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

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Abstract

Soil salinity in Australian agriculture is the largest land degradation causing numerous problems for plant growth and development which results in abnormal physiological functions (Metcalfe and Bui, 2016). Almonds are considered a suspectable crop when it comes to soil salinity with large yield losses correlated to increases in soil salinity (Phogat et al., 2018). Applications of amino acids have been proven to have positive effects on the growth and production of almonds through accumulation of osmolytes (Sudmalis et al., 2018). These osmolytes assist in improving abiotic stresses in plants such 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 (Effect of Amino Acids on Plants, 2021). In this trial, young almond trees planted on previously salinity effected soil were treated with an amino acid and biologically complex formulation known as Re-Leaf® which is designed to assist stressed plants by providing useful and immediately available amino acids and also improving the availability of nutrients in the soil for improved tree nutrition promoting a healthier tree through chelation in the soil. It was found that almond trees treated with Re-Leaf® had denser and greener canopies compared to control trees, increased whole nut weight, hull weight and kernel weights (significantly higher), better out-turns and increased nutrition levels of available macro and micro elements in the soil and in plant tissues.

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

1. 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 (Brown, 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 (Cuevas et al., 2019). However, these management practices are not a 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 (48.2%) and biologically active organic molecules – BAOM (32.8%). 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 (Sudmalis et al., 2018).

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.

2. Objectives

The specific objectives of this trial were to:

1. Evaluate the effect of fertigation with Re-Leaf® in improving the nutrient status of almond trees growing on salt effected soil.
2. Evaluate the effect of Re-Leaf® in improving crop vigour and canopy growth of almonds.
3. Evaluate the effect of fertigation with Re-Leaf® on the yield parameters of almonds; nut weight, kernel weight and total yield.

3. 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 a history of 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 affected by the soil salinity issues.

Two applications of Re-Leaf® was applied via scheduled fertigation across the trial site, with ten trees from three rows isolated using drip line taps which were considered the control plants. Ten trees from three adjacent rows were considered the treated trees and received applications of Re-Leaf®.

Table 1 highlights the application times and rates of Re-Leaf® applied at 2 key stages of the almond growing season at post flowering and at the fruit development stage.

Table 1: Application dates and rates of Re-Leaf®

Treatment	Application Date	Application rate (L/Ha)
Re-Leaf®	23/09/20 – Post Flowering	30 L/Ha
Re-Leaf®	14/10/20 – Fruit Development	30 L/Ha

4.Observations

Soil Nutrient Analysis

Soil samples (30cm deep) were taken in mid-January 2021 just prior to the beginning of commercial almond harvesting as requested by the orchards technical agronomist following correct soil sampling techniques. Soil samples were then sent to the Australian Precision Ag Laboratory (APAL) for a full soil nutrient profile analysis. See figure 1 for images of soil samples collection. The results were then analysed using GraphPad Prism software to determine any significant differences between the treatments.

Figure 1: Images of soil sampling for the Re-Leaf® trial. Soil samples were taken 30cm deep and sent to APAL for analysis.

Leaf Nutrient Analysis

Leaf samples were taken in mid-January 2021 just prior to the beginning of almond harvesting as requested by the orchards technical agronomist following correct leaf sampling techniques. 10 leaves from each tree/row were collected and the samples were then expressed posted to APAL for a full leaf nutrient analysis. See figure 2 for images of leaf collection. The results were then analysed using GraphPad Prism software to determine any significant differences between the treatments.

Figure 2: Images of leaf sampling. Leaves were taken from non-fruiting spurs at the 3rd leaf. Samples were sent to APAL for analysis.

Whole Nut weight, hull weight and hull nuts from nuts collected before harvest

Before commercial harvest, 10 nuts per tree (100 nuts per row) were collected from the trial blocks to get whole nut weight, hull weight and kernel weights. This was done to compare the out-turns calculated from nuts collected on the tree and also nuts collected at harvest from the ground. This data was also collected to compare weight between each component of the nut.

Nut collection at harvest for field weight (kg of nuts/tree)

Once the trees had been shaken and the nuts were on the ground, all the nuts from 18 trees (9 trees from Re-Leaf® rows and 9 from the control rows) were raked into rows, sifted using a slatted shovel and then weighed. This provided data on the quantity (kg) of nuts per tree. Trees which have similar canopy densities were chosen to weight nuts from. Figure 3 shows the methods used to gather the field weight data. A small sample of approximately 500 grams of nuts were also collected from each tree to make final out-turn calculations.

