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Seasol Irrigation Rate Trial

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Disclaimer

Executive Summary

This study investigated the effect of Seasol, a seaweed extract, on lettuce growth and water use efficiency under different irrigation regimes. The results showed that water-stressed treatments produced reduced yields compared to standard irrigation treatments, indicating successful manipulation of plant growth through irrigation volumes. The application of Seasol improved the water use efficiency of lettuce plants, as indicated by increased fresh and dry lettuce head weights under 60% irrigation. Without Seasol applied, there was a statistically significant ($p<0.05$) drop in fresh weight of 30.8g or 20.5% from standard 100% irrigation rate to 60% irrigation rate. However, there was a non-statistically significant drop in fresh weight of 4.2g or 2.8% from standard irrigation rate without Seasol to 60% irrigation rate with Seasol. The treated lettuce also had longer, heavier roots and a consistently higher chlorophyll content compared to untreated lettuce, indicating improved drought resistance.

The post-trial leaf tests showed lower concentrations of nitrate and total nitrogen in Seasol treated lettuce under water stress, likely due to nutrient dilution. Overall, the results suggest that Seasol treatment can counteract the negative effects of water stress on lettuce growth. These findings are consistent with previous research on the use of seaweed extracts to improve plant growth under drought stress.

Introduction

A water use efficiency trial was commissioned by Seasol to collect data on the effect of Seasol on plant yield and quality at different irrigation rates. The trial was conducted on cos lettuce in a pot trial at the Bosch glasshouse on the Sydney University campus from January to March 2023.

Methodology

Soil Collection and Analysis

Soil was collected from a commercial farming operation in Cobbity, NSW, which is on the Southwestern fringe of Sydney.

Two separate 3m x 2m areas (Figure 1, Figure 2) were sectioned off with plastic makers and 20 soil cores from were collected from each area, which were bulked and underwent representative soil analysis. Soil test results are attached as Appendix 1 and fertiliser recommendations are attached as Appendix 2.

Figure 1: Soil collection area - site 1. This soil was used for the Seasol trial.

Figure 2: Soil collection area - site 2. This soil was a backup and was not used for the Seasol trial.

Site 1 was selected as the most appropriate based on soil analysis and fertiliser recommendations. 1800kg of soil was collected on 10 January 2023 and promptly transferred to a glasshouse room (Figure 3). Soil was thoroughly mixed as it was moved into the glasshouse.

Figure 3: Soil transferred to a glasshouse room prior to potting.

Glasshouse Sanitation

Two glasshouse rooms were thoroughly cleaned and sanitised with 70% (w/v) ethanol on 09 January 2022 (Figure 4).

Figure 4: Glasshouse cleaned and sanitised prior to trial setup.

Pot Preparations and Treatment Applications

Soil was transferred to a clean wheelbarrow, thoroughly mixed again, before 2300g was weighed into to each pot, which filled pots to 11cm (Figure 5)

Pot dimensions: 14cm square x 22cm high.

The standard fertiliser recommendation was for 400 kg/ha Nitrophoska, which equates to 800mg per pot.

An additional 1900g of soil was weighed into a bucket, mixed with the appropriate amount of fertiliser (Figure 6) and transferred to each pot which filled pots an additional 10cm, which simulates the typical pre-plant fertiliser application depth.

400 cos lettuce seedlings (Leppington Speedy Seedlings) were screened, and any atypical plants were discarded. All Seedlings were carefully transplanted by hand to all pots in a random order (Figure 7).

Seasol was labelled with batch number 2226-SR-22234 and manufactured on 25/11/2022 (Figure 8). The Seasol treatment rate is 10L/ha per fortnight of retail grade Seasol, which is half the strength of Commercial grade Seasol. This equates to 5 L/ha per fortnight of commercial grade Seasol. Retail grade Seasol was diluted at the label rate to 30ml/9L (which equates to 3.33ml Seasol/L) (Figure 9) and 6ml of solution was applied via pipette as a soil drench to each applicable seedling (Figure 10).

Figure 5: Re-mixing soil and filling pots to 11cm with 2300g of soil.

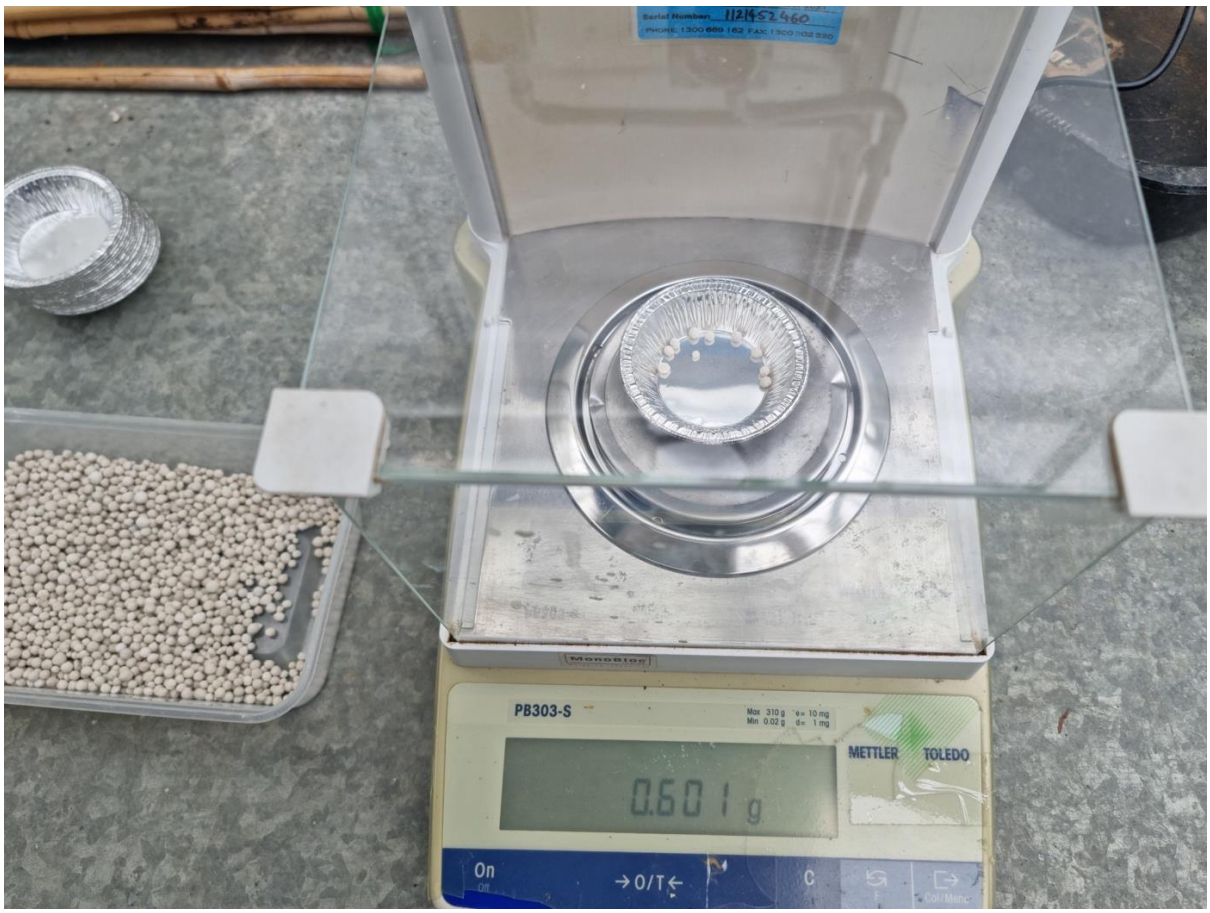


Figure 6: Fertiliser accurately weighed into aluminium trays.

Figure 7: Fertiliser prepared for mixing with soil.

Figure 8: Soil weighed to 1900g in preparation for fertiliser application.

Figure 6: Fertiliser added soil prior to mixing and final filling of pots.

