Effect of Seasol® treatment on post-harvest quality of strawberry

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Summary

A preliminary experiment was conducted to determine the effect of various combinations of Seasol® treatments in the nursery and fruit sectors on strawberry (cv. *Albion*) yield, postharvest disease development and quality. It was found that Seasol® treatments may contribute to higher strawberry fruit yields, better fruit visual quality and inhibition of fungal disease development on marketable fruit. In general, application of Seasol® in the nursery and fruit sectors (Treatment A) or in the fruit sector alone (Treatment C) was more effective in increasing yield and fruit quality than nursery application of Seasol® on its own (Treatment B). Over three harvests, Treatment A and C increased total marketable fruit yield by 10 to 15 % over the untreated control (Treatment D), but this difference was not statistically significant due to high variability among field plots within a treatment. Fruit yield obtained over three harvests may not be representative of the total fruit yield obtained in a field plot during a season where more than forty picks may be conducted. No significant difference in mean berry weight was found among fruit from treated and untreated plots, whilst Treatment A and B significantly improved berry visual quality relative to untreated fruit.

Although the effect of treatment on flesh firmness and overall berry colour was not significant fruit from Treatment C field plots had significantly greater red colour than fruit from all other treatments. Seasol® treatments did not significantly increase soluble solids concentration (SSC), titratable acidity or SSC to acid ratio. However, there were indications that both Treatment A and C may increase SSC, and SSC to acid ratio, relative to untreated fruit, and the effects of these treatments on SSC and TA need further investigation. Treatment A was also found to significantly increase the uniformity of fruit SSC within field plots which may be of commercial importance in providing consumers with strawberries of consistent sweetness. After cool storage for 7 days at 3 - 4 °C and incubation for 96 hours at 20 °C all Seasol® treatments significantly reduced mean fungal disease incidence relative to the untreated control among fruit picked at the 1st and 3rd harvest, but there was no significant effect of treatment on disease severity. Disease incidence in fruit from Treatment A plots was 11% lower than in untreated fruit. After incubation for 72 hours at 20 °C no significant difference in disease incidence or severity was observed between treatments among fruit picked at the 2nd harvest.

The potential interaction between marketable fruit maturity and treatment was explored by grouping fruit into statistically distinct hue angle and SSC classes. Comparison of berry firmness treatment means among each of three fruit hue angle classes revealed that Treatment A and C tended to increase berry firmness relative to the untreated control among fruit with greater colour and maturity. Higher berry flesh firmness in more mature fruit could be beneficial in prolonging postharvest storage life. Fruit from Treatment A plots also tended to remain firmer at higher SSC. This could be advantageous as production of sweeter fruit with relatively higher berry firmness and lower maturity could allow producers to pick fruit that is marginally less mature but with acceptable SSC and flavour. Furthermore, marketable fruit from Treatment A plots that had less colour development tended to have higher SSC than similar fruit picked from untreated plots. Higher SSC at marginally lower, but still marketable berry colour, may result in greater uniformity of SSC among fruit batches containing a range of maturities. **Note that positive trends due to treatment identified above should not be considered statistically significant or confirmed.**

Recommendations

Based on this preliminary experiment no definitive recommendations can be made for the use of Seasol® treatments to increase strawberry yield and improve fruit quality. Although any increases in berry yield and most berry quality parameters were not statistically significant, the experiment indicated that there were positive trends from Seasol® treatments relative to the untreated control. For example, a mean increase in berry yield of up to 15%, a significant decrease in postharvest disease incidence of 11 %, and a mean increase in SSC of 1 °Brix.

It is recommended that further experiments are conducted to confirm that trends in improved yield, fruit quality and reduced disease development are statistically significant and commercially important. Specifically, future studies should incorporate the following changes to experimental design and methodology:

1) The statistical power of the experiment should be increased to enable detection of a pre-specified effect size due to Seasol® treatment. For example the experiment could be designed to detect a commercially important increase in SSC of 1 to 1.5 °Brix if Seasol® treatments are effective. As another example a commercially important yield increase to cover the cost of commercial Seasol® treatment could be determined and used as the effect size to determine the number of field plots (replicates) required for the experiment. It is also recommended that the size of field plots should be increased so that larger quantities of representative fruit are picked within plots at each harvest.

2) Treatment effects on strawberry quality may be difficult to detect without considering the range of fruit maturities (based on fruit colour) within a field plot at any particular harvest. Results from this experiment indicate that Seasol® treatment effects may be dependent on fruit maturity and thus a future experiment should contain a fruit maturity factor at various levels within the experimental design to account for the interaction between treatment and fruit maturity. This can be achieved in two ways; if fruit quantities are relatively low then separate harvests can be conducted at which only immature, mature and ripe marketable fruit are picked; if fruit quantities are sufficient then marketable fruit can be segregated into three distinct maturity classes at each harvest.

3) Future studies should incorporate postharvest disease assessment at multiple incubation times to determine if treatment effects on disease development occur at low disease incidence and severity and thus are of commercial importance, and to determine whether treatments delay the onset of first appearance of disease among fruit. Furthermore the visual quality and colour of fruit prior to incubation should be quantified, with these quality parameters used as covariates when analyzing disease incidence and severity data.

Background

Research conducted in the strawberry nursery industry at Toolangi in 2013/14 and 2014/15 showed that treatment with Seasol® significantly increased transplant yields and root quality (Mattner et al., 2014 & 2015). However, the subsequent fruit yield and quality performance of transplants in the field produced via treatment with Seasol® has not been determined. Studies in the literature show that seaweed extracts applied to strawberry plants can increase fruit yields (Spenelli et al., 2010; Alam et al., 2013; El-Miniawy et al., 2014; Eshghi & Garazhian, 2015), but Boček et al. (2012) found no significant increase in strawberry fruit size or yield during two seasons of trials, and found no effect of seaweed extract on grey mould disease incidence in the field. The direct effect of Seasol® on fruit yield and fungal disease development requires further investigation.

El-Miniawy et al. (2014) found that seaweed extract foliar sprays improved fruit quality characteristics such as fruit firmness, soluble solids concentration, titratable acidity and SSC to acid ratio. Eshghi & Garazhian (2015) showed that relatively high concentrations of humic acid applied as a foliar spray increased strawberry SSC and vitamin C concentration relative to no treatment. But the effect of Seasol® treatment on fungal disease development after harvest and fruit quality as measured by soluble solids concentration (SSC), flesh firmness and titratable acidity, has not been experimentally verified, and the interaction of Seasol® treatment with fruit maturity measured via berry surface colour and SSC has not been determined.

Experimental objectives

The objectives of this preliminary experiment were to:

1. Determine whether Seasol® treatment increases marketable berry yield or fruit size compared to the untreated control;
2. Investigate the effect of Seasol® treatment on visual quality, flesh firmness and composition of marketable berries at harvest;
3. Determine whether Seasol® treatment reduces fungal disease incidence and severity compared to the untreated control after postharvest storage and incubation of fruit;
4. Understand the effect of Seasol® treatment on variation in marketable fruit quality within field plots at each harvest;
5. Investigate the effect of Seasol® treatment on berry eating quality at various levels of fruit maturity to inform future design of strawberry field experiments.

