Assessing the Efficiency of Re-Leaf® in Improving Almond Nut Production in Areas of Soil Salinity

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Soil salinity in Australian agriculture is the largest land degradation causing numerous problems for plant growth and development which results in abnormal physiological functions. Almonds are considered a suspectable crop when it comes to soil salinity with large yield losses correlated to increases in soil salinity. Applications of amino acids have been proven to have positive effects on the growth and production of almonds through accumulation of osmolytes. These osmolytes assist in improving abiotic stresses in plants which as salinity. Amino acids assist in maintaining turgor pressure, regulate stomatal opening and closing, scavenge for harmful reactive oxygen species (ROS), ensure normal metabolic functions and to provide the basic building blocks for protein production (Ali et al., 2019).

Key words: Soil salinity, almonds, abnormal physiological functions, amino acids, abiotic stress, osmolytes.

Introduction

Soil salinity and sodicity is one the largest contributing factors to agricultural land degradation in Australia. Dealing and mitigating saline soil in Australia is both a costly exercise and also has damaging effects on the production statistics in both dryland and irrigated agriculture. Currently approximately 5.7 million hectares of land is affected by salinity is Australia and by 2050, that number is expected to jump to 17 million hectares (Metcalfe and Bui, 2016).

Almonds are relatively sensitive to the presence of salts in the soil. Their soil salinity threshold is 1.5 mS/cm and once soil salinity reached 2.8 mS/cm there is a 25% in yield loss (Doll, 2016). Therefore, it is important to monitor the sources of salts which are used in agriculture. There salts sources can come from salts already present in the soil, fertilisers and composts and also irrigation water.

In order to manage soil salinity in Almond orchards, farms have four different management practices which would help in mitigating soil salinity. These include; managing salt build up through in-season leaching, displacement of salts through water amendments, leaching of salts through dormant leaching and also making educated pre-planting decisions on soil salinity resistant/tolerant root stocks (Doll, 2016). However, these management practices are not quick fix and constant monitoring is needed in order to ensure salinity levels are being lowered.

Dual Chelate Fertilizer Pty Ltd has developed an amino acid rich fertigated fertilizer called Re-Leaf. Re-Leaf is a formulation containing 17 different amino acids (50%) and biologically active organic molecules – BAOM (5%). The benefits of amino acids in improving abiotic stress tolerance in plants has been research with many positive trials conducted highlighting how plants use available amino acids to assist in the normal physiological functions in plants when exposed to stress such as salinity, drought, extreme temperatures and nutrient deficiencies. To overcome these adverse effects, plants accumulate and use different osmolytes (carbohydrates, betaine, proline and other amino acids) to maintain turgor pressure, regulate stomatal opening and closing, scavenge for harmful reactive oxygen species (ROS), ensure normal metabolic functions and to provide the basic building blocks for protein production (Ali et al., 2019).

In this study, the effect of soil applied applications of Re-Leaf to an almond block known to be affected by soil salinity will be assessed. Parameters observed will include yield analyses, nutrient leaf concentrations and out turn percentages.

Objectives

1. Evaluate the effect of fertigation with Re-Leaf® in improving the nutrient status of salt stressed almonds.

2. Evaluate the effect of Re-Leaf® in improving crop vigor and canopy growth of almonds grown in saline soil.

3. Evaluate the effect of fertigation with Re-Leaf® on the yield parameters of almonds; nut weight, kernel weight and total yield.

Materials and Methods

Site Selection and Trial design

This trial was conducted in an almond orchard within the Sunraysia region of Victoria. The selected block in the orchard had severe salinity issues in the past and as a result, areas of the effected block were replanted due to the loss of older almond trees which were severely affected by the soil salinity issues.

Soil injections of Re-Leaf® was applied across the sample site, with ten trees from three rows isolated using drip line taps. These where considered the control plants. Ten trees from three adjacent rows were considered the treated trees and received applications of Re-Leaf®.

Table 1 highlighted application times of Re-Leaf applied at 2 key stages of almond trees and nut development, post flowering and at fruit development stage.

Table 1: Application rate and timing of Re-Leaf®

Treatment	Rate/ha	Application timing
Control	0	
Transit Re-Leaf®	30 L/ha	After Flowering Fruit Development Stage

Observations

Soil analysis

Soil samples were taken prior to Re-Leaf applications and analysed by Analytical Laboratories & Technical Services Australia (ALTSA)

Leaf nutrient analysis

During the active growth stage, leaf samples were collected from each tree in both the control and treatment groups. These leaves were washed and analysed at ALTSA, Victoria - for the presence of: Nitrogen (N), Phosphorus (P), Potassium (K), Sulfur (S), Calcium (Ca), Magnesium (Mg), Sodium (Na), Aluminium (Al), Boron (B), Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn), Silicon (Si) and Molybdenum (Mo).

Kernel Weight, Hull weight and Nut Weight

A 2 metre transect of whole nuts was collected from each row, and the resulting nut weight and kernel weight measured and recorded.

Statistical Analysis

Analysis of variance was performed using Prism 7 (Graph Pad Software). Significant difference between the treatments was determined by comparing the replicate means using an unpaired t-test ($P<0.15$).