Figure 3: Images of nuts collected from shaken trees. Nuts were sifted to remove leaves, sticks and dirt then weighed to provide kg of nuts/tree.

Out-turn calculations

Out-turns are calculated to determine the percentage of kernel in a whole almond nut. The higher the percentage, the heavier the kernels are. Out-turns are crucial to determine profits made on almond orchards. In this trial, out-turns were calculated from almond nuts collected prior to harvest and also during harvest. Out-turns are calculated using the following equation:

Out-Turn% = (Kernel Weight/Whole Nut Weight) x 100

Statistical analysis

Statistical analyses (t-test and multiple t-tests) were done using GraphPad Prism 9. Significant difference ($P<0.15$) between treatments was determined by comparing the replicate means. Error bars were also used on graphs.

5. Results

Figure 4: Drone image of the block treated with Re-Leaf®. Rows with red rectangles were treated with applications of Re-Leaf® and rows with yellow rectangles were control rows.

Figure 5: A photo taken of almond trees treated with Re-Leaf® before harvest.

Figure 6: A photo taken of a control row before harvest which is adjacent to the Re-Leaf® treated row.

Figure 7: Almond tree treated with Re-Leaf® before harvest.

Figure 8: Control almond tree in the Re-Leaf® trial.

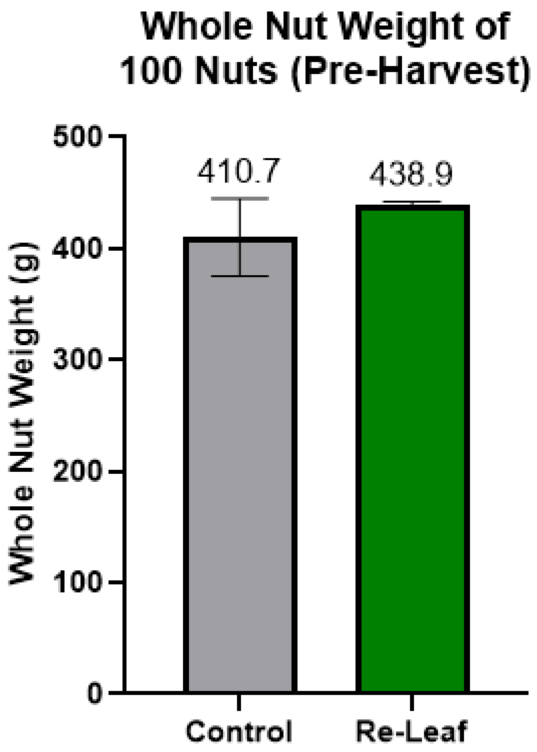

Figure 9 : The average whole nut weight of 100 nuts collected from almond trees in the Re-Leaf® trial. Nuts were picked randomly from trees.

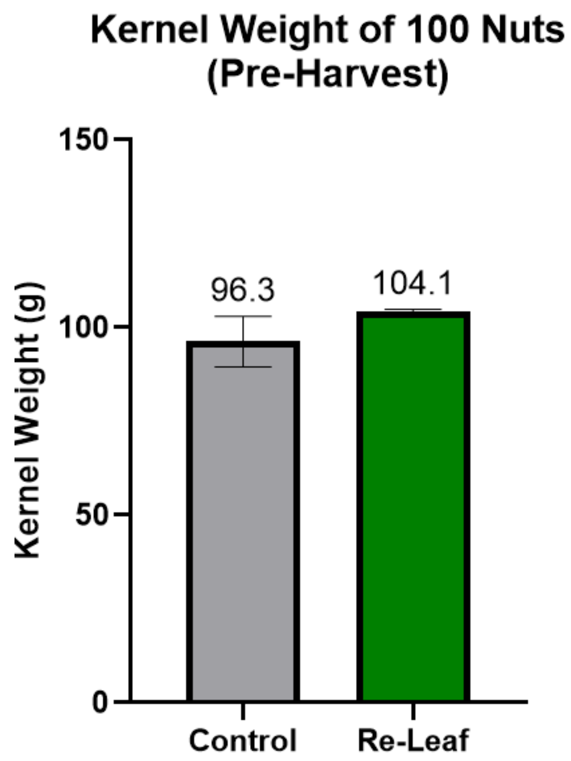

Figure 11: The average kernel weight of 100 nuts collected from almond trees in the Re-Leaf® trial. Nuts were picked randomly from trees. Significant difference (P<0.15).

