

Evaluating the Use of Biostimulants for Indoor Hydroponic Lettuce Production

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SUMMARY. Biostimulant products have various reported benefits for plant production in the field or using hydroponic systems in protected structures. However, limited information is available describing their potential use for indoor farming applications. Considering that lettuce (*Lactuca sativa*) is one of the most popular crops produced in commercial indoor farms, the objective of this study was to compare growth and quality of lettuce grown indoors using nine biostimulant products derived from humic substances, amino acids, hydrolyzed proteins, or seaweed extracts. ‘Monte Carlo’, ‘Fairly’, and ‘Lalique’ lettuce were grown hydroponically for 30 to 33 days under a daily light integral, day/night temperature, relative humidity, and carbon dioxide concentration of $\approx 13 \text{ mol}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$, 22/21 °C, 70%, and 800 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively. There were no positive effects from using any of the biostimulant products evaluated in our study as growth (leaf area, leaf number, shoot diameter, and shoot and root dry weight), yield (shoot fresh weight), and quality (bolting, tipburn index, leaf color, and SPAD index) of treated plants were generally similar to those from the untreated control. Applications from one seaweed extract caused slight negative growth effects, possibly due to phytotoxicity. Cultivar differences showed that Fairly plants had the highest susceptibility to tipburn and bolting, and none of the biostimulant products countered these symptoms. Overall, the products evaluated provided marginal advantages for indoor hydroponic lettuce production.

Indoor farms, also known as “vertical farms,” enable year-round plant production and offer many opportunities to reduce global challenges with climate, food, and water security (U.S. Department of Agriculture, 2019). The United States is one of the countries with the highest monetary investment in research, development, and business support for this young, expanding industry (Kubota, 2020). However, barriers that affect profitability still need to be overcome

by the industry to become a key part of U.S. agriculture (de Souza et al., 2022). Alternatives to maximize yield and quality of indoor-grown plants are being evaluated in many forms. There is potential to use biostimulant products indoors, which have many reported benefits in agriculture (Kozai et al., 2022). In recent reviews, Roupheal and Colla (2020) and Ertani et al. (2021) described how biostimulants can help improve various plant processes in the field or using hydroponic systems in high-tunnels or greenhouses. However, no studies have evaluated their potential use for indoor farming applications with sole-source lighting.

Humic substances are often derived from the decomposition of organic matter through the metabolic activity of soil microbes (Bulgari et al., 2019). They have been shown to cause auxin-like effects in plants and improve macro- and micronutrient uptake in many crop species (Bronick and Lal, 2005; Ferreras et al., 2006; Nardi et al., 2009; Puglisi et al., 2009; Scaglia et al., 2015). Humic and fulvic acids are two derived forms of humic substances that have been reported to increase antioxidant activity, chlorophyll

content, photosynthesis, and nucleic acid synthesis in various crops (Akladios and Mohamed, 2018; Canellas et al., 2015; Fan et al., 2014; Schiavon et al., 2010).

Seaweed extracts are commonly extracted from macroalgae. They contain various bioactive substances such as amino acids, vitamins, phytohormones, and antioxidants (Battacharya et al., 2015). When applied at low concentrations, seaweed extracts can improve seed germination and transplant establishment, provide resistance to biotic and abiotic stresses, and extend the postharvest shelf life of perishable plant products (Kulkarni, et al., 2019; McLachlan, 1992; Norrie and Keathley, 2005).

Organic nitrogen (N)-containing compounds include free amino acids and peptides of protein and nonprotein origins, polyamines, betaines, and related substances (du Jardin, 2015). Amino acid and peptide mixtures are generally obtained by chemical or enzymatic protein hydrolysis often using plant or animal by products (Colla et al., 2015; du Jardin, 2015; Schaafsma, 2009). Hydrolyzed proteins have been shown to increase iron and N metabolism, nutrient uptake, and water-use efficiency of several horticultural crops (Cristofano et al., 2021a; Dass et al., 2021; Ertani et al., 2009; Xu and Mou, 2017; Paradiković, et al., 2013).