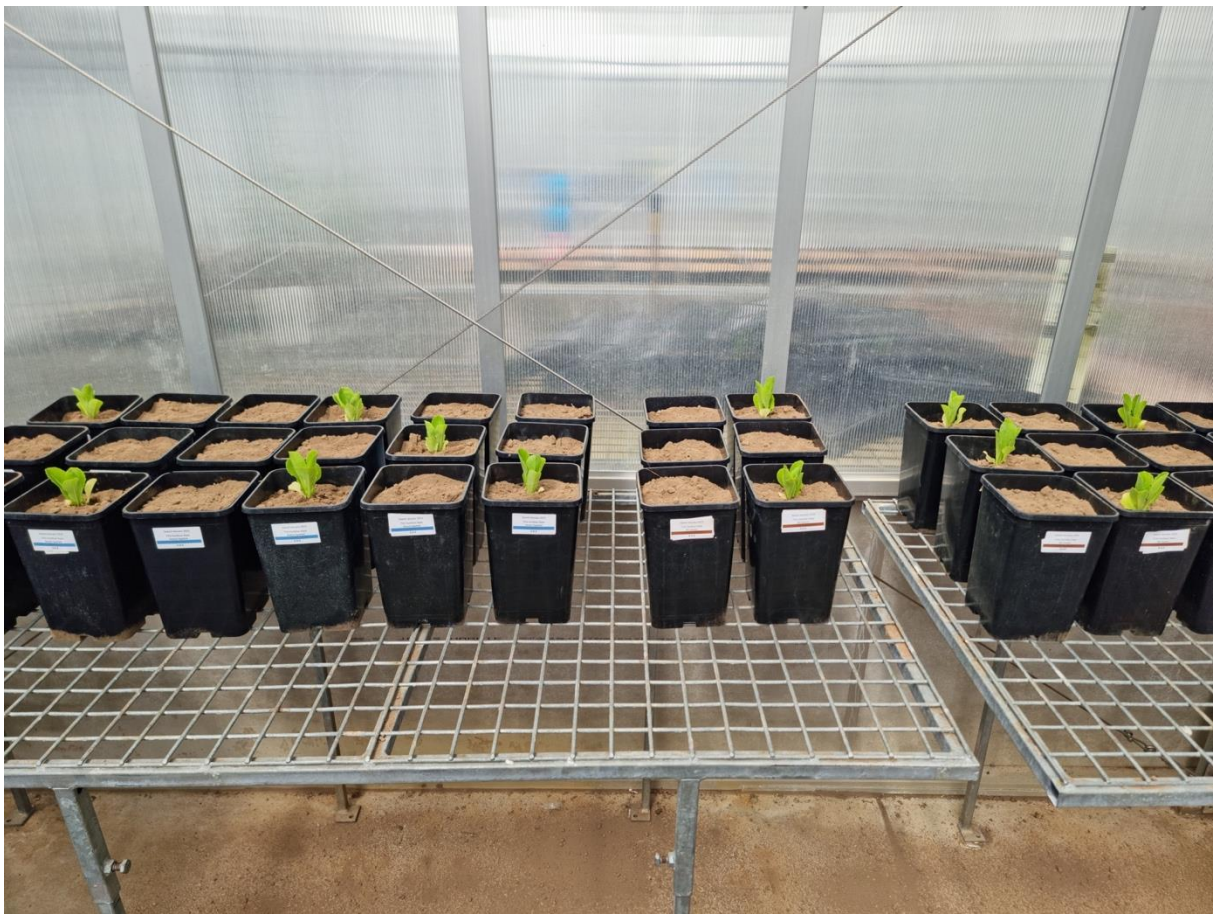


Figure 7: Lettuce seedlings transplanted randomly to prepared pots.

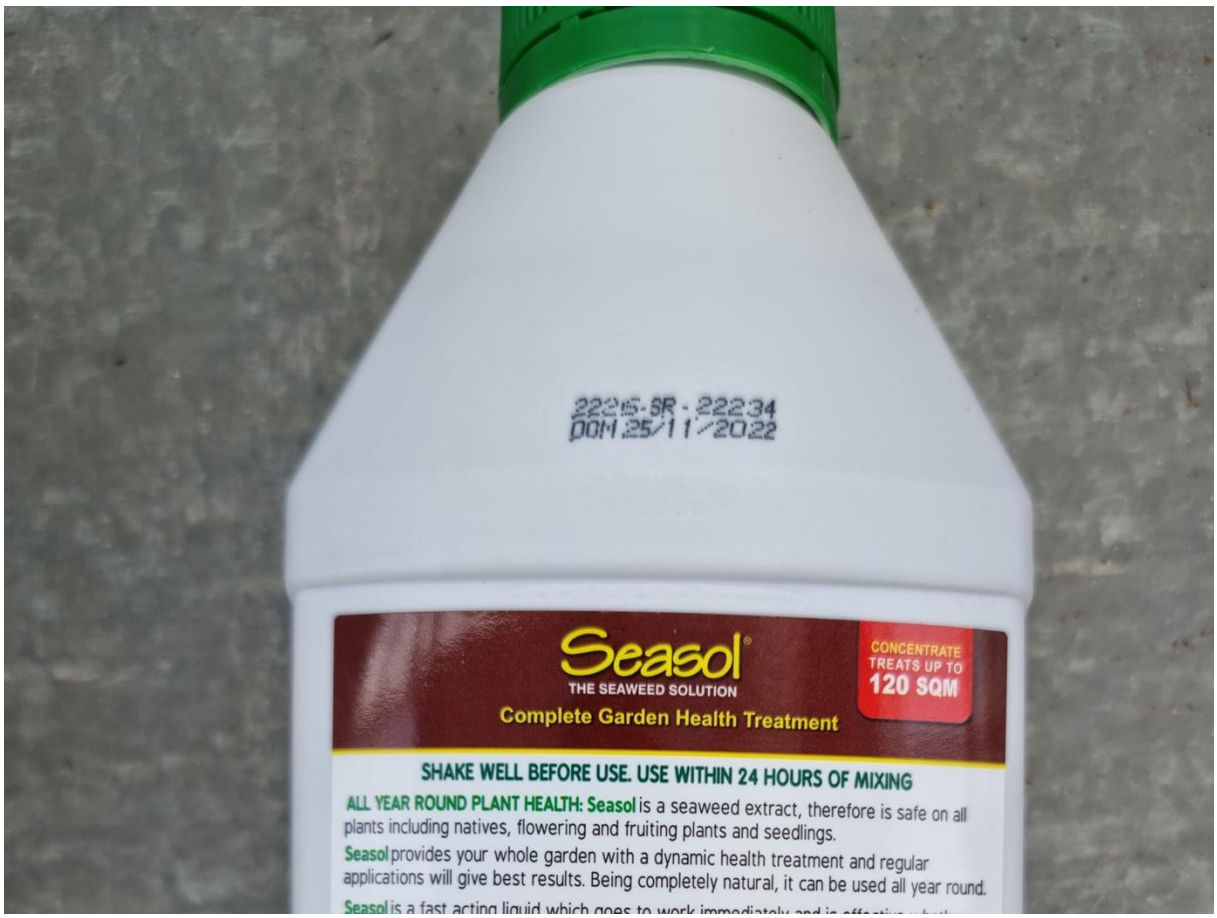


Figure 8: Recently manufactured Seasol used for day 1 treatments.

Figure 9: Seasol concentrate was diluted to 30ml per 9L or 3.33ml/L.

Figure 10: 5L/ha Seasol applied via pipette in a 4cm radius around each lettuce seedling. 3ml of solution (which equates 0.01 Seasol concentrate) applied to each applicable pot.

Hand Watering

Each pot was watered by hand every second day to ensure that accurate irrigation volumes were applied. Irrigation volumes were determined using soil moisture sensors installed in two pots that received 100% irrigation volumes. Daily water use was also verified by filling three spare planted pots (Figure 12) to saturation and measuring weight loss over two and four days. These pots were excluded from the trial.

Irrigation volumes were measured by appropriately sized glass beakers (Figure 11) or by measuring cylinder when required.

Irrigation volumes were recalculated regularly as the plants matured.

Figure 11: Each pot watered by hand with a measured volume.

