all the essential minerals. To examine the trunk accumulation of Na and Cl, trunk strip samples were taken from high-salt trees at the end of July 2014 without inflicting significant damage to the trees.

In addition, carbon isotope discrimination analysis was used to evaluate salinity-induced water stress. Due to physical (related to diffusion) and biochemical (enzymatic) reasons, plants discriminate against the heavier stable carbon isotope 13C in the atmosphere. However, when plants with C_3 metabolism close their stomata upon water stress, this discrimination is impaired. Changes in the isotopic carbon composition of plant tissues can therefore be used as a quantitative marker of water stress. Almond leaf samples collected at the beginning of June 2014 and July 2014 were analyzed for their carbon composition.

Experiments in 2015:

1. Experiments started in 2014:

   All of the five experiments, which were started in 2014, continued in the 2015 growing season. In order to study the carry-over effects of salinity on leaf mineral composition, leaf samples were collected in April 2015 before the salt treatments were restarted. The treatments were continued for 9 weeks, and then, the experiments were terminated. Tree growth and salt toxicity symptoms were monitored as described above. At the end, the most extensive tissue sampling was conducted for these experiments. The following samples were collected, dried and ground for mineral (including Na and Cl) and other analyses:

   - Mature leaves: These were also analyzed for carbon isotope discrimination.
   - Actively growing shoot tips: These were specifically used to evaluate the boron (B) status of the trees and examine if salt treatments had a significant effect on the tissue B levels. They were also analyzed for carbon isotope discrimination.
   - Rootstock trunk bark
   - Rootstock trunk wood (xylem)
   - Scion trunk bark
   - Scion trunk wood (xylem)

   In addition, another set of mature leaf samples were frozen at -80°C. They will be analyzed for compatible solutes (e.g. glycine betaine, proline) to study their accumulation in almond leaves in response to salinity and their possible contribution to salinity tolerance. Also, they will be analyzed for malondialdehyde as a measure of lipid peroxidation, which is, in turn, a measure of oxidative stress.

2. Rootstock experiment in solution culture:

The objective is to study the Na and Cl uptake and translocation traits of our rootstocks in the absence of rootstock-scion interactions and under controlled conditions. This 4-replicate experiment complemented the data obtained from the 2-year rootstock experiment on grafted almond trees. In this experiment, non-grafted rootstocks (Nemaguard, Hansen536, Empyrean-1 and Viking), which were rooted from hardwood cuttings, were grown hydroponically (in aerated static solution culture without any solid growth medium) under greenhouse conditions and then treated with different levels of NaCl (control, 30 mM, 60 mM) for 1 month. The leaves, stems and roots of all plants were harvested separately, dried, weighed and ground. They will be analyzed for mineral concentrations.

3. Split-root experiment:

Using solution culture: to study the effects of non-uniform salinity in the root zone on the water, Na, Cl, nitrate and B uptake of almond trees, this model 4-replicate split-root experiment was conducted with non-grafted rootstocks (Nemaguard, Hansen536, Empyrean-1 and Viking), which were rooted from hardwood cuttings and grown hydroponically under greenhouse conditions. Special split-root pots with two independent chambers were designed and constructed for this purpose. When they were ready for the treatments, they were divided in 3 groups: control/control, control/salt, salt/salt. For the salt treatment, 60 mM NaCl was added to the nutrient solutions. The treatments were continued for 3 weeks. During this period, the changes in 24-h water consumptions of the rootstocks were measured periodically. To study the nitrate and B uptake, the stable isotopes 15N and 10B were used. The stable N isotope was applied for an uptake period of 8 hours and the stable B isotope for an uptake period of 24 hours. The experiment was terminated 3 days after the stable isotope applications by harvesting the leaves, stems and roots of the plants separately. The samples were dried, weighed and ground. They were analyzed for total minerals, including Na and Cl, as well as for 15N and 10B.

Using substrate: to understand how non-uniform saline conditions affects the root zone of grafted trees in term of growth, salt tissue accumulation and plant water status. An experiment using Turface a substrate was conducted using Nonpareil grafted on three different rootstocks (Nemaguard, Hansen 536 and Empyrean I). For establishing the experiment, roots of the tested trees were carefully washed and pruned dividing the root system in two equal parts. Plants were placed on customized pots built for this experiment (each half of the root had 5 gal, giving a total of 10 gal per plant). Trees were growth for 2 months before starting the experiment. The treatment applied were: control/control, control/low salt, control/high salt, low salt/low salt, low salt/high salt and high salt/high salt. Low salt treatment consisted in the addition of 20 mM of NaCl and for high salts 40 mM of NaCl was added. Leaf samples were collected to track Na^+ and Cl^- accumulation, tree pictures were used for estimating plant growth and stem water potential was measured to verify plant water status. Treatments were applied for a period of 120 days. At the end of the experiment one replicate was harvested and the other 4 replicates were kept for continuing the experiment the season 2016.

4. Retranslocation and efflux experiment:

The objective of this experiment is to determine if retranslocation and root efflux of toxic ions (Na and Cl) may constitute an important tolerance and recovery mechanism for almond trees. For this purpose, young Nonpareil and Monterey trees grafted on Nemaguard were grown in pots under field conditions by using the same growth medium and the same irrigation and treatment system as in the 2-year experiments started in 2014. This was a two-stage experiment with 4 replicates. The first was the salt treatment stage while the second was the recovery stage. They were grouped in 2 harvest groups. The first group was harvested at the end of the salt treatment stage, and the second group was harvested at the end of the recovery treatment.

During the salt treatment stage, they were grown at 3 salinity levels for 2 months:

- Control (EC = ~0.8 dS/m),
- Low salt (EC = ~2.8 dS/m),
- High salt (EC = ~4.8 dS/m).

While the control solution contained only the complete set of mineral nutrients, 20 mM and 40 mM NaCl were added to the irrigation water for the low and high salt treatments, respectively.

During the 6-week recovery phase, the remaining former low-salt trees were irrigated with the control solution whereas the remaining former high-salt trees were irrigated with either the control or the low-salt solution.

For harvest, all the leaves and woody tissues (trunk and stems) above the graft union were collected separately, dried, weighed and ground and analyzed for Na and Cl concentrations. A net decrease in total Na or Cl contents (concentration x biomass) of the scions during the recovery phase will indicate the presence of significant Na or Cl retranslocation and efflux. Growth and dilution during the recovery phase were also evaluated as possible tolerance mechanisms.

5. Potassium-salinity interaction experiment:

Results from the 2014 growing season showed that KCl was more toxic than NaCl because the leaf Cl levels increased more rapidly in KCl-treated trees. As KCl is still a widely used K fertilizer in California and also other parts of the world, this finding is of critical importance. Other important aspects of K x salinity interaction, which were studied in other crops previously, include salinity-induced K deficiency and the use of K fertilizers to increase the Na tolerance. In order to elucidate the K x Cl and K x Na interactions in almond trees, Fritz almonds grafted on Nemaguard were grown in pots under field conditions by using the same growth medium and the same irrigation and treatment system as in the 2-year experiments started in 2014. In this factorial experiment with 4 replicates, there are 3 K (low: 0.3 mM; medium: 1.2 mM; high: 4.8 mM) and 3 salt (control; 40 mM NaCl; 40 mM Na in the form of Na_2SO_4) treatments. Leaf samples will be analyzed for K, Na and Cl concentrations.

Experiments in 2016:

1. Experiment 1: Salinity stress tolerance in almond: effects of rootstocks.
   To study the effect of almond rootstocks on salt tolerance, seven rootstocks were selected in consultation with well-known nurseries. One-year-old Nonpareil plants grafted on Nemaguard, Empyrean 1, Rootpac-R, Bright 106, Bright Hybrid, Corner Stone, and Krymsk 86 were planted in Turface in 7 gallon pots in open field. Each rootstock was replicated five times. After growing the plants for four months, salinity treatments have been started. Based on the result of experiment conducted in 2014 and 2015 only one salinity level is being used with a zero salt control. Salinity level of 5.0 ds/m was applied using 2 NaCl and 1 Na_2SO_4 to represent Na dominant salinity. Plants were irrigated 3 times daily with a complete nutrient solution. Salts were applied with irrigation water and a leaching fraction of at least 25% to keep the nutrient and salt levels in the pots constant.

   Leaf samples from the treated and the control plants were collected before starting the salt treatment. Pictures of the same plants were taken for growth comparison, which will continue at monthly interval. Additionally, tree trunk circumference was measured before starting treatment and will be recorded at monthly intervals, for growth comparison of the salt treated plants with untreated control. Leaf samples will be collected periodically at monthly intervals to monitor the concentration of Na and Cl in leaves and to correlate it with growth response.

2. Split root experiments:
   Using solution culture: This experiment has been started to study the effects of non-uniform salinity in the root zone on water, Na, Cl and nitrate uptake of almond trees. This experiment will allow determining if the effects of NaCl are solely a consequence of the osmotic influence or as a consequence of the specific effects of Na^+ or Cl^-. Non grafted seedling of Nemaguard were placed on special split-root pots with two independent chambers designed and constructed for this purpose. The split roots were grouped into the following 9 categories: control/control, control/nutrient+salt, nutrient/only salt, nutrient/nutrient+PEG, nutrient+PEG/nutrient+PEG, nutrient/only PEG, DI water/nutrient+salt and DI water/nutrient.