Experimental methods

Seasol® treatments in the nursery and fruit sectors

Four treatments comprising of Seasol applications in the nursery and fruit sectors of strawberry (cv. *Albion*) production were made. **Treatment A** consisted of monthly Seasol® drench applications in the nursery sector of production, and then drench and foliar applications in the fruit sector of production (Table 1). **Treatment B** consisted of monthly Seasol® drench applications in the nursery sector of production only. **Treatment C** consisted of monthly Seasol® drench and foliar applications in the fruit sector of production only. **Treatment D** was the control treatment with water rather than Seasol® applied in the nursery and in the fruit sector of production. Seasol® was applied as a 1:400 solution at 10 L/ha. Individual plots contained 16 plants and fruit were produced using standard agronomic practices. The trial was conducted on a commercial strawberry fruit farm at Yarra Glen, Victoria.

Table 1. Seasol® treatments used for strawberry yield and quality experiment.

TREATMENT	NURSERY TRT	FRUIT TRT
A	Seasol*	Seasol
B	Seasol	Nil
C	Nil	Seasol
D	Nil	Nil

* 10 L/ha, monthly, through drip and foliar spray

Harvest

At each of three harvests all marketable strawberry fruit were picked from each field plot. Harvests were conducted on the 1st, 10th and 21st of April 2016. At each harvest 15 to 20 fruit per field plot were carefully picked and placed in a ventilated strawberry punnet (Fig. 1). Strawberries were then placed in an esky containing Refreeze™ ice packs and transported to a postharvest laboratory where they were refrigerated overnight at 3 - 4 °C. Within 24 hours of harvest 8 fruit per punnet were randomly selected and removed for quality assessments whilst the remaining 6 to 9 fruit were placed back in cool storage to be used for fungal disease assessment.

Figure 1. Harvested fruit in replicate punnets each representing a field plot.

Experimental design

The experimental unit in the trial was a field plot which was represented by a single punnet once fruit were harvested. The experiment was a randomised complete block design with two factors (harvest and treatment) and three replicates (field plots) per treatment:

3 harvests x 4 treatments x 3 replicate plots x 14-17 fruit

The randomisation of treatments within experimental field plots is presented in Figure 2.

Marketable fruit picked from replicate plots at each harvest were segregated into two batches, the first consisting of eight fruit used for quality assessments and the second consisting of 6 to 9 fruit for fungal rot assessment after cool storage and incubation.

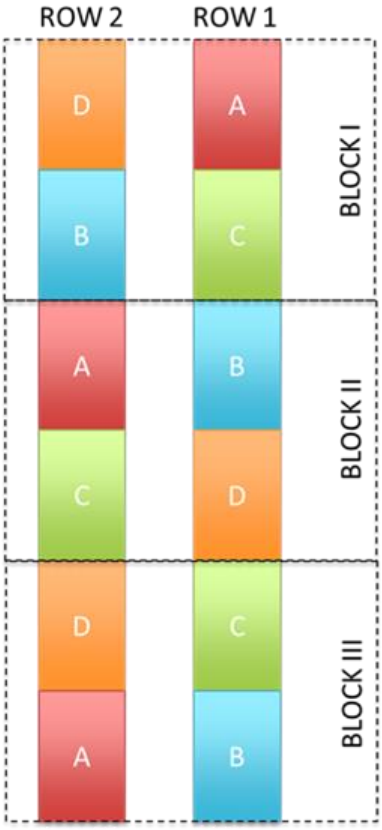

Figure 2. Layout of experimental field plots for Seasol® strawberry yield and quality experiment.

Fruit quality assessments

At each harvest fruit in each punnet were weighed and eight fruit per punnet (field plot) were randomly selected for quality assessments (Fig. 3). These eight fruit were weighed and after warming to 20°C each fruit was assessed for visual quality, colour uniformity, surface colour (hue), flesh firmness and soluble solids concentration. An unfiltered composite juice sample from the eight fruit per punnet was also collected for measurement of titratable acidity.

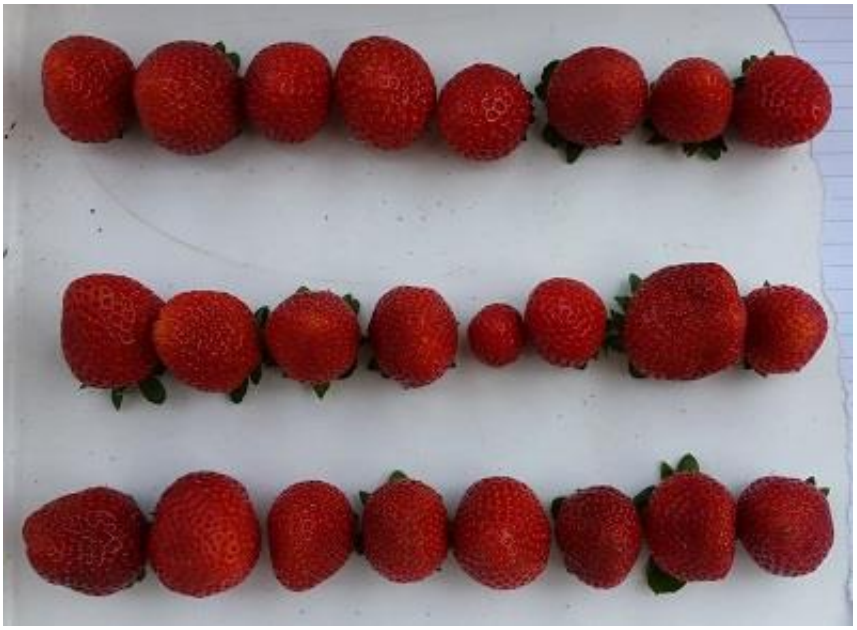

Figure 3. Strawberry fruit replicates prepared for quality assessments.

Visual quality

Each fruit was scored for overall visual quality using a 5-point rating scale where 5 = excellent, 4 = very good; 3 = good; 2 = poor; and 1 = very poor. Fruit with a score of 3 or less would be considered unmarketable. The main parameters considered when assessing loss of visual quality in each fruit were bruising or soft spots, poor colour uniformity, misshapen fruit and very small fruit.

Figure 4. Examples of strawberry fruit with reduced visual quality due to soft spots, poor colour uniformity and poor shape.

Colour uniformity

Each fruit was scored for uniformity of red surface colour as a percentage of fruit surface with 100% representing fully-uniform red colour (i.e., no green or light red colour on a fruit) (Fig. 5).

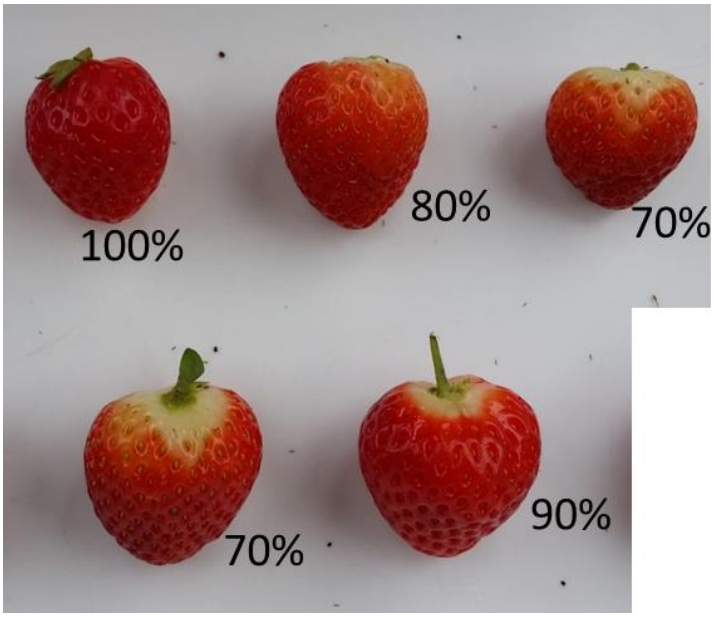

Figure 5. Example of uniform red colour range encountered in marketable strawberry fruit.

