Six years of strawberry trials in commercial fields demonstrate that an extract of the brown seaweed *Ascophyllum nodosum* improves yield of strawberries.

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Abstract

Strawberry (*Fragaria ananassa)* growers are often looking for sustainable products to enhance the overall health and yield of their crop. In trials over six years, one summer and five winter studies, conducted in the Oxnard area of California, it was shown that soil drip-tape applications of seaweed extract (*Ascophyllum nodosum)* (trade name Acadian LSC) enhanced yield of strawberries by an average of 4394 kg per hectare compared to the control. This improvement in yield is likely due to improvements in early growth and crown development as were seen in the five years where it was evaluated. Two-spotted spider mite (*Tetranychus urticae*) populations were high enough to rate in two of the trials with Acadian LSC suppressing mites in both trials. Charcoal Rot caused by the pathogen *Macrophomina paseolina* was present in the fourth year of this study (2013-2014). The seaweed extract treated plots had less mortality due to this disease compared to the control (27.4 vs 16.6 % plant mortality). Lastly, in the first year study there were high soil sodium levels and the seaweed extract treated plots had less phytotoxicity as well as lower sodium levels in the leaves. These results indicated that *Ascophyllum nodosum* extract significantly improves yield of strawberries however the mode of action is likely multifaceted and may involve improvements in growth, tolerance to environmental stresses, and tolerance to biotic stresses.

Keywords: Strawberries, yield, mites, crown number, *Macrophomena*, seaweed, A*scophyllum nodosum*

INTRODUCTION

Strawberries in California are intensely farmed with many growers open to new production methods to improve their yields. In addition, non-toxic products that improve yield are especially important because strawberries are often grown in highly populated areas which limits agricultural inputs. An extract from the marine plant, *Ascophyllum nodosum* has been demonstrated in prior research to improve yield of strawberries (Alam et al., 2012; Morales, 2006). However, this prior research has been conducted under extremely controlled conditions in small plots, pots, or greenhouse. The purpose of this work was to examine the effect of *A. nodosum* on yield of strawberries in commercial fields. The trial was conducted over 6 years and evaluates how *A. nodosum* performs under commercial conditions.

MATERIALS AND METHODS

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This study consisted of 6 field trials conducted over 7 years in commercial growers' fields. The first, second, fourth, fifth, and sixth trials were planted in the fall with harvests through the winter and ending in the spring. The third trial was planted mid-summer and harvested through December 2009. The treatments in these six trials included an untreated control (grower's normal program without *A. nodosum*) and the grower's normal program with a commercial extract of *A. nodosum* (Acadian LSC) applied every two weeks at 4.68 l/ha through the commercial drip irrigation beginning at planting and continuing until the grower shifted from fresh market to processing production. In the winter trials the applications began in November and ended in March totaling 9-10 applications. For the summer trials the applications began in July and ended in November totaling 6 applications.

Table 1: Application timing

Application Beginning and End dates		
	Applications began	**Applications ended**
2006-2007	November 1	March 14
2007-2008	November 1	March 27
2009	July 21	November 20
2013-2014	October 1	April 1
2014-2015	November 5	March 25
2015-2016	November 9	March 14

The treatments were injected into individual drip lines with a sub-injection point during regular irrigations every two weeks. Since the trials were conducted in commercial fields with multiple growers and locations, the grower standard practices varied by planting dates and location. The fields were prepared by applying 550-900 kg/ha of a controlled release 18-8-13 fertilizer per acre, fumigating with Telone/Cloropicrin or Chloropicrin alone, and laying plastic on 1.63 meter beds. Plant spacing was 30-38 cm. Trial design were non-randomized strip trial or two large side by side blocks with four to six subplots within each plot. The subplots for evaluation contained 24-40 plants per plot. The varieties were the grower's proprietary and University of California varieties. In the first, second and third trials, the initial Acadian LSC treatment was applied prior to planting bare root crowns by drenching with a 0.1% solution. This was done by pouring 38 liters of the 0.1% Acadian LSC solution into each plastic lined transplant box which contained 1000 plants each. The transplants were allowed to soak with the seaweed extract for ten minutes. The water was then drained from the box and the transplants immediately transplanted and watered in with overhead sprinklers. This at planting dip was not included in the last 3 trials. Numbers of crowns per plant were counted mid-season in five of the trials. Yield data was collected in all trials by having the subplots picked by a commercial picker at each commercial picking date. All fresh market fruit from each plot was weighed. Numbers of commercial flats were calculated by assuming 4.5 kg per flat. Economics were calculated at each harvest date based on shipping point process from the USDA website: Market News - Fruit and Vegetable - Search by Reports.