   For the salt treatment, 60 mM NaCl will be added and for the PEG treatment a concentration of 151.5 g/Kg of solution will be used. The treatments will have continued for 3 weeks. During this period, daily water consumption will be measured. To study the nitrate, Na^+ and Cl^- uptake form the solution, a nutrient depletion approach developed by Claassen and Barber will be used. Uptake will be tracked for a period of 8 hours. Plants will be harvested and separated into leaves, stems and roots. The samples will be dried, weighed and ground and will be analyzed for total N, Na^+ and Cl^-.

   Using substrate: (experiment started in 2015 and continue): To study the accumulative effect of NaCl on mineral composition of almond trees, leaf samples were collected in April 2016 before applying salt treatments. The treatments will continue for 120 days. At termination of the experiment tree growth, salt accumulation in tissue and tree water status will be accessed in the same way as the previous season. At 120 days, plants will be

harvested and divided into leaves, stem, trunk, rootstock and roots, and then nutrient concentration in the same organs will be determined.

3. Nitrate uptake experiments under solution culture
   a) Effect of salinity on nitrate uptake of Nemaguard seedlings not previously exposed to NaCl: in order to quantify the immediate effect of saline conditions on nitrate uptake kinetics of Nemaguard.
   b) Effect of salinity on nitrate uptake of Nemaguard pre-treated with salt: in this experiment we are studying the effect of saline conditions on nitrate uptake kinetics of Nemaguard grown under saline conditions after the plants has been exposed to NaCl for a period of 30 days.

      For both of the studies a factorial experiment will be used with 3 levels of salt (control, 30 mM NaCl and 60 mM NaCl) and 6 levels of nitrate (levels not defined yet) with each treatment replicated and 5 times. 60 seedlings of Nemaguard are being grown for each of the experiment. Nitrate uptake will be measured by nitrate depletion approach developed by Claassen and Barber. Further we will measurements water uptake of each plants on daily basis to quantify the effect of NaCl on water uptake over time.

Results and Discussion:

There was a great degree of variation among the rootstocks in terms of salinity tolerance. Nonpareil grafted on Nemaguard was the first one to exhibit necrosis in mature leaves. Severe stress caused defoliation after necrosis (**Figure 1**). Trees on Hansen536 were clearly more salt-tolerant. They started to show symptoms while trees on Nemaguard were almost completely defoliated. Trees grafted on Empryean-1 or Viking were totally free of symptoms throughout the season.

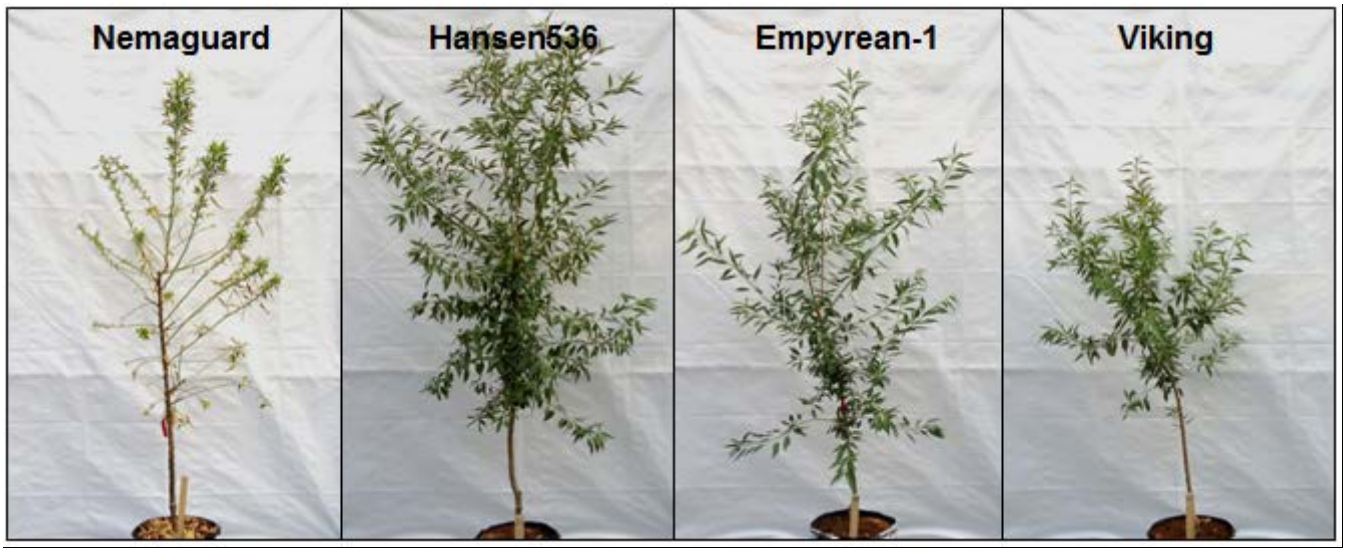

Figure 1. Nonpareil almond trees grafted on different rootstocks (Nemaguard, Hansen536, Empyrean-1 and Viking) two months after high salt treatment (40 mM NaCl; EC = ~4.8 dS/m)

As shown in **Figure 2**, the growth of Nonpareil was already inhibited at high salinity in the second month when the rootstock was Nemaguard. The canopy size of the trees on Nemaguard was significantly reduced at both the low and high salinity levels in the third month

due to defoliation while control trees continued to grow. At 105 days after commencement of the salinity treatments, trees in the highest saline treatments were removed from the high salinity treatment and changed to the control treatments to allow trees to recover from the severe stress. The recovery treatment had an immediate positive effect on the high-salt-treated trees on Nemaguard. Vigorous new growth due to the recovery treatment increased the estimated canopy size of former high-salt-treated trees above that of the low-salt-treated ones toward the end of the season. Nonpareil on Hansen536 rootstock was significantly less affected by salinity than Nonpareil on Nemaguard. At the low salinity level, trees on Hansen536 did not lose their leaves at all due to salinity but at the high salinity level, a significant growth depression was observed in the third month of treatment. High-salt-treated trees on Hansen536 did not respond to the recovery treatment as quickly as those on Nemaguard. Control and low-salt trees on Hansen536 stopped growing and started to lose some leaves toward the end of the season but this was not related to salinity. Nonpareil on Empyrean-1 and Viking rootstocks were completely unaffected by the salinity treatments used here.

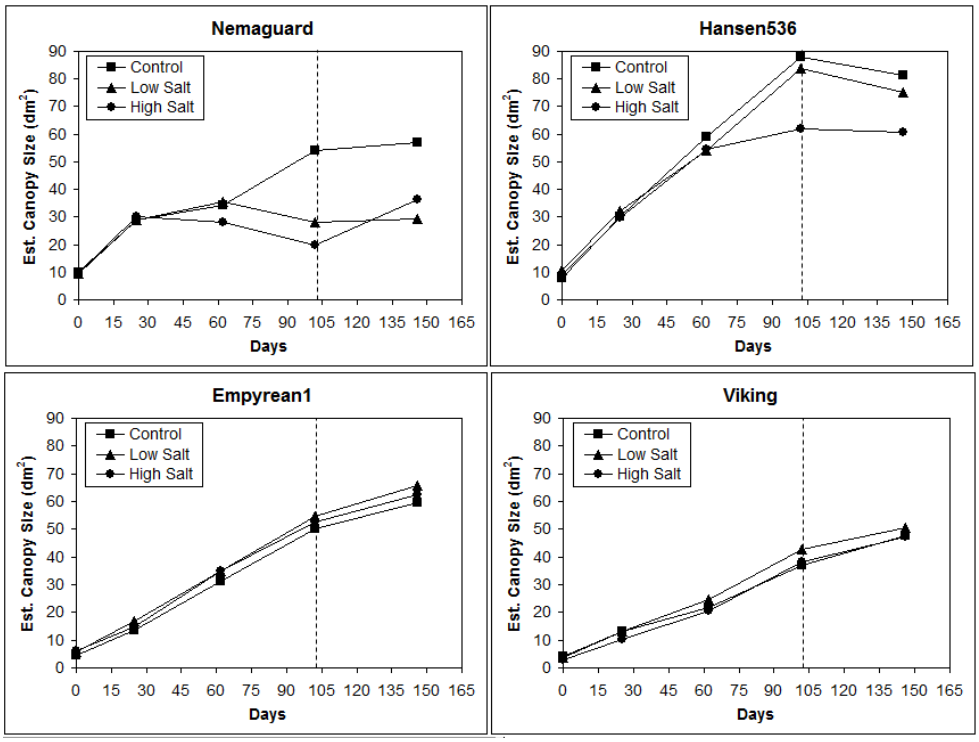

Figure 2. Growth of Nonpareil almond trees grafted on different rootstocks (Nemaguard, Hansen536, Empyrean-1 and Viking) based on canopy size estimated by digital image analysis under control (0 mM NaCl; EC = ~0.8 dS/m), low salt (20 mM NaCl; EC = ~2.8 dS/m) and high salt conditions (40 mM NaCl; EC = ~4.8 dS/m). The dashed line indicates the start of recovery treatment (application of control solution) for high-salt-treated trees.

