HortScience 58(8):917–921. 2023. https://doi.org/10.21273/HORTSCI17228-23

Photosynthetic Rate and Root Growth Responses to *Ascophyllum nodosum* Extract–based Biostimulant in Creeping Bentgrass under Heat and Drought Stress

Xunzhong Zhang
School of Plant and Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Zachary Taylor
Helena Agri-Enterprises LLC, Collierville, TN 38017, USA

Mike Goatley
School of Plant and Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Kehua Wang
College of Grass Science and Technology, China Agricultural University, Beijing 100083, China

Isabel Brown and Kelly Kosiarski
School of Plant and Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Keywords. abiotic stress, bioproduct, cool-season grass, kelp extract, nitrogen, summer stress, turfgrass

Abstract. **Creeping bentgrass (*Agrostis stolonifera*) experiences quality decline during summer in the United States transition zone and warmer regions. Various bioproducts have been used to improve creeping bentgrass performance and to mitigate effects of summer stress in the United States transition zone. This 2-year study was carried out to examine if foliar application of seaweed extract (SWE; *Ascophyllum nodosum*)-based biostimulant Utilize® could enhance creeping bentgrass nitrate reductase (NaR) activity, and root viability under heat and drought stress conditions. The Utilize® was sprayed biweekly on creeping bentgrass foliage at 0, 29, 58, 87, and 116 $\mu L \cdot m^{-2}$, with application volume of 815 $L \cdot ha^{-2}$. Two weeks after first application, plants were exposed to heat (35/25 °C, day/night) and drought stress (40% to 50% evapotranspiration replacement) conditions for 42 days in an environment-controlled growth chamber. In general, the abiotic stress caused turf quality reduction. Foliar application of Utilize® at 58, 87, and 116 $\mu L \cdot m^{-2}$ increased turf quality, leaf color ratings, leaf chlorophyll, carotenoid content, and net photosynthetic rate (Pn). Utilize® at 58, 87, and 116 $\mu L \cdot m^{-2}$ increased NaR activity by 26.5%, 16.3%, and 16.3%, respectively, when compared with the control. Utilize® at 58, 87, and 116 $\mu L \cdot m^{-2}$ increased root biomass, root length, surface area (SA), and root volume when compared with the control. Utilize® at 58 and 87 $\mu L \cdot m^{-2}$ improved root viability by 16.3% and 30.9%, respectively, when compared with the control. Our data indicate that the SWE-based biostimulant Utilize® improves nitrogen (N) metabolism and root viability. Utilize® treatment at 58 $\mu L \cdot m^{-2}$ biweekly can be considered an effective approach for improving creeping bentgrass performance during summer stress.**

Creeping bentgrass is an important cool-season turfgrass species widely used for golf course putting greens; however, it experiences summer stress and quality decline in the US transition zone and other regions with similar climates (Wang et al. 2011). Abiotic stress such as drought and heat may damage turfgrass through free radical–induced oxidative injury of photosynthetic apparatus (Ali et al. 2021; Deolu-Ajayi et al. 2022; Jiang and Huang 2001; Zhang and Schmidt 1997, 1999). Climate changes increase frequency and severity of abiotic stress and have a persistent negative impact on turfgrass quality (Chang et al. 2016; Zhang et al. 2015, 2022). Plants possess various defense systems to present or minimize abiotic stress–induced damage to plant cells and photosynthetic function. It has been documented that plants possess different defense mechanisms to cope with abiotic stresses including metabolism (nitrogen, hormones, antioxidants) alteration, osmotic adjustment, stomatal behavior, and saturation level of cell membrane lipids (Huang et al. 2014; Merewitz and Liu 2019; Zhang and Schmidt 1999).