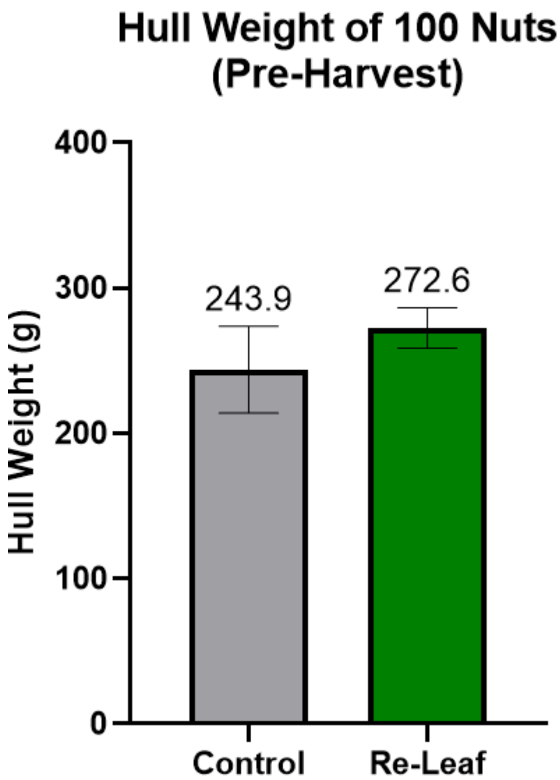

Figure 10: The average hull weight of 100 nuts collected from almond trees in the Re-Leaf® trial. Nuts were picked randomly from the trees.

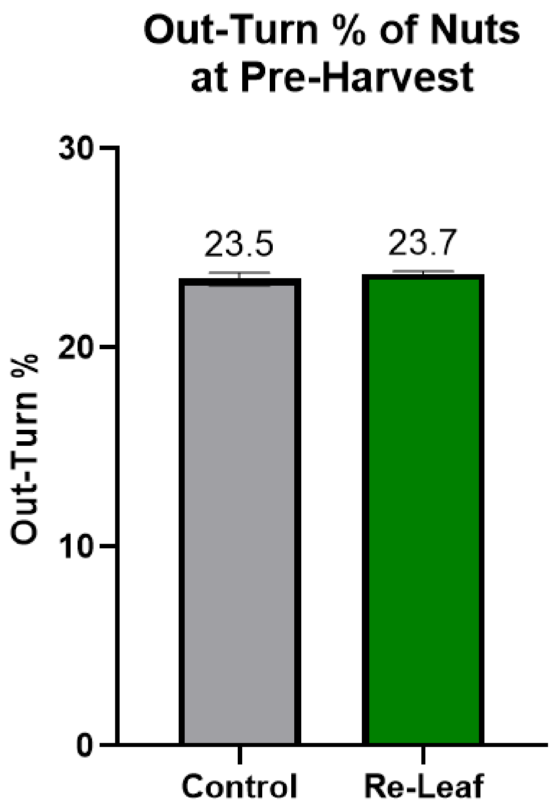

Figure 12: Out-turn % of nuts collected on the trees before harvest began.

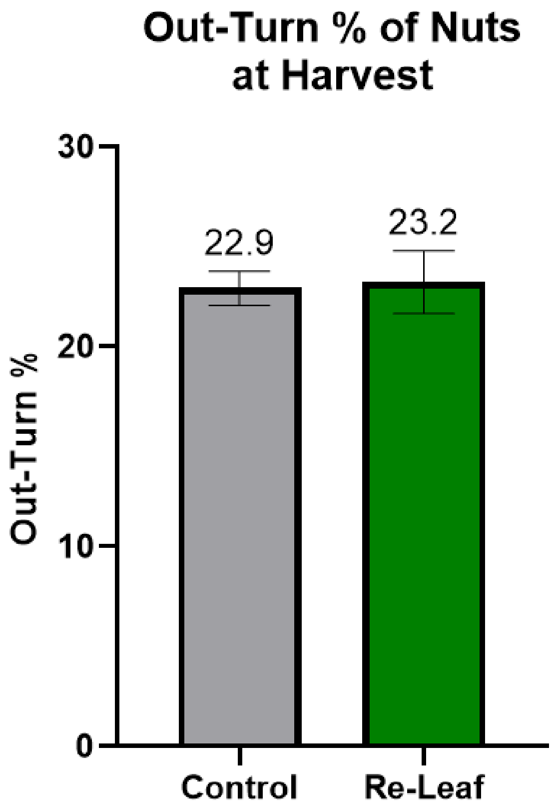

Figure 13: Out-turn % of almonds at harvest.