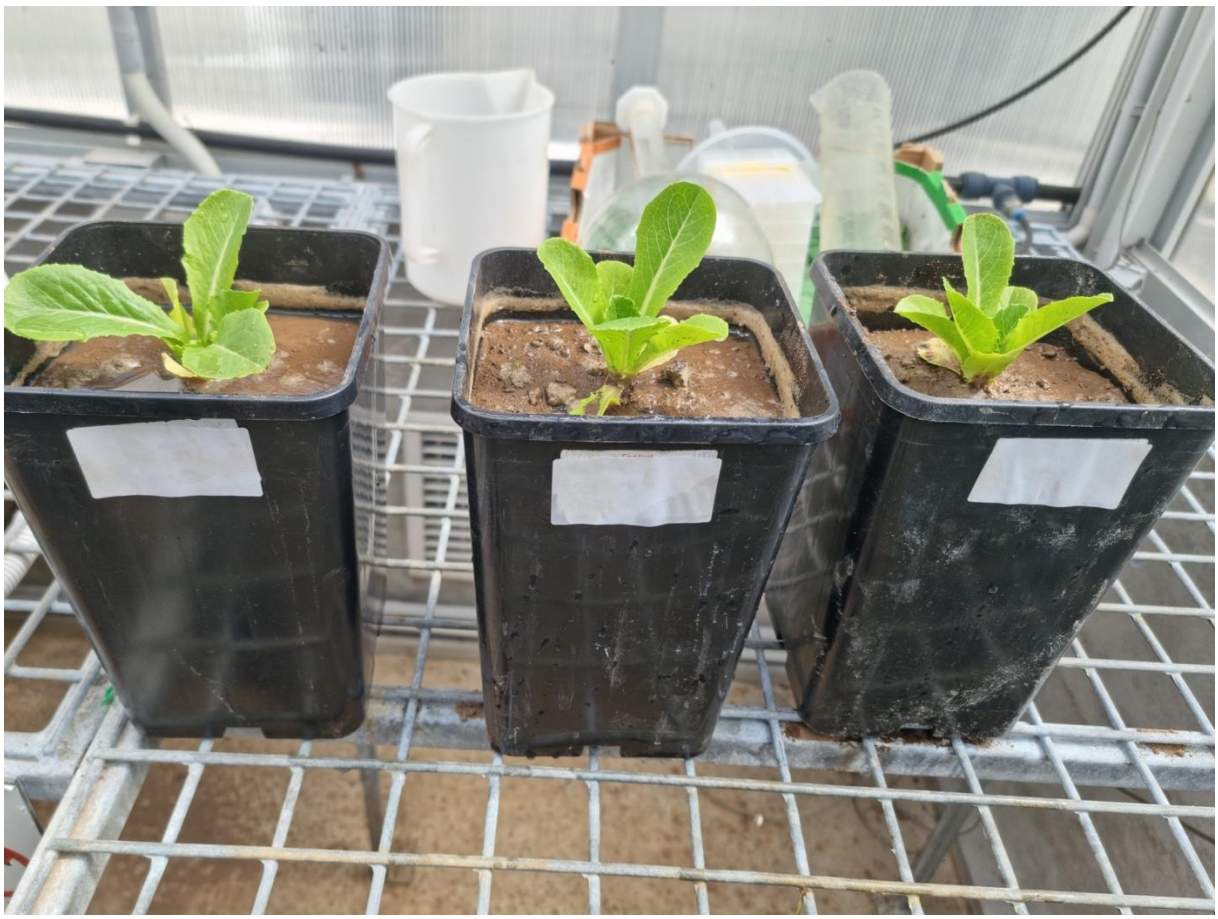


Figure 12: Three spare pots at saturation are being dried down to calculate daily water use.

Watering Log

Each of the treatments received the following volumes of water throughout the trial, as shown in (Table 1):

1. Standard Irrigation Rate: 8.7L or 446mm
2. 80% Irrigation Rate: 7.0L or 358mm
3. 60% Irrigation Rate: 5.3L or 270mm

Table 1: Watering log

Date	100% Volume (ml)	80% Volume (ml)	60% Volume (ml)
12/1/23	100	100	100
14/1/23	180	144	108
16/1/23	180	144	108
18/1/23	180	144	108
19/1/23	100	80	60
21/1/23	150	120	90
23/1/23	150	120	90
25/1/23	200	160	120
27/1/23	200	160	120
29/1/23	200	160	120
31/1/23	200	160	120
02/2/23	200	160	120
04/2/23	300	240	180
06/2/23	300	240	180
08/2/23	300	240	180
10/2/23	300	240	180
12/2/23	300	240	180
14/2/23	400	320	240
16/2/23	500	400	300
18/2/23	500	400	300
20/2/23	500	400	300
22/2/23	500	400	300
24/2/23	500	400	300
26/2/23	500	400	300
28/2/23	500	400	300
02/3/23	500	400	300
04/3/23	400	320	240
06/3/23	400	320	240
Total (L)	**8.7**	**7.0**	**5.3**
Total (mm)	**446**	**358**	**270**

Seasol Treatment Applications

Seasol was labelled with batch number 2226-SR-22234 and manufactured on 25/11/2022 (Figure 8). The Seasol was applied at 10L/ha per fortnight of retail grade Seasol, which is half the strength of Commercial grade Seasol. Seasol was diluted at the label rate to 30ml/9L (which equates to 6.66ml Seasol/L) (Figure 9) and 3ml of solution was applied via pipette as a soil drench to each applicable seedling (Figure 10) in the first two applications. The remaining Seasol applications were applied at 6ml per pot (Table 2).

All treated pots received identical volumes of diluted Seasol solution. Untreated pots did not receive any Seasol or supplementary irrigation, therefore the untreated pots received an additional 24ml of solution, compared to treated pots. This equates to an additional 0.28%, 0.34% and 0.45% solution applied to treated pots at 100%, 80% and 60% irrigation rates respectively.

Table 2: Seasol application rates and volumes.

Date	Seasol Rate – Retail Grade (Commercial Grade)	Volume Seasol solution per pot	Dilution
12/1/23	5L/ha (2.5L/ha)	3ml	30ml/9L
19/1/23	5L/ha (2.5L/ha)	3ml	30ml/9L
25/1/23	10L/ha (5L/ha)	6ml	30ml/9L
10/2/23	10L/ha (5L/ha)	6ml	30ml/9L
22/2/23	10L/ha (5L/ha)	6ml	30ml/9L
Total (ml)		**24ml**	

Trial Layout

Pots have been laid out in a randomised block design. There are six blocks for each trial, which corresponds to the six replicates in each trial, for a total of 12 blocks. There are three pots of each treatment assigned to each block. There are 18 pots per block in the irrigation trial (Figure 13) and 24 pots per block in the fertiliser trial (Figure 14). A list of all pots is provided in Appendix 3.

Figure 13: One of six randomised blocks in the irrigation trial. There are 18 pots per block.

Figure 14: One of six randomised blocks in the fertiliser trial. There are 24 pots per block.

Monitoring

The following data was being monitored:

1. Pot moisture via two soil moisture probes
2. Room air temperature of two rooms via four temperature loggers
3. Flying insect loads of two rooms via four sticky fly traps. This will also help control insect loads.

Maintenance

The trial will be watered and checked every two days. Maintenance tasks are:

1. Irrigate plants as per trial treatments
2. Check pest traps
3. Remove and weeds from pots
4. Check plants for pest damage

Assessments

There were three assessments carried out on the lettuce trial:

- 8th February: Plant height
- 21st February: Plant height
- 6th March: Plant height, plant fresh weight, plant dry weight, root length, root dry weight, leaf SPAD, root assessments.

Root Cleaning and Photography

Each pot was randomly selected for root cleaning and destructive analysis over a two-day period. Replicates 5, 4, 3 were assessed on day 1 and replicates 6, 2, 1 were assessed on day 2.

Pots were gently inverted and emptied, taking care not to disturb the soil and root mass. The plant and soil mass were laid out to be photographed on a stainless steel mesh (Figure 15). Soil was gently washed away from the roots using a hose until there was no visible soil remaining. Soil washed from roots were collected for nutrient analysis (**Figure 17**).