In the cultivar experiment, all of the four cultivars tested were very sensitive to NaCl salinity when grafted on Nemaguard although there was some variation. Nonpareil was the first cultivar to experience extensive leaf loss and exhibit a net decrease in canopy size in the second month of the high salinity treatment (**Figure 3**). Mission, Monterey and Fritz followed Nonpareil. All cultivars responded well to the recovery treatment for the high-salt trees but Nonpareil and Monterey had the most vigorous new growth during the recovery phase.

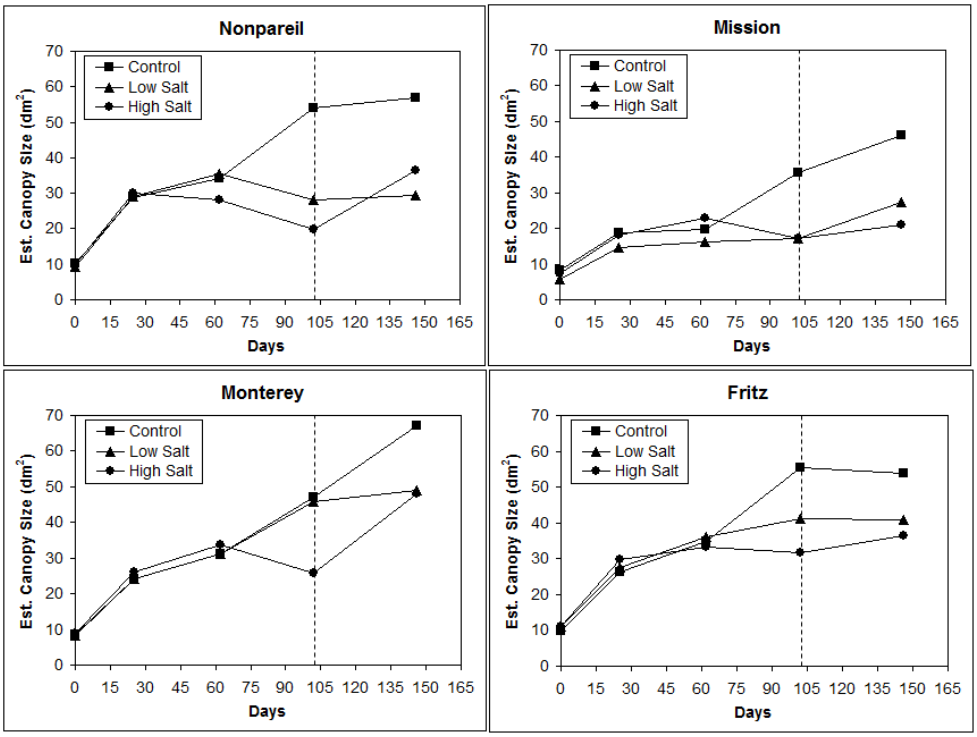

Figure 3. Growth of different almond cultivars (Nonpareil, Mission, Monterey and Fritz) grafted on Nemaguard based on canopy size estimated by digital image analysis under control (0 mM NaCl; EC = ~0.8 dS/m), low salt (20 mM NaCl; EC = ~2.8 dS/m) and high salt conditions (40 mM NaCl; EC = ~4.8 dS/m). The dashed line indicates the start of recovery treatment (application of control solution) for high-salt-treated trees.

As can be seen in **Figures 4 and 5**, the effects of salinity depended predominantly upon the rootstock in our rootstock-cultivar interaction experiment. When grafted on Nemaguard, both Nonpareil and Fritz were markedly more salt-sensitive than when grafted on Hansen536. The salt responses of Fritz on Hansen536 in terms of growth depression and defoliation were similar to those of Nonpareil on Hansen536. This demonstrates that the rootstock selection is particularly critical for salinity tolerance in almond trees.

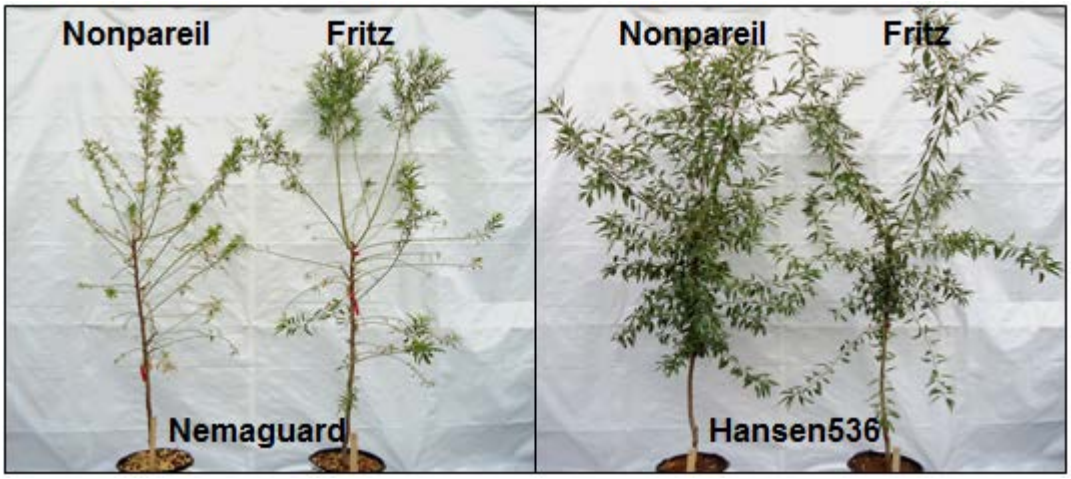

Figure 4. Leaf symptoms of Nonpareil and Fritz almonds grafted on Nemaguard and Hansen536 rootstocks two months after high salt treatment (40 mM NaCl; EC = ~4.8 dS/m)

In the salt type experiment, the leaves on the KCl-treated trees started to turn necrotic along the margins at least two weeks before those of the NaCl-treated ones. As a result, two months after salt treatments, Nonpareil trees on Nemaguard treated with 40 mM KCl were 90% defoliated while those treated with 40 mM NaCl had lost 75% of their leaves (**Figure 6**). Trees treated with Na_2SO_4 showed no stress or toxicity symptoms even though they received the same level of Na as the NaCl-treated trees.

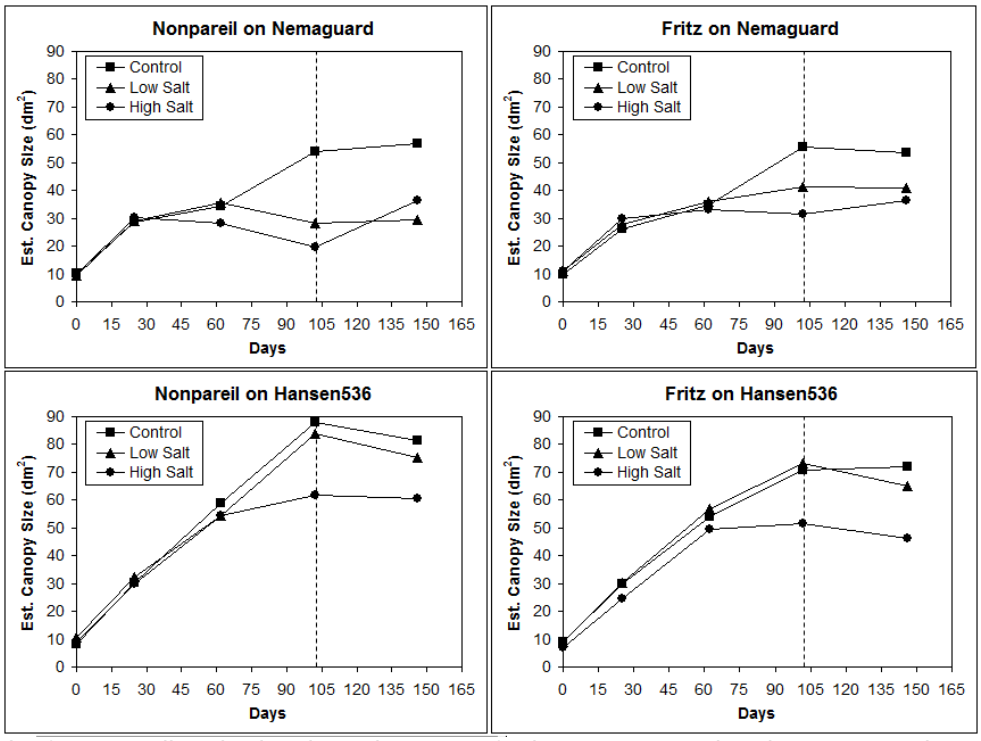

Figure 5. Growth of Nonpareil and Fritz almond trees grafted on Nemaguard and Hansen536 based on canopy size estimated by digital image analysis under control (0 mM NaCl; EC = ~0.8 dS/m), low salt (20 mM NaCl; EC = ~2.8 dS/m) and high salt conditions (40 mM NaCl; EC = ~4.8 dS/m). The dashed line indicates the start of recovery treatment (application of control solution) for high-salt-treated trees.