Although studies have shown potential for the use of biostimulants in agriculture, most have focused on alleviating stresses such as drought, extreme temperatures, high salinity, or nutrient deficiencies (Botta, 2012; Cristofano et al., 2021b; de Vasconcelos and Chaves, 2019; du Jardin, 2015; Lucini et al., 2015; Roupheal and Colla, 2020). Biostimulants have not been extensively studied in conditions like those used in commercial indoor farms, where plants are typically grown free from environmental stress (Kozai, 2022). Considering that lettuce (*Lactuca sativa*) is one of the most popular crops produced by the indoor farming industry, the objective of this study was to quantify and compare growth and quality of hydroponic lettuce plants grown indoors using different biostimulant products. We hypothesized that under optimal growing conditions for indoor hydroponic lettuce production, limited benefits would be measured

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when comparing different products with an untreated control treatment.

Materials and methods

Seeds of ‘Monte Carlo’ (Vilmorin-Mikado, Salinas, CA), ‘Fairly’ (Rijk Zwaan, De Lier, The Netherlands), and ‘Lalique’ (Rijk Zwaan) lettuce, which are categorized as romaine, butterhead, and leaf lettuce types, respectively, were sown into 200-cell trays of stabilized propagation plugs (34-mL individual cell volume) composed of peatmoss and a polymer binder. Seedlings were grown inside a walk-in growth chamber (C6 Control System with EcoSys Software; Environmental Growth Chambers, Chagrin Falls, OH) at the University of Florida (Gainesville, FL) using a daily light integral of $\approx 13 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ($180 \pm 5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for $20 \text{ h}\cdot\text{d}^{-1}$) provided by light-emitting diode fixtures (GP150; Signify, Somerset, NJ) with peak wavelengths of 660 and 448 nm, which delivered 87% red (700 to 800 nm) and 13% blue (400 to 500 nm) light. Ambient temperature, relative humidity, and carbon dioxide were set at 24°C , 70%, and $800 \mu\text{mol}\cdot\text{mol}^{-1}$, respectively. Seedlings were subirrigated every other day using a commercial water-soluble fertilizer providing 16N–1.7P–14.1K (OASIS Hydroponic Fertilizer 16–4–17; Oasis Grower Solutions, Kent, OH) dissolved in tap water (electrical conductance of $0.4 \text{ mS}\cdot\text{cm}^{-1}$, pH of 8.3, and $40 \text{ mg}\cdot\text{L}^{-1}$ calcium carbonate) at a concentration of $150 \text{ mg}\cdot\text{L}^{-1}$ N, resulting in an EC of $1.2 \text{ dS}\cdot\text{m}^{-1}$.

The experiment was replicated twice over time. Although there was a slight offset in the timing between the two replications, treatments were applied when plants were at the same developmental stages in both replications over time. At 9 or 12 d after sowing for each replication over time, respectively, a uniform seedling of each cultivar was transplanted into one of 80 cylindrical deep-water culture hydroponic systems (2 gal). Each hydroponic system had a white plastic lid with three openings that held 2-inch-diameter net cups placed 20 cm apart. Individual hydroponic systems were regarded as an experimental unit with a single plant from all three cultivars. Plastic barriers were placed inside each reservoir to separate plant roots. A black plastic tube attached to an air pump [320 gal/h

(Dual Diaphragm Air Pump; General Hydroponics, Santa Rosa, CA)] was placed at the bottom of each reservoir to provide continuous aeration to the nutrient solution.

