Journal of Applied Phycology
https://doi.org/10.1007/s10811-023-02979-0

RESEARCH

The biostimulant effect of an extract from *Durvillaea potatorum* and *Ascophyllum nodosum* is associated with the priming of reactive oxygen species in strawberry in south-eastern Australia

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Received: 31 January 2023 / Revised: 20 April 2023 / Accepted: 22 April 2023

Abstract
Strawberry is an important horticultural crop in Victoria, Australia. Grey mould caused by *Botrytis cinerea* reduces marketable yield and post-harvest quality of strawberry fruit in the region. We conducted two field experiments in Victoria (Coldstream and Warburton) to evaluate the effectiveness of a commercial seaweed extract from *Durvillaea potatorum* and *Ascophyllum nodosum* (Seasol®) on the yield, revenue, and post-harvest rot of strawberry fruit. We applied the extract to strawberry crops (cv. Albion) monthly as a combined drench (10 L ha^{-1}) and foliar spray (1:400), with water as a control. Application of the seaweed extract significantly increased strawberry fruit yields by 8-10% and revenue by AU$0.37-0.59 per plant. Furthermore, the extract significantly reduced the incidence and severity of post-harvest rots in strawberry fruit by 52-87%, respectively. The extract did not affect the firmness, soluble solids concentration (SSC), titratable acidity, or SSC:acid of strawberry fruit. In a separate laboratory experiment, we found that growing strawberry in the seaweed extract (1:400) increased the concentration of peroxidase by 50% and doubled H_2O_2 in roots soon after treatment. Increases in reactive oxygen species are an indicator of a suite of pathways associated with resistance and tolerance of biotic and abiotic stresses. Overall, the results demonstrate that the seaweed extract can act as a commercially-viable biostimulant for strawberry fruit production in south-eastern Australia.

Keywords *Fragaria* × *ananassa* · Seasol® · Seaweed extract · *Botrytis cinerea* · Post-harvest quality · Revenue

Introduction

Strawberry (*Fragaria* × *ananassa*) is an important horticultural crop in Australia and the industry was recently valued at AU$420 million per annum (Mattner et al. 2018; Horticulture Innovation Australia 2022). In addition to its commercial importance, strawberry fruit is recognized for its high organoleptic and nutraceutical qualities (Samykanno et al. 2013). Strawberry production occurs in every Australian state, with major regions in Victoria, Queensland, and Western Australia (Horticulture Innovation Australia 2022). The industry in Victoria mostly uses day-neutral cultivars of strawberry (e.g., 'Albion'), which fruit in response to mild temperatures over summer (from spring to autumn, up to 9 months of production). Planting occurs in autumn/winter using freshly-dug transplants (bare-rooted runners) or plug plants, and in summer using cold-stored (frigo) runners. Production mostly occurs in open fields in fumigated soils sealed with black plastic mulch (low density polyethylene), although there is an increasing trend towards hydroponic systems under tunnels. These contrasting conditions in Victoria expose strawberry plants to a range of biotic (e.g., *Botrytis cinerea* and other pathogens and pests) and abiotic (e.g., extreme temperatures over summer) stresses.

Botrytis cinerea is a necrotrophic, fungal pathogen that infects over 500 crops, including berries, and results in economic losses across the globe of up to US$100 billion

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Published online: 12 May 2023

(Hua et al. 2018). *Botrytis cinerea* causes grey mould of strawberry, which is a serious post-harvest disease because it reduces the quality and marketability of fruit. In Victoria *B. cinerea* can reduce commercial yield of strawberry fruit by up to 16% (Washington et al. 1992), and cause post-harvest rots of up to 70% (Washington et al. 1999). Currently, growers use a range of fungicides to manage grey mould of strawberry, and these strategies are highly effective compared with non-treated controls (Washington et al. 1992, 1999; Menzel et al. 2016). However, the continued use of these chemistries increases the risk of development of resistance to fungicides in populations of *B. cinerea*. For example, researchers in Australia have identified strains of *B. cinerea* from strawberry that are resistant to the fungicides benzimidazole, fenhexamid, iprodione, procymidone, and pyrimethanil (Washington et al. 1992; Menzel et al. 2016). Furthermore, many fungicide chemistries are under threat of withdrawal due to their impact on human and/or environmental health. For example, the fungicide benzimidazole was withdrawn from use in Australia in 2006 following concerns over its effects on human reproduction and development (Australian Pesticides and Veterinary Medicines Authority 2023). Therefore, industry urgently needs more sustainable methods that complement or offset the use of fungicides to manage fruit rots and other diseases, and to increase the proportion of marketable fruit.

Recent literature reviews provide evidence that the use of extracts from brown seaweeds as plant biostimulants can condition strawberry for increased tolerance of biotic and abiotic stresses and result in improved growth, yield, plant health and post-harvest quality of fruit (Righini et al. 2018; Garza-Alonso et al. 2022). Du Jardin (2015) defined a plant biostimulant as 'any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content'. A distinguishing feature of biostimulants from synthetic fertilizers is their effectiveness at low rates of application. Indeed, many commercial biostimulants derived from brown seaweeds have low nutrient concentrations and contain several plant growth regulators (Tay et al. 1985, 1987; Wite et al. 2015).