Fruit surface colour

Surface colour was measured on both cheeks of each fruit at its widest point with a hand-held tristimulus reflectance colorimeter (model CM-2600d, Minolta Corp.). Colour was recorded using the CIE L*a*b* uniform colour space (CIE Laboratories), where L* indicates lightness, a* indicates chromaticity on a green (-) to red (+) axis, and b* chromaticity on a blue (-) to yellow (+) axis. Numerical values of a* and b* for each fruit were averaged and then the average hue angle calculated using H° = arctan (b*/a*) (Fig. 6).

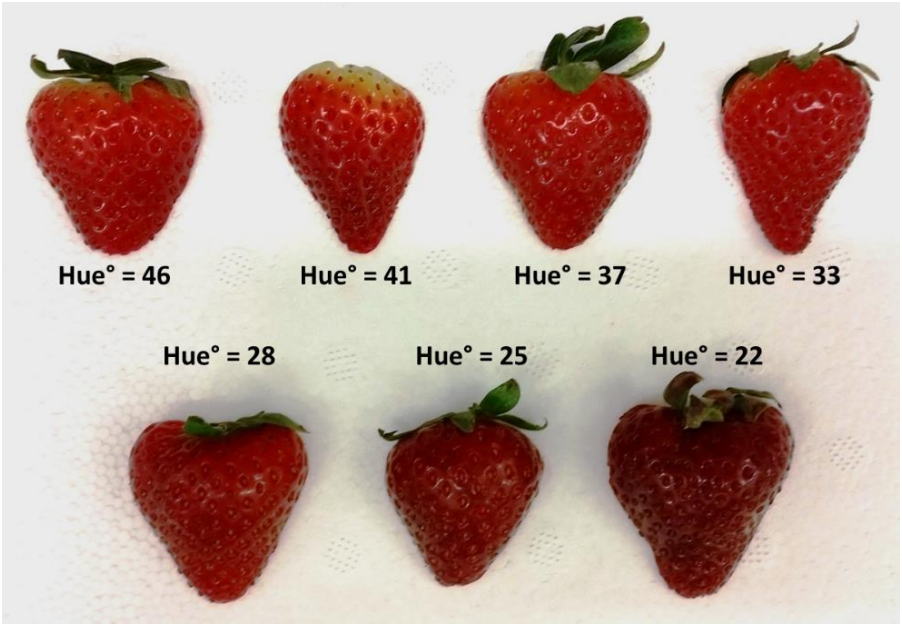

Figure 6. Common surface hue angle (°) range encountered in marketable strawberry fruit.

Flesh firmness

Flesh firmness was measured on both cheeks of each fruit at its widest point with a hand-held Agrosta® Durofel DFT 100 digital firmness tester using the Shore A hardness 0 to 100 scale where 0 = extra soft, 20 = soft, 40 = medium soft, 70 = medium hard and 90 = hard. During firmness measurements soft spots on fruit were avoided. The firmness tester was calibrated to zero prior to measurements at each harvest.

Soluble solids concentration (SSC)

SSC in °Brix was measured by slicing the tip of each fruit with a knife and squeezing fruit to release approximately 0.5 ml of juice onto the lens of a temperature-compensated digital refractometer (ATAGO PAL-1) with a measurement accuracy of ± 0.2 °Brix. The refractometer was calibrated with distilled water prior to SSC measurements at each harvest.

Titratable acidity

After SSC measurements the eight fruit per punnet were crushed in a plastic bag by hand, 8 ml of juice collected in an eppendorf tube, and juice frozen at -20°C until completion of all three harvests. All juice samples were thawed at 20°C and 3 ml of juice from each sample diluted in 5 ml of distilled water once juice temperature in all samples was above 15 °C. Titratable acidity of each sample was then measured via endpoint titration to pH 8.2 with 0.1 M NaOH using an automatic titrator (Steroglass Titre X), AS23 Micro autosampler and Hamilton® Slimtrode pH electrode. Mean titratable acidity for fruit in each punnet was calculated as grams of citric acid equivalent per litre of juice using the NaOH titre volume. Sugar to acid ratio for each punnet (field plot) was calculated from mean SSC and titratable acidity measurements using the formula; SSC to acid ratio = SSC ÷ titratable acidity × 10.

Fungal disease assessments

Six to nine fruit per punnet (field plot) were stored for 7 days at 3 - 4°C and 70 ± 5 % RH, and then incubated at 20°C for 72 hours (2nd harvest) or 96 hours (1st and 3rd harvest). Prior to cool storage fruit within a punnet were positioned so that no fruit was touching adjacent fruit. After incubation each fruit in a punnet was assessed for fungal disease symptoms and their severity. Disease severity was scored on each infected fruit using a four-point rating scale for percent of fruit surface infected where: 1 = <10%; 2 = 11 - 25%; 3 = 26 - 50%; and 4 > 50%. Mean disease incidence per punnet was calculated as; % Incidence = number of fruit with disease ÷ total fruit in the punnet × 100. For each punnet mean disease severity (DS) was calculated using the Townsend-Heuberger formula (Townsend & Heuberger, 1943):

$$DS\ (\%) = \sum(dn) \div DN \times 100$$; where

d = degree of infection according to severity scoring scale (i.e., 1, 2, 3 or 4)

n = number of fruit per disease severity category

D = highest degree of infection possible

N = total fruit within a punnet assessed for disease symptoms

Statistical analyses

To determine the main and interaction effects of harvest and treatment on yield, fruit quality and fungal disease incidence and severity, data were analysed as a factorial experiment with three replicates using two-way ANOVA in GenStat 17 (VSN International Ltd., Oxford, UK).

Violations of the ANOVA assumption of normality in the data, such as non-normality (Skewness, Kurtosis) or heterogeneity of treatment variance, were assessed using residual error plots, skewness and kurtosis tests of normality, and Bartlett's test of homogeneity of variance. Where necessary the appropriate data correction transformation was applied to data prior to ANOVA based on optimal values of lambda calculated from Box-Cox analysis in Genstat.

A two-way analysis of variance (ANOVA) was performed to determine main treatment and interaction effects on fruit quality and disease development at each harvest. Multiple comparisons of treatment means were only conducted if the ANOVA treatment P-value was significant (i.e., $P < 0.05$). Multiple comparisons of treatment means were conducted at each harvest using Fisher's Least Significant Difference (LSD) test with statistical differences between means determined at a 5% significance level ($P = 0.05$). Note that in the report the term 'significant' refers to statistical significance rather than to effects that may be commercially significant.