On exposure to heat and drought stress, plant cells may have an energy imbalance. The plant cells may absorb more energy through the light harvesting complex than what can be dissipated or transduced by photosystem II (Merewitz and Liu 2019; Wang et al. 2012). Excess energy may be accepted by O_2 and causes accumulation of toxic free radicals or reactive oxygen species (ROS) (Huang et al. 2014; Wu et al. 2017). In addition, stomatal closure induced by drought stress would reduce CO_2 and O_2 exchange through guard cells, resulting in excess O_2 and thus ROS production in cells. Drought-induced oxidative stress occurs in perennial ryegrass (Zhang et al. 2015), creeping bentgrass (Merewitz and Liu 2019; Zhang and Schmidt 1999; Zhang et al. 2022), tall fescue [*Festuca arundinacea* (Schreb.) S.J. Darbyshire; Man et al. 2011] and Kentucky bluegrass (*Poa pratensis* L.; Jiang and Huang 2001).

N assimilation is one of the most important metabolic processes of higher plants (Wang et al. 2011). N is the mineral nutrient that is needed by grass plants in the largest amounts (3% to 5% dry leaf tissues). Because N is the important component of chlorophyll and many metabolites, proteins, and nucleic acids, which are associated with plant tolerance to stress, plants with appropriate N nutrition would have better turfgrass color ratings, growth rate, and greater capacity to tolerate abiotic stress. Nitrate (NO_3^-) and ammonium (NH_4^+) are the common forms of N available for plants. A large amount of metabolic energy is required by plants to uptake inorganic N and subsequently assimilate it into organic N. Nitrate is the most commonly available form of N for grasses and it has to be reduced to nitrite (NO_2^-) and then ammonium, which is incorporated into amino acid biosynthesis (Wang et al. 2013). Nitrate reductase (NaR) is the important enzyme in N metabolism, catalyzing the conversion from nitrate to nitrite. Plants with greater NaR activity may have greater N use efficiency and chlorophyll biosynthesis, especially when plants are grown under abiotic stress and N deficiency conditions (Zhang et al. 2022).

Plant biostimulants have been used for improving turfgrass performance in creeping bentgrass putting greens (Al-Juthery et al. 2020; Ali et al. 2021; Cox 2019; Zhang and Schmidt 1997, 1999). Various plant biostimulants, such as extracts of brown seaweed (*A. nodosum*), humic acid, fulvic acid, protein hydrolysates, beneficial microorganisms, and small organic molecules, have been used for turfgrass management (du Jardin 2015; Storer et al. 2016; Zhang and Schmidt 1999; Zhang

---

Received for publication 25 Apr 2023. Accepted for publication 30 May 2023.
Published online 19 Jul 2023.
X.Z. is the corresponding author. E-mail: xuzhang@vt.edu.

et al. 2022). Although various hormones and other hormone-like compounds have been identified in SWE (Craigie 2011), the hormonal effects of extracts of *A. nodosum* are explained to a large extent by the up- and downregulation of hormone biosynthetic genes in plant cells, and to a lesser extent to the hormonal content of SWE themselves (du Jardin 2015; Wally et al. 2013).

The SWE treatments may improve chlorophyll, and photosynthetic activity, reduce leaf tissue senescence, and also enhance root growth and viability (Ali et al. 2021; Alikaabi et al. 2022; Deolu-Ajayi et al. 2022; Zhang et al. 2022). Cytokinins can improve NaR activity and N assimilation (Sakakibara et al. 2006). Recent study showed that the SWE-based biostimulant Utilize® improved photosynthesis, N metabolism, and root viability in bermudagrass under heat and drought stress conditions (Zhang et al. 2022). The SWE-based biostimulant Utilize® is derived from extracts of brown seaweed (*Ascophyllum nodosum*) and is formulated exclusively for Helena Agri-Enterprises by Goemar (Saint Malo Cedex, France).

The objectives of this study were to examine effects of foliar application of the SWE-based biostimulant Utilize® on N metabolism, photosynthesis, and root growth and determine the optimum rate of Utilize® for improving creeping bentgrass tolerance to heat and drought stress and quality.