During each replication over time, plants were grown inside one of two walk-in growth chambers, each equipped with two opposite shelving units. Each shelving unit had an upper and a lower compartment (61 cm width \times 183 cm length \times 94 cm height). Each compartment held 10 randomly placed hydroponic systems with different treatments, for a total of four treatment replications per growth chamber and eight treatment replications during each experimental run. The same conditions described earlier were used throughout the study, except that ambient day (from 0200 to 2200 hr) and night (from 2200 to 0200 hr) air temperatures were set to 22 and 21°C , respectively. To minimize issues with tipburn, two fans (AXIAL 1238; AC Infinity, City of Industry, CA) were placed within each compartment above plants and provided constant air flow in opposite directions. Reservoirs were refilled with half strength nutrient solution every time they reached 75% of the volume capacity and completely replaced once before treatments were applied 9 or 12 d after transplanting, which corresponded with the recommended timing in the product labels (Table 1). Solution pH was adjusted weekly to a 5.8 to 6.2 range using an acid or a base (pH Down or pH Up, General Hydroponics), which added either phosphoric acid and citric acid, or potassium carbonate and potassium silicate, respectively. All hydroponic systems were randomly rotated once weekly within each treatment compartment to minimize location effects in the experimental area. Plants were harvested at 30 or 33 d after sowing during the first and second replication over time, respectively, once they had reached marketable size.

TREATMENTS. Nine commercial products were evaluated in the study, with different application methods, timing, and dosages according to the product labels (Table 1). The treatments applied included two humic substances [Huma Pro; Bio Huma Netics, Gilbert, AZ (HS-1) and Mr.Fulvic; Mr.Fulvic, Tallahassee, FL (HS-2)], two amino acid products

[Fortify, OASIS Grower Solutions (AA-1) and Megafol; Valagro USA, Coral Gables, FL (AA-2)], three hydrolyzed proteins [Macro-Sorb; Macro-Sorb Technologies, Mount Laurel, NJ (HP-1), Brown’s Fish Hydrolysate; C.R. Brown Enterprises, Andrews, NC (HP-2), and On-Gard + On-Gard Calcium; BioWorks, Victor, NY (HP-3)], and two seaweed extracts [Kelpak; Kelp Products USA, Modesto, CA (SE-1) and VIVA, Valagro USA (SE-2)]. An untreated control treatment was also included for comparison. As indicated in Table 1, the various products had different active ingredient, and some had already been evaluated in previous studies using hydroponics or for lettuce production in the field.

DATA COLLECTED. Data were collected from all plants grown in the study. One day before harvest, relative chlorophyll content was measured on three points of a fully expanded leaf using a SPAD meter (SPAD-502; Konica Minolta Sensing, Osaka, Japan). Leaf color was measured on the same leaf using a portable colorimeter (CR-200, Konica Minolta Sensing), where a^* indicates the ratio between greenness and redness (green: $a^* = -60$; red: $a^* = +60$), b^* indicates the ratio between blueness and yellowness (blue: $b^* = -60$; yellow: $a^* = +60$), and on a circular scale, hue angle (h°) or tone indicates redness (0°), yellowness (90°), greenness (180°), or blueness (270°) (Owen and Lopez, 2015). Tipburn severity was rated using a subjective scale modified from Frantz et al. (2004), where 0 = no signs of tipburn; 1 = central leaflets with small necrotic spots; 2 = 5 to 10 leaves with small necrotic spots and misshapen leaf margins; and 3 = malformed leaves and meristem death in $>60\%$ of leaves. Similarly, bolting severity was assessed using a subjective scale modified from Beretta et al. (2013) and Holmes (2017), where 0 = no internode elongation; 1 = leaf erection; and 2 = stem elongation. At harvest, the total number of leaves ($>1 \text{ cm}$) were counted. Shoot height and diameter were measured with a ruler, and the area of the sixth fully expanded leaf was measured using a leaf area meter (LI-3100C; LI-COR Biosciences, Lincoln, NE). Shoots were weighed immediately after harvest with an electronic balance to obtain fresh weight (FW). Shoot and roots were oven-dried separately at

Table 1. Biostimulant products evaluated for hydroponic lettuce production indoors.