Treatment means that were back-transformed from transformed data used for ANOVA are indicated in results tables. Fungal disease incidence and severity data among fruit picked at 1st and 3rd harvest and incubated for 96 hours were analysed separately to data collected for 2nd harvest fruit that was only incubated for 72 hours prior to assessment.

To compare the degree of variation among treatments for important fruit quality parameters using ANOVA, the coefficient of variation within field plots was calculated based on eight fruit per plot using: Coefficient of variation (CV) % = plot standard deviation ÷ plot mean × 100.

Results

Strawberry fruit quality at harvest

Marketable yield and visual quality

Treatment A (nursery sector Seasol® + fruit sector Seasol®) and Treatment C (nil nursery sector + fruit sector Seasol®) increased total marketable fruit yield during three harvests by approximately 15% relative to the untreated control (Table 2), but these differences were not statistically significant due to high variability in yields among field plots within a treatment (Table 3). On average marketable yield in all treatments was significantly higher at the 3rd harvest in comparison to the first two harvests.

Table 2. Treatment effect on mean total marketable fruit yield over three harvests.

Treatment	Total marketable yield (g/plot)	
A	917	
B	880	
C	914	
D (Control)	792	
F prob	Treatment	0.090 (ns)

Table 3. Harvest and treatment effect on mean marketable fruit yield.

	Marketable yield (g/plot)#			
Treatment	**1st harvest**	**2nd harvest**	**3rd harvest**	**Overall**
A	232	326	350	301
B	254	281	338	290
C	269	267	332	289
D (Control)	217	294	331	279
F prob	Treatment	0.702 (ns)		
	Harvest	<0.001		
	Treatment x Harvest	0.343		

#Back-transformed means calculated from transformed data used for ANOVA

At each harvest the effect of treatment on mean berry weight was not significant, but on average fruit from Treatment A plots were marginally larger than fruit from untreated plots (Table 4). Within a harvest no treatment consistently resulted in fruit with the highest mean berry weight. Although there was no significant treatment effect on mean berry colour uniformity at each harvest fruit from Treatment A plots on average had consistently higher colour uniformity than fruit from untreated plots (Table 5).

At both the 2nd and 3rd harvests fruit from Treatment A and Treatment B (nursery sector Seasol® + nil fruit sector) plots had a significantly higher mean visual quality score than fruit from untreated plots (Table 6). The mean visual quality score among fruit from Treatment A plots was also significantly higher than in fruit from Treatment C plots. On average fruit from Treatment A and Treatment B plots had significantly higher visual quality than fruit from Treatment C plots and untreated plots.

Table 4. Harvest and treatment effect on mean berry weight.

	Berry weight (g)#			
Treatment	**1st harvest**	**2nd harvest**	**3rd harvest**	**Overall**
A	15.9	20.1	19.6	18.6
B	17.4	18.2	18.2	17.9
C	18.4	17.5	18.9	18.2
D (Control)	15.2	18.5	19.3	17.7
F prob	Treatment	0.814 (ns)		
	Harvest	0.053 (ns)		
	Treatment x Harvest	0.458		

#Back-transformed means calculated from transformed data used for ANOVA

Table 5. Harvest and treatment effect on mean berry colour uniformity.

	Berry colour uniformity (%)			
Treatment	**1st harvest**	**2nd harvest**	**3rd harvest**	**Overall**
A	96.7	95.0	94.6	95.4
B	95.8	90.8	91.3	92.6
C	94.2	93.3	93.8	93.8
D (Control)	94.6	89.2	92.5	92.1
F prob	Treatment	0.196 (ns)		
	Harvest	0.102 (ns)		
	Treatment x Harvest	0.842		

Table 6. Harvest and treatment effect on mean berry visual quality score. Treatment means with same letters within a column are not significantly different (*P* = 0.05).

	Berry visual quality score			
Treatment	**1st harvest**	**2nd harvest**	**3rd harvest**	**Overall**
A	4.8 a	4.8 a	4.5 a	4.7 a
B	4.8 a	4.3 b	4.6 a	4.6 a
C	4.8 a	4.2 bc	4.2 b	4.4 b
D (Control)	4.8 a	4.1 c	4.1 b	4.3 b
F prob	Treatment	0.002		
	Harvest	<0.001		
	Treatment x Harvest	0.135		

Figure 7. Comparison of visual quality among fruit from Treatment A plots (Top; VQ score = 4.8) and fruit from untreated plots (Bottom; VQ score = 4.1); Fruit picked at the 2nd harvest.

Berry surface colour

At each harvest the effect of treatment on mean berry colour measured as surface hue was not significant with very little difference in surface colour among fruit from treated and untreated plots (Table 7). This result is not surprising as berry colour is the main indicator of mature and marketable fruit, and only fruit with sufficient colour development were picked from each field plot.

Although mean berry hue wasn't significantly different among treated and untreated plots indicating similar overall surface colour among treatments, fruit from Treatment C plots had on average significantly greater red colour compared to other treatments based on the mean chromaticity a* value (i.e., colour on the green to red axis) (Table 8). Within a harvest no treatment consistently resulted in fruit with the greatest mean red colour based on chromaticity a*.

Table 7. Harvest and treatment effect on mean berry surface hue angle.

	Berry surface hue (°)			
Treatment	**1st harvest**	**2nd harvest**	**3rd harvest**	**Overall**
A	29.9	29.5	28.7	29.4
B	29.5	31.1	29.3	30.0
C	30.0	30.4	28.9	29.8
D (Control)	29.5	31.3	29.3	30.0
F prob	Treatment	0.719 (ns)		
	Harvest	0.004		
	Treatment x Harvest	0.453		

Table 8. Harvest and treatment effect on mean berry surface a* [chromaticity on the green (-) to red (+) axis]. Treatment means with same letters within a column are not significantly different (*P* = 0.05).

	Berry surface chromaticity a* (°)			
Treatment	**1st harvest**	**2nd harvest**	**3rd harvest**	**Overall**
A	35.8 ab	36.7 a	34.6 a	35.7 b
B	34.2 a	36.5 a	35.4 a	34.2 a
C	36.7 b	38.0 a	35.0 a	36.7 c
D (Control)	35.3 ab	37.6 a	35.2 a	35.3 b
F prob	Treatment	0.044		
	Harvest	0.004		
	Treatment x Harvest	0.652		

Berry firmness and composition

At each harvest the effect of treatment on mean berry flesh firmness measured using the Shore A hardness scale was not significant with no consistent pattern in flesh firmness among treatments within a harvest (Table 9). At the 2nd harvest flesh firmness was significantly higher in fruit from treated plots compared to fruit picked at the 1st and 3rd harvest. At the 3rd harvest untreated fruit and Treatment B fruit had a non-significant but substantially higher flesh firmness than Treatment A and C fruit and this was associated with a lower SSC, higher TA, and lower SSC to acid ratio.

Mean fruit SSC was very similar among treatments at the 1st and 2nd harvest, with very little difference in overall mean SSC among treatments (Table 10). At the 3rd harvest Treatment A and C fruit were higher in SSC than fruit from Treatment B and untreated plots, and this difference was associated with a lower flesh firmness and higher maturity in fruit among Treatment A and C plots.

Table 9. Harvest and treatment effect on mean berry flesh firmness.