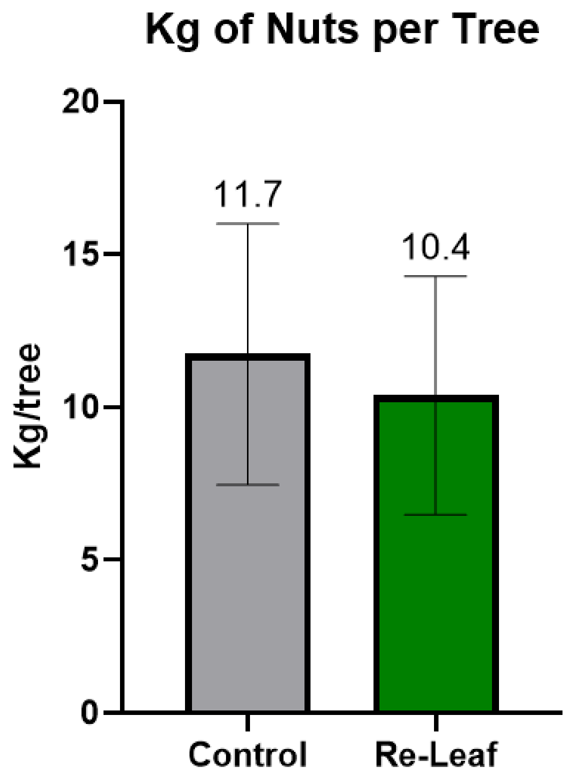

Figure 14: The average quantity of nuts per tree between Re-Leaf® and control trees.