Field and pot experiments conducted in different regions around the world have found that extracts from brown seaweeds increased fruit yields of strawberry by 8–50% (Masny et al. 2004; Roussos et al. 2009; Spenelli et al. 2010; Alam et al. 2013; Eshghi et al. 2013; El-Miniawy et al. 2014; Kapur et al. 2018a; Mattner et al. 2018; Al-Shatri et al. 2020; Mufty and Taha 2021; Popović et al. 2022; Rana et al. 2022; Ashour et al. 2023). For example, in a comprehensive series of six field experiments in California from 2006 to 2016, Holden and Ross (2017) showed that fortnightly drip application to soil of a commercial extract from the brown seaweed *Ascophyllum nodosum* increased the yield of strawberry fruit by an average of 15%, which resulted in an increase in revenue of US$ 869 ha^{-1} compared with the control. Most of the seaweed extracts evaluated in the literature are from a single-origin, and the potential use of multi-origin species of seaweeds in extracts has not been thoroughly considered for strawberry production. Moreover, evaluation of seaweed extracts for use in specific crops like strawberry is required under real-world conditions (Ricci et al. 2019) due to the variation in yield responses across different regions, cultivars, and production systems, combined with differences in the composition and manufacturing of the extracts themselves. Also, generation of revenue data from the use of seaweed extracts is important to support the sustainable and economic adoption of these products by growers.

Righini et al. (2018) and Garza-Alonso et al. (2022) identified the issues of resistance to pathogens and post-harvest quality of fruit as key gaps for further research on the effects of seaweed extracts in strawberry. Washington et al. (1999) showed that foliar application of extracts from brown seaweeds reduced the post-harvest incidence of grey mould in strawberry fruit by up to 40%. Similarly, treatment of strawberry plants with seaweed extracts have increased some parameters of fruit quality and functionality in strawberry including soluble solids concentration (SSC) (El-Miniawy et al. 2014; Kapur et al. 2018a, b; Rana et al. 2022), SSC to acid ratio (Al-Shatri et al. 2020; Rana et al. 2022), polyphenolic compounds (Soppelsa et al. 2019; Tajdinian et al. 2022), flavonoids and anthocyanin (Mufty and Taha 2021; Popović et al. 2022; Tajdinian et al. 2022), firmness (El-Miniawy et al. 2014) and antioxidant activity (Tajdinian et al. 2022). Yet, several other studies show few or opposite effects of seaweed extracts on fruit quality in strawberry (Masny et al. 2004; Roussos et al. 2009; Spenelli et al. 2010; Eshghi et al. 2013; Kapur et al. 2018a, b; Soppelsa et al. 2019; Popović et al. 2022).

Researchers have postulated that the stimulatory effects of extracts of brown seaweeds on plant growth and health are due to the complex of hormones and other compounds they contain (Khan et al. 2009). However, insights from research using *Arabidopsis* mutants suggests that the phytohormone levels present in seaweed extracts are insufficient to achieve the significant plant responses reported in the field (Wally et al. 2013). Instead, their research found evidence that seaweed extracts modulate plant pathways for biosynthesis of phytohormones. Subsequent research approaches using transcriptomics, metabolomics, and cellular biology have characterised the responsiveness of many plant pathways, including phytohormones, due to application of seaweed extracts (Islam et al. 2020, 2021; Staykov et al. 2021; Tran et al. 2023).

In addition, there is increasing evidence that seaweed extracts can act as priming stimuli for induction of adaptive defence against biotic and abiotic stress factors in plants (Fleming et al. 2019; Islam et al. 2020, 2021; Rasul et al.

2021; Cocetta et al 2022). Defence priming is considered a low fitness cost to the plant because the response is only partially and transiently activated (Martinez-Medina et al. 2016). Garza-Alonso et al. (2022) described the biochemical mechanisms of perception of biostimulants by strawberry cells to transduction, signalling and gene regulation. Reactive oxygen species (ROS) such as H_2O_2 function as important signallers in this process due to their reactivity and ability to oxidize and modify protein molecules and influence redox balances in the cell (e.g., Waszczak et al. 2018). ROS also activate mitogen-activated protein kinases and their signalling cascades effect gene regulation (Garza-Alonso et al. 2022). Due to their systemic signalling role, changes in concentrations of ROS are indicators of defence priming in plant cells and of enhanced plant cross-tolerance mediated by previous exposure to stress (Perez and Brown 2014).

We conducted field experiments in the strawberry industry that aimed to test the hypothesis that a commercial extract from two brown seaweeds, *Durvillaea potatorum* and *Ascophyllum nodosum*, increase fruit yields and revenue from strawberry, and reduce post-harvest rot. We also conducted an experiment in the laboratory on the effect of preconditioning strawberry plants with the seaweed extract on the activation of ROS in root cells and induction of defence priming.

Materials & methods

Strawberry field experiments

Two field experiments were conducted on strawberry farms in the Yarra Valley, Victoria, Australia, across contrasting planting seasons. The first trial was from November (spring) to June (winter) at Coldstream, Victoria (37°41´04.0´´S, 145°26´00.6´´E) (short-season crop). The second trial was from May (winter) to June (winter) at Warburton, Victoria (37°44´57.9´´S, 145°38´18.4´´E) (long-season crop). Planting material in the experiments were bare-rooted runners of the cultivar Albion, which had been cold-stored (frigo plants) (Coldstream trial) or freshly-dug (Warburton trial) from the nursery (Toolangi Certified Strawberry Runner Growers Co-operative, Toolangi, Victoria).