	Berry flesh firmness (Shore A score)#			
Treatment	**1st harvest**	**2nd harvest**	**3rd harvest**	**Overall**
A	49.6	59.8	46.4	51.5
B	46.9	60.8	52.4	53.0
C	53.7	59.4	48.0	53.4
D (Control)	44.3	61.5	59.6	54.2
F prob	Treatment	0.852 (ns)		
	Harvest	<0.001		
	Treatment x Harvest	0.079		

#Back-transformed means calculated from transformed data used for ANOVA

Table 10. Harvest and treatment effect on mean berry soluble solids concentration (SSC).

	Berry SSC (°Brix)#			
Treatment	**1st harvest**	**2nd harvest**	**3rd harvest**	**Overall**
A	9.5	9.5	10.1	9.7
B	9.7	9.2	9.7	9.5
C	9.5	9.3	10.6	9.8
D (Control)	9.9	9.3	9.6	9.6
F prob	Treatment	0.777 (ns)		
	Harvest	<0.001		
	Treatment x Harvest	0.051		

#Back-transformed means calculated from transformed data used for ANOVA

At each harvest the effect of treatment on mean berry titratable acidity (TA) was not significant but on average fruit from untreated plots had marginally lower TA than fruit from among treated plots (Table 11). Within a harvest there was no consistent pattern in mean TA among treatments. There was also little difference in mean fruit TA within a treatment when compared across three harvests.

Although overall mean SSC to acid ratio as a measure of berry flavour did not vary significantly among treatments, fruit from Treatment C plots had on average consistently higher SSC to acid ratio at each harvest than untreated fruit (Table 12). At the 3rd harvest SSC to acid ratio in fruit from Treatment A and C plots was over 2 units higher than in fruit from Treatment B and untreated plots, indicating that Seasol® treatment during the fruit production sector may be beneficial in improving berry flavour in comparison to no treatment.

Table 11. Harvest and treatment effect on mean berry titratable acidity.

	Berry titratable acidity (g citric acid/L juice)#			
Treatment	**1st harvest**	**2nd harvest**	**3rd harvest**	**Overall**
A	8.1	7.7	6.8	7.5
B	7.4	9.1	7.3	7.8
C	8.0	7.3	6.9	7.4
D (Control)	6.9	7.1	7.4	7.1
F prob	Treatment	0.689 (ns)		
	Harvest	0.382		
	Treatment x Harvest	0.555		

#Back-transformed means calculated from transformed data used for ANOVA

Table 12. Harvest and treatment effect on mean berry SSC to acid ratio.

	SSC to acid ratio#			
Treatment	**1st harvest**	**2nd harvest**	**3rd harvest**	**Overall**
A	11.8	12.5	15.5	13.3
B	13.3	11.8	12.9	12.7
C	13.3	12.5	15.1	13.6
D (Control)	12.8	11.6	12.9	12.4
F prob	Treatment	0.464 (ns)		
	Harvest	0.101		
	Treatment x Harvest	0.767		

#Back-transformed means calculated from transformed data used for ANOVA

Fungal disease incidence and severity

Fruit infection was not observed during 7 days of cold storage at 3-4 °C and 70% relative humidity. After fruit was taken out of cold storage all Seasol® treatments significantly reduced mean fungal disease incidence relative to untreated fruit after incubation for 96 hours at 20 °C (Table 13). The fungal diseases observed were Grey Mould (*Botrytis cinerea*), Phytophthora fruit rot (*Phytophthora* spp.) and Rhizopus rot (*Rhizopus* spp.) with *B. cinerea* the pathogen observed in higher levels during the three assessments.

Following 96 hours incubation at 20 °C a high incidence of disease was observed at both the 1st and 3rd harvest as would be expected after incubation at 20 °C. At the 3rd harvest there was no significant difference in disease incidence among treatments but at the 1st harvest both Treatment A and C significantly reduced disease incidence relative to untreated fruit that had 100% disease incidence (Fig. 8a).

After incubation for 96 hours at 20 °C overall mean fungal disease severity was high in fruit from all treatments with no significant difference in disease severity among treatments (Table 14). Mean disease severity was significantly higher in all treatments at the 3rd harvest when compared to disease severity at the 1st harvest.

Table 13. Harvest and treatment effect on mean berry fungal rot incidence in fruit picked at the 1st and 3rd harvest after cool storage for 7 days at 3 - 4 °C and incubation for 96 hours at 20 °C. Treatment means with same letters within a column are not significantly different (*P* = 0.05).

	Disease incidence (%)		
Treatment	**1st harvest**	**3rd harvest**	**Overall**
A	73.8 a	100.0 a	86.9 a
B	88.9 bc	92.6 a	90.7 a
C	85.7 ab	96.3 a	91.0 a
D (Control)	100.0 c	96.3 a	98.1 b
F prob	Treatment	0.008	
	Harvest	0.036	
	Treatment x Harvest	0.094	

Table 14. Harvest and treatment effect on mean berry fungal rot severity in fruit picked at the 1st and 3rd harvest after cool storage for 7 days at 3 - 4 °C and incubation for 96 hours at 20 °C.

	Disease severity (%)#		
Treatment	**1st harvest**	**3rd harvest**	**Overall**
A	54.4	75.5	64.1
B	58.0	68.8	63.2
C	58.7	68.5	63.4
D (Control)	65.0	70.0	67.5
F prob	Treatment	0.068 ns)	
	Harvest	0.006	
	Treatment x Harvest	0.373	

#Back-transformed means calculated from transformed data used in ANOVA

After incubation for 72 hours at 20 °C moderate mean fungal disease incidence was observed in all fruit picked at the 2nd harvest with no significant difference in disease incidence among treatments (Table 15). Although fruit from Treatment C plots had 13% lower disease severity than untreated fruit this difference was not significant due to the high variability in disease severity among replicate punnets within a treatment (Fig. 8b). As expected both disease incidence and severity were substantially lower when fruit were incubated for 72 hours rather than 96 hours.

Table 15. Treatment effect on mean berry fungal rot incidence and severity in fruit picked at the 2nd harvest after cool storage for 7 days at 3 - 4 °C and incubation for 72 hours at 20 °C.

Treatment	**Disease incidence (%)#**	**Disease severity (%)#**
A	43.6	26.5
B	40.5	32.6
C	41.8	21.1
D (Control)	40.9	34.1
Treatment Fprob	0.998 (ns)	0.823 (ns)