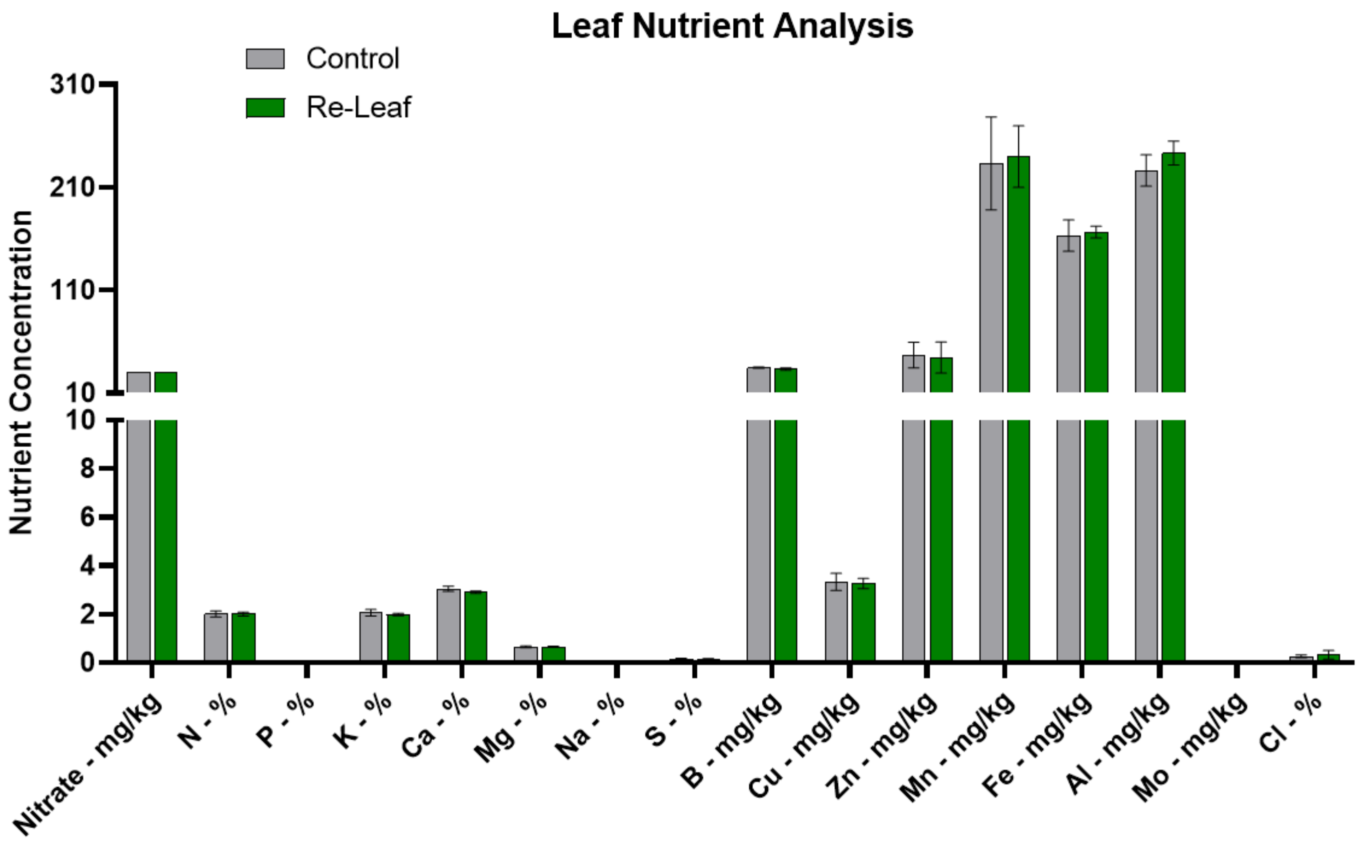

Figure 15: Leaf nutrient analysis of control almond trees and Re-Leaf® treated almond trees. Leaf samples were taken in mid-January 2021 in accordance with correct leaf sampling times. Samples were analysed by APAL.