Results

Figure 1: A photo taken of almond trees treated with Re-Leaf (treatment) just before shaking.

Figure 2: A photo taken of control almond trees just before shaking. Note that trees are not as dense and seem to be sparse with their branches.

Figure 3: Almond tree treated with Re-Leaf.

Figure 4: Control almond tree in the Re-leaf trial.

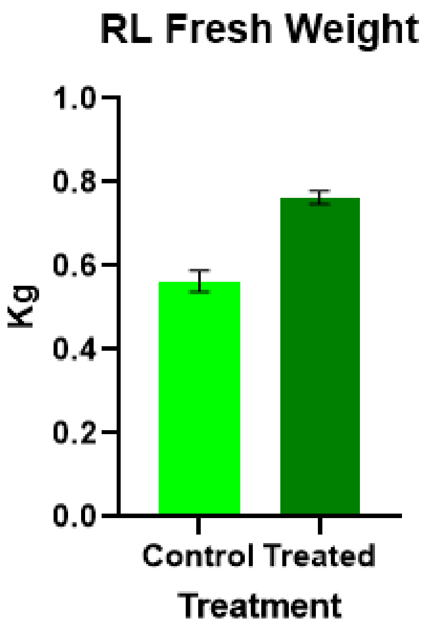

Figure 5: The average fresh weight of almonds collected from almond trees in the Re-Leaf Trial (P<0.15). Average weight of 200 nuts per row in both treated and control sections.

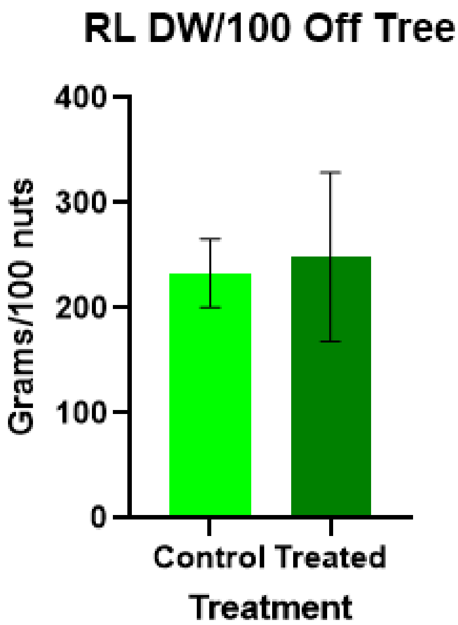

Figure 6: The average dry whole weight of 100 nuts collected from almond trees in the Re-Leaf Trial. (P<0.15)

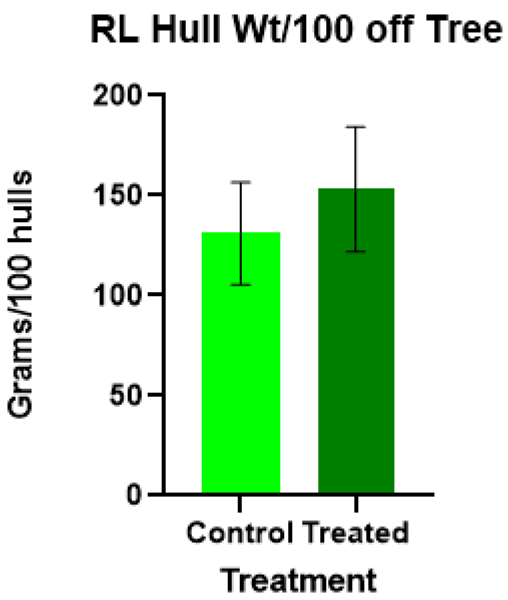

Figure 7: The average hull weight of 100 nuts collected from almond trees in the Re-Leaf Trial. (P<0.15)

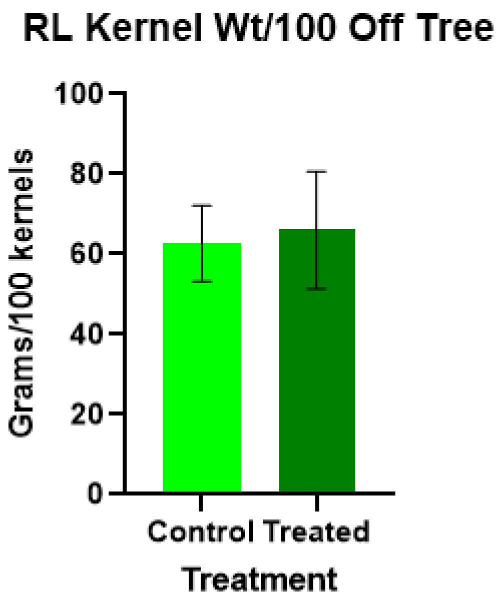

Figure 8: The average kernel weight of 100 nuts collected from almond trees in the Re-Leaf Trial. (P<0.15)