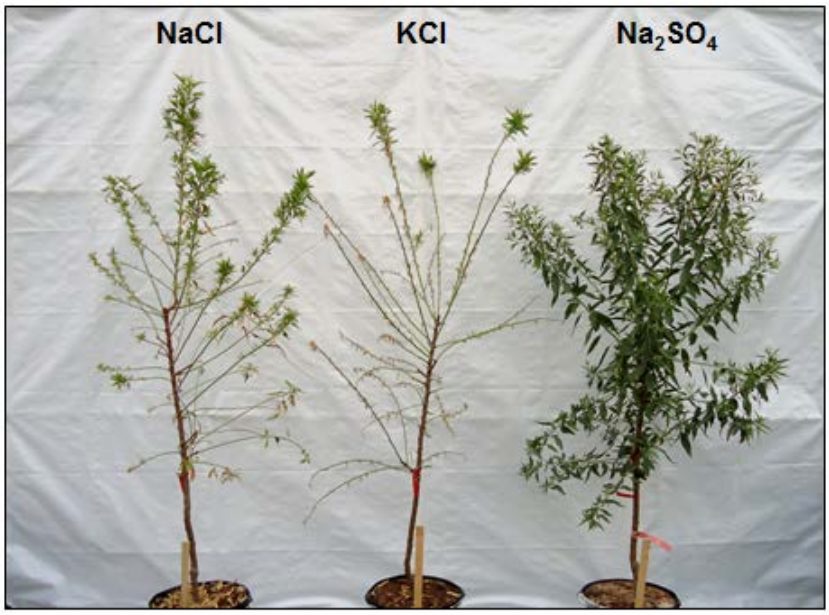

Figure 6. Effect of salt type (NaCl, KCl or Na_2SO_4) on the leaf symptoms and defoliation observed in Nonpareil grafted on Nemaguard two months after high salt treatment (40 mM Na and/or Cl)

The canopy size data were in agreement with these observations (**Figure 7**). In the second month, a much steeper decrease in estimated canopy size was observed for trees treated with high KCl than for those treated with high NaCl. Similarly, the effect of the low salt treatment was clearly harsher for the KCl-treated trees in the third month than for the NaCl-treated ones. In the recovery phase, the former high-KCl trees could not recover nearly as well as the former high NaCl trees. Apparently, Na_2SO_4 treatments did not adversely affect the growth of the trees. These results demonstrated that Cl and not Na was responsible for the symptoms as the predominant toxic ion in our experiments.

Secondary (trunk) growth data correlated well with primary (canopy) growth data. In the rootstock experiment, only the trunk growth of the trees grafted on Nemaguard was significantly impaired by the salt treatments (**Figure 8**). While the high salinity treatment also tended to reduce the secondary growth of the trees on the other rootstocks the effects were not significant. In the cultivar experiment, the trunk diameters of Nonpareil and Fritz were markedly reduced by the salinity treatments whereas those of Monterey and Mission were affected to a lesser extent. The apparently insignificant effect of salinity on Mission could be due to micronutrient imbalance (data not shown), which affected Mission more than the other cultivars and limited the growth of control trees. In the rootstock-cultivar interaction experiment, the effect of salinity on the trunk growth of the trees on Hansen536 was markedly smaller than its effect on the trunk growth of the trees on Nemaguard. When grafted on Hansen536, Nonpareil appeared to be more salt-tolerant than Fritz. Finally, NaCl and KCl limited the secondary growth drastically in the salt type experiment while the effect of Na_2SO_4 was insignificant.

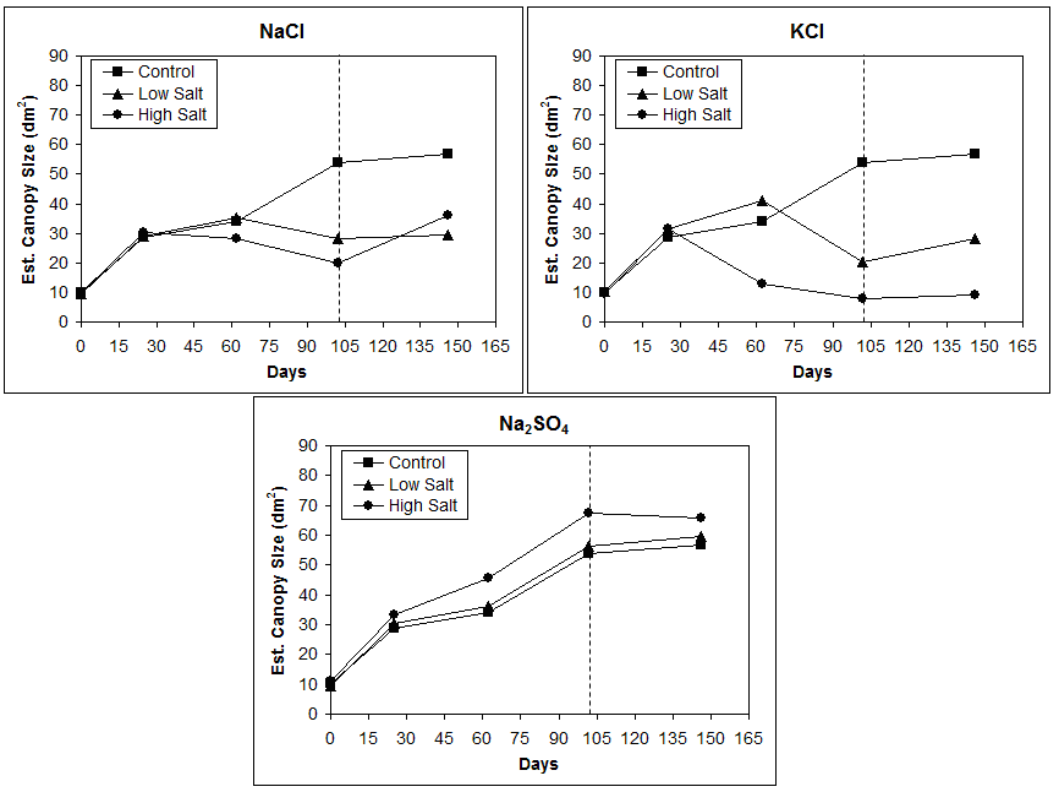

Figure 7. Effect of salt type (NaCl, KCl or Na_2SO_4) on the growth of Nonpareil grafted on Nemaguard based on canopy size estimated by digital image analysis at high salinity (40 mM Na and/or Cl). The dashed line indicates the start of recovery treatment (application of control solution) for high-salt-treated trees.

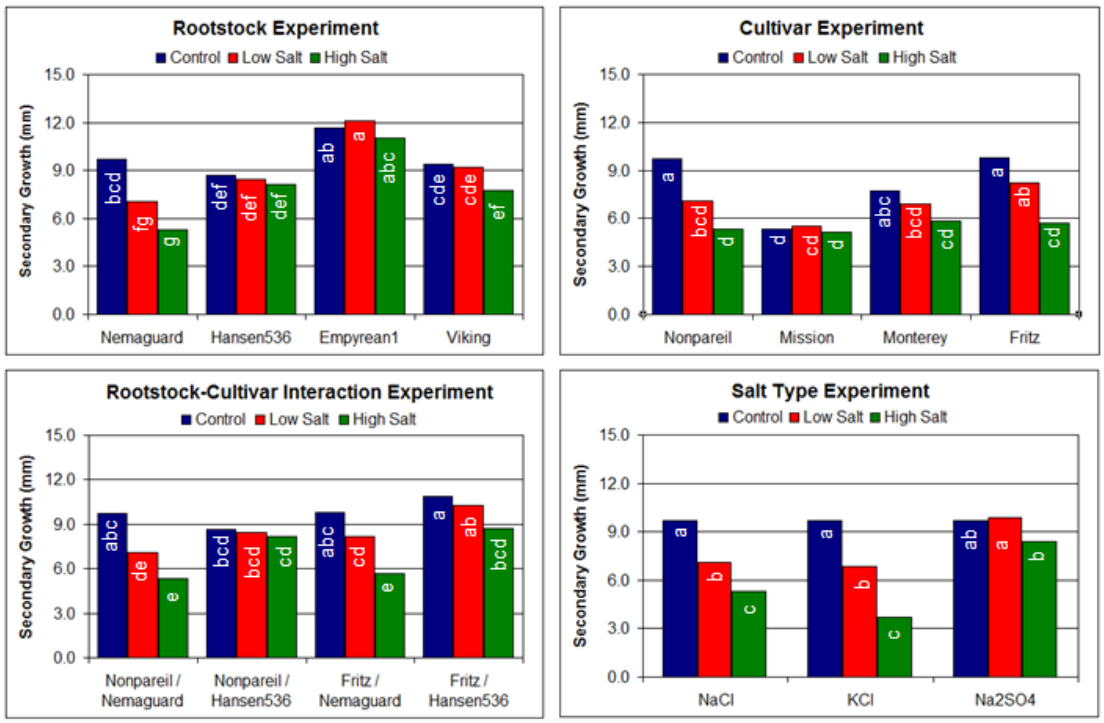

Figure 8. Secondary growth as measured by the increase in trunk diameter 15 cm above the graft union from the start of the salt treatments (control, low and high) to the start of the recovery treatment for the high-salt trees. Data are shown for the rootstock, cultivar, rootstock-cultivar and salt type experiments. Different letters indicate significant differences according to Tukey's HSD test ($P < 0.05$).