Soil at both trial sites was prepared by rotary hoeing to depth of 25 cm and raising beds. Soil was fumigated with chloropicrin (300 kg ha^{-1}) and beds covered with black plastic film (low-density polyethylene). During soil fumigation, two bands of a fertilizer blend (Pivot 800, Incitec Pivot, Victoria) were chiselled into soil to a depth of 10 cm at a rate of 800 kg ha^{-1} and trickle-irrigation tape laid under the plastic.

Four weeks after fumigation, runners were planted by hand into plots through holes in the plastic (November at Coldstream, and May at Warburton). Prior to planting, half the runners in both trials were soaked overnight in a 1:400 concentration of the seaweed extract and half in water (control). Beds were 1.02 or 0.80 m wide and there were four or two rows of strawberry plants per bed at the Coldstream and Warburton sites, respectively. Individual plants were spaced 40 cm apart. Plants were watered by overhead irrigation during establishment, after which plants were mostly irrigated by drip irrigation through the trickle tape. All other agronomic procedures in the trial followed standard industry practices. This included application of a standard rotation of fungicides (captan, fenhexamid, iprodione, thiram, cyprodinil/fludioxinil and pethiopyrad) every fortnight through the fruiting season at label rates.

The seaweed extract used in the trials was an alkaline hydrolysis product from *Durvillea potatorum* and *Ascophyllum nodosum* with a soluble solid level of 16% (w/w) to standardise applications (Seasol®; Seasol International, Bayswater, Victoria, Australia). The undiluted extract has a pH of 10.5 and contains 0.2 % (w/v) N, 0.02% P, 3.7 % K, 0.3 % S, 458 mg L^{-1} Ca, 972 mg L^{-1} Mg, 115 mg L^{-1} Fe, 2 mg L^{-1} Mn, 15 mg L^{-1} B, and 5 mg L^{-1} Zn. In addition, Wite et al. (2015) reported the undiluted extract contained 7 % (w/v) total laminarins, 154 µg L^{-1} total auxins, 36 µg L^{-1} total cytokinins (including zeatin, dihydrozeatin, isopentenyl-adenine, and their corresponding ribosides and glucosides; Tay et al. 1985, 1987) and 382 µg L^{-1} total betaines. Half of the plots and plants in the trials were treated with the seaweed extract (1:400 concentration) as a monthly drench (10 L ha^{-1}) to the soil, combined with a foliar spray (to the point of runoff). The other half of plots and plants were drenched and sprayed with equivalent volumes of farm irrigation water (control). The irrigation water at Warburton and Coldstream had a pH of 7.2 and 6.9, and electrical conductivities of 87 and 109 µS cm^{-1}, respectively. The first application of the treatments was at planting and the last application was 2 weeks before the final harvest (June). In total, there were seven applications of the seaweed extract at the Coldstream trial (i.e. a total 0 (control) and 70 L of the extract ha^{-1}), and thirteen applications at the Warburton trial (i.e. a total 0 (control) and 130 L of the extract ha^{-1}). The trials were conducted as randomised complete block designs with six blocks of the two treatments (seaweed extract and control) at the Coldstream trial, and eight blocks at the Warburton trial. There were 20 plants per plot at both trials.

Fruit yield and revenue

Marketable fruit from plants in each plot were picked, counted, and weighed 2-3 times per week from January to June at the Coldstream trial, and from October to June at the Warburton trial. Revenue from strawberry fruit for each pick

was calculated from wholesale prices for strawberry fruit at the Melbourne, Victoria market (FreshLogic, Hawthorn, Victoria).

Post-harvest fruit quality and rot

At two sample harvests in the Warburton trial (January, after three applications of the seaweed extract; and March after six applications of the seaweed extract), up to 25 pieces of marketable fruit were randomly selected from each plot. The fruit samples were placed in ventilated strawberry punnets and cooled to 4ºC in a commercial cool-room at the farm site, and then immediately transported in an esky with ice packs (Refreeze™) to a post-harvest laboratory where fruit was refrigerated at 4ºC at 70% ± 5 % RH.

Within 24 hours of harvest, 10 fruit per punnet (equivalent to one plot) were allowed to warm to 18°C and were measured for parameters of post-harvest quality and maturity. 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. Soluble solids concentration (SSC) in °Brix was measured by slicing the tip of each fruit with a knife and squeezing 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 fruit were then crushed in a plastic bag by hand, and juice (3 mL) from each sample was diluted in 5 mL of distilled water. Titratable acidity of each sample was 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 (Sadler and Murphy 2010). 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.

The remaining fruit in each punnet were left refrigerated and later assessed for incidence and severity of rot diseases after storage for 3 and 7 day (January harvest) or 5 and 10 days (March harvest). Fungi causing the rots were identified using a light microscope and taxonomic keys. Rot severity was assessed on each infected fruit using a score for the surface area affected by disease, where: 1 = 1 to 5%; 2 = 6 to 15%; 3 = 16 to 25%; 4 = 26 to 50%; and 5 = > 50% fruit area infected. Mean disease severity (DS) was calculated using the Townsend-Heuberger formula: DS (%) = $\sum$(dn) ÷ DN × 100; where d = degree of infection according to severity scoring scale (i.e., 1, 2, 3, 4, or 5), n = number of fruit per disease severity category, D = highest degree of infection possible and N = total number fruit assessed (Townsend and Heuberger 1943). Mean disease incidence was calculated as a percentage of the number of infected fruit per punnet.