#Back-transformed means calculated from transformed data used in ANOVA

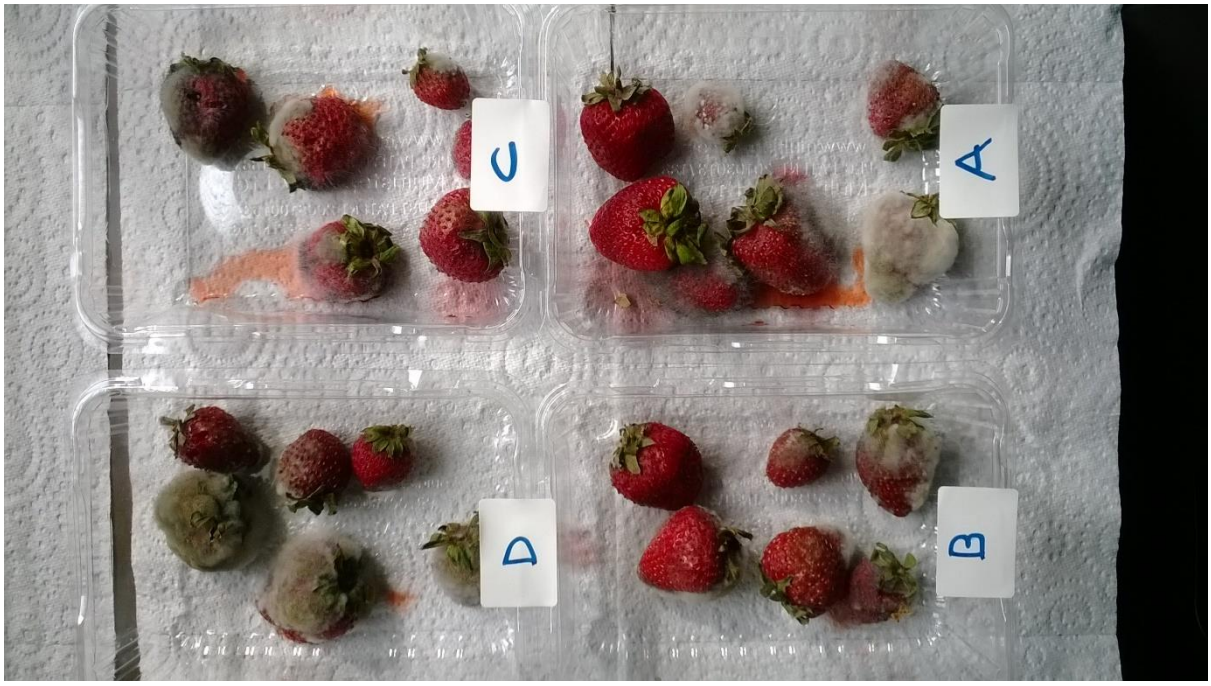

Figure 8a. Comparison of fungal disease incidence and severity among fruit from the four Treatments (D = untreated plots), picked at the 1st harvest and incubated for 72 hours at 20 °C after cool storage for 7 days at 3 - 4 °C.

Figure 8b. Comparison of fungal disease incidence and severity among fruit from Treatment C plots (top) and fruit from untreated plots (bottom), picked at the 2nd harvest and incubated for 72 hours at 20 °C after cool storage for 7 days at 3 - 4 °C.

Variability in berry quality

The variability in berry surface colour, flesh firmness and SSC among plots within a treatment was quantified by calculating the coefficient of variation (CV). The effect of treatment on berry quality variation among field plots measured by the CV was then determined using ANOVA. As the CV is a standardized measure of variability it also enabled comparison of variation among the various measured fruit quality parameters.

The mean CV within a field plot for hue angle, chromaticity a* and SSC was below 6%. This indicates that variability in berry colour and SSC among fruit within a field plot was very low, with no significant difference among treatments in mean hue angle CV or chromaticity a* CV (Table 16). SSC was significantly less variable in fruit from Treatment A plots compared to untreated fruit, and fruit from Treatment C plots. This result indicates that Treatment A fruit had more uniform SSC than untreated fruit of similar maturity as measured by berry colour.

Mean berry flesh firmness was two to three times more variable among field plots in comparison to berry colour and SSC as measured using the CV. There was no significant difference in berry firmness CV among treatments. As found for SSC, fruit among Treatment A plots had on average more uniform flesh firmness than fruit among untreated plots. These results suggest that Treatment A may reduce variability in berry firmness and SSC of marketable fruit picked at a relatively uniform berry colour.

Table 16. Main treatment effect on mean coefficient of variation within field plots for berry surface hue angle, a* chromaticity, flesh firmness and SSC. Treatment means with same letters within a column are not significantly different (P = 0.05).

		Mean coefficient of variation (%)			
Treatment		**Hue angle #**	**Chromaticity a***	**Flesh firmness #**	**SSC #**
A		3.3	3.5	11.2	4.2 a
B		4.1	3.8	13.1	4.6 ab
C		4.0	4.0	12.4	5.3 b
D (Control)		3.7	3.4	13.9	5.0 b
F prob	Treatment	0.336 (ns)	0.542 (ns)	0.140 (ns)	0.048
	Treatment x Harvest	0.944	0.288	0.768	0.989

#Back-transformed means calculated from transformed data used in ANOVA

Interaction between treatment and berry maturity

The relationship between major berry quality parameters (i.e., berry colour as hue angle, firmness and SSC) as influenced by treatment were explored by grouping fruit into statistically-distinct hue angle and SSC classes. Thus the influence of treatment on flesh firmness within each colour and SSC class, and on SSC within each colour class, could be assessed separately providing further insights regarding the interaction between treatment and fruit quality to inform future research studies.

Berry hue classes were created by arranging colour data in descending order for 72 fruit within a treatment picked over three harvests. Fruit were then segregated into three groups of 24 fruit. The mean hue angle and standard error was calculated for each group of 24 fruit within a treatment. Two-tailed t-tests were conducted to determine if there were significant differences in mean hue angle among treatments within a class, and to determine if the three hue angle classes were statistically-distinct. The same classification and statistical procedure was conducted on fruit SSC data. **It should be noted that only potential trends due to treatment could be identified using this technique as the experimental design did not include an explicit fruit maturity factor. Thus positive trends due to treatment identified below should not be considered statistically significant or confirmed.**

Within each berry hue angle and SSC class there was no significant difference between treatment means. This indicates that treatment means within a class were similar, and thus comparisons of fruit quality parameters between treatments within a class were valid. The three berry hue angle and SSC classes established from fruit quality data were statistically-distinct (i.e., significantly different at $P = 0.05$). Therefore each berry colour and SSC class represented a clearly different level of fruit maturity within which treatment comparisons could be made. Mean hue angle representing each berry colour class was 26° (most red), 30°, and 33° (least red), and these classes spanned the range of berry colour measured in marketable fruit during the trial. Mean SSC representing each berry SSC class was 8.3 °Brix (least sweet), 9.6 °Brix, and 11.1 °Brix (most sweet), and again these classes spanned the SSC range measured during the trial.

Comparison of berry firmness treatment means among each of three hue angle classes revealed that Treatment A and C may increase berry firmness among fruit with greater colour (i.e., higher maturity) relative to untreated fruit (Fig. 9). However, this trend was not statistically significant. Greater berry flesh firmness in more mature fruit (as measured by hue angle) could be advantageous in terms of prolonging postharvest storage life and in reducing postharvest losses. In contrast there seemed to be no influence of treatment on berry firmness in fruit of less colour, with untreated fruit being marginally firmer than treated fruit when berries were relatively less mature.

Comparison of berry firmness treatment means among each of three SSC classes revealed that fruit from Treatment A plots tended to remain firmer at higher SSC but again this effect was not statistically significant. In contrast, there was no trend amongst treatments in less mature fruit with lower SSC (Fig. 10). No consistent increase in berry firmness due to Treatment B or C was observed in sweeter fruit within the 9.6 °Brix and 11.1 °Brix classes. Production of sweeter fruit with higher berry firmness could be advantageous allowing producers to pick fruit that is marginally less mature but that still has acceptable SSC, flavour and consumer acceptance.