Salinity stress has several components among which induced water stress and specific ion toxicities are generally considered the most important ones. Induced water stress would cause reductions in growth parameters. In our experiments, however, salinity treatments were not associated with growth impairment when there were no leaf toxicity symptoms. In terms of canopy and trunk growth, the performance of salt-treated trees was impaired only in cases where leaves turned necrotic due to ionic toxicity and fell off the trees (**Figures 1-8**). This indicates that under our conditions, trees did not suffer significantly from salinity-induced water stress and all the observed effects were mainly due to ionic toxicity. Nevertheless, in order to more closely investigate the water stress component, carbon isotope discrimination analysis was performed on mature leaf samples collected 34 and 65 days after salinity treatments. This analysis provides an integrated measure of water stress over time and is therefore more advantageous than instantaneous gas exchange or water potential measurements. The carbon discrimination, denoted by Δ, is expected to decrease under water stress. In the rootstock experiment, the trees grafted on Nemaguard had higher Δ values than the others, irrespective of the salinity treatment (**Table 1**). Salinity did not have any effect on the Δ values 34 days after treatment while high salinity did not decrease but slightly increased the Δ values 65 days after treatment.

Table 1. Carbon isotope discrimination of Nonpareil almonds grafted on different rootstocks (Nemaguard, Hansen536, Empyrean1 and Viking) 34 and 65 days after treatment with control (0 mM NaCl; EC = ~0.8 dS/m), low salt (20 mM NaCl; EC = ~2.8 dS/m) and high salt solutions (40 mM NaCl; EC = ~4.8 dS/m)

Rootstock	Δ (‰) (34 Days after Treatment)			
	Control	**Low Salt**	**High Salt**	**Mean**
Nemaguard	21.6 ± 0.3	21.9 ± 0.2	21.8 ± 0.6	**21.8 A**
Hansen536	21.1 ± 0.5	20.7 ± 0.3	20.8 ± 0.3	**20.9 B**
Empyrean1	20.7 ± 0.5	20.9 ± 0.5	21.0 ± 0.4	**20.9 B**
Viking	21.3 ± 0.2	21.2 ± 0.2	21.1 ± 0.6	**21. 2 B**
Mean	**21.2 a**	**21.2 a**	**21.2 a**	
Rootstock	Δ (‰) (65 Days after Treatment)			
	Control	**Low Salt**	**High Salt**	**Mean**
Nemaguard	22.2 ± 0.3	22.2 ± 0.1	22.8 ± 0.2	**22.4 A**
Hansen536	21.8 ± 0.2	21.7 ± 0.2	21.9 ± 0.2	**21.8 B**
Empyrean1	21.4 ± 0.6	21.7 ± 0.6	21.7 ± 0.4	**21.6 B**
Viking	21.5 ± 0.1	21.6 ± 0.2	22.3 ± 0.5	**21.8 B**
Mean	**21.7 b**	**21.8 b**	**22.2 a**	

Means followed by different letters are significantly different according to Tukey's HSD test ($P < 0.05$).

In the cultivar experiment, there were also some statistically significant differences between the cultivars, irrespective of the salt treatments, Mission exhibited the highest carbon discrimination (**Table 2**). As in the rootstock experiment, Δ was unaffected by the salinity treatment 34 days after treatment, and the slight but statistically significant effect of high salinity observed 65 days after treatment was not negative but positive. These data confirmed that our salinity treatments did not induce any significant water stress.

Table 2. Carbon isotope discrimination of different almond cultivars (Nonpareil, Mission, Monterey and Fritz) grafted on Nemaguard 34 and 65 days after treatment with control (0 mM NaCl; EC = ~0.8 dS/m), low salt (20 mM NaCl; EC = ~2.8 dS/m) and high salt solutions (40 mM NaCl; EC = ~4.8 dS/m)

Cultivar	Δ (‰) (34 days after treatment)			
	Control	**Low Salt**	**High Salt**	**Mean**
Nonpareil	21.6 ± 0.3	21.9 ± 0.2	21.8 ± 0.6	**21.8 C**
Mission	23.6 ± 0.4	23.0 ± 0.4	22.6 ± 0.7	**23.1 A**
Monterey	22.4 ± 0.4	22.2 ± 0.3	22.5 ± 0.3	**22.4 B**
Fritz	22.2 ± 0.3	22.1 ± 0.5	22.3 ± 0.2	**22.2BC**
Mean	**22.4 a**	**22.3 a**	**22.3 a**	
Rootstock	Δ (‰) (65 days after treatment)			
	Control	**Low Salt**	**High Salt**	**Mean**
Nonpareil	22.2 ± 0.3	22.2 ± 0.1	22.8 ± 0.2	**22.4 A**
Mission	22.9 ± 0.7	22.1 ± 0.4	22.1 ± 0.3	**22.4 A**
Monterey	21.6 ± 0.3	21.8 ± 0.5	22.2 ± 0.3	**21.8 B**
Fritz	21.6 ± 0.3	21.9 ± 0.4	22.6 ± 0.3	**22.0AB**
Mean	**22.1 b**	**22.0 b**	**22.4 a**	

Means followed by different letters are significantly different according to Tukey's HSD test ($P < 0.05$).

The leaf Na and Cl concentration data for the rootstock experiment demonstrated significant differences between the rootstocks in Na and Cl accumulation (**Figure 9**). At both low and high salinity levels, leaves of Nonpareil had the highest Na and Cl concentrations at all of the three time points when the rootstock was Nemaguard. Trees on Hansen536 had up to 50% lower leaf Na and Cl concentrations than trees on Nemaguard. The Empyrean-1 and Viking rootstocks appeared to be the most efficient Na and Cl excluders among those tested. Trees grown on either of these two rootstocks had significantly lower leaf Na and Cl concentrations than those grown on Hansen536 or Nemaguard. These data explain why trees on Nemaguard were the first ones to show leaf burn symptoms and lose leaves in the rootstock experiment while trees on Empyrean-1 and Viking were symptom-free throughout the season (**Figures 1 and 2**). Another very important aspect of the data shown in **Figure 9** is that the leaf Cl levels were always about one order of magnitude higher than the leaf Na levels. The highest Na concentration measured was around 0.4% whereas the leaf Cl concentrations exceeded 4.0%.

From these data, it can be concluded that Cl accumulates markedly faster than Na in the leaves when the irrigation water contains comparable levels of Na and Cl. In addition, under these conditions, Cl appears to be primarily responsible for the toxicity symptoms. Other perennial crops known to be Cl-sensitive include grapevine, avocado and Citrus species. Nevertheless, Na toxicity may be more important than Cl toxicity where the Na concentration in the soil and/or irrigation water is higher than the Cl concentration.