Product name	Treatment code	Type	Main active ingredient	Nitrogen content (%)	Application method	Application time ^z	Dose (mL·L ⁻¹) ^y	Reported in the literature ^x
Huma Pro16 (HUMA GRO, Gilbert, AZ)	HS-1	Humic substance	Humic acid	Not reported	Mixed in nutrient solution	After transplanting	1.13	–
Mr.Fulvic (Mr.Fulvic, Tallahassee, FL)	HS-2	Humic substance	Fulvic acid and amino acids	Not reported	Mixed in nutrient solution	After transplanting	0.26	–
Fortify (Oasis Grower Solutions, Kent, OH)	AA-1	Amino acid	Amino acids (glycine, L-glutamic acid, L-alanine)	Not reported	Mixed in nutrient solution	9 or 12 d after transplanting	0.26	–
Megafol (Valagro USA, Coral Gables, FL)	AA-2	Amino acid	Nitrogen and potash	3.0	Foliar spray	At sowing	2.00	–
Macro-Sorb (Macro-Sorb Technologies, Mount Laurel, NJ)	HP-1	Hydrolyzed protein	Free amino acids and nitrogen	0.1	Foliar spray	After transplanting	1.00	Paradiković et al., 2013
Brown's Fish Hydrolysate (C.R. Brown Enterprises, Andrews, NC)	HP-2	Hydrolyzed protein	Nitrogen and phosphorous	2.3	Drench	9 or 12 d after transplanting	2.00	Botta, 2012
On-Gard + On-Gard Calcium (BioWorks, Victor, NY)	HP-3	Hydrolyzed protein	Nitrogen and calcium	5.0	Foliar spray	At sowing	3.00	Xu and Mou, 2017
Kelpak (Kelp Products USA, Modesto, CA)	SE-1	Seaweed extract	Soluble potash	Not reported	Drench	After transplanting	3.00	–
VIVA (Valagro USA)	SE-2	Seaweed extract	Nitrogen and soluble potash	3.0	Mixed in nutrient solution	9 or 12 d after transplanting	4.66	Kulkarni et al., 2019

^zTransplanting occurred at 9 or 12 d after sowing for each replication over time, respectively. Within each row, the first and second number represent the application time during the first and second replication over time, respectively.

^ymL·L⁻¹ = 1000 ppm.

^xCited studies evaluated the corresponding biostimulant product in hydroponics or for lettuce production in the field.

70 °C for 72 h to determine shoot and root dry weight (DW), respectively. The shoot FW:DW ratio and shoot:root DW ratio were calculated by dividing shoot FW by shoot DW, and shoot DW by root DW, respectively.

EXPERIMENTAL DESIGN AND DATA ANALYSES. The experiment used a randomized complete block design where each chamber during an experimental replication over time was regarded as a block, each with four experimental units (hydroponic system) that served as treatment replications. Data were pooled between replications over time, as the variances between experiments were not different and the statistical interactions between treatment and replication over time were not significant ($P \geq 0.05$). The influence of the two different categorical independent variables (i.e., treatment and cultivar), and their possible interaction on each of the continuous dependent variables were analyzed using a two-way analysis of variance. Data are presented as main

effects ($n = 48$ and 160 for treatment and cultivar, respectively) except for leaf area and shoot FW, in which data for each cultivar are presented separately ($n = 16$) because of the significant treatment \times cultivar interaction ($P \leq 0.05$). Mean separation for all response variables was performed using Tukey's honestly significant difference test at $P \leq 0.05$. All data were analyzed using statistical software (JMP Pro ver. 16.0.0; SAS Institute Inc., Cary, NC).