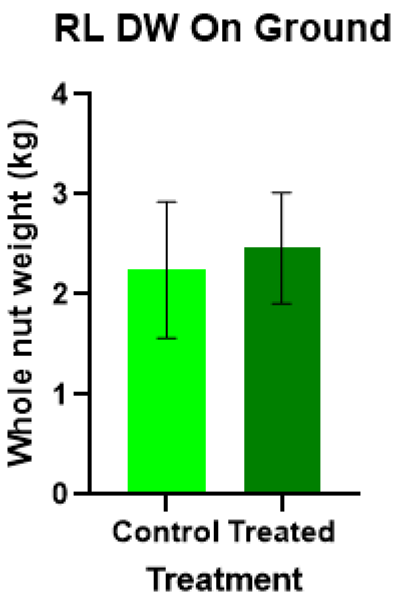

Figure 9: The average whole nut weight of nuts collected off the ground in a 2 meter transect. Nuts collected from almond trees in the Re-Leaf Trial after shaking. (P<0.15)

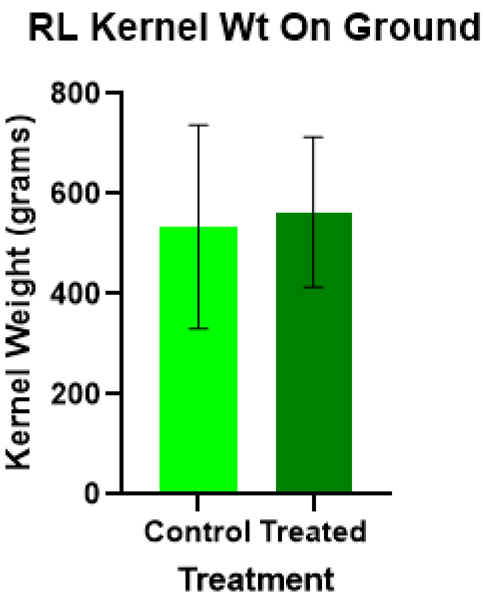

Figure 10: The average kernel weight of nuts collected off the ground in a 2 meter transect. Nuts collected from almond trees in the Re-Leaf Trial after shaking. (P<0.15)

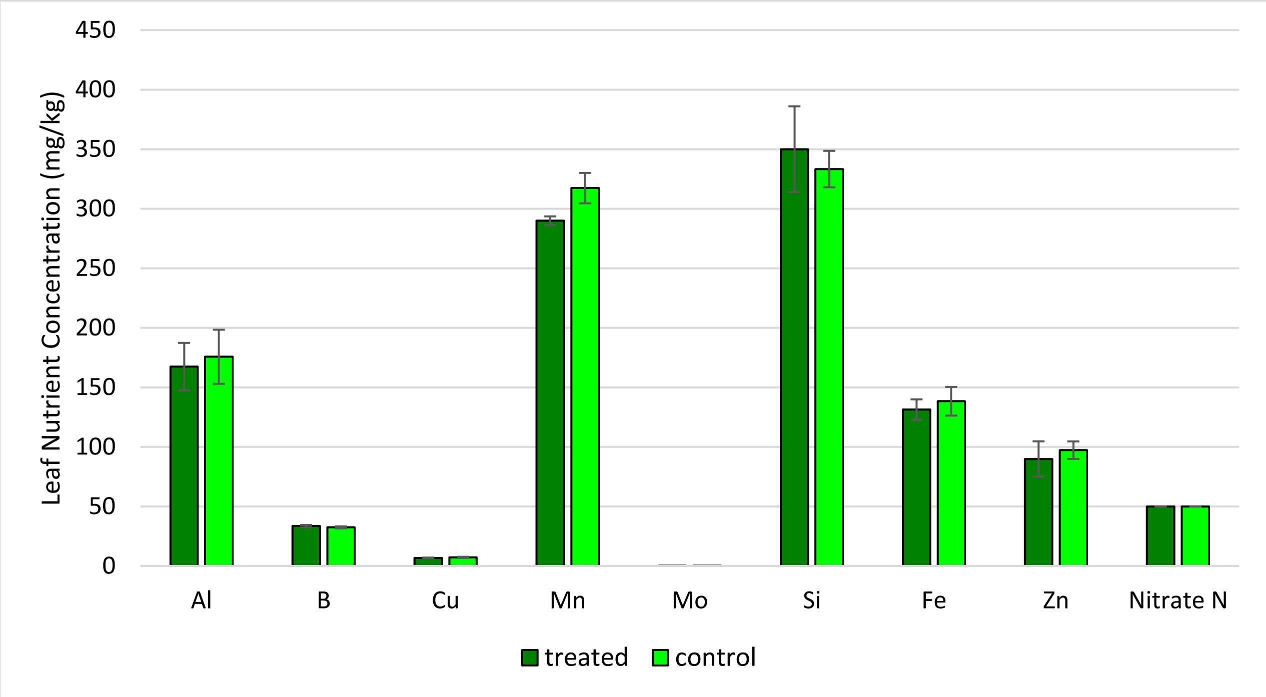

Figure 11: Leaf nutrient concentrations of almond leaves in the Re-Leaf trial. Measurements are taken in mg/kg. A t-test was performed to determine the significant difference between the control vs treated, different superscripts show significant difference (P<0.15). The t-test was performed with Prism 7 (Graph Pad Software).