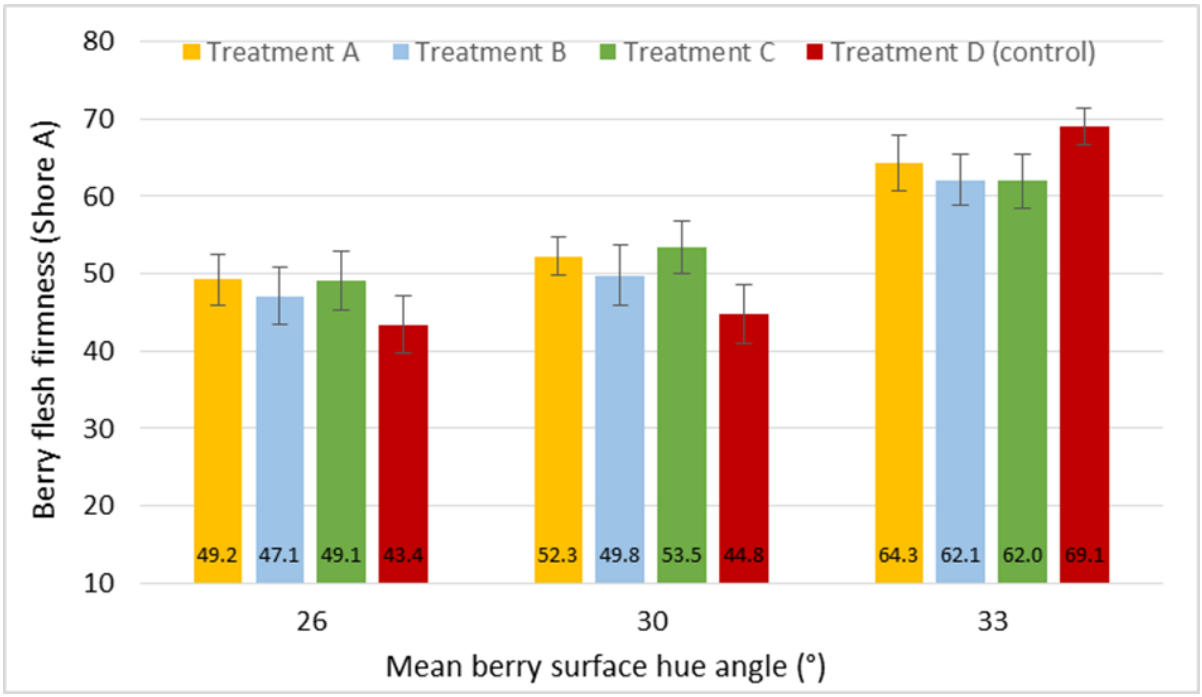

Figure 9. Effect of treatment on mean berry flesh firmness within each of three distinct berry hue classes (n = 24 fruit per treatment within a hue class). Bars represent the standard error of treatment means.

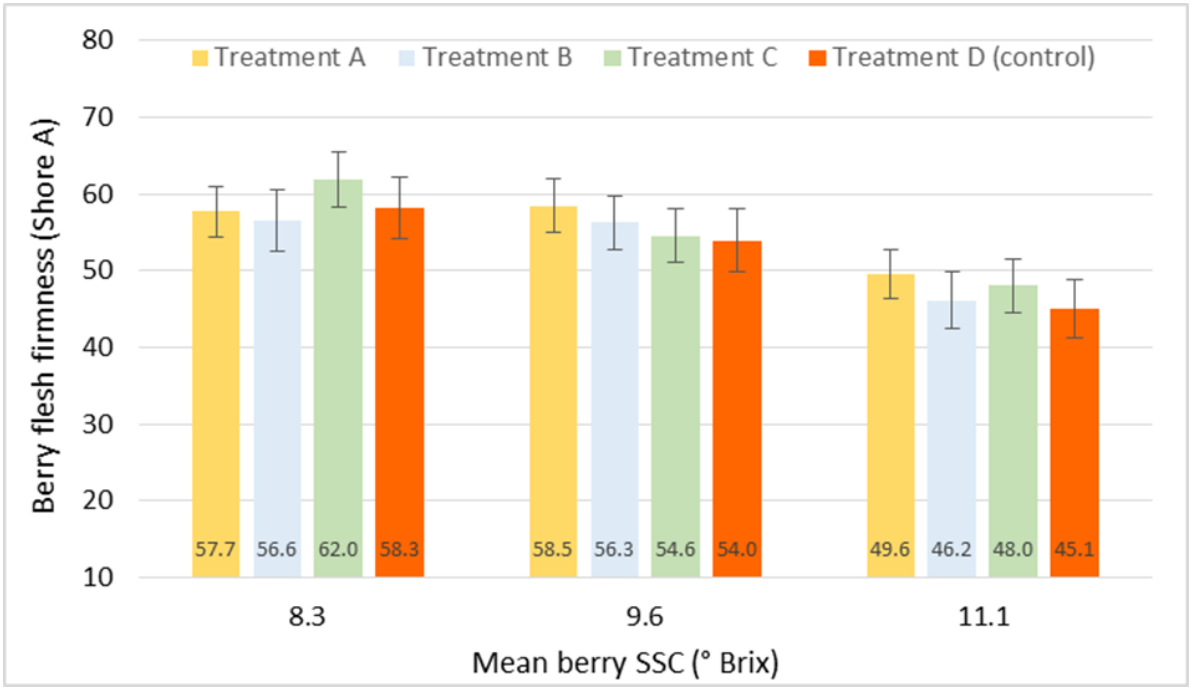

Figure 10. Effect of treatment on mean berry flesh firmness within each of three distinct berry SSC classes (n = 24 fruit per treatment within a SSC class). Bars represent the standard error of treatment means.

Comparison of berry SSC treatment means among each of three berry hue angle classes showed that fruit from Treatment A plots tended to have higher SSC when less coloured (i.e., at a hue angle (°) of 30 and 33) than fruit from untreated plots (Fig. 11). As with the positive trends due to treatment noted above, this trend was not statistically significant. No consistent increase in berry SSC due to Treatment B or C was observed in less coloured fruit within the 30° and 33° hue angle classes. At harvest, higher SSC at marginally lower berry colour is likely to result in greater uniformity of SSC among marketable fruit that contain a range of maturities. These results suggest that among the three Seasol® treatments, Treatment A (nursery sector Seasol® + fruit sector Seasol®) may contribute to an increase in SSC in marketable fruit within a pick that has relatively less colour and higher firmness.