Results and discussion

There were no positive effects from using any of the biostimulant products evaluated in our study as growth, yield, and quality of treated plants were generally similar to those from the untreated control (Fig. 1; Tables 2 and 3). In contrast to our findings, Cristofano et al. (2021a) and Miceli et al. (2021) reported increases in growth and yield of hydroponic lettuce plants treated with hydrolyzed proteins and seaweed

extracts, respectively, compared with untreated control plants. However, both studies were conducted in a greenhouse where daily temperatures reached up to 34 to 36 °C. Considering that lettuce has an optimal day/night temperature close to 24/19 °C (Choi et al., 2000; Seginer et al., 1991), the positive effects found in those studies are plausibly attributed to a heat stress tolerance advantage provided by the biostimulant treatments. Accordingly, others have shown that amino acids and signaling peptides help regulate the expression of transcription factors and biosynthesis of osmolytes that confer tolerance mechanisms to plants in response to abiotic stresses such as heat (Botta, 2012; Kim et al., 2021). Similarly, seaweed extracts provide protective compounds such as antioxidants, which help regulate endogenous stress-responsive genes in plants (du Jardin, 2015).

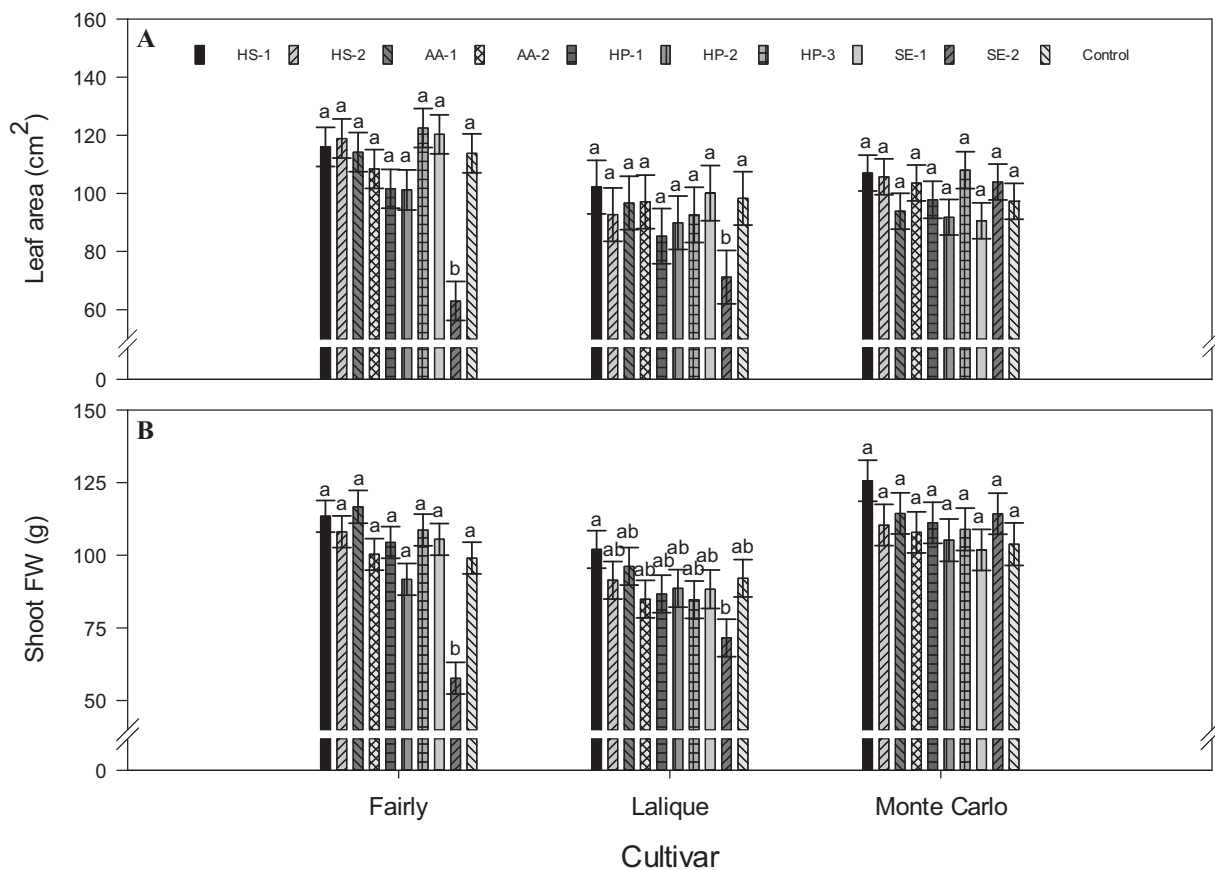


Fig. 1. (A) Leaf area (measured on the sixth fully expanded leaf) and (B) shoot fresh weight (FW) of three lettuce cultivars grown hydroponically indoors using different biostimulant products. Bars represent the mean \pm SE for each cultivar ($n = 16$). Within each cultivar group, means with the same letter are not different based on Tukey's honestly significant difference test at $P \leq 0.05$. Table 1 describes the code, trade name, application method, timing, and dosage for each treatment; $1 \text{ cm}^2 = 0.1550 \text{ inch}^2$, $1 \text{ g} = 0.0353 \text{ oz}$.