Photographs of all pots are available here:
https://www.dropbox.com/sh/a5bzl52jevl0cei/AADZ7HZK_Nd0afn__05J4c6Qa?dl=0

Figure 15: Lettuce plant and root structure laid out prior to root cleaning.

Figure 16: Roots were gently cleaned with a hose.

Figure 17: Soil was collected from each pot for nutrient analysis.

Plant Height and Root Lengths Assessments

Lettuce head heights were measured with a tape measure from the root crown the highest leaf (**Figure 18**). Measurements were recorded to the nearest millimetre.

Plant roots were cut from the lettuce head at the root crown and the length of the root mass measured with tape.

Figure 18: Lettuce plant and root mass laid out for length measurements and photography.

Plant Fresh Weights

Lettuce heads were separated from soil, washed and root cut at the crown before weights were measured by benchtop scales (**Figure 19**) (d=0.1g).

Figure 19: Lettuce head fresh weights measured immediate after harvest.

Leaf SPAD

Leaf SPAD was measured at 10 points on each lettuce head. Each measurement was taken on a unique young and mature leaf using a *Minolta SPAD 502 Chlorophyll Meter*, which was regularly recalibrated throughout the assessments (**Figure 20**).

Figure 20: SPAD measurements were collected from 10 leaves on each lettuce head.

Soil Nutrients

Representative soil samples were collected from each pot for a bulked analysis of each of the six treatments by Phosyn Analytical for the following nutrients (**Figure 21**):

pH (H_20), pH ($CaCl_2$), EC, S, P, Na, K, Ca, Mg, Al, Cl, Cu, Zn, Mn, Fe, B, NH4-N, NO3-N, Organic Matter

Figure 21: Soil samples were collected from every pot and bulked into their respective treatments.

Plant Dry Weights

Lettuce were bagged, labelled and dried for 72 hours at 65°C in a fan forced plant dehydrator (**Figure 22**) before being re-weighed with benchtop scales (d=0.1g) for dry weight measurements. Leaf matter was sampled for nutrient analysis.

Figure 22: Lettuce heads were individually bagged for drying in a fan-forced plant dehydrator.

Root Dry Weights

Roots were bagged, labelled and dried for 72 hours at 65°C in a fan forced plant dehydrator before being re-weighed with benchtop scales (**Figure 23**) (d=0.1g) for dry weight measurements.

Figure 23: Roots were weighed after drying.

Leaf Nutrients

Representative dried leaf samples were collected from each plant for a bulked analysis of each of the six treatments by Phosyn Analytical for the following nutrients (**Figure 24**):

N, S, P, K, Ca, Mg, Cu, Zn, Mn, Fe, B, Na, Mo, Cl, NO_3

Figure 24: Bulked leaf samples were collected for nutrient analysis.

Root Arbuscular Mycorrhizal Fungi

Three sets of roots from each treatment were randomly collected for arbuscular mycorrhizal fungi assessments. Roots were carefully collected (**Figure 25**), washed (**Figure 26**) and stored in 70% alcohol (**Figure 27**) before staining and assessment by Ryan Hall under a microscope.

Figure 25: Roots were carefully collected prior cleaning of the root mass.

Figure 26: Roots were gently dipped in water for cleaning

Figure 27: Roots were stored in 70% alcohol prior to staining.

Results

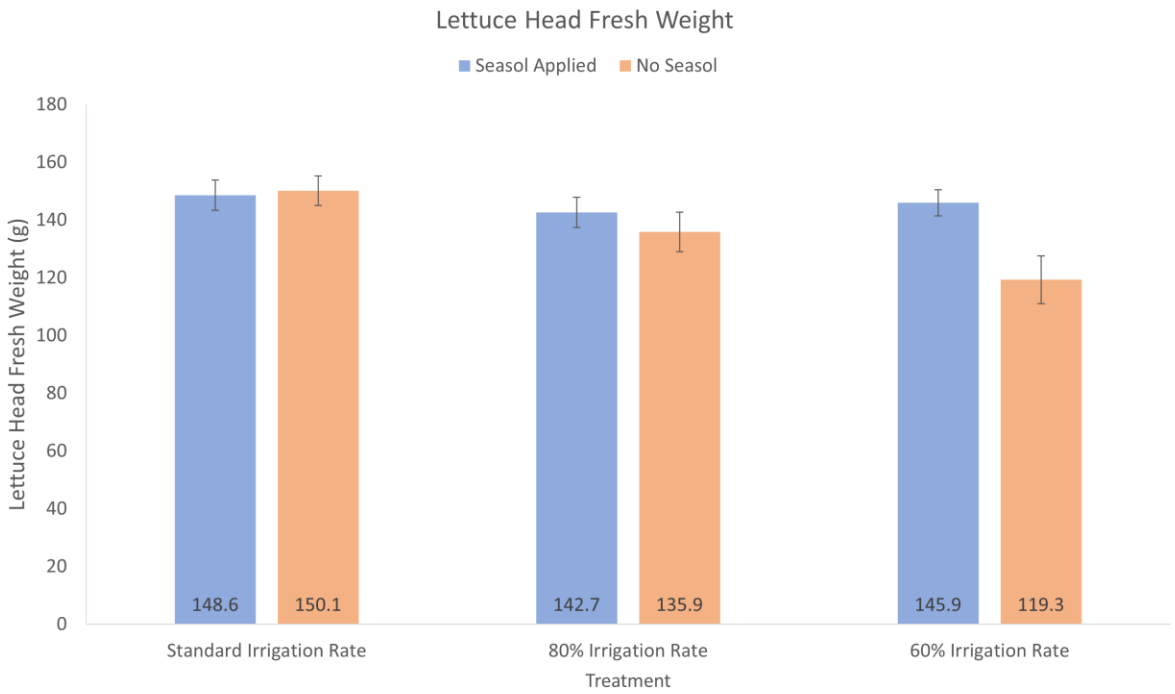


Figure 28: Cos lettuce head fresh weights at maturity. There is a significant ($p < 0.05$) difference between Seasol treated and untreated lettuce at 60% irrigation volume.

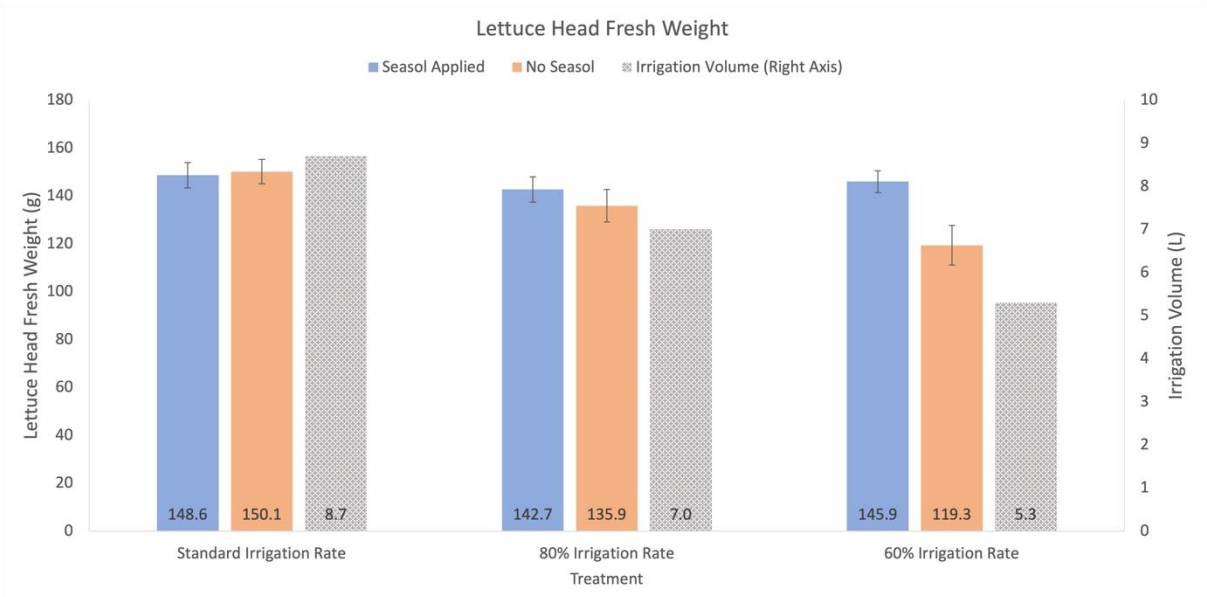


Figure 29: Cos lettuce head fresh weights at maturity (left axis) and irrigation volumes (right axis). There is a significant ($p < 0.05$) difference between Seasol treated and untreated lettuce at 60% irrigation volume.