If significant insects or disease were present in the studies, it was rated. Two-spotted spider mites (*Tetranychus urticae)* were present in two of the years and *Macrophomena* Charcoal Rot was present in one year. Mites were rated by counting all of the mites on five leaves per plot. *Macrophomena* Charcoal Rot was rated by counting the number of dead plants per plot and calculating a percentage.

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Across trial yield summary was conducted using each year as a replicate. Data were analyzed using a T-TEST across each year. The significance of the treatment differences was p=.002 (EXCEL software). Significance for individual years is not presented because the treatments were applied to extremely large plots using commercial irrigation and were either arranged in strips or as an individual large block that did not have a random design with six subplots evaluated within.

Across trial crown summary was also conducted using each year as a replicate with data analyzed using a T-Test across each year. The significance of the treatment differences were ≤ 0 0.05.

Mite data was rated by counting 5 randomly selected mid-tier leaves from each subplot and counting both motiles and eggs. *Macrophomena phaseolina* data were collected by counting the number of dead plants in 6 random locations within each treatment and percent mortality calculated. Because the design of these trials being an extremely large plot with 6 subplots sampled within each large plot, the statistics for individual years should be considered with caution, however both mite and *Macrophomena* data are presented with statistics for individual years because an across trial summary was not possible due to the pest being present only in one or two years.

Leaf sodium was evaluated by taking random mid-tier leaf samples from each subplot three times during the growing season and sending them to Fruit Grower's Laboratory in Santa Paula, California for analysis.

RESULTS AND DISCUSSION

Total yield was enhanced by the *A. nodosum* applications all six years. The average yield increase was 4393 kg/ha or a 15% yield increase (Table 2).

Table 2: Yield per hectare

	Number of Commercial Trays Per Hectare						
	2006-2007	**2008-2009**	**2009**	**2013-2014**	**2014-2015**	**2015-2016**	**Average**
Control	1051.5	2050.4	1106.3	654.8	422.1	1029.6	1052.2b
A. nodosum	1249.4	2164.6	1201.7	781.85	577.1	1291.6	1211.1a

Economics were calculated using an average price of $7.00 per 4.53 kg tray. This equates to an average revenue increase of $869 per hectare per year (Table 3).

Table 3: Gross revenue per hectare

	Revenue Per Hectare						
	2006-2007	**2008-2009**	**2009**	**2013-2014**	**2014-2015**	**2015-2016**	**Average**
Control	$7,360	$14,352	$7,744	$2,940	$2,101	$7,534	$6,891
A. nodosum	$8,745	$15,152	$8,412	$3,660	$2,832	$9,277	$7,760

The numbers of crowns per plant were measured mid-season in 5 of the studies. The *A. nodosum* treated plants averaged 2.77 crowns per plant. The control plants averaged 2.14 crowns per plant (Table 4)

Table 4: Number of crowns per plant

	Number of Crowns Per Plant				
	2006-	**2008-**	**2009**	**2014-**	**2015-**	**Average**

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	2007	2009		2015	2016	
Control	1.80	2.80	2.81	1.70	1.60	2.14b
A. nodosum	2.60	3.80	3.84	1.80	1.80	2.77a

Two-Spotted spider Mites were present in two of the 6 years. In both trials there were significantly less mites per leaf in the *A. nodosum* treated compared to the control (Table 5).

Table 5: Number of Two -spotted Spider Mites per leaf

	Mites Per Leaf 2013-2014	 2014-2015	 Average
Control	15.3a	35.3a	25.3
A. nodosum	4.0b	22.0b	13.0

Macrophomena phaseolina, Charcoal Rot, was present in 2013-2014. By the final rating (242 DAP) there were significantly less dead plants in the *A. nodosum* treated plots (Table 6)

Table 6: Percent mortality due to *Macrophomena phaseolina* at 242 days after planting, 2013-2014

	Percent Dead Plants 2013-2014
Control	27.4a
A. nodosum	16.6b

The initial year that these trials were conducted was in a field high in sodium. Soil salinity was 5.22 mmhos/cm. Optimum soil salinity for strawberries is 0-2 mmhos/cm. Leaf tissue tests through the season consistently demonstrated lower sodium levels in the *A. nodosum* treated plants compared to the control (Table 7). In addition root dry weight was improved in the *A. nodosum* treated plants indicating improved vigor despite the adverse soil conditions (Table 8).