Analysis of reactive oxygen species in strawberry roots

We used a laboratory rather than a field method to assess the effect of the seaweed extract on accumulation of ROS in strawberry tissue. Previous developmental research on the method showed that use of field grown plants can confound the effect of treatment on ROS accumulation. This is because washing and removing soil particles from roots resulted in accumulation of ROS independent of any treatment (data not shown). In the method, strawberry plug plants (cv. Albion, 3-month old, 115 cm^3 cell) in soil-less media (coir / composted pine bark) were obtained from a commercial nursery at Toolangi, Victoria (Toolangi Certified Strawberry Runner Growers Co-operative). The seedlings were placed in a plant growth chamber (Thermoline Scientific, Australia) under cool white fluorescent lights (100 µmol photons m_{-2} s^{-1}) with a 16:8 h (day: night cycle) at 21 ± 2°C for full period of the experiment. To prepare the plants for ROS staining, the seedlings were carefully removed from the tray, roots washed free of media, and plants transferred into a beaker containing 1 L of distilled water, for 2 days. The distilled water was then replaced by the seaweed extract (1:400 dilution) or distilled water as a control. There were three replicates (each with three plants) of each treatment. After 4 and 5 days of plant growth in the beaker, the roots were detached and stained with 3,3´-diaminobenzidine (DAB) for H_2O_2 detection. This staining was optimized using different concentrations and incubation times with DAB (data not shown). Optimal staining occurred when the roots were placed in a petri-dish and submerged in 20 mL of DAB (0.5 mg mL^{-1}) for 10 min in the dark at room temperature. This staining method was subsequently used for all treatments in the experiment. Root pieces were then thoroughly washed before the tips being visualized using a light microscope under bright field for the presence of a reddish-brown precipitate indicating H_2O_2. Images were captured with a digital camera mounted on the microscope.

Hydrogen peroxide and peroxidase were extracted from root tissues of strawberry plants at 0, 1, 4 and 5 days after treatment with the seaweed extract as described by Islam et al. (2017). Briefly, roots (ten roots per replicate) were frozen in liquid N, ground to a fine powder with a mortar and pestle and then 500 µL of a 40 mM potassium phosphate buffer (pH 6.5) was immediately added to create a homogeneous mix. When the mix became liquid, it was then taken up into a 1.5 mL Eppendorf and centrifuged for 15 min at 13,000 × g at 4ºC. Hydrogen peroxide and peroxidase were quantified from the supernatant using a commercial kit

(Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit, Life Technologies, Australia) as per the manufacturer's protocols, and the resulting fluorescence was measured using a microplate reader (Varioskan LUX multimode microplate reader, Thermo Scientific, Australia). To measure the quantity of the compounds, linear equations for H_2O_2 and peroxidase were generated using serial dilutions of known concentrations of H_2O_2 and horseradish peroxidase (HRP) and using the Amplex Red kit. Data were expressed as μm H_2O_2 gFW^{-1} and mU peroxidase gFW^{-1}.

Statistical analysis

Except where otherwise stated, data from the trials were statistically analysed using ANOVA on Genstat 18th Ed. (VSN International). Homogeneity of variance was determined by examining plots of fitted values versus residuals, while histograms of residuals were examined for normality of distribution. Where variance was heterogeneous across treatments, appropriate data transformations were made to restore homogeneity. Fisher's least significant difference (LSD) test was used to identify differences between treatment means. The level of significance used was $p \leq 0.05$.

Results

Fruit yields and revenue

Application of the seaweed extract significantly increased total fruit yield by 8% and 10% compared with the untreated control at the Coldstream and Warburton trials, respectively (Table 1). This corresponded with a significant increase in total revenue from fruit of AU$ 0.37 and AU$ 0.59 $plant^{-1}$, respectively (Table 1). The size of strawberry fruit was not significantly different in the seaweed extract treatment and the untreated control at either trial site (Table 1).

Table 1 Commercial fruit yields and revenue from strawberry (cultivar Albion) treated with a seaweed extract from *Ascophyllum nodosum* and *Durvillaea potatorum* in field experiments at Coldstream and Warburton, Victoria, Australia

Location	Treatment	Total Fruit Yield (g $plant^{-1}$)	Total Revenue (AU$ $plant^{-1}$)	Fruit Size (g $berry^{-1}$)
Coldstream	Seaweed Extract	545.8	4.10	10.33
	Control	498.5	3.73	10.31
	LSD (p = 0.05)	21.2	0.21	ns
Warburton	Seaweed Extract	966.7	5.84	16.8
	Control	872.9	5.25	16.7
	LSD (p = 0.05)	63.1	0.36	ns

Post-harvest quality and fruit rot

There was no significant difference in the firmness, SSC, titratable acid, or SCC:acid between fruit from the two treatments in the trial at Warburton (Table 2).

The incidence and severity of fruit rot was greater in the assessment in March than in January (Table 3). Rots increased the longer fruit was in storage. Isolation and microscopic examination showed that rots were mostly caused by *B. cinerea* (> 90%), and to a lesser extent by *Rhizopus stolonifer*.

In the January assessment, treatment with the seaweed extract reduced the incidence and severity of fruit rot, but not to significant levels (Table 3). In the March assessment, the use of the extract significantly reduced the incidence and severity of fruit rots by 52-87%.

