Potential of Mycorrhizal Inoculation to Mitigate Water Stress in Almond

Project No.: **15-STEWCROP5-Gaudin/Volder**

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Objectives:

Mycorrhizal fungi are a key component of soil ecosystems and symbioses with tree crops can provide many benefits: while trees provide carbon to the mycorrhizae, fungal hyphae help with exploitation of a greater volume of soil and have been shown to enhance resource acquisition and uptake in many tree species [1-3]. However, very little is known about mycorrhizae associations with almond trees and the potential benefit of inoculation to growers. In fact, many California growers inoculate their trees but the extent to which Prunus rootstocks are mycorrhizal and the impact of management practices on root colonization by endogenous or applied mycorrhizae population remain unknown. Although improved water relations under deficit irrigation as a result of mycorrhizal symbiosis have also been reported[1,3-9], the functional significance of inoculation to almond water status when irrigation water is limited remains unclear. *This project seeks to elucidate whether almond roots effectively form beneficial* *association in common rootstocks, identify management practices that promote symbiosis and characterize their potential to help mitigate water stress in young trees.*

Specific objectives are to:
- Objective 1 - Survey the extent of almond tree symbiotic relationships during the growing season across soil type and management practices.
- Objective 2 - Determine the aptitude for mycorrhizal root colonization of different common rootstocks
- Objective 3 - Monitor root colonization rates upon inoculation of seedling and mature trees.
- Objective 4 - Assess the potential of commercial inoculants to improve tree water status and alleviate water stress during early plant establishment.

Interpretive Summary:

We provide the first detailed assessment of arbuscular mycorrhizal symbiosis in almond orchards of California as affected by management and investigate the biophysical drivers of mycorrhizal presence and colonization. We show that, albeit endogenous mycorrhizae populations are ubiquitous, higher colonization rates are found in non-fumigated and organically managed orchards and lowest rates in conventional orchards. Potential differences between rootstocks in terms of colonization rate and responsiveness to symbiosis are being investigated. We found sharp increase in colonization rates upon inoculation of young trees in field setting and further assessed how inoculation with mycorrhizae may alter nonbearing almond host water movement into, though, and out of almond tree while under well-watered or low-watered conditions. Preliminary results indicate that when plants were exposed to extreme drought stress an increase in stomatal conductance occurred in inoculated trees. Inoculation and soil health-building management practices which promote root colonization by mycorrhizae may improve tree tolerance to drought episodes and assist the almond industry in its expansion, especially in areas where seedlings must withstand periods of water deficit.

Materials and Methods:

Objective 1, 2 and 3- Mycorrhizae survey: We sampled roots in 6 orchards in 2015 and 13 orchards in 2016 in different soils and rainfall zones (**Table 1**). In particular, we focused our efforts on measuring mycorrhizal colonization in orchards (1) under conventional and organic management, (2) with or without planted/natural vegetation cover crop, (3) with or without fumigation, along a (4) carbon (C) input gradient (none, composts, biochar, hulls or whole tree inputs), (5) in soils of different types and soil organic matter (SOM) content and in (6) different rootstocks. We also followed colonization dynamics after inoculation in bearing and non-bearing orchards inoculated with commercial inoculants. A mature orchard in Esparto for which a baseline soil and mycorrhizal colonization was established in 2015 (Brian Paddock, Organic grower, Capay Hills Orchards) was re-sampled in 2016 after inoculation of cover crop at blooming. We also sampled Garry Gliddon's nonbearing orchard (Treevine Consulting) where a large trial was setup with Valent to compare growth of inoculated with non-inoculated trees upon establishment.

Root and soil samples from the top 0-20cm were collected from 10-20 trees per treatment in September 2015 and between June and August 2016. Roots were gently washed and preserved in 50% ethanol. A microscopy quantification method was developed and optimized for almond roots (**Figure 1A**) as follows: Preserved fine lateral roots were trimmed to 2mm segments, placed in microcassettes and autoclaved in 10% KOH solution to clear tannins. Cleared root samples were rinsed and bleached twice in 0.6% NaOCl for 5 minutes, submerged in sterile 0.25% white vinegar for 5 minutes and rinsed again. Cassettes were hot-bathed at 90°C in staining solution (5% black Schaeffer ink to white vinegar) for 5 minutes. Cassettes were then rinsed thoroughly and stored at 25°C in sterile acidified water overnight to improve contrast. Stained roots were quantified with grid-marked petri dishes under a dissecting microscope at 1.6X. Approximately 30 roots segments were placed in petri dishes with acidified water. The number of roots presenting blue-stained mycorrhizae intersecting with grid lines was counted three times after reshuffling with a hand held tally on one subsample per tree. A compound microscope (100X magnification) was used to confirm colonization on subsamples (**Figure 1A**).