Table 7: Sodium content in the leaves in the trial conducted 2006-2007

	Leaf Sodium, 2006-2007 Dec 18	 March 1	 April 18	 Average
Control	0.016	0.014	0.016	0.015
A. nodosum	0.014	0.013	0.015	0.014

Table 8: Root dry weight 9 weeks after planting

	Root Dry Weight, 2006-2007
Control	1.3b
A. nodosum	2.1a

The yield results seen in the *A. nodosum* treated plots were likely due to significant improvements in the numbers of crown divisions, however there may have been other factors involved. Tolerance to drought stress has been seen with applications of *A. nodosum*

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in citrus (Spann and Little, 2011). A downregulated gene responsible for tolerance to salinity in *Arabadopsis* was described by Jatish et al, 2012. This type of environmental stress tolerance may have contributed to the improved yields seen in these strawberry trials. Additionally, higher chlorophyll levels and antioxidant capacity has also been observed in *A. nodosum* treated spinach, broccoli, mungbeans, and cabbage (Di Fan et al., 2013; Battacharyya et al., 2015). It is possible that higher antioxidant capacity also contributed to enhanced tolerance to environmental stress in the strawberries. Improved tolerance to environmental stress may have not only contributed to improved yields but also resulted in reduced mite populations.

Enhanced disease tolerance has been observed in other crops such as carrots, okra, onions, cucurbits, and tomatoes (Jayaraman et al.. 2010; Battacharyya et al., 2015). The mode of action behind this was elucidated by Subramanian et al, 2011. They demonstrated that the jasmonic pathway was elicited by applications of *A. nodosum* and resistance to *Pseudomonas Syringae* was induced. This defense pathway is also associated with stress and insect tolerance which may have contributed to the mite suppression and salinity tolerance seen in these strawberry trials (Santino et al., 2013).

Higher levels of key foliar nutrients (K, Mg, Ca, Cu, Fe, Zn, Mn) have been reported in lettuce, tomato, tomato, banana, and grapes (Battacharyya et al., 2015). These nutrients observed to be influenced by *A. nodosum* are especially important with many California strawberry growers supplementing Ca, Zn, and Fe. Although these strawberry trials were not focused on fertility, enhanced nutrient levels likely occurred which could have contributed to the observed yield increases.

When considering the potential mode of action seen in these trials, the review paper by Dhriti Battacharyya et al. at Dalhousie University on the activity and potential bioactive compounds of seaweed extract provides excellent insight across many crops (Battacharyya et al., 2015). They attributed improved abiotic stress tolerance to the regulation of key genes (RD29A, RD22, SOS, C8F3, COR15A), the accumulation of osmolytes such as proline, sorbitol, and betaines, as well as reduced transpiration and better regulated stomatal conductance. Acadian LSC contains elicits key sugars such as mannitol which are known to be osmoprotectants. Acadian also contains natural betains, as well as elicits the production of higher levels of betains. The stress tolerance attributed to *A. nodosum* is very likely caused by numerous naturally occurring osmoprotectants, elicited osmoprotectants such as proline and betaines, as well as the presence of antioxidants in the product such as fucose-containing polysaccharides and elicited higher antioxidant capacity.

The natural sugars contained in *A. nodosum* extract may act as natural chelators for nutrients such as the reported Zn, Fe, Mn, Ca, and Cu. In addition, unpublished trials by Acadian Seaplants Limited have demonstrated that foliar levels of some nutrients such as Zn, Ca and N are enhanced with foliar applications of *A. nodosum* with no added nutrients in the mix with the product. In this scenario chelation of nutrients is not possible which indicates that the mode of action surrounding nutrient uptake is still not fully described however likely involves the upregulation of genes responsible for nutrient translocation.

Reports of disease suppression most often hypothesize that plant responses are elicited via compounds such as: oligo- or polysaccharides, peptides, proteins, lipids, cell wall debris, carrageenans, laminarin, β 1-3 glucans or sulfated fucans or combinations thereof (Lizzi et al., 1998; Patier et al., 1993; Subramanian et al., 2011) Marine algae in general are a rich source of bioactive compounds. For example, the cell wall is composed of unique polysaccharides that can be potential elicitors of plant defense responses. The brown alga, *A. nodosum* contains laminarin (β-D-(1→3) glucan) that acts as an elicitor and has shown to play a key role in plant growth and defense response by the induction of antimicrobial phytoalexins (Patier et al., 1993).

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CONCLUSIONS

This series of 6 trials conducted under commercial growing conditions in California constitutes a very thorough look at what growers can legitimately expect from incorporating *A. nodosum* (Acadian LSC) extract in their production program. Trials were conducted both in the winter and summer production and very consistently demonstrated a yield increase in the *A. nodosum* treated plots each year compared to the control. This is the first published report on this type of biostimulant in large scale ``real world`` situations over multiple seasons and should give growers a high level of confidence in incorporating this type of biostimulant in their commercial program. The mode of action is likely multifaceted involving improvements in growth, tolerance to environmental stresses, and tolerance to biotic stresses.

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