Analysis of reactive oxygen species in strawberry roots

Roots treated with the seaweed extract and stained with DAB showed the presence of a reddish-brown precipitate, which indicated the presence of H_2O_2. Only a slight basal level of the precipitate was detected in roots grown in water as a control, while the roots not stained with DAB (used as a negative control) showed no coloration (Fig. 1A). Concentrations of H_2O_2 significantly increased (doubled) in roots at 5 days after treatment with the seaweed extract. Similarly, concentrations of peroxidase significantly increased in roots treated with the extract, by up to 50%, at 1 day after treatment (Fig. 1B).

Table 2 Parameters of fruit quality of strawberry (cultivar Albion) treated with a seaweed extract from *Ascophyllum nodosum* and *Durvillaea potatorum* in a field experiment at Warburton, Victoria, Australia

Parameter	Treatment	January	March
Firmness (Shore A)	Seaweed Extract	54.2	44.6
	Control	55.4	44.9
	LSD (p = 0.05)	ns	ns
Soluble Solid Concentration (SSC) (°Brix)	Seaweed Extract	8.4	9.2
	Control	8.3	9.1
	LSD (p = 0.05)	ns	ns
Titratable Acidity (g citric acid L^{-1} juice)	Seaweed Extract	7.4	8.3
	Control	7.8	8.5
	LSD (p = 0.05)	ns	ns
SSC:acidity	Seaweed Extract	11.4	11.1
	Control	10.8	10.8
	LSD (p = 0.05)	ns	ns

Table 3 Incidence and severity of post-harvest rot of strawberry fruit (cultivar Albion) following treatment of plants with a seaweed extract from *Ascophyllum nodosum* and *Durvillaea potatorum* in a field experiment at Warburton, Victoria, Australia

Rot Disease Measurement	Treatment	January		March	
		3 days storage	7 days storage	5 days storage	10 days storage
Incidence (%)	Seaweed Extract	0.96	2.24	2.78	24.52
	Control	2.92	5.07	15.42	51.70
	LSD ($p = 0.05$)	ns	ns	6.90	7.46
Severity (%)	Seaweed Extract	0.58	2.11	0.56	15.23
	Control	1.76	4.26	4.48	33.70
	LSD ($p = 0.05$)	ns	ns	2.14	6.62

Discussion

This research demonstrated the capacity for a commercial seaweed biostimulant made from multi-origin species to increase yield and revenue of strawberry and reduce post-harvest rot. It is also the first study to demonstrate that treatment with a seaweed extract can increase concentrations of ROS in strawberry cells, which are important signallers for defence priming against biotic and abiotic stress.

Effect of the seaweed extract on strawberry fruit yield and economics

Our field trials showed that application of the seaweed extract as a combined soil drench and foliar treatment increased strawberry yield by 8% in a short-season crop and 10% in a long-season crop. These results concur with previous research in open field soils that showed application of extracts from brown seaweeds increased strawberry fruit yields by 8-30% (Masny et al. 2004; Alam et al. 2013; El-Miniawy et al. 2014; Mattner et al. 2018; Mufty and Taha 2021; Popović et al. 2022; Ashour et al. 2023). In particular, the results from the current study closely align with a series of field experiments in California where drip application of a seaweed extract from *A. nodosum* increased strawberry yields by an average of 15% (Holden and Ross 2017). Californian data are particularly relevant to conditions in south-eastern Australia because production systems and cultivars of strawberry are similar. Moreover, the yield responses in strawberry fruit in the current trial corresponded with those in nursery plants in Victoria using the same seaweed extract, where runner numbers increased by 8-19% (Mattner et al. 2018). More broadly, the response of strawberry in the current experiments is comparable with yield and growth responses with the same seaweed extract, application method and rate in other cropping systems in Australia (Mattner et al. 2013; Arioli et al. 2015, 2021; 2022; 2023). In contrast, an experiment conducted in Europe showed that a mixture of brown seaweed extract and amino acids had no effect on strawberry fruit yields in an organic system (Boček et al. 2012). This trial, however, used a low concentration formulation of extract (24% extract of *A. nodosum* in the concentrate), only treated plants as a foliar spray, and applied the extract less frequently (five times through the cropping cycle) than in the current experiments. Similarly, Soltaniband et al. (2022) found no effect of fortnightly drip applications of an extract from *A. nodosum* on strawberry yield in Canada, but unlike the current study their experiments were conducted in soil-less media.

Several studies have found that strawberry genotype influenced the yield response to seaweed extracts (Masny et al. 2004; Mattner et al. 2018; Mufty and Taha 2021; Rana et al. 2022). For example, Alam et al. (2013) found that the cultivar used in the current experiments (Albion) was the least responsive to seaweed extracts of the four strawberry genotypes they tested. This may partially explain why some studies that used other strawberry cultivars (e.g., El-Miniawy et al. 2014; Popović et al. 2022; Ashour et al. 2023) showed greater yield responses to seaweed extracts than the current experiments. Furthermore, the yield response of the strawberry cultivar Albion has varied when grown under protected culture, in different regions and using different concentrations and types of seaweed extract (Alam et al. 2013; Al-Shatri et al. 2020; Mufty and Taha 2021).