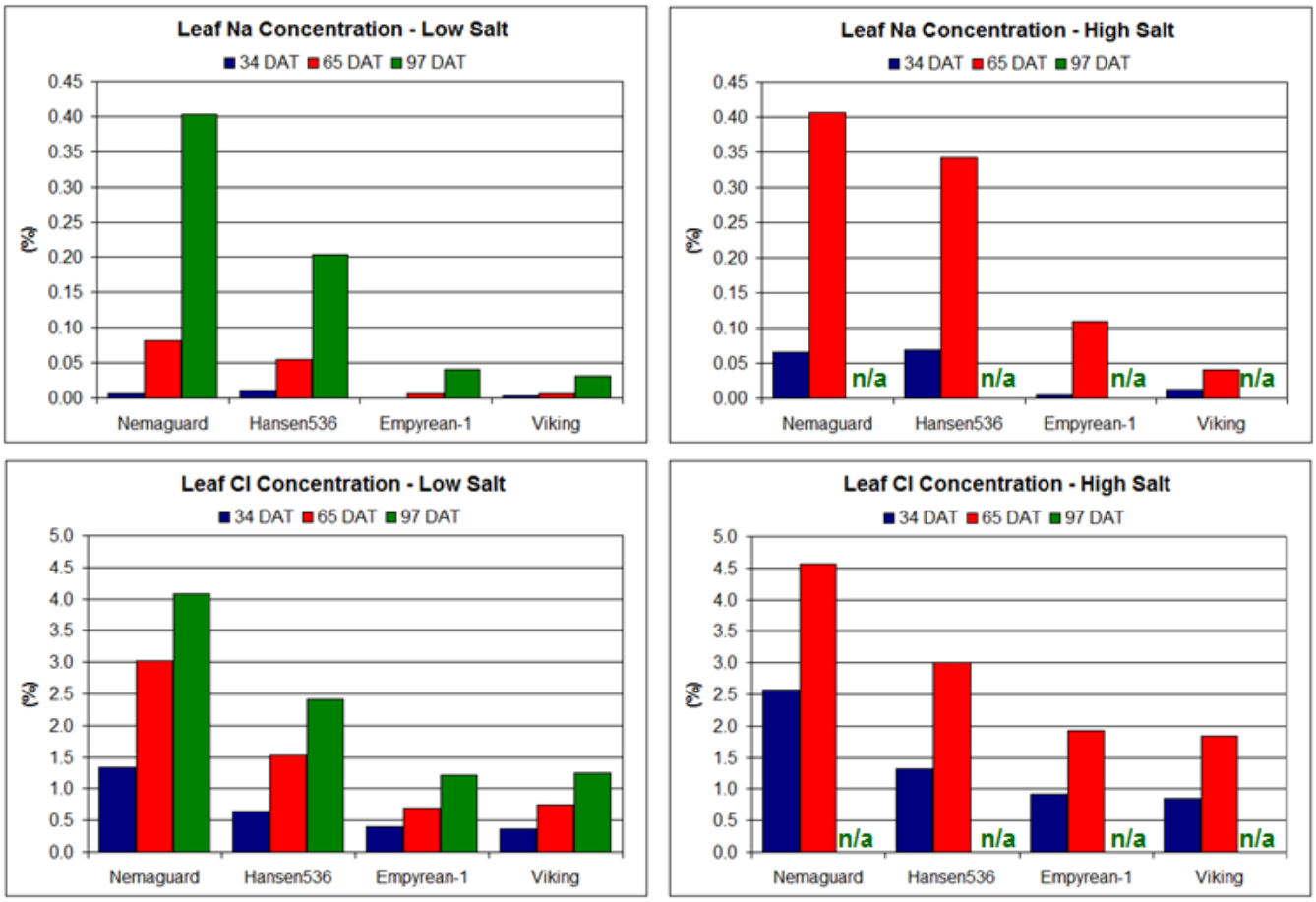

Figure 9. Na and Cl concentrations of older leaves of Nonpareil grafted on different rootstocks (Nemaguard, Hansen536, Empyrean-1 and Viking) 34, 65 and 97 days after treatment (DAT) with low salt (20 mM NaCl; EC = ~2.8 dS/m) and high salt solutions (40 mM NaCl; EC = ~4.8 dS/m). 97 DAT data are not available (n/a) for the high salinity treatment because of nearly complete defoliation of the trees grafted on Nemaguard.

In the cultivar experiment, Fritz had the highest leaf Na concentrations among all cultivars grown on Nemaguard at all time points and at both salinity levels whereas Nonpareil and Mission had the lowest leaf Na concentrations (**Figure 10**). Monterey exhibited an intermediate performance between Nonpareil and Fritz at high salinity. With respect to leaf Cl concentration, Nonpareil, Monterey and Fritz were not different from each other. Mission had lower leaf Cl concentrations, especially at low salinity. However, as discussed above, the data shown for Mission in the cultivar experiment may have been impaired by micronutrient imbalance. The strikingly high Cl concentrations measured in the leaves of all cultivars grown on Nemaguard explain why all cultivars appeared to be similarly salt-sensitive in our experiment (**Figure 3**).

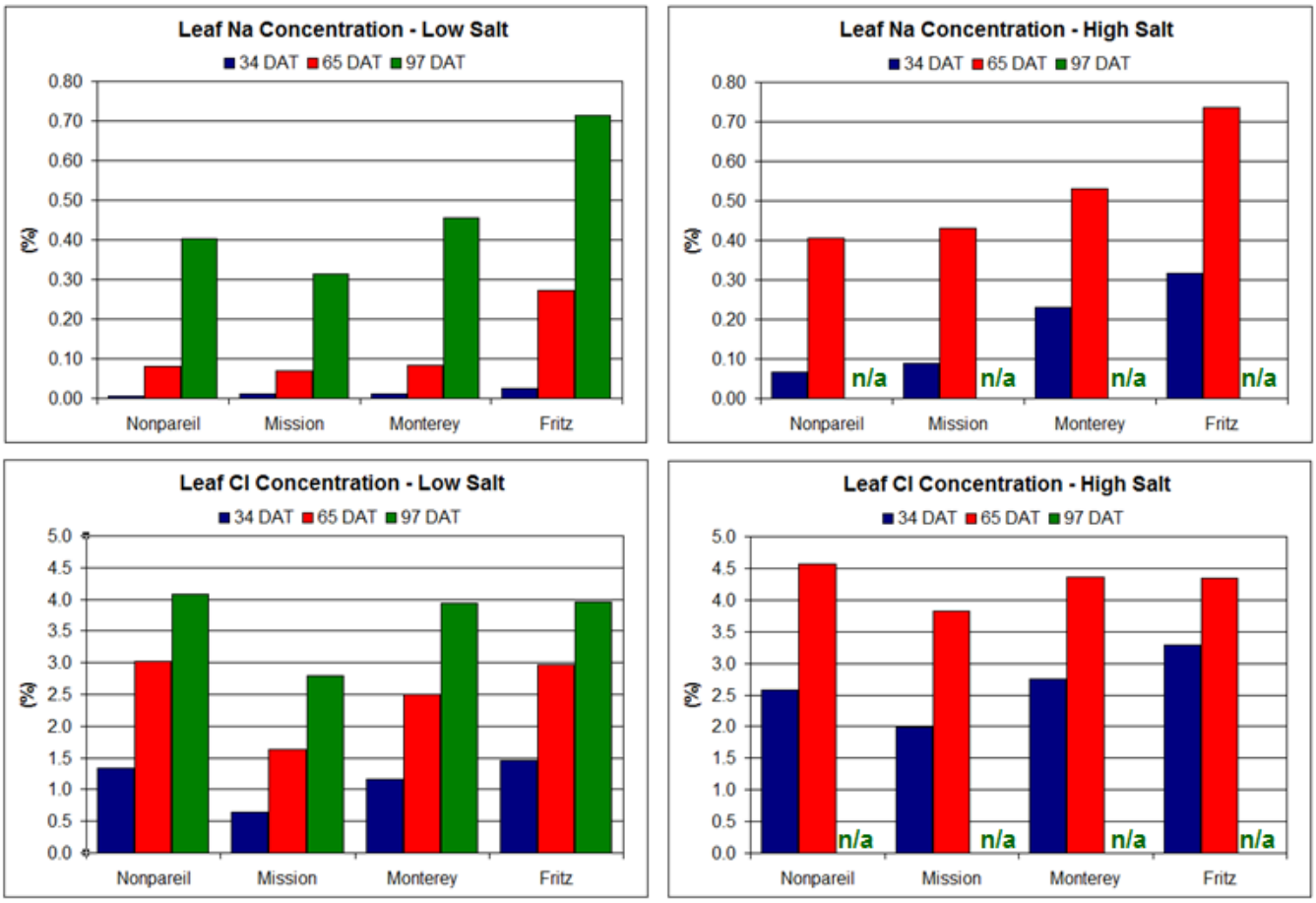

Figure 10. Na and Cl concentrations of older leaves of different almond cultivars (Nonpareil, Mission, Monterey and Fritz) grafted on Nemaguard 34, 65 and 97 days after treatment (DAT) with low salt (20 mM NaCl; EC = ~2.8 dS/m) and high salt solutions (40 mM NaCl; EC = ~4.8 dS/m). 97 DAT data are not available (n/a) for the high salinity treatment because of nearly complete defoliation of the trees.

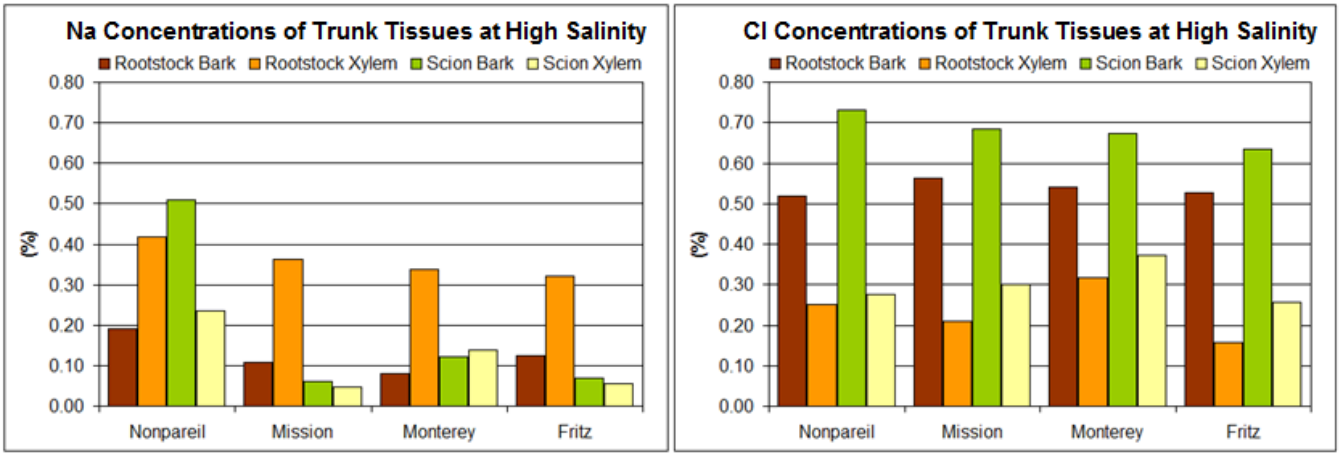

Figure 11. Na and Cl concentrations of trunk tissue samples (rootstock bark, rootstock xylem, scion bark, scion xylem) obtained from different almond cultivars (Nonpareil, Mission, Monterey and Fritz) grown on Nemaguard 84 days after treatment with high salt (40 mM NaCl; EC = ~4.8 dS/m).