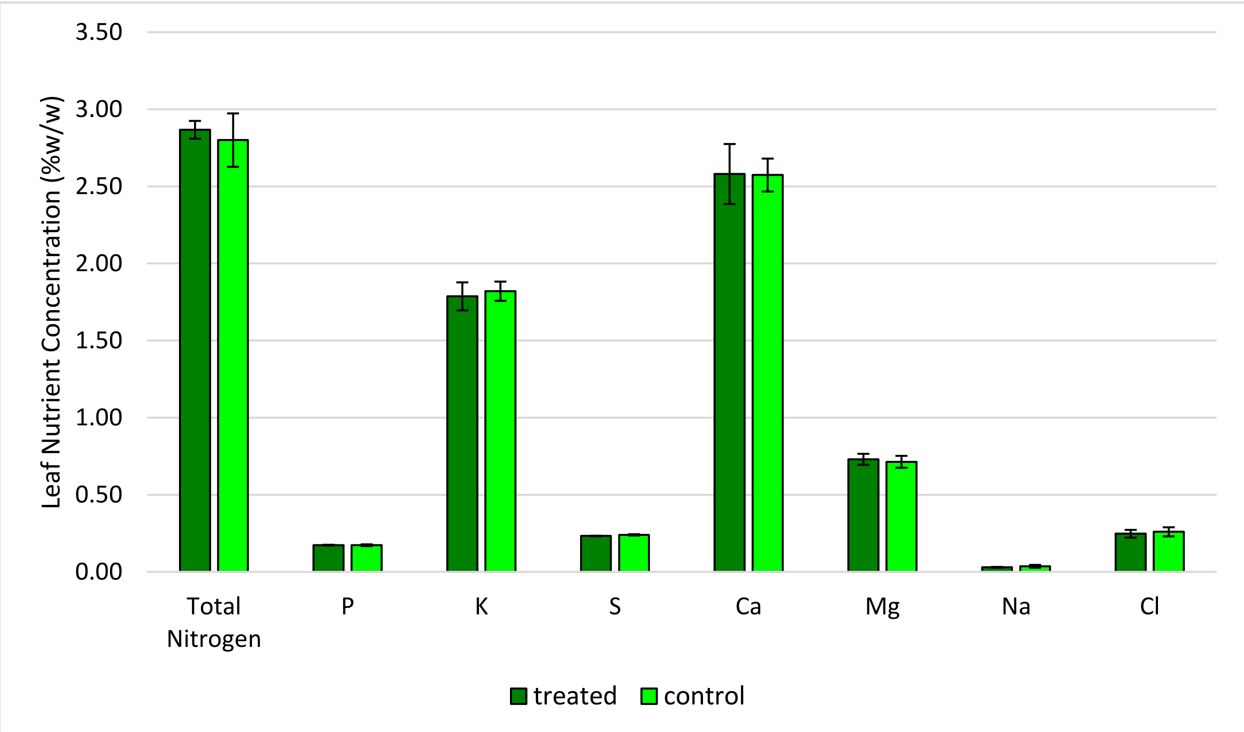

Figure 12: Leaf nutrient concentrations of almond leaves in the Re-Leaf trial. Measurements are taken in %w/w. A t-test was performed to determine the significant difference between the control vs treated, different superscripts show significant difference (P<0.15). The t-test was performed with Prism 7 (Graph Pad Software).