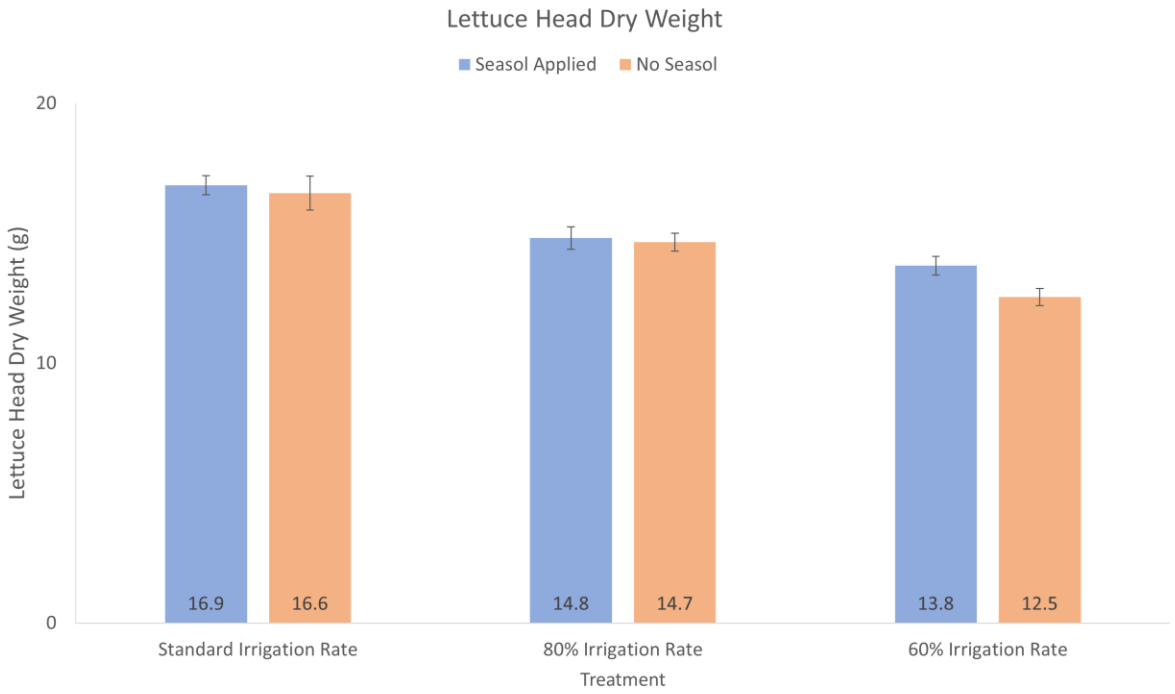


Figure 30: Cos lettuce head dry weights at maturity. There is a significant ($p < 0.05$) difference between Seasol treated and untreated lettuce at 60% irrigation volume.

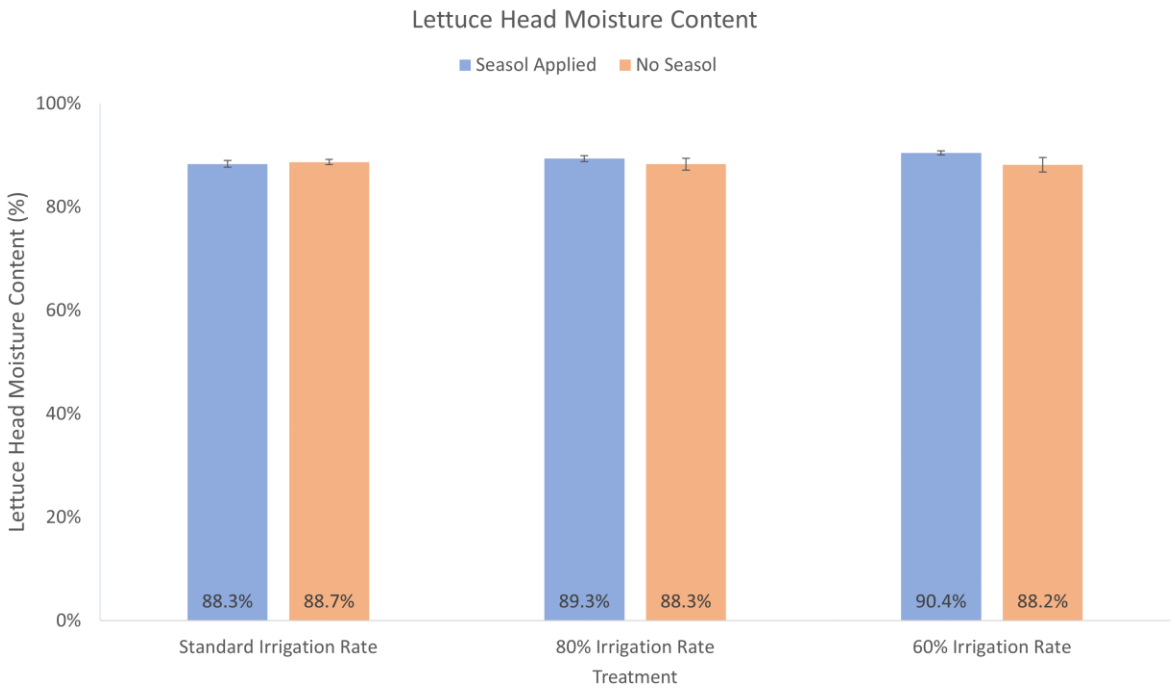


Figure 31: Cos lettuce moisture content at maturity. There is no significant difference between treatments.

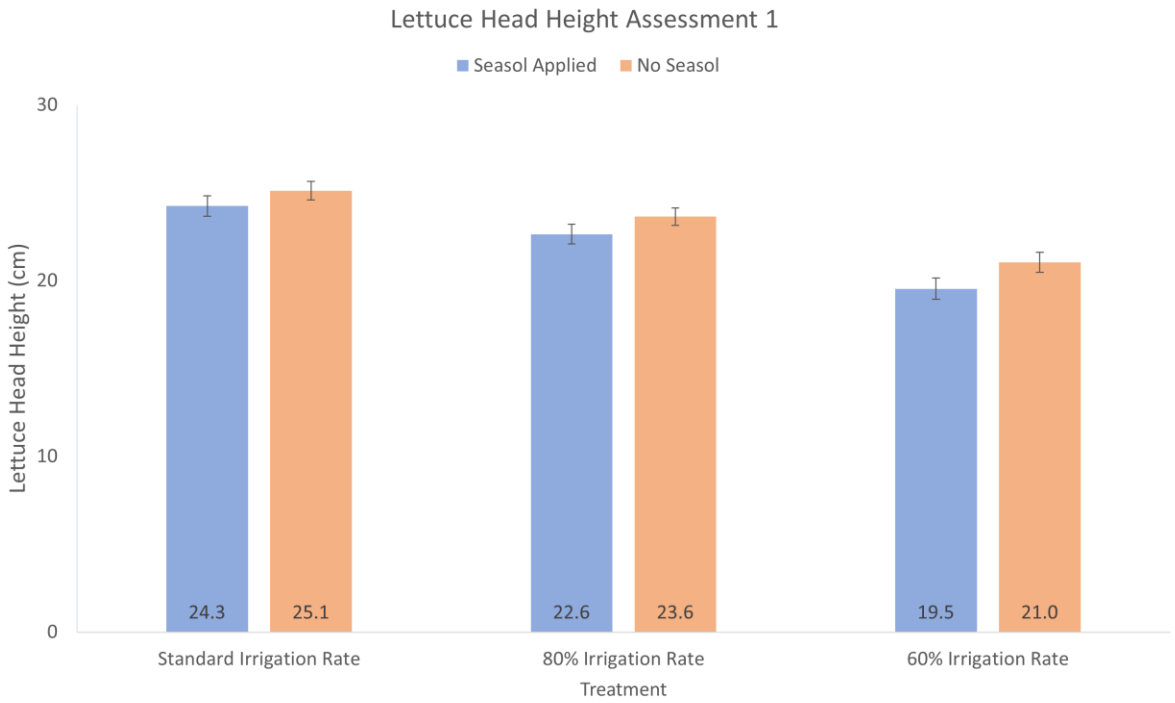


Figure 32: Cos lettuce head heights four weeks after transplanting. There is no significant difference between treatments.