The analysis of trunk tissue samples collected from the high-salt trees in the cultivar experiment revealed that the Nonpareil scion accumulated markedly more Na in its bark and xylem than the others (**Figure 11**). In contrast, the bark and xylem tissues of Mission and Fritz scions had much lower Na concentrations. Such differences in trunk Na storage capacity may affect the Na sensitivity of cultivars. The exceptionally high trunk Na storage capacity of Nonpareil may translate into lower leaf Na concentrations and thus higher Na tolerance while the lack of Na storage in the trunk of Fritz and Mission may result in higher leaf Na levels and thus higher Na sensitivity. The common rootstock Nemaguard also had high concentrations of Na in its trunk tissues, particularly in its xylem tissue. Similar Cl concentrations were measured in the trunk tissues of all cultivars grafted on Nemaguard. Although they were higher than the trunk Na levels, they were very low when compared to the leaf Cl concentrations (**Figure 10**). This and the lack of any difference between the cultivars in this respect indicate that trunk storage of Cl is not an important Cl tolerance mechanism. Finally, it is noteworthy that the bark was significantly richer in Cl than the wood. If this is related to Cl redistribution via the phloem needs to be investigated.

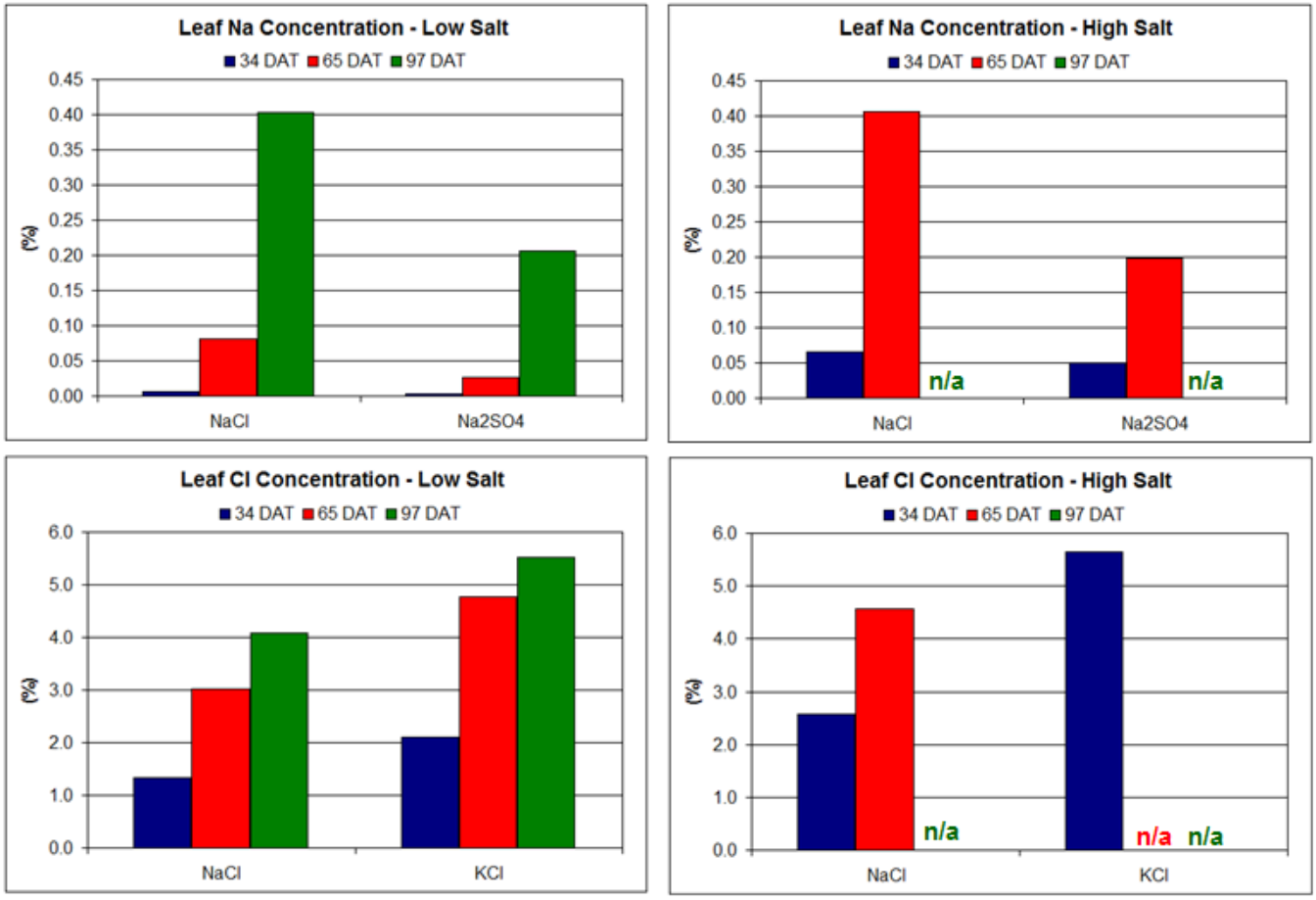

Figure 12. Na and Cl concentrations of older leaves of Nonpareil grafted on Nemaguard 34, 65 and 97 days after treatment (DAT) with low (20 mM Na and/or Cl) and high salinity (40 mM Na and/or Cl). Some data for the high salinity treatment are not available (n/a) due to defoliation of NaCl- and KCl-treated trees.

In the salt-type experiment, the leaf Na concentrations of NaCl-treated trees were twice as high as those of Na_2SO_4-treated ones at each time point and at both salinity levels although the Na concentrations in the irrigation water were the same (**Figure 12**). Salt type also

dramatically affected the leaf Cl levels. The KCl-treated trees accumulated Cl much more rapidly in their leaves than the NaCl-treated ones, which received the same amount of Cl. This explains why the KCl-treated trees were injured earlier and more severely by the salt treatments than the NaCl-treated ones. Apparently, the counter ions affect the uptake rates of Na and Cl. Chloride stimulates Na uptake more than SO_4 while excess K stimulates Cl uptake more than Na. This may be related to the cation-anion balance in the plant. The practical consequence of this finding is that the exact ionic composition of any saline soil or irrigation water is critical because it will determine how fast each toxic ion will accumulate in the plant tissues and thus which toxic ion will be primarily responsible for salt damage.

Table 3. Na and Cl concentrations of older leaves of Nonpareil and Mission scions double-grafted on Nemaguard 29, 61 and 93 days after treatment (DAT) with control (0 mM NaCl; EC = ~0.8 dS/m), low salt (20 mM NaCl; EC = ~2.8 dS/m) and high salt solutions (40 mM NaCl; EC = ~4.8 dS/m)

Scion	NaCl	Na (mg/kg)		
		29 DAT	61 DAT	93 DAT
Nonpareil	None	19 ± 10	23 ± 8	18 ± 1
	Low	78 ± 7	563 ± 425	808 ± 450
	High	454 ± 229	3181 ± 1892	- ± -
Mission	None	24 ± 8	28 ± 12	24 ± 15
	Low	78 ± 31	583 ± 307	1637 ± 619
	High	852 ± 624	6071 ± 3035	- ± -

Scion	NaCl	Cl (%)		
		29 DAT	61 DAT	93 DAT
Nonpareil	None	0.24 ± 0.06	0.30 ± 0.03	0.32 ± 0.02
	Low	0.60 ± 0.14	1.58 ± 0.39	2.07 ± 0.58
	High	1.29 ± 0.20	3.12 ± 0.82	- ± -
Mission	None	0.26 ± 0.05	0.34 ± 0.02	0.35 ± 0.04
	Low	0.63 ± 0.10	1.60 ± 0.12	2.26 ± 0.28
	High	1.56 ± 0.41	3.74 ± 0.87	- ± -

In the double-graft experiment, there were some impressive differences in the leaf Na accumulation patterns of the Nonpareil and Mission scions on Nemaguard rootstock. At low salinity, both scions had the same Na concentrations in their leaves in the first two months after treatment (**Table 3**). Three months later, however, the average leaf Na concentration of Mission was twice as high as that of Nonpareil. At high salinity, differences were clear within one month after treatment and leaf Na levels increased rapidly to levels tenfold higher than those measured at low salinity. At each time point, Mission leaves contained twice as much Na as Nonpareil leaves. This difference cannot be explained by root uptake of Na because in this experiment, the two scions share the same rootstock and thus the same root system. The difference is caused by different tissue distribution patterns of Na in the two cultivars. As shown in **Figure 11** and discussed above, the woody tissues of Nonpareil appear to have a high Na storage capacity whereas those of Mission do not store Na. In terms of leaf Cl concentrations, the two scions behaved similarly.