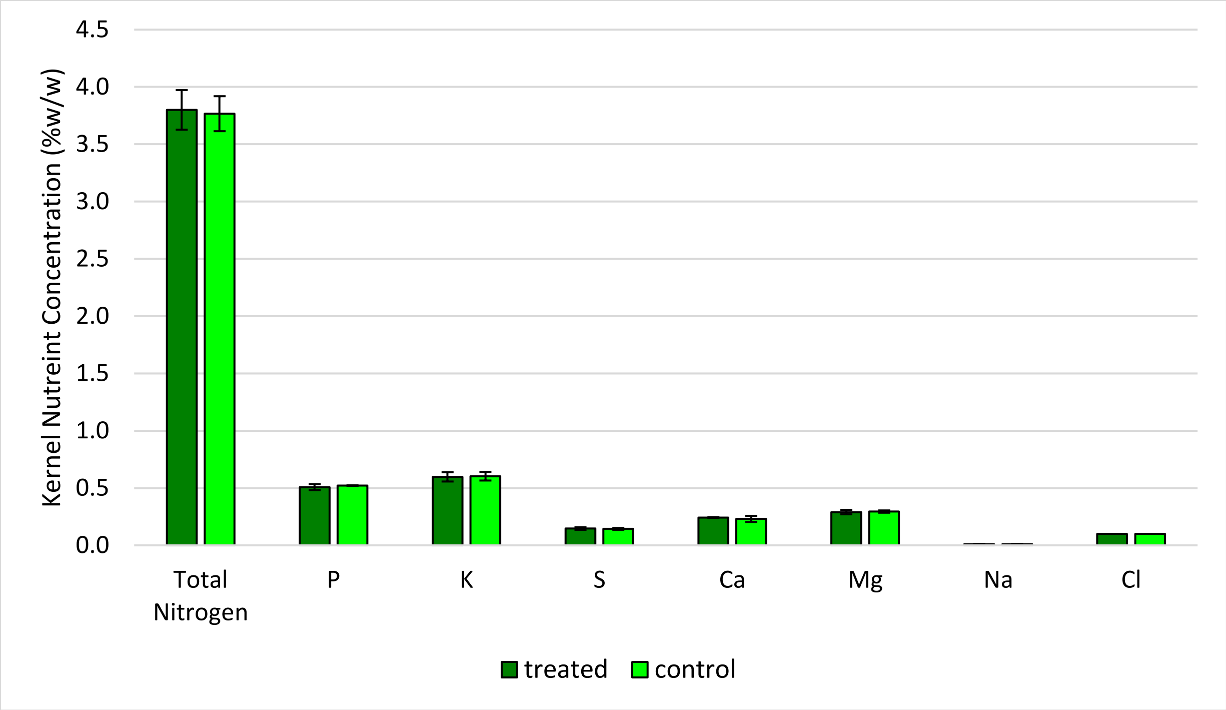

Figure 13: Kernel nutrient concentrations of almonds in the Re-Leaf trial. Measurements are taken in %w/w. A t-test was performed to determine the significant difference between the control vs treated, different superscripts show significant difference (P<0.15). The t-test was performed with Prism 7 (Graph Pad Software).

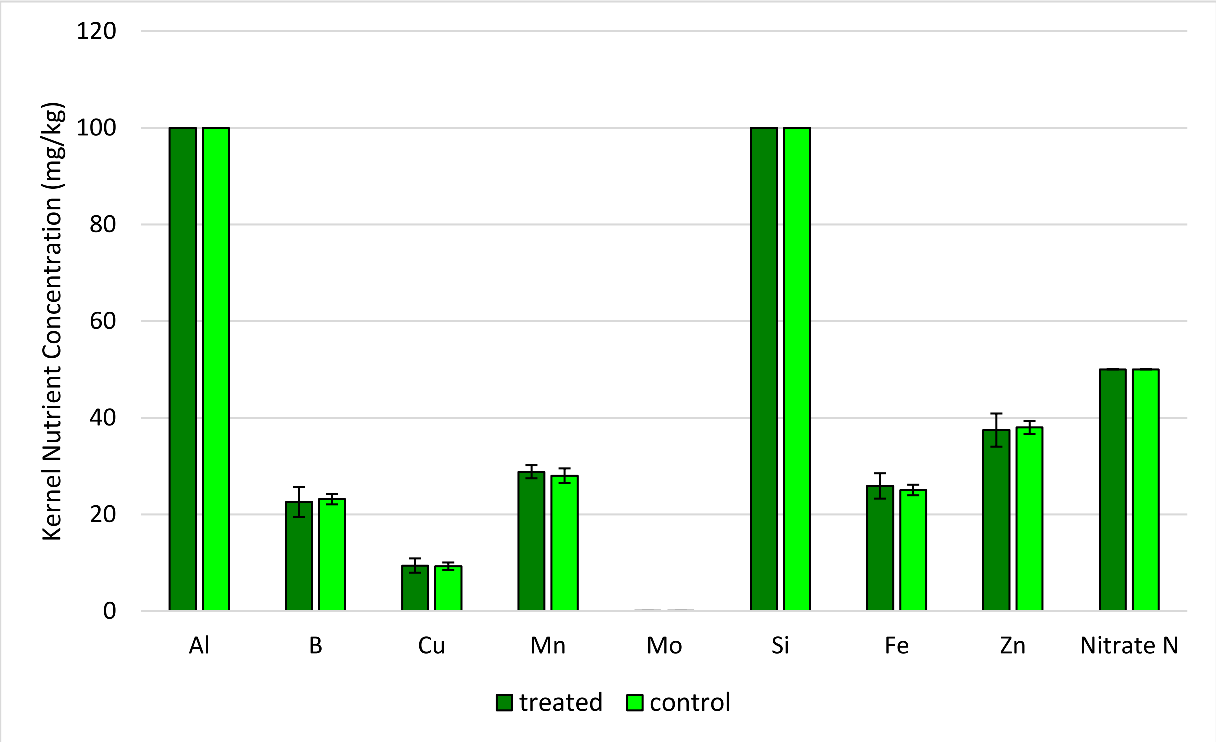

Figure 14: Kernel nutrient concentrations of almonds in the Re-Leaf trial. Measurements are taken in mg/kg. A t-test was performed to determine the significant difference between the control vs treated, different superscripts show significant difference (P<0.15). The t-test was performed with Prism 7 (Graph Pad Software).