Results from the current trials indicate that the economics of using the seaweed extract in strawberry production can be favourable for growers in Victoria. We showed the use of the seaweed extract increased revenue from fruit by AU$ 0.37 and AU$ 0.59 plant^{-1} while the cost of the treatment was AU$ 0.01 and AU$ 0.03 plant^{-1} in the Coldstream and Warburton trials, respectively. Most strawberry growers in Victoria have the equipment to apply the seaweed extract through their fertigation system and as additives to their spray programs, and therefore there would be negligible additional infrastructure and labour costs from the treatment. The intangible benefits of using extract for strawberry growers are reduced post-harvest fungal decay, increased shelf-life of fruit,

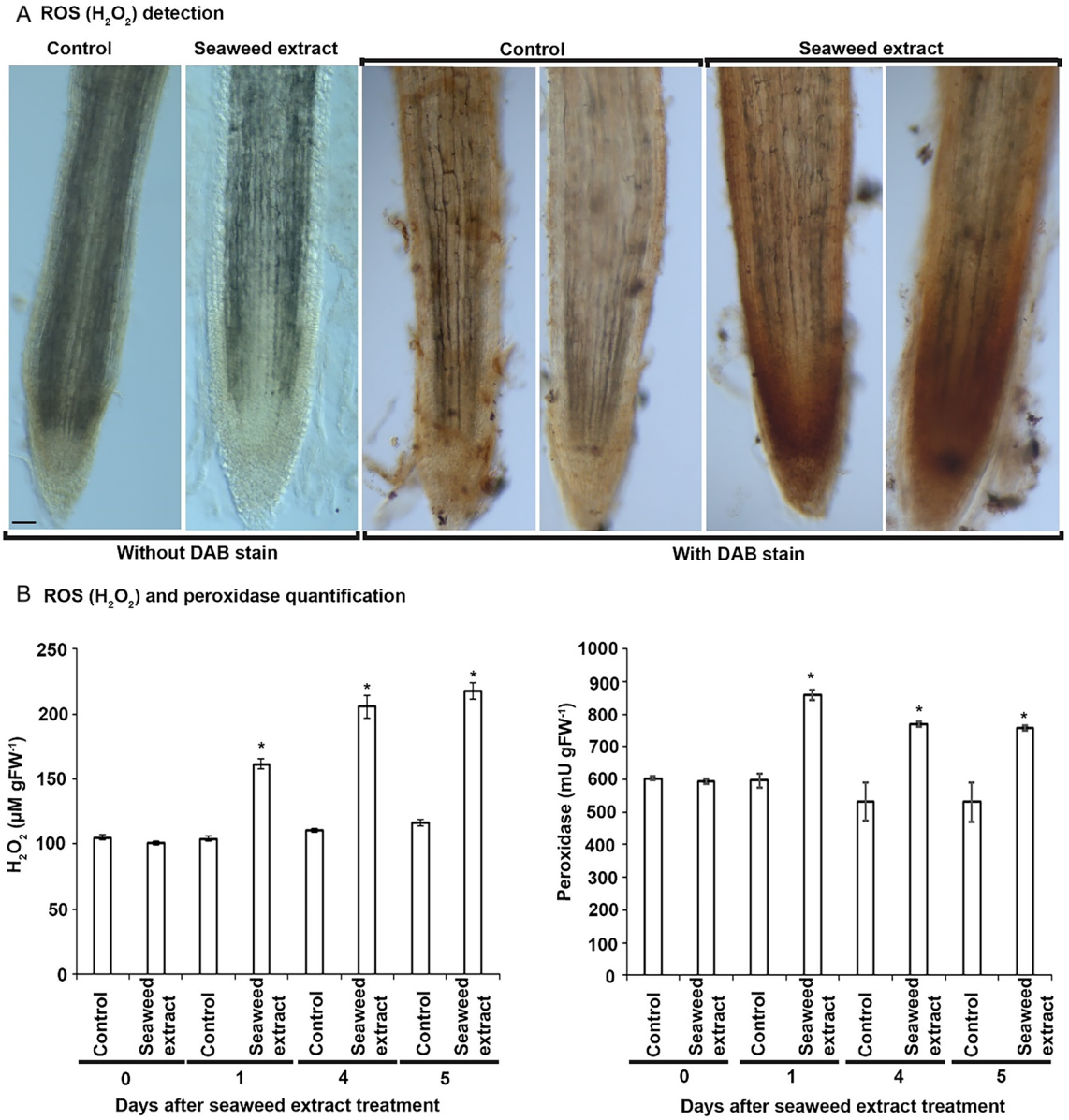

Fig. 1 A. Hydrogen peroxide (H_2O_2) detection using the DAB stain in the root tip cells of strawberry grown in a solution containing a seaweed extract or water as the control (scale bar = 50 µm). The reddish-brown precipitate in the seaweed extract treatment indicates the presence of H_2O_2. B Concentrations of H_2O_2 and peroxidase detected in root tip cells of strawberry. Data shown are the mean of three independent biological replicates and bars represent the standard errors of means. * denotes the significant difference in the treatment compared with the control at $p \leq 0.05$

and associated retailer and consumer confidence in the quality of the product. Holden and Ross (2017) also found revenue increases from improved fruit production from the use of seaweed extracts in commercial strawberry trials in California. Furthermore, studies with the same seaweed extract used in the current study showed positive economic outcomes in other cropping systems in Australia such as avocado, wine grapes, and sugar cane (Arioli et al. 2021, 2022, 2023).