Management practices were recorded at each orchard (rootstocks, irrigation regime, and fertility inputs) and bulk soils are being analyzed for physiochemical properties (pH, organic matter content, soil texture, soil bulk density, moisture and nutrient content).

Objective 4- Pot study: We assessed the potential of commercial inoculants to improve tree seedling water status under deficit irrigation in a pot experiment. In June 2015 and May 2016, young trees (Nonpareil on Harding 536 rootstock) were transplanted into forty -6 gal pots. Half the pots were inoculated at transplant (MycoApply Ultrafine Endo® inoculant) and trees remained well watered until the first week of August, when water was withheld from half of the trees (n= 10 for each mycorrhizal by water treatment combination). Leaf gas exchange, stomatal conductance, and stem water potential were measured periodically during the dry-down period. Trees were harvested in September for 2015, and separated into roots and shoots. Leaf, stem, and root biomass were measured, as well as total leaf area. Root characteristics including specific root length of fine roots mycorrhizal infection rate are currently being determined for 2015. For 2016, we increased the amount of inoculant used, and increased the severity of the low-water treatment. Trees have not yet been harvested.

Results and Discussion:

We surveyed mycorrhizal colonization in common rootstocks across management practices in growers' fields and research trials to identify the management practices and soil properties instrumental to enhance symbiotic relationship in commercial orchards. We first used molecular approaches (RT-qPCR) to develop a high throughput method to quantify mycorrhizal fungi in almond roots collected at the various sites and pot experiment. This approach proved challenging and needed to be validated using established protocols. Therefore, we focused our efforts on developing an efficient and reliable microscopy protocol to quantify colonization rates of fine roots.

To date, we have analyzed one-third of our samples and significant trends are starting to emerge. Mycorrhizae are ubiquitous and, on average, the non-inoculated conventional almond tree roots analyzed are colonized at ~72%. Organically managed orchards sampled show significantly higher root colonization rates (82%) (**Figure 1B**). Highest colonization rates were found in non-fumigated and organically managed orchards and lowest rates in conventional orchards, whether trees were planted after previous orchard was burned or returned to the soil (**Figure 1C**). Fumigation did not affect colonization rates in the trial located in Ballico and this orchard presented high rates of colonization across treatments. However, quantification is far from complete (n=3 of 10 in all teatment presented), the potential differences between rootstocks nested in these results are not known, and soils remain to be analyzed. Albeit ubiquitous, the formation and function of mycorrhizal relationships may change with tree age, root growth dynamics, soil properties, moisture environment, rootstock/scion combination and fungal species. Quantifying colonization in all treatments will help fully exploit the potential of mycorrhizal association and multivariate analysis of the complete dataset should also help identify soil properties and management practices promoting colonization.

We show that inoculation significantly increased colonization of young trees by 27% compared to non-inoculated control during establishment of a commercial orchard (**Figure 1C**). However, the potential of this practice to improve water relations and young tree resilience to irrigation water shortages is not clear. Various studies have shown that external mycelium of mycorrhizal fungi directly transport water to the host plant, enhance nutrient uptake under water stress, and increase water flow into roots by increasing root hydraulic conductance and aquaporin function [4-6,10-13]. It was also recently shown that mycorrhizal associations increase plant water uptake under conditions of low water availability by increasing stomatal conductance [10]. We conducted a pot study to test whether inoculation of young trees enhance growth, leaf gas exchange, and stomatal conductance rates under drought conditions.

In the potted almond experiment in 2015, we found that under mild water stress (**Figure 2**) during the majority of the experiment, caliper growth (**Figure 3**) and leaf physiology were not significantly affected by inoculation. However, under extreme water stress at the end of the growth period, stomatal conductance (**Figure 4**) and photosynthetic assimilation were significantly increased for plants in the low-water treatment that were inoculated compared to the plants that were not inoculated, when the stem water potential was ten or more bars below the baseline (**Figure 5**). Maintaining higher conductance rates at low stem water potential allowed more water to be moved through inoculated plants than their non-inoculated counterparts. Under field conditions, where hyphal networks are more extensive and explore more soil volume through their ability to penetrate very small micropores they could confer a water advantage to plants by allowing stomates to stay open longer under adverse conditions. This provides benefits both for prolonged carbon uptake and reduced leaf temperature.