Materials and Methods

Plant materials and growth conditions

The experiment was conducted from 11 Jun through 17 Sep 2020. The experiment was repeated from May 18 through 25 Aug 2021 in the same growth chamber at Virginia Tech, Blacksburg, VA, USA. Mature ‘Penn A4’ creeping bentgrass (10 years old) plugs (10 cm, diameter, 5 cm deep) were transplanted into plastic pots (16 cm diameter, 15 cm deep) filled with The United States Golf Association–specified sand with 10% peat (by volume). Plants were grown in growth chambers (Conviron, model PGC20) with temperatures at 22/18 °C (day/night), 70% relative humidity, 12-h photoperiod, and photosynthetically active radiation at 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plants were fertilized at 0.73 $g \cdot m^{-2}$ N (28N–8P–18K plus micronutrients) biweekly; cut height was 5 cm. The grass was irrigated three times per week to maintain appropriate soil moisture level.

Heat and drought stress treatments

Plants were cultured in growth chambers for 6 weeks. On 23 Jul 2020 and 30 Jun 2021 (day −14), treatment solutions were sprayed to the grass foliage as follows: 1) Control; 2) Utilize® at 29 $\mu L \cdot m^{-2}$; 3) Utilize® at 58 $\mu L \cdot m^{-2}$; 4) Utilize® at 87 $\mu L \cdot m^{-2}$; and 5) Utilize® at 116 $\mu L \cdot m^{-2}$. The product was diluted in distilled water and the application solution volume at 815 $L \cdot ha^{-1}$. There were five treatments with four replications, with a total of 20 pots. The treatments were applied with a CO_2-pressured sprayer delivering a volume of 815 $L \cdot ha^{-1}$ and at a pressure of 290 kPa biweekly for a total of four applications. The rates for this trial were selected based on the label rate (58 $\mu L \cdot m^{-2}$), bioassay rates, the turfgrass species, and management level. Because Utilize® contains 5% N from Urea, additional N from Urea was added to all treatments so that N rate was equalized across treatments. N was applied to all pots at 0.73 $g \cdot m^{-2}$ biweekly. With this approach, possible N effect other than SWE in the product was eliminated. Utilize® exhibited biostimulant activity, containing an equivalent 3.33 $\mu g \cdot mL^{-1}$ of plant active cytokinins based on a radish cotyledon expansion bioassay, which was performed with a 1.0% solution according to the method by Yopp et al. (1986). Fourteen days after the first application, turfgrass was subjected to heat and drought stress treatment with temperatures at 35/25 °C (day/night). The grasses were irrigated every day to compensate for 40% to 50% of gravimetrically measured evapotranspiration (ET) loss. The pots were weighed daily and the amount of water added was calculated based on ET loss.

Measurements

The stress treatment lasted for 42 d. Fresh leaf samples were collected on days 0, 14, 28, and 42 for analysis of NaR, chlorophyll, carotenoids, and plant tissue nutrients. Day 0 was the date when the stress was initiated. The in vivo NaR activity was analyzed immediately after sampling. A part of fresh leaf samples were frozen in liquid N and kept at –80 °C before analysis of leaf pigments. At the end of the experiments, roots were removed from pots and rinsed free of soil. A small portion of fresh roots (0.2 g) was sampled for root viability assay, and the rest was analyzed for root characteristics using WinRhizo technology. The roots were then dried in an oven at 65 °C for 48 h and weighed.

Turf quality and leaf color. Turf quality was rated on a visual scale from 1 to 9, with 1 representing completely dead or brown leaves, 6 representing minimum acceptability, and 9 indicting turgid and green leaves, with optimum canopy uniformity and density (Morris 2022; Zhang et al. 2022).

Leaf color was visually rated on a scale from 1 to 9, with 1 indicating completely dead or brown leaves and 9 indicting dark green color (Morris 2022; Zhang et al. 2022).

Leaf Pn and leaf pigment content. Pn was determined by using a portable photosynthetic system (LI-6400XT; LI-COR Corporation, Lincoln, NE, USA) as described by Zhang et al. (2022).