Effect of the seaweed extract on strawberry fruit rot and quality

Reducing fungal decay in strawberries is critical in minimizing losses during commercial storage and marketing, and for increasing retailer and consumer confidence in the quality of the product and its shelf-life. Results from this experiment showed that monthly application of the seaweed extract reduced the incidence and severity of post-harvest rots in

strawberry fruit by up to 52-87%, depending on how long they were stored. The effect of the extract in reducing the incidence and severity of rots in fruit was greater in March, when disease incidence was highest, than in January. This may be due to the cumulative effect of more treatments with the extract by March, or seasonal effects. The effect of the extract in the current trials could not be attributed to differences in the maturity or quality of fruit because berry firmness, SSC, titratable acidity and SSC:acid were the same between treatments. Furthermore, our previous research showed that the seaweed treatment did not affect the proportion of rejected fruit at harvest (Mattner et al. 2018), indicating that the main effect of the extract on rot was in reducing post-harvest decay from fungal infection and increasing shelf-life of fruit. Previous field research conducted in Victoria using the same seaweed extract as the current trials also found that the treatment reduced the post-harvest incidence of grey mould of a different cultivar of strawberry (Selva) by 40% (Washington et al. 1999). In contrast, Masny et al. (2004) showed that two seaweed extracts from *Ecklonia maxima* and *A. nodosum* had no effect on the incidence of grey mould in strawberry at harvest. However, their study did not incubate fruit to determine post-harvest rots as in the current experiment. Boček et al. (2012) found that foliar application of a seaweed extract from *A. nodosum* reduced the incidence of grey mould in strawberry by 40-58%, but the effect was not statistically significant due to the relatively low and variable levels of disease in the controls.

Several studies have shown that the use of seaweed extracts can improve attributes of strawberry quality, including fruit firmness (El-Miniawy et al. 2014), SSC (El-Miniawy et al. 2014; Kapur et al. 2018a, b; Rana et al. 2022), and SSC:acidity (Al-Shatri et al. 2020; Rana et al. 2022). Still, other studies show no effects of seaweed extracts on these strawberry parameters (e.g., Roussos et al. 2009; Spenelli et al. 2010; Eshghi et al. 2013; Soppelsa et al. 2019; Popović et al. 2022). We also found no difference in the firmness, SSC, TA or SSC:acidity of fruit from strawberry plants treated with the seaweed extract and the control. Clearly, variable results between studies may relate to extract type and application method, and strawberry genotype, region, and production system. Given the importance of the organoleptic and nutraceutical properties of strawberry, however, we concur with Garza-Alonso et al. (2022) that more research is required to quantify and understand the possible mechanisms of seaweed extracts on fruit quality of strawberry. As a minimum, this will require studies with much greater sample sizes of fruit than the current experiment.

ROS signalling and other possible mechanisms stimulated by the seaweed extract

There is increasing evidence that seaweed extracts have properties that improve stress tolerance in treated plants through the priming of defence (Islam et al. 2020, 2021; Kerchev et al. 2020; Rasul et al. 2021; Shukla et al. 2021; Sujeeth et al. 2022). Plant priming is an adaptive response mechanism in plants against biotic and abiotic stress (Martinez-Medina et al. 2016). *Arabidopsis* transcriptomics and cellular biology experiments showed that the same seaweed extract used in the current study resulted in the up-regulation of a suite of genes (e.g., *PR1*, *PR5*, *NPR1*, *MLO*) associated with direct or indirect plant defence (e.g., systemic acquired resistance and those regulated by salicylic acid, jasmonic acid and ethylene) (Islam et al. 2020, 2021). Using identical techniques as the current study, these changes in gene expression were associated with temporal increases in H_2O_2 and peroxidases in the plant roots (Islam et al. 2017, 2020; 2021). Similarly, Cook et al. (2018) showed increased production of H_2O_2 in *Arabidopsis* following treatment with an extract from *Ascophyllum nodosum*, which was associated with up-regulation of *PR1* and other defence-related genes. Other studies also showed that various individual components of seaweed extracts, including those in the extract used in the current study, such as alginates (Dey et al. 2019), laminarins (Gauthier et al. 2014) and algal saccharides (Abouraïcha et al. 2017) stimulate production of H_2O_2 in host plants challenged by different pathogens. These studies also support the broader notion that seaweed extracts can act as elicitors of defence responses in plant hosts (Khan et al. 2009; Sharma et al. 2014; Shukla et al. 2016, 2021).

Originally, ROS were only considered harmful metabolic by-products and excessive levels are often the result of adverse environmental conditions, ultimately causing oxidative stress and cell death (Kerchev et al. 2020; Kerchev and Van Breusegem 2022). Indeed, many studies showed that seaweed extracts can reduce excessive ROS concentrations and moderate oxidative stress caused by salinity, drought, and extreme temperatures (Shukla et al. 2019; Sujeeth et al. 2022). However, moderate concentrations of ROS compounds, particularly H_2O_2, are increasingly recognised as important signallers in plant priming (Kerchev et al. 2020; Kerchev and Van Breusegem 2022) and can modulate defence-related enzymes like peroxidases that are involved in multiple physiological processes in the host that may be activated during pathogenesis (e.g., lignification, suberization, auxin catabolism). For example, Bajpai et al. (2019) showed that spray application of an extract from *A. nodosum* significantly increased concentrations of peroxidases and other defence-related enzymes in the leaf of strawberry, and this was associated with reduced severity of powdery mildew caused by the biotrophic pathogen *Podosphaeria aphanis*. In a laboratory experiment, we found that the seaweed extract increased and primed concentrations of H_2O_2 and peroxidase in strawberry root cells not challenged by a pathogen, as soon as one day after treatment.