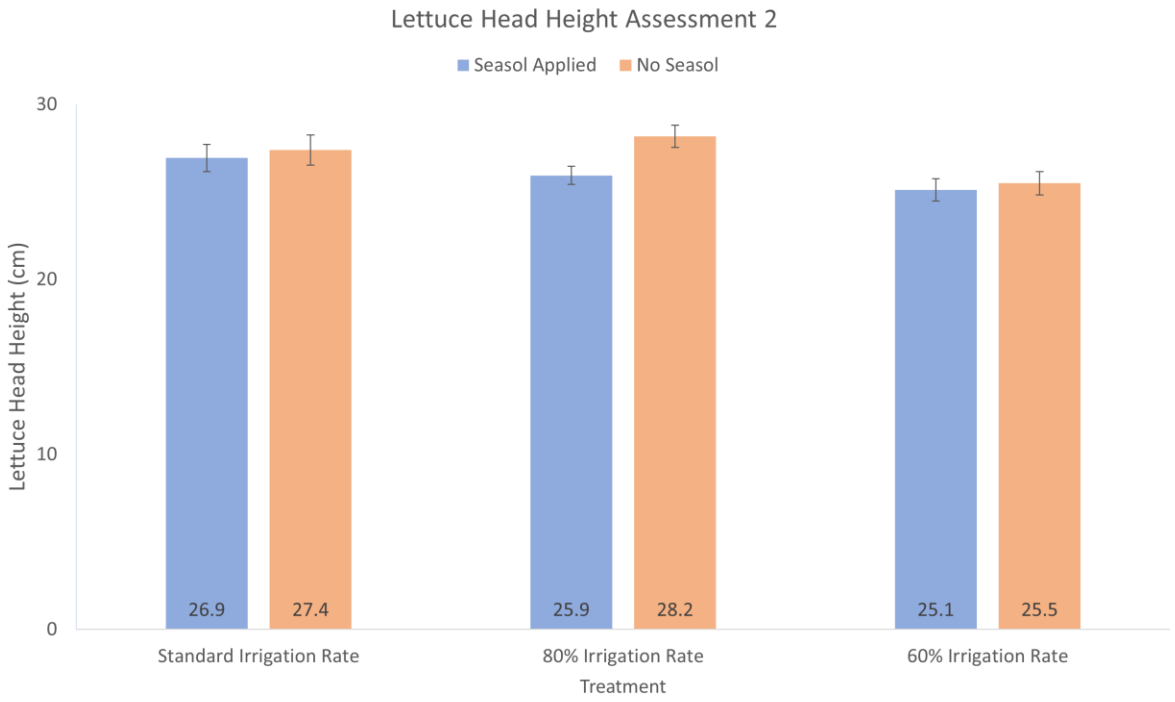


Figure 33: Cos lettuce head heights six weeks after transplanting. There is no significant difference between treatments.

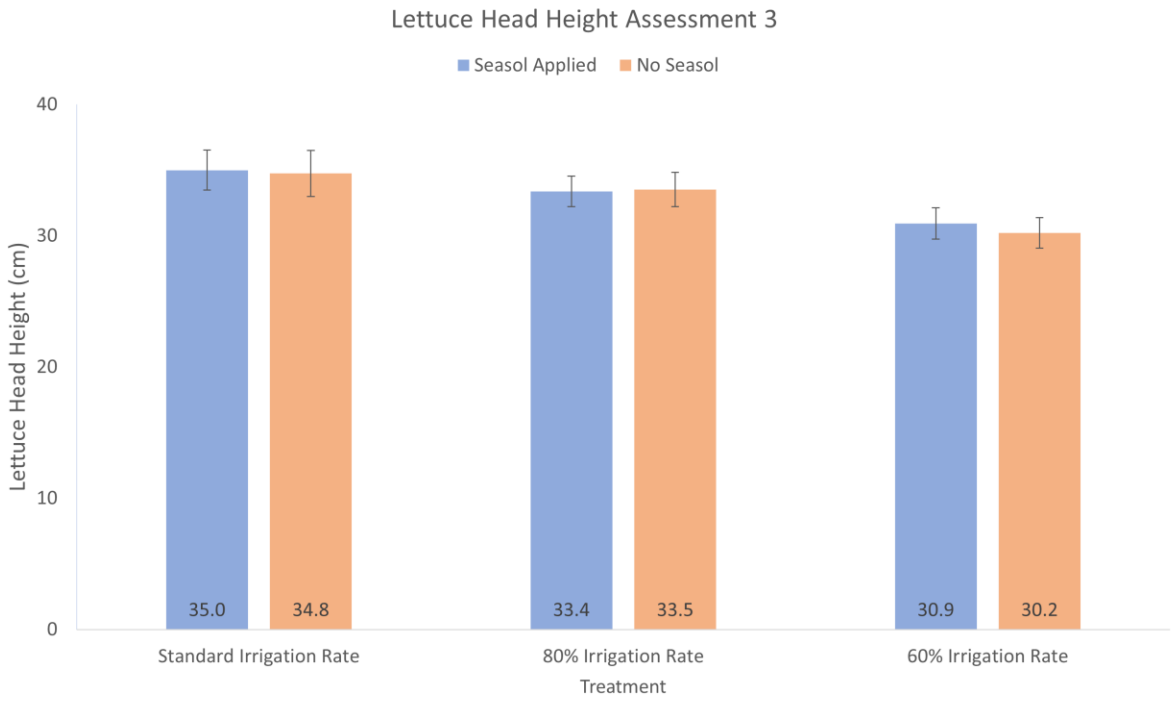


Figure 34: Cos lettuce head heights at maturity. There is no significant difference between treatments.