Table 2. Growth parameters measured on three lettuce cultivars grown hydroponically indoors using different biostimulant products.

Source	Leaves (no.)	Shoot diam (cm ²) ^z	Shoot ht (cm) ^z	Shoot DW (g) ^z	Root DW (g)	Shoot FW:DW (ratio)	Shoot:Root DW (ratio)
Treatment ^y							
HS-1	30.2 a ^x	21.3 a	13.3 a	4.9 a	2.0 a	23.5 a	2.4 a
HS-2	29.0 ab	20.5 a	12.6 a	4.5 a	1.9 ab	23.1 a	2.3 a
AA-1	29.7 a	21.3 a	12.8 a	4.8 a	2.0 ab	23.0 a	2.4 a
AA-2	28.8 ab	21.3 a	12.6 a	4.4 a	2.0 ab	22.7 a	2.2 a
HP-1	29.0 ab	21.3 a	12.5 a	4.6 a	2.0 ab	22.7 a	2.3 a
HP-2	28.9 ab	19.9 ab	12.8 a	4.3 a	1.9 b	22.6 a	2.3 a
HP-3	29.3 a	21.2 a	12.6 a	4.5 a	2.0 ab	22.6 a	2.3 a
SE-1	28.8 ab	21.0 a	12.4 a	4.4 a	1.9 b	22.7 a	2.3 a
SE-2	27.0 b	18.1 b	10.4 b	3.5 b	1.9 ab	21.6 a	1.8 b
Control	28.6 ab	20.5 a	12.8 a	4.5 a	1.9 ab	22.5 a	2.3 a
SE	0.5	0.4	0.3	0.1	0.02	0.6	0.01
Cultivar							
Fairly	33.6 a	4.0 b	11.8 c	4.0 b	1.9 b	25.0 a	2.1 b
Lalique	29.2 b	3.9 b	12.4 b	3.9 b	1.9 b	22.9 b	2.0 b
Monte Carlo	24.1 c	5.4 a	13.3 a	5.4 a	2.0 a	20.2 c	2.7 a
SE	0.3	0.2	0.1	0.1	0.01	0.3	0.01
Treatment	**	***	***	***	NS	NS	***
Cultivar	***	***	***	***	***	***	***
Treatment × cultivar	NS	NS	NS	NS	NS	NS	NS

^z1 cm² = 0.1550 inch², 1 cm = 0.3937 inch, DW = dry weight, 1 g = 0.0353 oz, FW = fresh weight.

^yTable 1 describes the code, trade name, application method, timing, and dosage for each treatment.

^xWithin row, treatment and cultivar means followed by the same letter are not different based on Tukey's honestly significant difference test at $P \leq 0.05$. Treatment means represent the average of 16 replications and three cultivars ($n = 48$). Cultivar means represent the average of 16 replications and 10 treatments ($n = 160$).

***, **, *, ns indicate statistical significance at the 0.001, 0.01, and 0.05 $P \leq$ level and not significant, respectively.