Therefore, inoculation may help in the development of ecology-based management tools to improve planting success, resource uptake and tree tolerance to drought episodes and assist the almond industry in its expansion, especially in areas where seedlings must withstand periods of water deficit.

Research Effort Recent Publications:

A poster was presented at the Annual Almond Board conference held in Sacramento on December 8th-10th 2015. Two research publications are currently in preparation: One paper lead by Tamara McClung and Astrid Volder will focus on assessing how inoculation of young trees impacts physiology and growth of young almond trees in response to water stress (to be submitted in the fall 2016). A second one, lead by Cristos Vasilikiotis and Amélie Gaudin will focus on results of the survey approach and impact of floor management practices and soil properties on root colonization rates (to be submitted in the fall 2016 to Mycorrhiza).

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Table and Figures:

Table 1. Survey sites in 2015 and 2016 field season.

Season	Treatment	Treatment	Rootstock	Farm - Research Site
2016	Compost addition	Control, no compost	Nemaguard	Roger Duncan Compost Trial
2016		Green waste compost	Nemaguard	Roger Duncan Compost Trial
2016		Manure compost	Nemaguard	Roger Duncan Compost Trial
2016		Control, no compost	Nemaguard	Roger's Green waste orchard
2016		Green waste compost	Nemaguard	Roger's Green waste orchard
2016	Conventional management	Conventional	Marianna	Jimmy Bertana
2016-2015		Conventional	Lovell	Rolston -FreeHeart Farm
2016-2015		Conventional	Nemaguard	Nickels Soil lab
2016		Conventional	Nemaguard	North Valley Nut-Hart Farm
2015	Fumigation	C-35	Nemaguard	Ballico trial, Doll
2015		Non-Fumigated	Nemaguard	Ballico trial, Doll
2015		Methyl Bromide	Nemaguard	Ballico trial, Doll
2016		ASD + Sudan	Nemaguard	Kearny - Greg's ASD Trial
2016		ASD, no sudan	Nemaguard	Kearny - Greg's ASD Trial
2016		CK + Sudan	Nemaguard	Kearny - Greg's ASD Trial
2016		CK, no sudan	Nemaguard	Kearny - Greg's ASD Trial
2016		Fumigated, no sudan	Nemaguard	Kearny - Greg's ASD Trial
2016	Fumigation x rootstock	Fumigated	Nemaguard	David Doll Rootstock Trial
2016		Fumigated	Hansen 536	David Doll Rootstock Trial
2016		Fumigated	Empyrean-1	David Doll Rootstock Trial
2016		Non-Fumigated	Nemaguard	David Doll Rootstock Trial
2016		Non-Fumigated	Hansen 536	David Doll Rootstock Trial
2016		Non-Fumigated	Empyrean-1	David Doll Rootstock Trial
2016	Inoculation	Inoculated	Nemaguard	Gary, Non bearing orchard
2016		Non-Inoculated	Nemaguard	Gary, Non bearing orchard
2016	Organic	Organic	Lovell	Massa organics
2016		Organic	Nemaguard	Nickels Soil lab
2015		Organic	Nemaguard	Nickels Soil lab
2016	Organic, Inoculation	Organic, inoculated	Lovell	Brian Paddock
2015		Organic, non inoculated	Lovell	Brian Paddock
2016	Replant management	Burn	Nemaguard	Kearny - Orchard Recycling Trial
2016		Tree incorporation	Nemaguard	Kearny - Orchard Recycling Trial
2016	Rootstock	Atlas	Atlas	Nickels Soil lab - Rootstock Trial
2016		Lovell	Lovell	Nickels Soil lab - Rootstock Trial
2016		Nemaguard	Nemaguard	Nickels Soil lab - Rootstock Trial
2016		Nickels	Nickels	Nickels Soil lab - Rootstock Trial