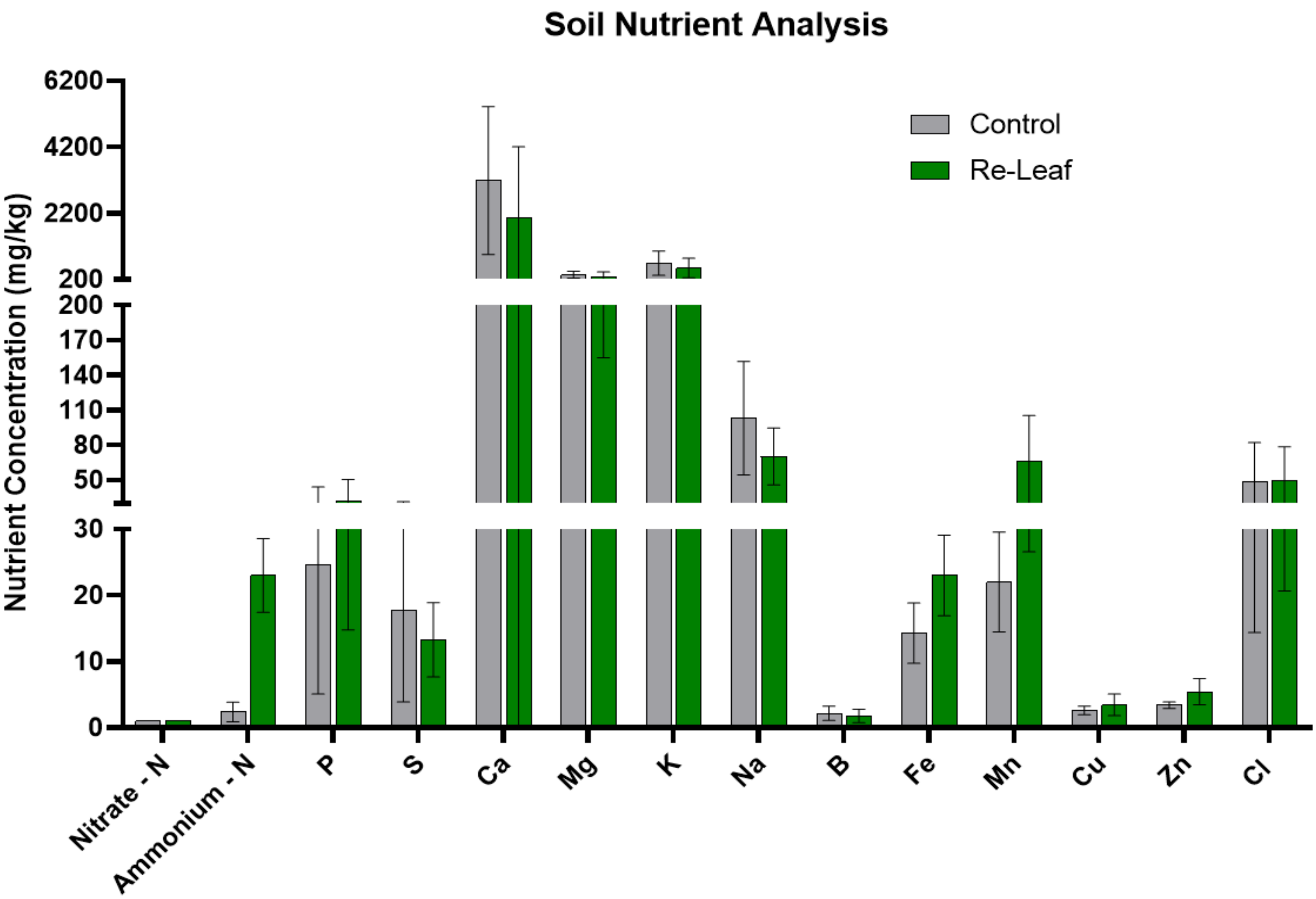

Figure 146: Soil nutrient analysis from control almond trees and Re-Leaf® treated almond trees. Samples were analysed by APAL

6. 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. The amino acids and BAOM also improve the availability of nutrients in the soil through natural chelation and also improves root growth increasing the uptake of nutrients in the soil.

Image Comparisons

Figure 4 shows a drone image of the trial block with rows highlighted in red rectangles were treated with a total of 60L/ha of Re-Leaf®. The rows highlighted with yellow rectangles were the control rows which were isolated using manual isolation taps on the drip lines to prevent any Re-Leaf® from being applied to these areas. When examining this image, it is difficult to notice any differences between then treated and control rows, however subtle differences can be seen when comparing figure 5 and 6 which show a control and treated row which are adjacent to one another.

When comparing figures 5 and 6, it can be seen that the almond trees treated with Re-Leaf® (figure 5) had a much denser canopy compared to the control trees seen in figure 6. The trees treated with Re-leaf® also seemed to have greener leaves than the control trees which has a slight yellow tinge to them. This increase in canopy density could be from the amino acids which 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 protein which are also necessary to support new growth.

Figure 7 and 8 also show similar observations with the almond trees treated with Re-Leaf® having a denser canopy with more vegetative growth compared to the control tree seen in figure 8. Having more vegetative growth is very beneficial as the photosynthetic capacity of the tree is also increased which provides the plant with sugars and energy required to grow more almonds.

Pre- Harvest Analysis of Nuts

Figures 9 to 12 show multiple different analyses done on almond nuts collected from the trees before harvest. Whole nut weight, hull weight, kernel weight and out-turn % (pre-harvest) measurements and calculations were made to see if there were any differences in weight from nuts collected on the tree and nuts collected at harvest. These nuts were collected off the tree shortly before harvest to minimise and moisture differences.

100 nuts from each row were collected and measured and it was found that almond trees treated with Re-Leaf® produced heavier nuts compared to control nuts. Figure 9 shows that trees treated with Re-Leaf® produced nuts which were 6.1% heavier than the control nuts. Since the whole nut weight was heavier, the hull and kernels weights of treated nuts were also increased by 11.8% and 8.1% respectively. When statistically analysing these results, it was found that trees treated with Re-Leaf® produced kernels which were statistically heavier than the control trees which is highlighted in figure 11. This increased nut growth is due to the amino acids provide by Re-Leaf®. Since amino acids are provided directly to the plant by Re-Leaf®, the plants don't have to expel energy to synthesise amino acids from the primary elements. By providing these amino acids directly to the plant, they can be used to make more proteins from the amino acids. These proteins can be used all over the plant system to improve function, increase yields and increase the overall quality of the almond trees. Re-Leaf® also contains amino acid nitrogen which can also be directly used to support new cell growth.

Since the kernel weights were higher in the treated trees compared to the control, the out-turn % (figure 12) was also higher for trees treated with Re-Leaf® compared to the control. Although it is only a slight increase, higher out-turn % are also extremely important as it relates directly back to return on investments.