Others have also reported increases in hydroponic lettuce yield under salinity stress using bacterial, amino acid, or seaweed extract biostimulants (Guinan et al., 2013; Moncada et al., 2020; Orsini et al., 2018). However, two of those studies found no yield increases from the use of biostimulants when plants were grown under no stress (Guinan et al., 2013; Moncada et al., 2020) (Fig. 1; Tables 2 and 3). Further, plants in the study conducted by Orsini et al. (2018) were also exposed to radiation stress from excessive heat and light in the greenhouse, which could further explain the benefits measured from the use of amino acid biostimulants. Under the conditions evaluated in our study, growth and yield advantages from the biostimulant products were marginal. Although biostimulants have been proposed as potential treatments to increase productivity in commercial indoor farms (Kozai et al., 2022), our findings indicate that when stressors are minimal or nonexistent, the benefits from using biostimulant products are limited.

Plants treated with SE-2 generally produced fewer and smaller leaves, shorter plants, and lower shoot FW and DW than those treated with most

other treatments (Fig. 1, Table 2). A few necrotic spots were also observed in most plants treated with SE-2, possibly indicating a phytotoxicity (Table 1). Correspondingly, others have shown that phytotoxicity reduces height and biomass production of hydroponic lettuce (Hoque et al., 2007). Further, according to Djidonou and Leskovar (2019), nutrient requirements of lettuce can be cultivar specific. This could explain the fact that leaf area and shoot FW of 'Monte Carlo' were not negatively affected by SE-2. In contrast, means for those two variables were generally lowest in 'Fairly' and 'Lalique' plants treated with SE-2, although for 'Lalique', only plants treated with HS-1 produced a significantly higher shoot FW than those with SE-2. It is likely that fertilizer formulations may need to be adjusted to accommodate the application of some biostimulant products in hydroponics, particularly considering cultivar-specific requirements of elements such as N.

In general, 'Monte Carlo' plants produced wider, taller shoots with a higher shoot and root DW than 'Fairly' and 'Lalique' (Table 2). Similarly, shoot FW of 'Monte Carlo' was generally highest, ranging from 102

to 126 g, whereas shoot FW of 'Fairly' and 'Lalique' ranged from 58 to 117 g and 72 to 102 g, respectively (Fig. 1B). The shoot FW:DW was highest for 'Fairly' plants, followed by 'Lalique' and 'Monte Carlo'. In contrast, shoot:root DW was highest for 'Monte Carlo', suggesting that these plants allocated more energy toward shoot rather than root growth.

Except for b*, which indicates leaf yellowness and was generally lower in plants treated with SE-1, there were no treatment differences in any of the quality parameters evaluated in our study (Table 3). Cultivar differences showed that Fairly plants were most susceptible to tipburn and had a slight bolting severity, followed by those of Monte Carlo. In contrast, 'Lalique' plants were not susceptible to either disorder. 'Monte Carlo' plants also had the highest SPAD index, a*, and h°, which correspond with our visual assessment of color in that this cultivar produced darker green leaves than 'Fairly' and 'Lalique'.

It is widely accepted that head and leaf characteristics determine the level of susceptibility to tipburn among different lettuce cultivars (Birlanga et al., 2021). As mentioned before,

Table 3. Quality parameters measured on three lettuce cultivars grown hydroponically indoors using different biostimulant products.