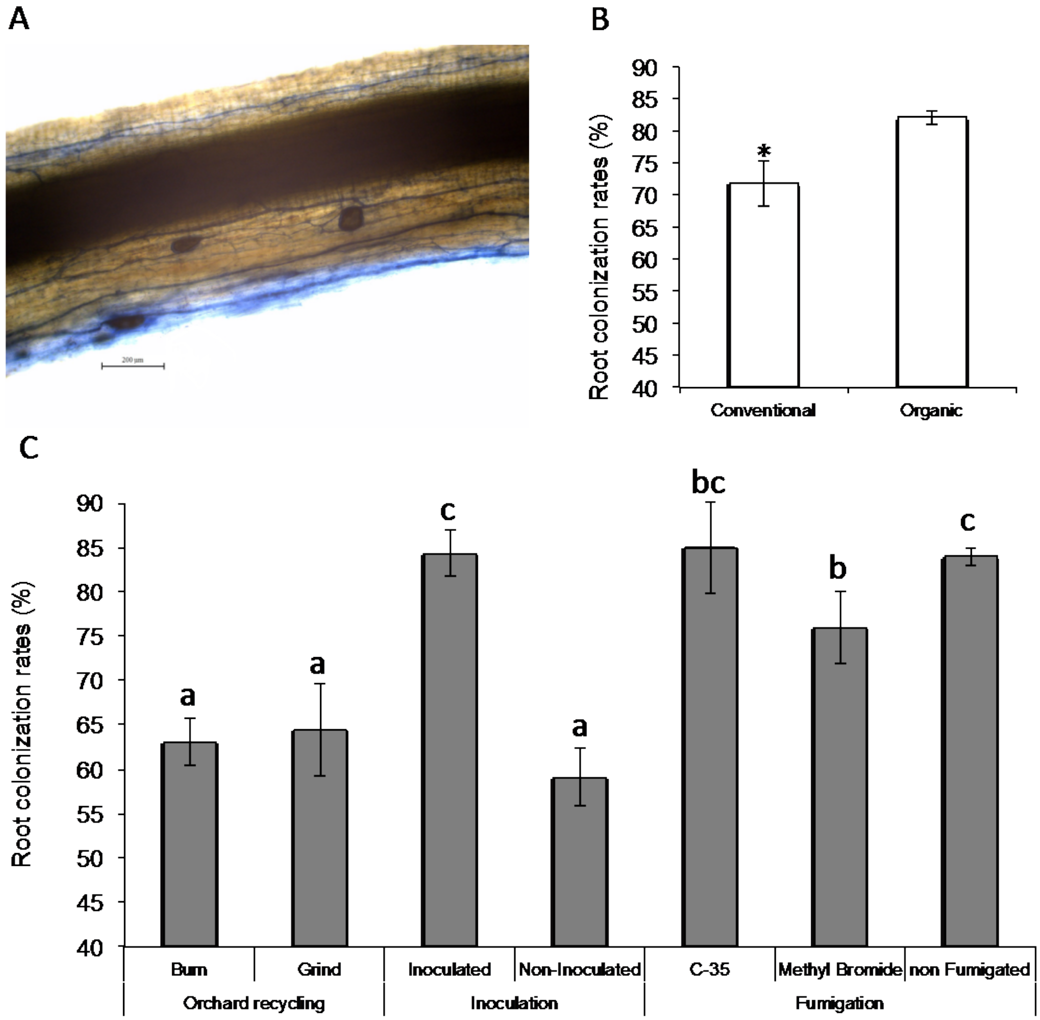

Figure 1. Mycorrhizal colonization in common rootstocks across management practices in growers' fields and research trials. **A-** Representative pictures of arbuscular mycorrhizae colonization of root cells. Vesicules are shown. **B-C** Colonization rates in (**B**) conventional and organic orchards (n=3) and (**C**) conventional orchards under different management practices. Orchard recycling = Kearny whole orchard recycling trial established in 2008 (B. Holtz), inoculation= inoculation of young orchard upon establishment with MycoApply, Fumigation = non-fumigated control and C35 and methyl bromide fumigation trial in Ballico (D. Doll).