In the case of the split root experiments under substrate, a decrease of toxic symptoms on canopy was observed over time in all the non-uniform saline treatments in which saline conditions were given to one side of the root system and good nutritional conditions were given to the another side (**Figure 13**). Those observations were consistent with the measurements of canopy growth (**Figure 15**) and trunk growth (not presented data) in which the non-uniform saline conditions did not have significant differences respect the control/control treatment.

Those results are preliminary and for the current season a small set of trees under substrate is being tested to validate the results of the previous season and also to have a deeper understanding of the plant response under non-uniform condition in terms of water uptake and root growth.

Figure 13. Effect of non-uniform conditions (control/control, control/high salt, high salt/high salt) on leaf symptoms and defoliation observed in Nonpareil grafted on Nemaguard two months after treatment applied (high salt = nutrient + 40 mM NaCl, control = only nutrients)

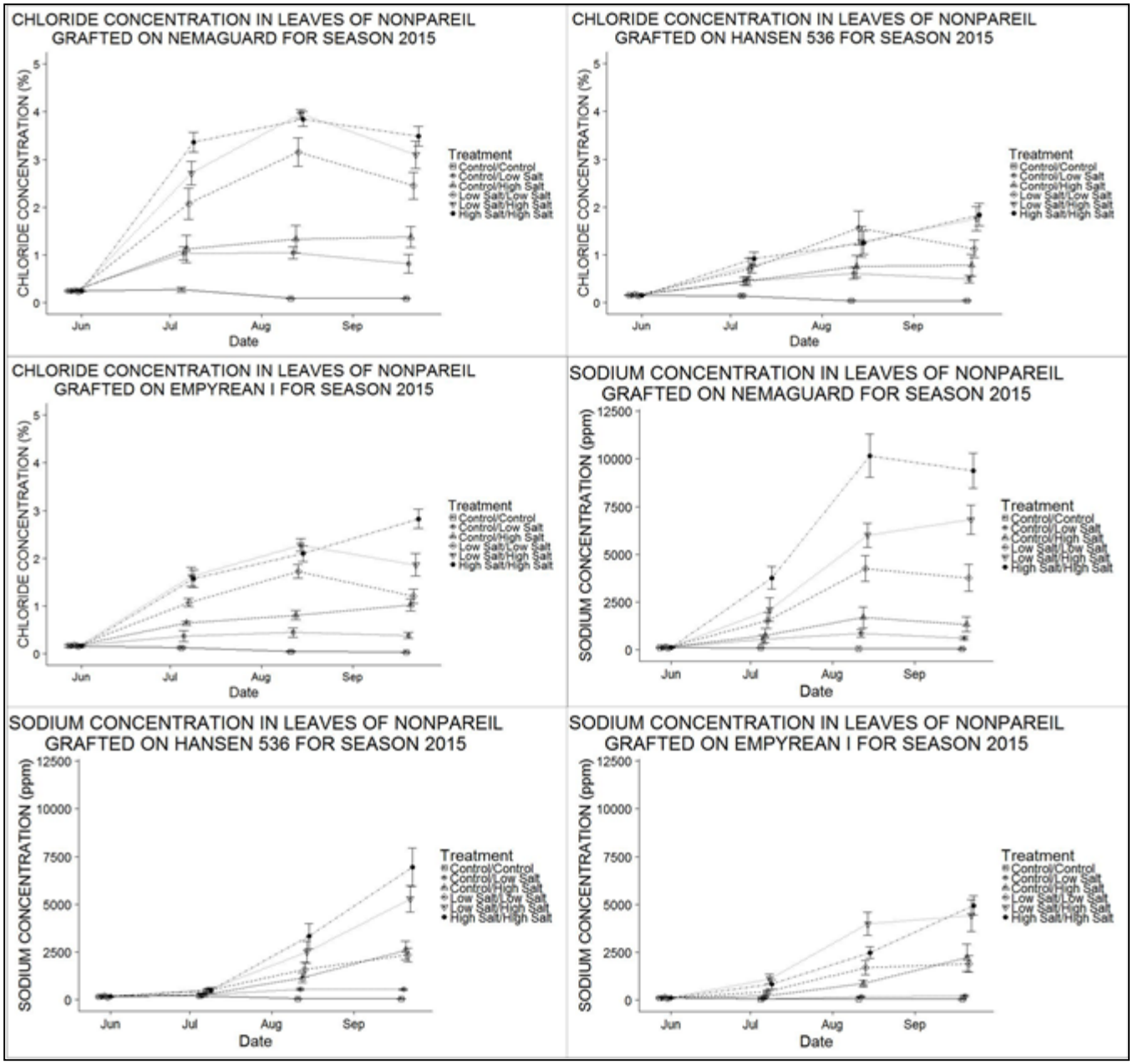

Figure 14. Na and Cl concentrations of leaf tissue samples over season 2015 obtained from Nonpareil cultivar grafted on different rootstocks (Nemaguard, Hansen 536 and Empyrean I). Where: control= 0.6 dS/m, low salt= 2.6 dS/m and high salt=4.6 dS/m.

The leaf Na and Cl concentration data from the split root experiment demonstrated the presence of significant differences between the rootstocks in Na and Cl accumulation which validate the results of experiments above (under uniform conditions) and demonstrated a decrease of the salt accumulation in leaf tissue over time when partial balanced nutritional conditions are present in the root system (**Figure 14**).

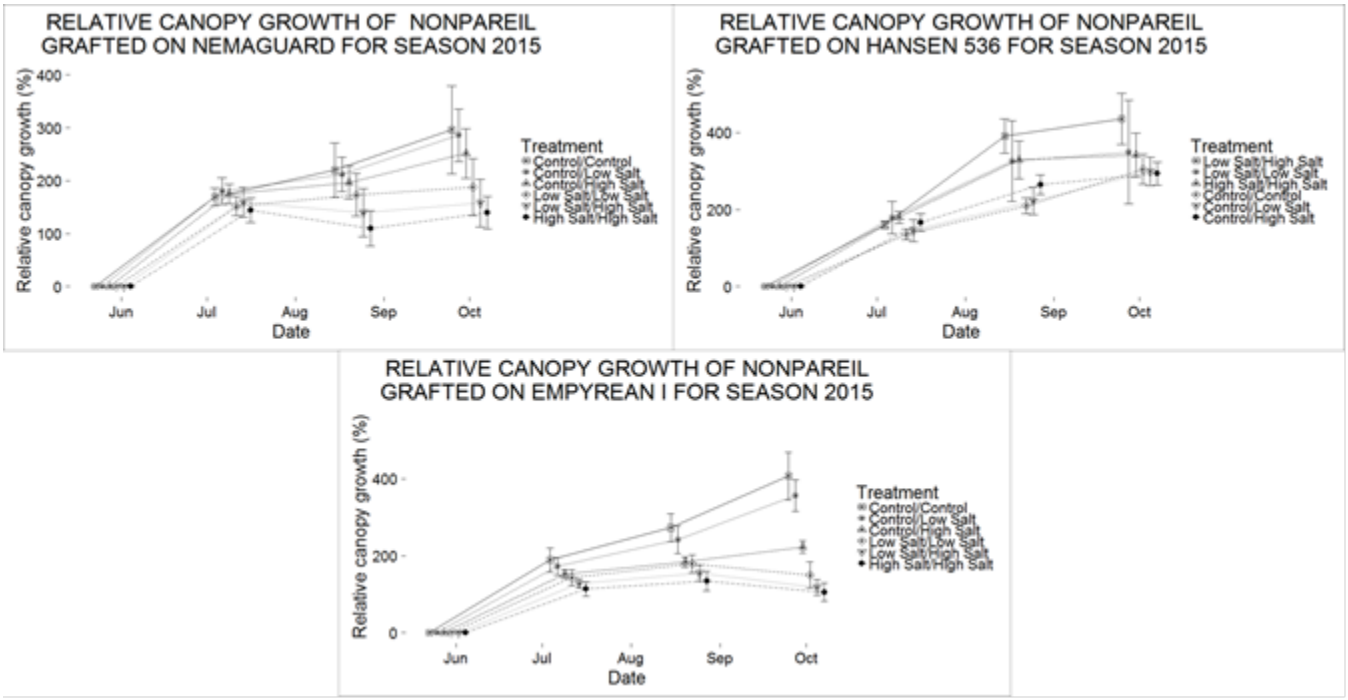

Figure 15. Relative growth of Nonpareil almond trees grafted on different rootstocks (Nemaguard, Hansen536, Empyrean-1 and Viking) under non-uniform conditions. Estimated performed by digital image analysis under control (0 mM NaCl; EC = ~0.6 dS/m), low salt (20 mM NaCl; EC = ~2.6 dS/m) and high salt conditions (40 mM NaCl; EC = ~4.6 dS/m).

The above results were obtained in the first two years of experimentation. Sampling and data analysis for the third year are underway.

We would like to thank Almond Board of California for funding this research project.