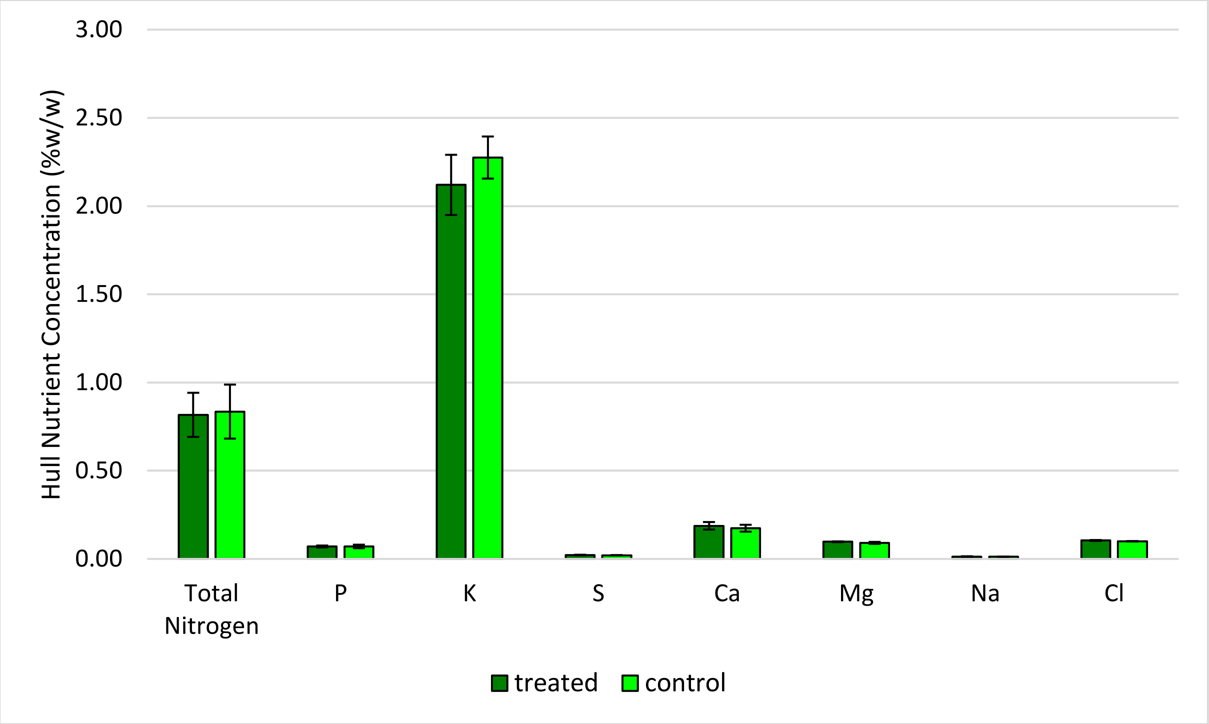

Figure 15: Hull nutrient concentrations of almonds in the Re-Leaf trial. Measurements are taken in %w/w. A t-test was performed to determine the significant difference between the control vs treated, different superscripts show significant difference (P<0.15). The t-test was performed with Prism 7 (Graph Pad Software).