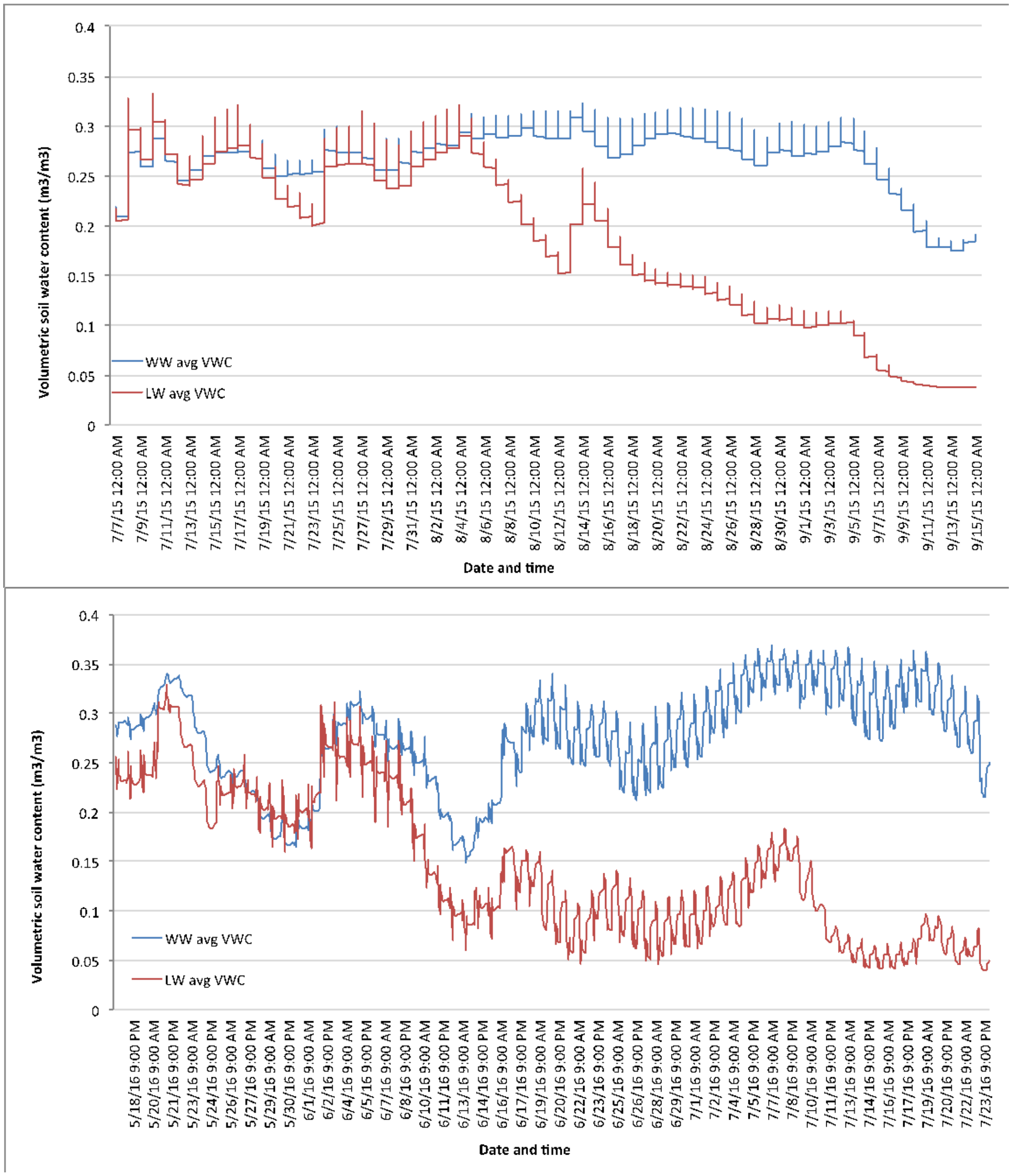

Figure 2. Volumetric water content in pots in (top) 2015 and (bottom) 2016.