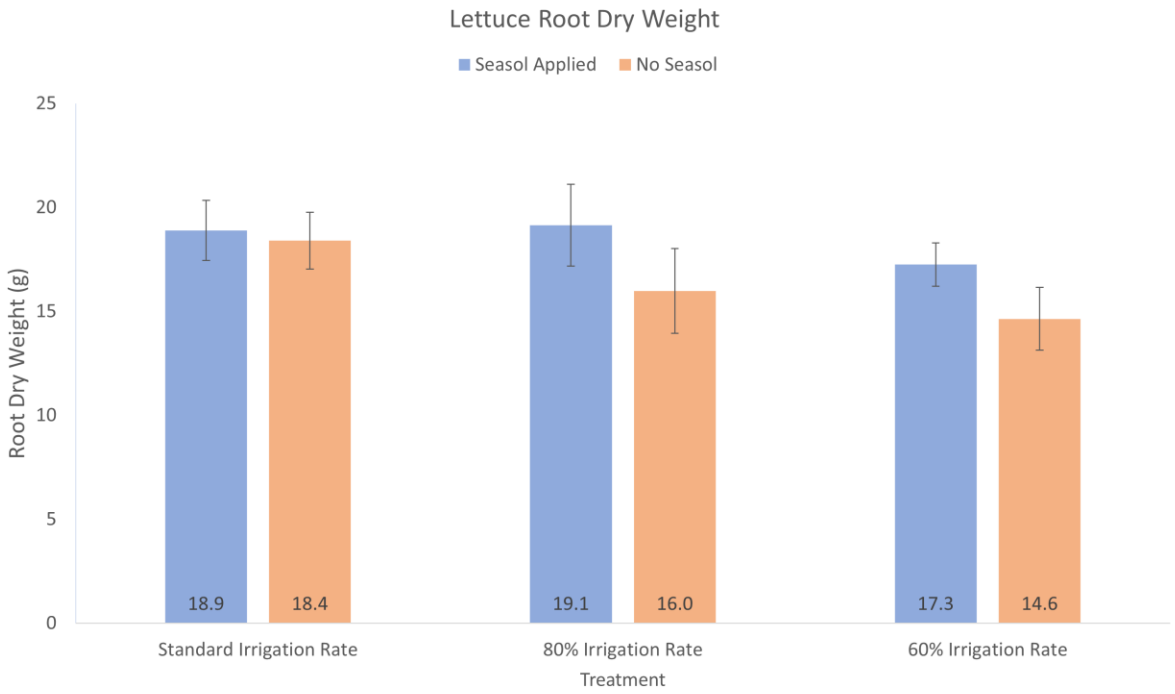


Figure 35: Cos lettuce root dry weights at maturity. There is a significant ($p < 0.05$) difference between Seasol treated and untreated lettuce at 60% irrigation volume.

Figure 36: Cos lettuce maximum root lengths at maturity. There is no significant difference between treatments.

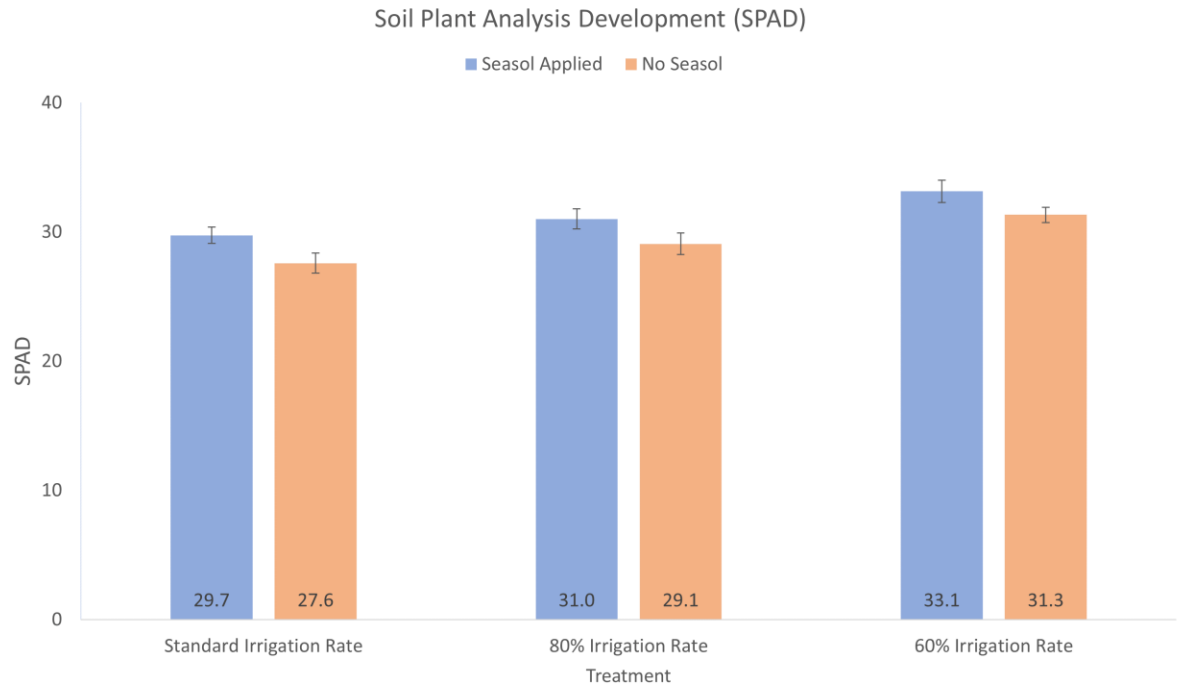
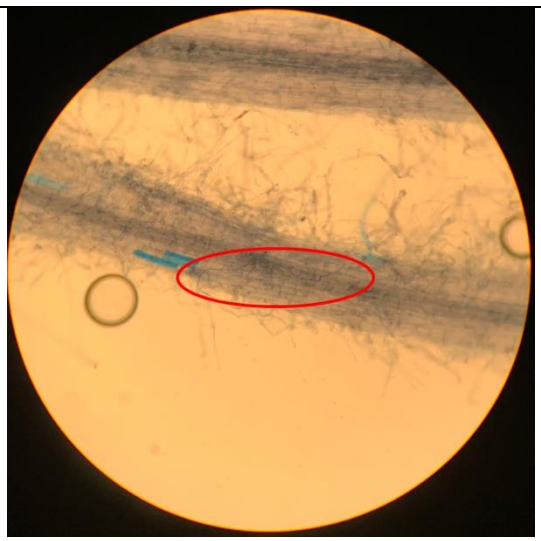


Figure 37: Cos lettuce leaf SPAD levels at maturity. There is significant difference ($p < .10$) at all irrigation volumes.

Table 3: Mycorrhizal root assessment scores at maturity. There is no significant difference between treatments.

	100% irrigation Seasol	**80% irrigation Seasol**	**60% irrigation Seasol**	**100% irrigation no Seasol**	**80% irrigation no Seasol**	**60% irrigation no Seasol**
Hyphae	0.77%	0.00%	4.20%	1.82%	1.25%	5.24%
Vesicle	0.00%	0.00%	0.96%	0.00%	0.00%	0.00%
Arbuscule	0.00%	0.00%	0.00%	0.91%	0.00%	0.61%

Table 4: Mycorrhizal root assessment images and analyses.

60% irrigation rate, Seasol applied • 100x magnification • No arbuscular mycorrhizal fungi present in this field of vision of the root fragment	
60% irrigation rate, Seasol applied • 400X magnification • Stained fungi, not arbuscular mycorrhizal fungi (AMF) due to presence of septa (red circle). All AMF fungi are aseptate, meaning they have no segments and are continuous.	
60% irrigation rate, Seasol applied • 100x magnification • Stained fungi Hyphae fragment, potentially arbuscular mycorrhizal fungi	

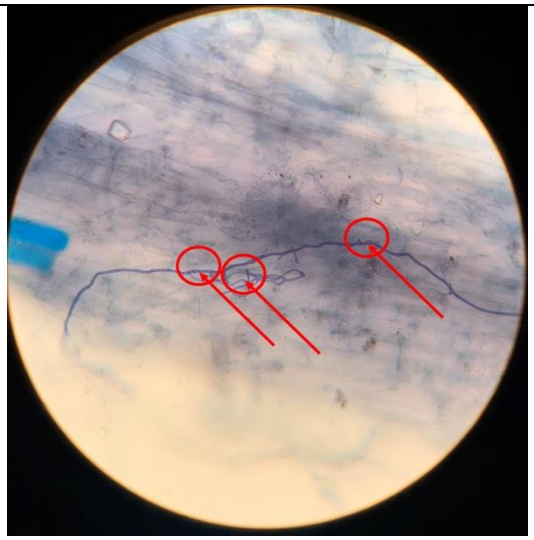
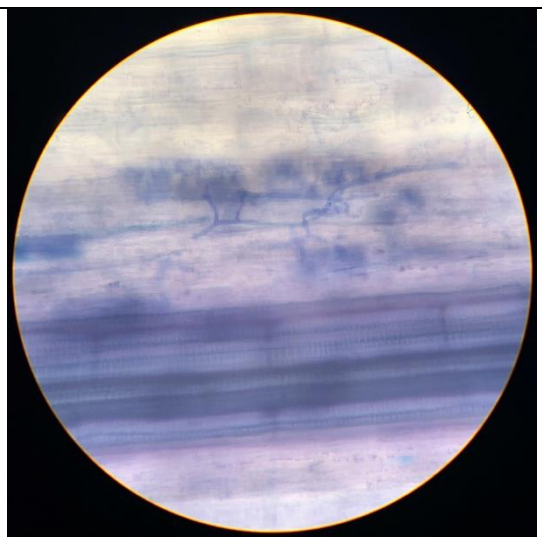
60% irrigation rate, Seasol applied • 400x magnification • Stained fungi Hyphae fragment, not arbuscular mycorrhizal fungi, Due to septa being present	
60% irrigation rate, Seasol applied • 400x magnification • Red circle indicates non- arbuscular mycorrhizal fungi (AMF) due to septa (red arrow) • Green circle represents possible AMF, due to no septa being present. However, due to the prevalence of other non-AMF in close proximity, it is possibly a non-AMF fragment	
100% irrigation rate, no Seasol applied • 400x magnification • Stained fungi Hyphae fragment, Arbuscles present (blue blur around fungi fragments)	

Table 5: Analysis of variation (ANOVA) P-values of results.