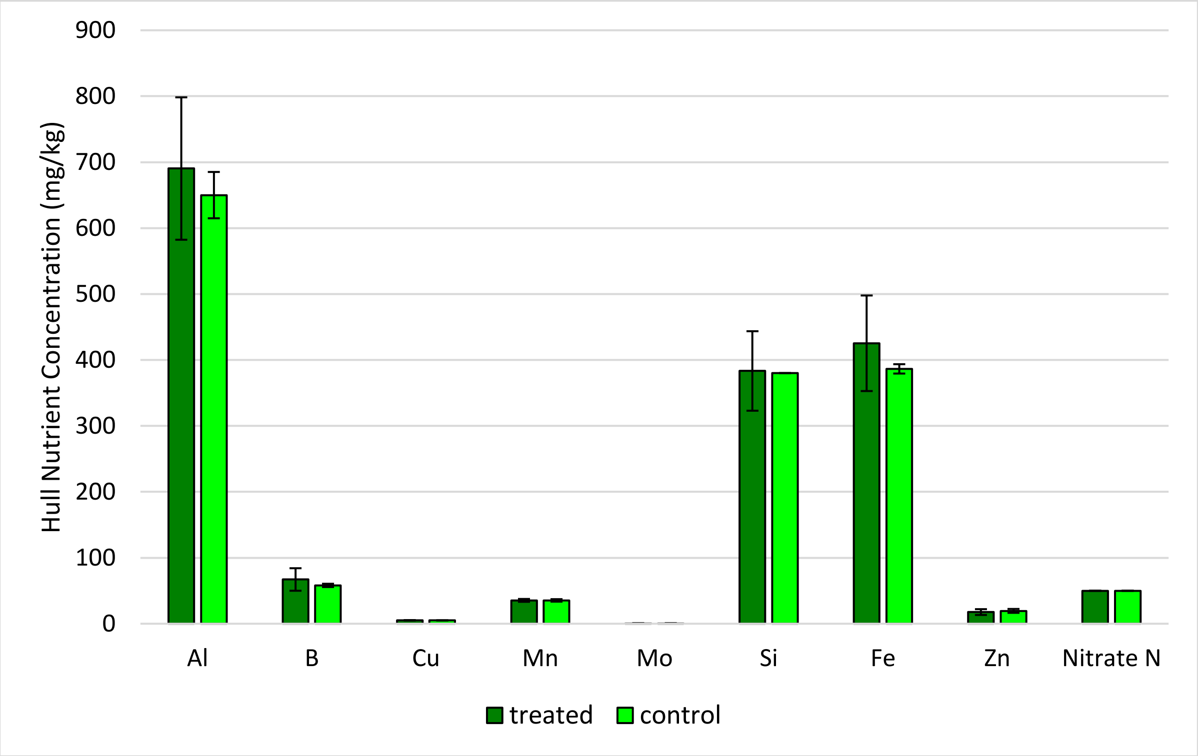

Figure 16: Hull nutrient concentrations of almonds in the Re-Leaf trial. Measurements are taken in mg/kg. A t-test was performed to determine the significant difference between the control vs treated, different superscripts show significant difference (P<0.15). The t-test was performed with Prism 7 (Graph Pad Software).

Table 2: The out-turn percentage of the control and treated almonds in the Re-Leaf trial.

Out-Turn %	
Control	26.86
Treated	26.54

Discussion

Re-Leaf is a fertiliser/bio-stimulant which is prominently made from amino acids and also some biologically active organic molecules (BAOM). These active ingredients work together to assist plants by increasing their stress tolerance to abiotic stresses such as soil salinity which this block has had a history.

When looking at the images highlighted figures 1 and 2, it can be seen that there is a visual difference between the amount of vigor between the Re-Leaf treated trees and the control trees. Re-Leaf treated trees (figure 1) had a fuller and denser canopy than the control trees. This indicates that Re-leaf treated trees had more vegetative shoots and spurs which could be correlated to the readily available amino acids. These amino acids assist in the production of growth regulators such as Auxin. BAOM also assist in the availability and movement of macro and micro nutrients around the plant which can be used to make carbohydrates and proteins which are also necessary for new growth and development.

Figure 3 and 4 also show the visual differences between the number of nuts on the treated and control trees. Almond trees treated with Re-Leaf (figure 3) visually had more nuts compared to the control trees shown figure 4. This can also be confirmed when looking at figure 9 which refers to the average whole nut weight in a 2-meter ground transect from treated and control trees. Figure 9 shows that control trees on average had a 2 meter transect weight of 2.25kg whereas treated trees had a 2 meter transect weight of 2.46kg which represents a percentage increase of 9.3%. Although the difference between the control and treated weights are not significant, it is an important variance to highlight.

Shortly before harvest, 200 nuts per row in both treatments were collected to measure their fresh weight, dry weight, hull weight and kernel weight which is represented in figures 5,6,7 and 8 respectively. In each of these figures, the treated trees produced nuts which were heavier than the control resulting in heavier hulls and kernels. Again, these differences were not significantly different except for figure 5 which shows that the fresh weights between the treated and control trees had a weight percentage increase of 41%. This is interesting to note as when looking at the kernel (figure 13 and 14) and hull (figures 15 and 16) nutrient analyses, there are no significant differences in the concentrations of elements between the two treatments. Therefore, the increased weight in the almond nut parameters for Re-Leaf treated trees is mostly likely a result of reduced stress through applications of Re-Leaf. When almond trees are less stressed, they are able to increase the production of endosperms and hulls resulting in heavier nuts.

Finally, when analyzing leaf nutrient concentrations presented in figures 11 and 12, there are no statistically significant differences between the control and treated almond trees. However, the trees treated with Re-leaf on average had higher levels of B, Si, total nitrogen, Mg and Ca. Again, even though there are limited nutrient concentration differences, on average the Re-leaf treated trees did produced heavier nuts and kernels overall. This could also be explained through the use of biologically active organic molecules and amino acids used in the manufacturing of Re-Leaf which assist in chelating nutrients and increasing transport of nutrients throughout the plant from where the nutrients are available to where they are required.

When comparing the out-turn percentages in table 2, there is no significant difference between the treatment and control out-turn. This calculation was derived using the dried whole nut weight and kernel weight from 100 nuts collected from the two treatments. The out-turn percentages are higher than industry standard which is most likely a result of an insufficient sampling size.

Conclusion

In conclusion this trial was conducted to assess the efficiency of Re-Leaf in improving almond nut production in areas of soil salinity. The results gathered from this trial demonstrate that through applications of Re-Leaf® at key growth stages in almond development, increases in the yield of almonds along with other parameters such as hull, kernel weight can be increased in soil effected by salinity.

When studying the figures presented it can be found that almond trees treated with Re-Leaf showed the following improvements:

- Enhanced tree vigor and tree fullness along with an increased density of nuts on treated trees.
- Increased fresh weight of almonds by 41% through the application of Re-Leaf.
- Increased the weights of hulls and kernels by 17% and 5.2% respectively through applications of Re-Leaf.
- Increased the yield of almonds collected in a 2-meter ground transect by 9% in rows treated with Re-Leaf compared to the control rows.
- Increased the leaf nutrient concentration of some elements such as Boron, Silicon, total Nitrogen, Calcium and Magnesium

References

Ali, Q., Athar, H., Haider, M., Shahid, S., Aslam, N., Shehzad, F., Naseem, J., Ashraf, R., Ali, A. and Hussain, S., 2019. Role of Amino Acids in Improving Abiotic Stress Tolerance to Plants. *Plant Tolerance to Environmental Stress*, pp.175-204.