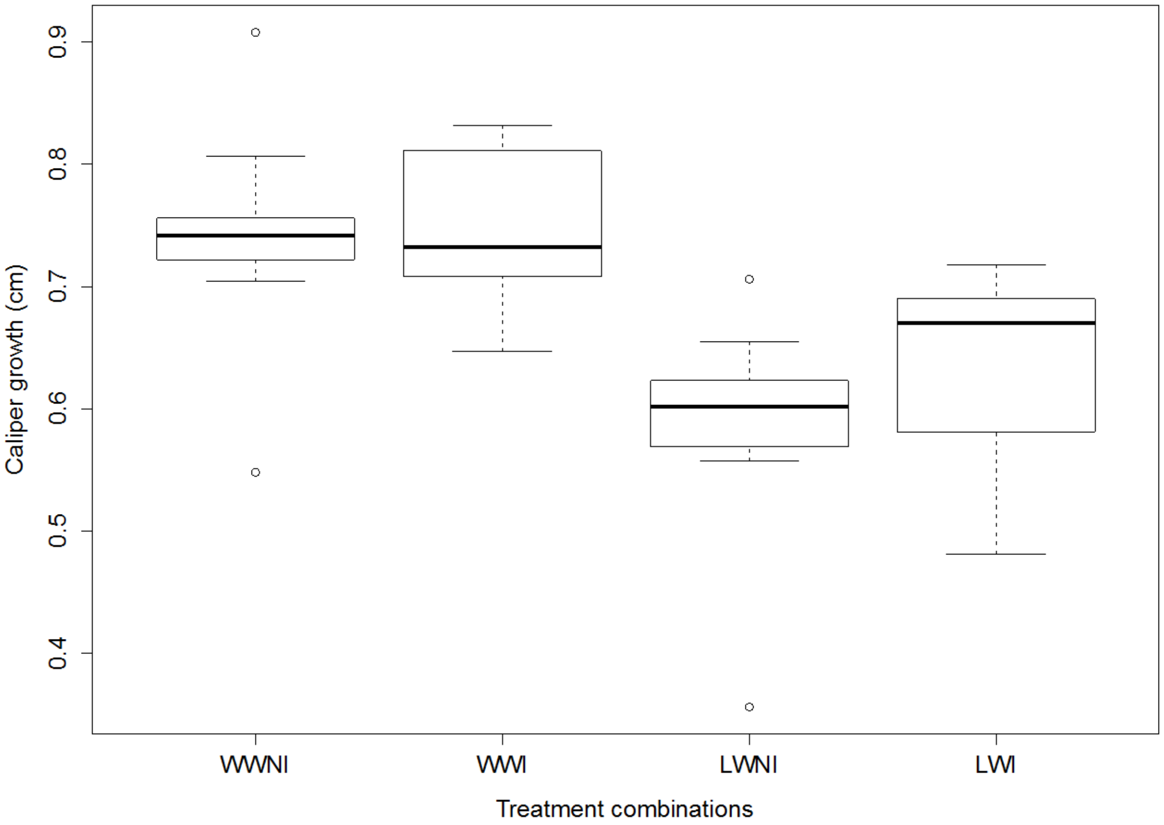

Figure 3. Caliper growth (final – initial) over the duration of the 2015 potted almond experiment.

Treatment combinations are well-watered non-inoculated (WWNI), well-watered inoculated (WWI), low-watered non-inoculated (LWNI), low-watered inoculated (LWI). Dark horizontal bars represent the median, and the boxes contain the middle 50% of the data, with whiskers extending to the highest and lowest data points. Outliers are more than 1.5 times the interquartile range represented by the box. No significant difference was found between treatments.

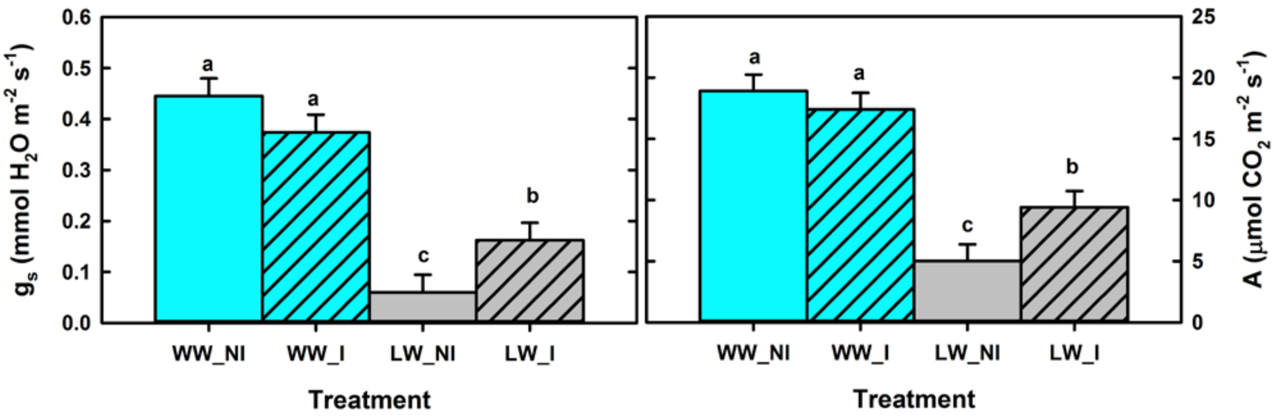

Figure 4. Physiological response of almond tree to inoculation under well-watered and water stress conditions

Stomatal conductance to water vapor (left) and photosynthetic assimilation rate (right) for the potted almond experiment in 2015, in the last week of the experiment when plants had the most negative stem water potential. Conductance and photosynthesis were significantly higher for plants in the low-water treatment that were inoculated.