ANOVA P-Values	Root Dry Weight	Dry Weight	Head Height	Fresh Weight	Moisture Content	SPAD	Root Length
Standard Irrigation Rate	0.81	0.69	0.92	0.84	0.66	**0.04**	0.78
80% Irrigation Rate	0.27	0.77	0.94	0.44	0.41	**0.097**	0.16
60% Irrigation Rate	0.16	**0.02**	0.66	**0.01**	0.13	**0.09**	0.53

Table 6: Tukey HSD analysis of Lettuce Head Fresh Weights at harvest.

Pair	Difference	SE	Q	p-value
x1-x2	5.9389	5.9303	1.0015	0.981
x1-x3	2.6611	5.9303	0.4487	1.000
x1-x4	1.5232	6.0168	0.2532	1.000
x1-x5	12.7389	5.9303	2.1481	0.653
x1-x6	29.2886	6.0168	4.8678	**0.011**
x2-x3	3.2778	5.9303	0.5527	0.999
x2-x4	7.4621	6.0168	1.2402	0.951
x2-x5	6.8	5.9303	1.1467	0.965
x2-x6	23.3497	6.0168	3.8807	**0.076**
x3-x4	4.1843	6.0168	0.6954	0.996
x3-x5	10.0778	5.9303	1.6994	0.835
x3-x6	26.6275	6.0168	4.4255	**0.027**
x4-x5	14.2621	6.0168	2.3704	0.551
x4-x6	30.8118	6.1022	5.0493	**0.007**
x5-x6	16.5497	6.0168	2.7506	0.381

Table 7: Tukey HSD Group numbers used in analysis of Lettuce Head Fresh Weights at harvest.

Group #	Treatment
1	Standard Irrigation Rate with Seasol
2	80% Irrigation Rate with Seasol
3	60% Irrigation Rate with Seasol
4	Standard Irrigation Rate without Seasol
5	80% Irrigation Rate without Seasol
6	60% Irrigation Rate without Seasol

Based on the results from a Tukey HSD test (Table 6) on lettuce head fresh weights, the following treatments are significantly different at a 95% confidence:

1. Standard Irrigation Rate **with** Seasol x 60% Irrigation Rate **without** Seasol ($p<0.01$)
2. 60% Irrigation Rate **with** Seasol x 60% Irrigation Rate **without** Seasol ($p<0.03$)
3. Standard Irrigation Rate **without** Seasol x 60% Irrigation Rate **without** Seasol ($p<0.01$)

Discussion

All water-stressed treatments produced reduced yields compared to standard irrigation treatments, indicating the experimental design successfully manipulated plant growth through irrigation volumes.

The results of the trial show that Seasol treatment improves the water use efficiency of lettuce plants, as indicated by fresh and dry lettuce head weights. When subjected to 60% irrigation, Seasol-treated lettuce showed a statistically significant ($P<0.05$) increase in both fresh weight (20% increase) and dry weight (10% increase) compared to untreated lettuce (Figure 28, Figure 29). Additionally, the Seasol-treated lettuce had a consistent yield across all irrigation rates. These findings are supported by the work of Xu and Leskovar (2015), who reported that seaweed extracts had no effect on spinach growth under full irrigation, but under drought stress, application of seaweed extract improved spinach leaf growth.

Without Seasol applied, there was a statistically significant ($P<0.05$) drop in fresh weight of 30.8g or 20.5% from standard 100% irrigation rate to 60% irrigation rate. However, there was a non-statistically significant drop in fresh weight of 4.2g or 2.8% from standard irrigation rate without Seasol to 60% irrigation rate with Seasol.

The root length data (Figure 36), although variable, further demonstrates the growth-stimulating effects of Seasol, showing that Seasol-treated lettuce had longer roots at the 80% and 60% irrigation rates. This is also supported by the dry root weights (Figure 34) which although also highly variable, follow a trend that Seasol-treated lettuce had heavier roots at the same irrigation rates. These root results are supported by the research of Chen et al (2023), which found that the application of seaweed extract significantly promoted root growth in deeper soil, changed root architecture, and improved water use efficiency of roots, ultimately enhancing drought resistance of sugarcane plants.

The trial also found that lettuce leaves treated with Seasol at all three irrigation rates displayed a significantly higher ($P<0.10$) Soil Plant Analysis Development (SPAD) level compared to untreated lettuce leaves (Figure 37), indicating a consistently higher chlorophyll content in the leaves of Seasol-treated lettuce. This difference is most pronounced at 80% and 60% irrigation rates, where the SPAD level of the Seasol-treated lettuce leaves was 8.4% and 4.5% higher respectively. SPAD levels were highest in the 60% irrigation treatment, followed by the 80% irrigation treatment, with the standard irrigation treatment featuring the lowest SPAD levels, regardless of Seasol application.

Higher irrigation volumes resulted in larger lettuce plants, therefore lower SPAD levels recorded on the larger plants are likely due to the nutrient dilution effect. The SPAD results are consistent with the findings of Blunden et al (1996) who demonstrated that the application of seaweed extract to soil increased chlorophyll concentrations in the leaves of treated plants, with positive effects observed in tomato, dwarf French bean, wheat, barley, and maize.

The lettuce subjected to 60% irrigation displayed the highest levels of arbuscular mycorrhizal fungi of all irrigation volumes, regardless of Seasol treatment. However, untreated lettuce exhibits overall higher levels of arbuscular mycorrhizal fungi than Seasol-treated lettuce (Table 3: Mycorrhizal root assessment scores at maturity. There is no significant difference between treatments., Table 4**:** Mycorrhizal root assessment images and analyses.). In this trial, Seasol treatment appears to have had little to no influence on the levels of arbuscular mycorrhizal fungi. The post-trial soil test results showed no significant differences between the Seasol-treated and untreated lettuce.

The post-trial leaf nitrate (ppm) and post-trial leaf total nitrogen (%) concentrations were higher in the 80% and 60% irrigation treatments without Seasol. All other treatments had similar levels of leaf nitrate and leaf total nitrogen, falling within the low-moderate range for Cos lettuce. This is an interesting result and is most likely due to the Seasol treatment increasing the growth of the water-stressed lettuce in the 80% and 60% irrigation treatments compared to the non-Seasol low irrigation treatments.

The total amount of nitrogen available to the plant was diluted in the Seasol treatment, hence the lower leaf concentrations. This is a well-known phenomenon which occurs when nutrients are in limited supply, as discussed by Broadley et al (2000). The most important observation is that the Seasol treatments result in increased growth of the deficit irrigated lettuce plants, counteracting the effects of water stress.

Conclusion

Seasol application effectively improved the water use efficiency of lettuce plants and increased their growth and yield under water-stressed conditions. The Seasol-treated lettuce had longer roots, heavier dry root weights, and consistently higher chlorophyll content than untreated lettuce. The findings suggest that Seasol could be an effective method for the management of lettuce in water-limited conditions. Further research is needed to investigate the underlying mechanisms of Seasol's growth-promoting effects on plants subjected to water stress. The water use efficiency effects of Seasol could also be studied in open field conditions with other crop types.

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