Harvest Analysis of Nuts

Figures 13 and 14 both show the data collected from the almond trees at harvest time. Once the trees had been shaken, all the nuts around 18 trees were swept using a hand rake and each trees total kg of nuts was weighed. The trees on the trial block are young and therefore there are a lot of weeds under and surrounding the trees. It was therefore quite difficult to rake all the nuts up from under each tree and many nuts would get caught in the weeds making it hard to retrieve them. This could explain why the average kg/tree for the Re-Leaf® is lower than the control shown in figure 14. Each tree had a small sample collected at harvest and out-turn % calculations were then done. Figure 13 shows the out-turn percentages of the treated and control trees at harvest and it was calculated that control trees had an out-turn 22.9% and treated trees had an out-turn % of 23.2% which is a percentage increase on 1.3%. This increase in out-turn suggests that trees treated with Re-Leaf® produced kernels which made up a higher ratio of the whole nut weight compared to control trees.

This again can be explained through the concentrated amino acids provided by Re-Leaf®. The amino acids have many effects on plants, not only do they provide amino acids for protein synthesis, but also improve the rate of photosynthesis which causes more carbohydrate synthesis for energy, chelate micronutrients for faster absorption and transportation inside the plant and beneficial plant hormones production is induced through methionine, tryptophan and arginine (Effect of Amino Acids on Plants, 2021). Together, these key plant processes all help in enhancing crop yields and boost production.

Leaf and Soil Nutrient Analysis

Figures 15 and 16 show graphs of the leaf and soil concentrations respectively. In the leaf nutrient analysis shown in figure 15, it was found that trees treated with Re-Leaf® had higher levels of Magnesium, Sulphur, Manganese, Iron and Molybdenum compared to the control.

In the soil nutrient analysis shown in figure 16, it was found that soil that was treated with Re-Leaf® had higher levels of Ammonium N (significantly higher), Phosphorus, Iron (significantly higher), Manganese (significantly higher), Copper and Zinc compared to the control.

These higher levels of nutrients in the leaf tissue and soil are a result of the amino acids and BAOM in Re-Leaf® which act as natural chelating agents and improve the absorption and transportation of nutrients within the plant. This increase in nutrition observed in the trees treated with Re-Leaf® correlates directly to the yield and out-turn increases highlighting the importance of providing biological molecules and amino acids in improving plant growth and yields.

Soil Salinity Analysis

When comparing the sodium concentration levels in soil between the treated and control blocks, it was found that soil treated with Re-Leaf® had less sodium in the soil compared to untreated soil (table 2). It was calculated that after applications of Re-Leaf®, there was approximately 32% less sodium in soil treated with Re-Leaf® compared to the control soil. The reason why there is a decrease in the sodium concentration in soil treated with Re-Leaf® is because the amino acids have the ability to chelate the sodium ions and make these ions easier to leach out of the soil. It has also been researched that other organic acids such as humic acid can significantly reduce the soil sodium concentration due to the fact that humic acids can absorb significant amounts of water which can dilute the salt which also facilitates the leaching of the soluble salts (Kenawy, 2017).

Table 2: The soil sodium differences between soil treated with Re-Leaf and the untreated soil.

Treatment	Sodium in Soil
Re-Leaf®	70.33 mg/kg
Control	103.00 mg/kg

In conclusion this trial was conducted to evaluate how soil applied applications of Re-Leaf® effected soil salinity and the positive effects that Re-Leaf® has on tree health and yield increases. Many parameters were considered and measured when coming to this conclusion including visual tree analyses, nut analyses before and after harvesting and also analysing out-turns between treated and control trees which relate directly to the orchards return on investment.

When studying the figures presented, it can be found that almond trees treated with applications of Re-Leaf® had the following results:

- Trees treated with Re-leaf® had a denser canopy and greener leaves than the control trees due to amino acids proving the precursor for auxin development.
- At pre-harvest, trees treated with Re-leaf® had higher whole nut, hull, and kernel weights compared to the control which have percentage increases of 6.1%, 11.8% and 8.1% respectively.
- At harvest, trees treated with Re-Leaf® produced higher out-turns compared to control trees with a percentage increase of 1.3% (22.9% vs 23.2%).
- Leaves collected prior to harvest showed higher levels of Magnesium, Sulphur, Manganese, Iron and Molybdenum in Re-Leaf® treated trees compared to the control trees.
- Treated soil collected prior to harvest showed higher concentrations of Ammonium N (significantly higher), Phosphorus, Iron (significantly higher), Manganese (significantly higher), Copper and Zinc compared to the control soil.