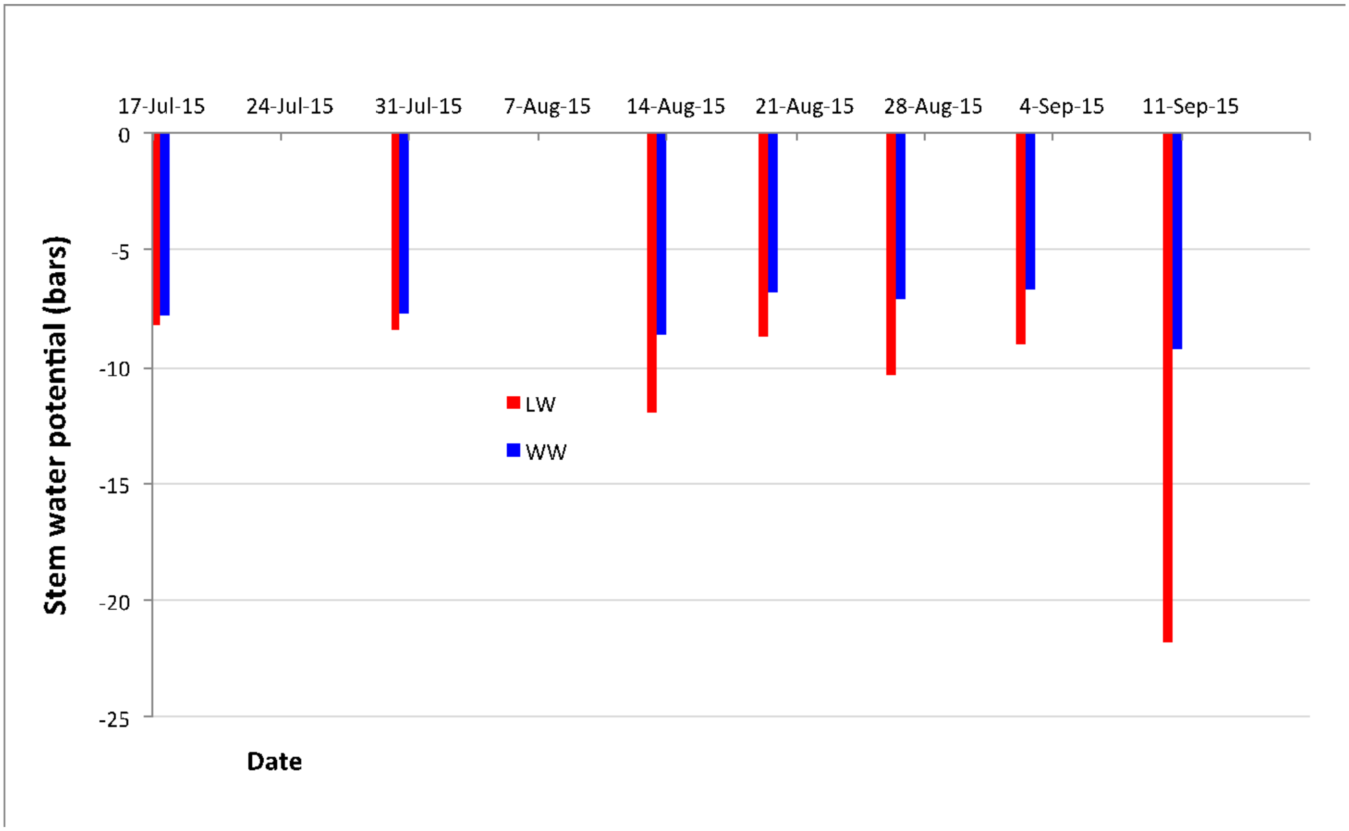

Figure 5. Stem water potential across duration of 2015 potted almond experiment for well-watered (WW) and low-watered (LW) plants. Low-water treatment was only under mild drought stress until the last date.