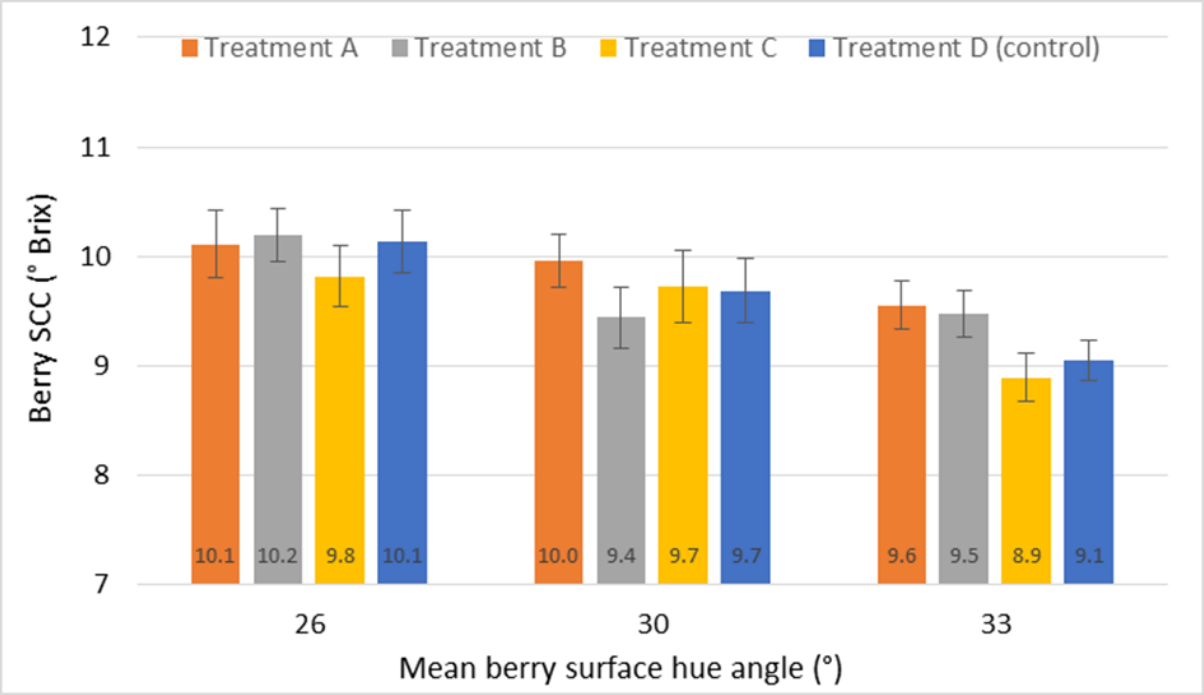

Figure 11. Effect of treatment on mean berry SSC within each of three distinct berry hue classes (n = 24 fruit per treatment within a hue class). Bars represent the standard error of treatment means.

Discussion

Strawberry yield and visual quality

Previous studies have demonstrated that seaweed extracts applied to strawberry plants can increase fruit yields (El-Miniawy et al., 2014; Alam et al., 2013; Spenelli et al., 2010). In the current experiment application of Seasol® in the nursery and fruit sectors increased average total marketable fruit yield by 10 to 15 % over three harvests relative to the untreated control. This difference was not statistically significant due to high variability in yields among field plots within a treatment. This result suggests that Seasol® treatment may provide commercially important increases in strawberry yield, and full data sets from the current trial are required to verify this result.

Berry visual score at two of the three harvests was significantly higher in fruit from Treatment A and C plots than in fruit from untreated plots, with treated fruit having more consistent and uniform colour and fewer soft spots. On average Treatment A and C increased visual quality score at harvest by 0.4 units when using a 1 to 5 scoring scale, and this effect size may be commercially important if fruit is cool stored or if there is a delay in retailing of fruit.

Little difference in mean berry colour measured as surface hue was found among treatments and this result was unsurprising as berry colour is the main indicator of mature and marketable fruit, and only fruit with sufficient colour development was picked from each field plot. However, fruit from Treatment C plots had significantly greater red colour compared to other treatments based on the mean chromaticity a*, although this did not translate into associated decreases in flesh firmness, or increases in SSC.

Strawberry disease development

After cool storage and incubation for 96 hours at 20°C all Seasol® treatments significantly reduced the incidence of fungal rots on fruit by 7 to 11 % relative to untreated fruit. When fruit was incubated for 72 hours at the 2nd pick there was no significant difference in disease incidence between treatments. Seasol® treatments did not significantly reduce disease severity relative to the untreated control. These results suggest that Seasol® treatment may inhibit development of fungal rots on fruit, but the effect may also be influenced by the incubation period. Future studies should incorporate disease assessment at multiple incubation times to determine if treatment effects on disease development are commercially significant. For example, whether Seasol® treatment may delay the onset of fungal rots on fruit.

Strawberry firmness and composition

There were significant differences in mean berry flesh firmness measured using Shore A hardness between harvests. However, within individual harvests there was no significant difference in berry firmness or consistent pattern in firmness between treatments. At the 3rd harvest untreated fruit and Treatment B fruit tended to have higher flesh firmness than Treatment A and C fruit. This trend was associated with lower SSC, higher titratable acidity (TA) and lower SSC to acid ratio.

Fruit flavour as measured by SSC, TA and SSC to acid ratios was not significantly different between treatments, with no consistent pattern in SSC and TA among treatments at each harvest. SSC and sugar to acid ratio at the 3rd harvest tended to be higher in Treatment A and C fruit compared to untreated fruit. There was a trend of a 1 °Brix difference in mean SSC between Treatment C and untreated fruit and a 2.6 unit difference in mean sugar to acid ratio between Treatment A and untreated fruit.

These results indicate that Seasol® treatment may improve berry flavour at harvest with a 1 to 1.5 °Brix difference in fruit SSC generally considered distinguishable by consumers and thus of potential commercial importance. A mean increase in SSC to acid ratio of 2 or greater is also likely to be important in meeting consumer requirements for balanced flavour in fruit, particularly in the case of strawberry, that at maturity has relatively high titratable acidity relative to soluble solids concentration. This potential positive effect should be explored further in future trials using greater replication.

Influence of fruit maturity on treatment

The potential interaction between marketable fruit maturity and treatment was explored by grouping fruit into statistically distinct hue angle and SSC classes. By utilising measurements on individual fruit the influence of a treatment among fruit of a specific maturity could be examined and provide guidance for the experimental design of future studies.

Comparison of berry firmness treatment means among each of three fruit hue angle classes revealed that Treatment A and C tended to increase berry firmness among fruit with greater colour and maturity relative to untreated fruit. Higher berry flesh firmness in more mature fruit could be beneficial in prolonging postharvest storage life and in reducing postharvest losses. It was also observed that fruit from Treatment A plots tended to remain firmer at higher SSC, whilst there was no trend amongst treatments in less mature fruit with lower SSC. Production of sweeter fruit with relatively higher berry firmness and lower maturity could allow producers to pick fruit that is marginally less mature but with acceptable SSC and flavour.

Furthermore fruit from Treatment A plots tended to have higher SSC when less coloured than fruit from untreated plots. Higher SSC at marginally lower but marketable berry colour is likely to result in greater uniformity of SSC among marketable fruit with a range of maturities. In this trial fruit SSC was found to be least variable among Treatment A plots suggesting that this treatment may increase uniformity of berry sweetness within a harvest. **Note that positive trends due to treatment identified above should not be considered statistically significant.** These positive trends require confirmation by conducting larger trials with greater replication.

Conclusions

Results from this preliminary experiment indicate that Seasol® treatments may contribute to higher strawberry yields, better fruit visual quality and inhibition of fungal disease development. Among the three Seasol® treatments, Treatment A (nursery sector Seasol® + fruit sector Seasol®) may potentially increase SSC, and its uniformity, in marketable fruit that has relatively lower colour and higher firmness.

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