Source	Bolting severity (0–2 scale) ^z	Tipburn (no. leaves)	Tipburn severity (0–3 scale) ^y	SPAD index	Leaf color ^x		
					a*	b*	h°
Treatment ^w							
HS-1	0.4 a ^v	0.6 a	0.2 a	38.5 a	–23.9 a	33.4 a	126.2 a
HS-2	0.4 a	1.5 a	0.4 a	39.4 a	–22.3 a	33.1 a	124.1 a
AA-1	0.3 a	0.9 a	0.2 a	37.6 a	–22.7 a	30.6 ab	125.7 a
AA-2	0.3 a	1.1 a	0.3 a	40.6 a	–23.1 a	32.7 a	125.3 a
HP-1	0.4 a	0.9 a	0.3 a	39.9 a	–23.6 a	32.6 a	125.7 a
HP-2	0.4 a	0.7 a	0.3 a	39.5 a	–23.1 a	33.4 a	125.2 a
HP-3	0.3 a	1.0 a	0.3 a	39.8 a	–22.6 a	32.8 a	124.5 a
SE-1	0.4 a	0.6 a	0.2 a	39.8 a	–21.6 a	26.2 b	127.4 a
SE-2	0.2 a	0.4 a	0.1 a	39.2 a	–23.0 a	32.8 a	125.2 a
Control	0.3 a	1.4 a	0.4 a	40.6 a	–22.2 a	32.3 a	126.7 a
SE	0.1	0.3	0.1	0.9	0.3	0.6	0.5
Cultivar							
Fairly	0.7 a	2.0 a	0.5 a	34.6 b	–22.9 c	32.0 a	125.8 b
Lalique	0.0 c	0.0 c	0.0 c	30.8 c	–17.5 b	25.2 b	125.3 b
Monte Carlo	0.3 b	0.6 b	0.2 b	53.1 a	–16.9 a	16.9 c	133.6 a
SE	0.04	0.2	0.04	0.5	0.2	0.3	0.3
Treatment	NS	NS	NS	NS	NS	*	NS
Cultivar	***	***	***	***	***	***	***
Treatment × cultivar	NS	NS	NS	NS	NS	NS	NS

^z0 = no internode elongation, 1 = leaf erection, 2 = stem elongation.

^y0 = no signs of tipburn, 1 = central leaflets with small necrotic spots, 2 = 5 to 10 leaves with small necrotic spots and misshapen leaf margins, 3 = malformed leaves and meristem death in >60% of leaves.

^xa* and b* = positive directions indicate redness and yellowness, respectively, whereas negative directions indicate greenness and blueness, respectively; h° = hue angle or tone indicates redness (0°), yellowness (90°), greenness (180°), or blueness (270°).

^wTable 1 describes the code, trade name, application method, timing, and dosage for each treatment.

^vWithin row, treatment and cultivar means followed by the same letter are not different based on Tukey's honestly significant difference test at $P \leq 0.05$. Treatment means represent the average of 16 replications and three cultivars ($n = 48$). Cultivar means represent the average of 16 replications and 10 treatments ($n = 160$).

***, **, *, NS indicate statistical significance at the 0.001, 0.01, and 0.05 $P \leq$ level and not significant, respectively.

'Fairly' and 'Monte Carlo' are categorized as butterhead and romaine lettuce types, respectively, both of which are also known as head lettuce. In contrast, 'Lalique' is a frilly, open lettuce type often referred to as leaf lettuce. The closed canopy structure of both head lettuce cultivars seems to have contributed to their susceptibility to tipburn, which is a localized calcium deficiency that affects rapidly developing young leaves (Table 3). Lettuce meristems are located in the interior of old, mature leaves, hindering active transpiration of late-forming leaves under certain conditions and thus increasing susceptibility to tipburn (Frantz et al., 2004). The lack of treatment differences in quality suggests that under our experimental conditions, none of the biostimulant products evaluated had positive effects to counter tipburn incidence in susceptible cultivars. However, considering that tipburn often occurs when lettuce heads are beginning to reach maturity (Currey et al., 2017), it is possible that biostimulant

applications at different times from the ones used in this study could provide advantages to counter the disorder.

In conclusion, there were no positive effects from using any of the biostimulant products evaluated in our study; growth, yield, and quality of treated plants were generally similar to those from the untreated control. Applications from one seaweed extract caused slight negative growth effects. Cultivar differences showed that Fairly plants had the highest susceptibility to tipburn and bolting, and none of the biostimulant products helped counter these disorders. There are numerous commercial biostimulant products available in the market, some of which have been reported to offer yield-enhancing properties to various horticultural crops. However, positive effects from the use of biostimulants are likely greater when plants are exposed to some level of stress, which is not a common occurrence in indoor plant production systems (Kozai, 2022).

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