Furthermore, H_2O_2 production was visualized in cells in the root tip of strawberry treated with the extract using a DAB staining technique. Due to their signalling role, changes in concentrations of ROS are potential indicators of defence priming in plant cells. The result that the seaweed extract increased ROS signallers in strawberry cells in the current study adds weight to the body of evidence that plant priming is a key mode of action of seaweed biostimulants. We hypothesize that the changes in ROS and peroxidase in the current experiment are associated with and partially explain the yield increases and rot suppression observed in the field experiments. Clearly, this hypothesis needs further research to confirm under field conditions and to demonstrate that increases in ROS occur more systemically across strawberry plant tissues, and not at levels that cause oxidative stress. It is also important to recognise that increases in ROS causing localized plant cell death in the host's hypersensitive response can promote infection by necrotrophic pathogens like *B. cinerea* (Nakajima and Akutsu 2014; Rossi et al. 2017). Indeed, *B. cinerea* can itself produce ROS and peroxidase to kill host cells in advance of infection (Nakajima and Akutsu 2014; Hua et al. 2018). However, increases in ROS and peroxidase in the plant host can more broadly indicate the initiation of a cascade of other mechanisms associated with resistance that may reduce infection by necrotrophic pathogens.

There is also some evidence that exogenous application of seaweed extracts has the capacity to directly inhibit fungal growth. For example, De Corato et al. (2017) showed that crude extracts from brown seaweeds inhibited the growth of *B. cinerea* in the laboratory and the development of grey mould in strawberry fruit. Furthermore, the seaweed extract used in the current study directly suppressed the growth of *Sclerotinia minor* in a series of bioassays (Mattner et al. 2014). Despite this, we did not observe inhibition of *B. cinerea* in disk-diffusion bioassays with 1:400 dilutions of the same seaweed extract used in the current study (unpublished data). There is no evidence, however, that the yield and rot responses of strawberry in the current experiments were due to a nutritional effect by the seaweed extract. This is because the nutrient content of the extract is low (Wite et al. 2015), trials were conducted under conditions of high nutrition, and treatment with the seaweed extract has had no significant effect on soil chemistry in previous strawberry trials (Mattner et al. 2018).

Implications for integrated management of fruit rots

Currently growers in Victoria use a range of fungicides to manage grey mould and other rots of strawberry. These methods are effective (Washington et al. 1992, 1999; Menzel et al. 2016), but their continued use increases the risk of development of resistance to fungicides in populations of *B. cinerea* and other pathogens. Fungicides are also under threat of withdrawal over concerns about their impact on human and/or environmental health. In the current trial, the use of fungicides was integrated with the application of monthly drenches and foliar spray with a seaweed extract. The use of the extract complemented the use of fungicides in reducing the development of post-harvest rots in fruit and increasing yield. Further trials are warranted to determine the potential for seaweed extracts to offset the number of fungicide applications required to manage fruit rots in strawberry production. There is also a strong need to investigate the integrated use of seaweed extracts with other non-chemical controls for fruit rots, such as spray-induced gene silencing (Niño-Sánchez et al. 2022), exposure to UV-C (Janisiewicz et al. 2016), biological agents (Abbey et al. 2018), nanotechnologies (Khan et al. 2021) and other biostimulants (Garza-Alonso et al. 2022), to effect more consistent and sustainable control. This could have the benefits of reducing growers' reliance on individual fungicides, reducing the costs of production, and increasing the sustainability of the industry.

Conclusions

The use of a combined drench and foliar application of a commercial extract from the seaweeds *D. potatorum* and *A. nodosum* increased strawberry yield and revenue from fruit and reduced post-harvest rot. Treatment with the extract increased concentrations of H_2O_2 and peroxidase in strawberry root cells and may indicate the initiation of defence priming against biotic and abiotic stress. We hypothesize that this response is associated with and may partially explain yield increases and rot suppression in strawberry plants treated with the seaweed extract.

Acknowledgments The authors acknowledge the growers who assisted so the trials could be conducted scientifically at Australian strawberry farms. We also acknowledge technical assistance from Larissa Coridas who picked and recorded fruit yields at the trials and Dr Donovan Garcia-Ceron who provided constructive feedback on the manuscript.

Authors' contributions All co-authors contributed to the final version of the work and approved the manuscript for publication. SWM: conducted the field research, analysis and interpretation of data and writing the manuscript; TA: project leader, analysis and interpretation of data, and writing the manuscript; ONV performed all fruit quality research, analysis and interpretation of fruit quality data, and writing the manuscript; MT: conducted laboratory research on reactive oxygen species, analysis and interpretation of data, and editing the manuscript; DJM conducted field research and writing the manuscript; DC provided expert input, oversight of laboratory research and editing the manuscript.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions Research funding was provided by Seasol International Pty Ltd (Australia).

Data availability Available data is provided in the publication.

Declarations

Conflicts of interest/Competing interests Seasol International (SI) is the manufacturer of the seaweed extract in Australia. TA is an employee of SI and an Adjunct Associate Professor at Deakin University. All other authors are not employees of SI. The authors declare that the research was conducted in the absence of any financial relationship that could be construed as a potential conflict of interest.

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