Doll, D., 2016. *Managing Orchard Salinity*. Almond Board of California. Available at: <https://www.almonds.com/sites/default/files/managing_orchard_salinity_1_0.pdf>

Metcalfe D, Bui E (2016). Land: Soil: Salinity and acidification. In: Australia state of the environment 2016, Australian Government Department of the Environment and Energy, Canberra, https://soe.environment.gov.au/theme/land/topic/2016/soil-salinity-and-acidification, DOI 10.4226/94/58b6585f94911

Key Findings	Description
Increased denseness of canopy in the Re-Leaf treated almond trees	Trees treated with Re-Leaf had tree canopies which displayed a more filled out appearance and denser than the control which displayed a spindlier look (figure 1 and 2)
Trees treated with **Re-Leaf** displayed **more nuts** which were more evenly spread around the tree in comparison to the control trees.	Trees treated with Re-Leaf had more nuts on their trees and more evenly spaced out compared to the control trees which had less nuts (figure 3 and 4).
Re-Leaf treated trees had **heavier** nut **fresh weights** (significant), **dry weights**, **hull weights, kernel weights** and **more nuts** collected from a **2 meter transect.**	Trees treated with Re-Leaf had a: • Significant percentage increase of **36%** in the **fresh weight** of almonds (figure 5). • Percentage increase of **7%** in the **dry weight** of almonds (figure 6). • Percentage increase of **17%** in the **hull weight** of 100 almonds (figure 7). • Percentage increase of **5%** in the **kernel weight** of 100 almonds and almond collected from the ground (figure 8 and 10 respectively). • Percentage increase of **9%** in the weight of **nuts collected in a 2 meter transect** (figure 9).
Re-Leaf treated trees had an out-turn of **26.86%** and **control** trees had an out-turn of **26.54%.**	When comparing the out-turn differences between the Re-Leaf and control trees, there was not much of a significant difference between the treated or control out-turns (table 2).
Re-Leaf trees had a higher percentage increase of **total Nitrogen, P, Ca, Mg, B and Si** in the leaves of almond trees compared to the control trees.	Trees treated with Re-leaf contained higher concentrations of total nitrogen, phosphorus, calcium, magnesium, boron and silicon which can be explained through the amino acids and BAOM contained in Re-Leaf which act as organic chelating agents, assisting in the uptake of nutrients (figures 11 and 12).

Appendix 1. Statistical Analysis of Results

Table 1: Analysis of yield parameters with reference to control and treated (Re-Leaf) almond trees. Values are given mean ± standard deviation. P value <0.15 was considered to be statistically significant.

Parameter	Treatment		*P*-Value	Significance	% increase
	Control	Re-Leaf			
Fresh Whole Nut Weight (kg) **Figure 5**	0.563 ± 0.025	0.763 ± 0.015	0.0003	Yes	36.52
Dry Whole Nut Weight (g) (100 Nuts) **Figure 6**	233 ± 32.40	249 ± 80.50	0.775	No	6.87
Hull Weight (g) (100 Nuts) **Figure 7**	131 ± 25.60	153 ± 31.20	0.399	No	16.79
Kernel Weight (g) (100 nuts) **Figure 8**	62.7 ± 9.45	66 ± 14.7	0.758	No	5.26
Whole Nut weight - 1m transect (kg) **Figure 9**	2.25 ± 0.679	2.46 ± 0.555	0.691	No	9.33

Table 2: Analysis of different nutrient levels in the leaves with reference to Control and Re-Leaf. P value <0.15 was considered to be statistically significant.

Nutrient	**Treatment (Mean)**		***P* Value**	**Significance**	**% increase**
	Control	**Re-Leaf**			
Total Carbon (%w/w)	45.7	45.3	0.519	No	-0.73
Total Nitrogen (%w/w)	2.80	2.87	0.561	No	2.38
Phosphorus (%w/w)	0.173	0.173	0.931	No	0.19
Potassium (%w/w)	1.82	1.79	0.628	No	-1.83
Sulphur (%w/w)	0.240	0.232	0.0491	Yes	-3.06
Calcium (%w/w)	2.57	2.58	0.961	No	0.26
Magnesium (%w/w)	0.714	0.730	0.619	No	2.29
Sodium (%w/w)	0.0359	0.0292	0.293	No	-18.68
Aluminium (PPM)	176	167	0.658	No	-4.74
Boron (PPM)	32.5	33.5	0.256	No	3.08
Copper (PPM)	7.20	6.77	0.269	No	-6.02
Manganese (PPM)	317	290	0.0233	Yes	-8.61
Molybdenum (PPM)	0.500	0.500	N/A	N/A	0.00
Silicon (PPM)	333	350	0.502	No	5.00
Iron (PPM)	138	131	0.458	No	-5.06
Zinc (PPM)	97.2	89.9	0.485	No	-7.58
Nitrate N (PPM)	50.0	50.0	N/A	N/A	0.00
Chloride (%w/w)	0.259	0.247	0.611	No	-4.76