8. References

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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	% Change
	Control	Treated (Re-Leaf®)			
Whole Nut Weight (g) (100 nuts) Pre-Harvest **Figure 9**	410.7 ± 34.84	438.9 ± 3.78	0.235	No	6.87
Hull Weight (g) (100 Nuts) Pre-Harvest **Figure 10**	243.9 ± 29.94	272.6 ± 13.99	0.207	No	11.77
Kernel Weight (g) (100 Nuts) Pre-Harvest **Figure 11**	96.25 ± 6.77	104.1 ± 0.72	0.116	Yes	8.16
Out-turn % of Nuts Pre-Harvest **Figure 12**	23.45 ± 0.32	23.72 ± 0.12	0.248	No	1.15
Out-turn % of Nuts Harvest **Figure 13**	22.94± 0.86	23.24± 1.56	0.615	No	1.31
Kg of Nuts per Tree (kg) Harvest **Figure 14**	11.74 ± 2.28	10.39 ± 3.90	0.492	No	11.5

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

Nutrient	Treatment (Mean)		*P* Value	Significance	% Change (Control to Treated)
	Control	Treated (Re-Leaf®)			
Nitrate N (mg/kg)	30.00	30.00	-	N/A	0.00
Nitrogen (%)	2.0070	2.00	0.971	No	-0.17
Phosphorus (%)	0.10	0.097	0.0602	Yes	-3.00
Potassium (%)	2.060	1.98	0.404	No	-3.72
Calcium (%)	3.047	2.91	0.0975	Yes	-4.60
Magnesium (%)	0.65	0.65	0.899	No	0.51
Sodium (%)	0.031	0.032	0.881	No	4.30
Sulphur (%)	0.16	0.16	-	N/A	0.00
Boron (mg/kg)	34.67	33.33	0.147	Yes	-3.85
Copper (mg/kg)	3.33	3.267	0.791	No	-2.00
Zinc (mg/kg)	47.00	44.67	0.847	No	-4.96
Manganese (mg/kg)	233.33	240.0	0.842	No	2.86
Iron (mg/kg)	163.33	166.70	0.742	No	2.04
Aluminium (mg/kg)	226.67	243.30	0.206	No	7.35
Molybdenum (mg/kg)	0.048	0.051	0.820	No	5.56
Chloride (%)	0.26	0.32	0.572	No	25.97

Table 3: Analysis of different soil nutrient levels and properties in the Re-Leaf® trial. P value <0.15 was considered to be statistically significant.

Nutrient	Treatment (Mean)		*P* Value	Significance	% Change (Control to Treated)
	Control	Treated (Re-Leaf®)			
pH 1:5 Water	7.79	7.16	0.548	No	-8.21
pH CaCl2	7.23	6.56	0.547	No	-9.18
Organic C (%)	0.57	0.94	0.0225	Yes	65.29
Nitrate – N (mg/kg)	1.00	1.00	-		0.00
Ammonium – N (mg/kg)	2.38	23.00	0.00343	Yes	871.83
Colwell P (mg/kg)	24.67	32.67	0.629	No	32.43
Sulphur (mg/kg)	17.70	13.27	0.634	No	-25.05
Calcium (mg/kg)	3187.00	2059.00	0.563	No	-35.39
Magnesium (mg/kg)	341.70	292.00	0.639	No	-14.54
Potassium (mg/kg)	689.00	540.30	0.615	No	-21.58
Sodium (mg/kg)	103.00	70.33	0.356	No	-31.74
Boron (mg/kg)	2.16	1.77	0.664	No	-18.34
Iron (mg/kg)	14.30	23.00	0.118	Yes	60.84
Manganese (mg/kg)	22.00	66.00	0.130	Yes	200.00
Copper (mg/kg)	2.60	3.47	0.440	No	33.33
Zinc (mg/kg)	3.40	5.43	0.159	No	59.80
Ca:Mg ratio	5.27	3.57	0.538	No	-32.28
K:Mg Ratio	0.59	0.56	0.750	No	-6.18
ECEC (cmol/kg)	20.92	14.37	0.557	No	-31.31
Chloride (mg/kg)	48.33	49.67	0.961	No	2.76

Salinity EC 1:5 (dS/m)	0.17	0.17	-	N/A	0.00
(Ece dS/m)	2.30	2.50	0.780	No	8.70
Clay %	16.33	14.30	0.748	No	-12.45
Sand (+20 micron) %	77.33	78.33	0.910	No	1.29
Silt (2-20 micron) %	6.33	7.43	0.714	No	17.37