Frozen leaf tissues were ground into powder, weighed, and placed in a test tube with 3 mL acetone. The tubes with the samples were kept in the dark at 4 °C for 48 h before analysis. The extract was transferred into a cuvette and the absorbance was measured with a spectrophotometer. Chlorophyll and carotenoid concentrations were calculated based on the formula described by Zhang et al. (2022). The formulas for chlorophyll and total carotenoids calculation are as follows:

$$\text{Chl a}\left(\mu g \cdot mL^{-1}\right) = (11.24 * A661.6\ nm) - (2.04 * 644.8\ nm).$$

$$\text{Chl b}\left(\mu g \cdot mL^{-1}\right) = (20.13 * A644.8\ nm) - (4.19 * A661.6\ nm).$$

$$\text{Chl a+b}\left(\mu g \cdot mL^{-1}\right) = (7.05 * A661.1\ nm) + (18.09 * A644.8\ nm).$$

$$\text{Carotenoids}\left(\mu g \cdot mL^{-1}\right) = [(1000 * A470\ nm) - (1.90 * chl\ a - 63.14 * chl\ b)]/214.$$

Leaf NaR activity. The in vivo NaR activity was determined by using the procedure of Chanda (2003) with minor modifications as described by Wang et al. (2011) and Zhang et al. (2022).

Root growth characteristics and viability analysis. Root growth characteristics (root length, root SA, root diameter, and root volume) were determined using WinRhizo technology according to Wu et al. (2017). The root biomass was the sum of weights of all subsamples from each pot. The root viability was analyzed following the method as described by Zhang et al. (2022).

Table 1. Turf quality and leaf color response to Utilize® in creeping bentgrass subjected to heat and mild drought stress.

Treatment	Rate ($\mu L \cdot m^{-2}$)	Time of stress (d) −14	0	14	28	42
		Turf quality (1–9, 9 = best)				
Control	0	7.5 a	7.3 c	6.6 c	6.1 c	6.4 c
Utilize®	29	7.5 a	7.7 b	7.1 b	6.4 b	6.7 b
Utilize®	58	7.5 a	7.7 b	7.3 a	6.9 a	7.1 a
Utilize®	87	7.5 a	8.0 a	7.4 a	7.0 a	7.1 a
Utilize®	116	7.5 a	8.0 a	7.5 a	6.9 a	7.0 a
		Leaf color (1–9, 9 = dark green)				
Control	0	7.5 a	7.3 c	6.7 c	6.4 c	6.6 c
Utilize®	29	7.5 a	7.6 b	7.2 b	6.9 b	7.0 b
Utilize®	58	7.5 a	7.8 b	7.5 ab	7.2 a	7.3 a
Utilize®	87	7.5 a	8.0 a	7.7 a	7.3 a	7.3 a
Utilize®	116	7.5 a	8.0 a	7.6 a	7.1 a	7.2 ab

Means followed by same letters within same column for each data set are not significantly different at $P = 0.05$.

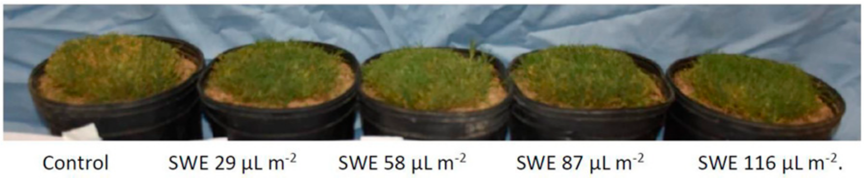

Fig. 1. Leaf color response of creeping bentgrass to seaweed extract–based biostimulant Utilize® under heat and drought stress conditions for 42 d.

Experimental design and statistical analysis

A randomized block design was used with four replicates for each treatment. Because no significant year × treatment interaction was found, the data from year 1 (2020) and year 2 (2021) were pooled and the average of each parameter across the two trials was used for statistical analysis. Effects of the two factors (year and treatment) and their interactions were analyzed with analysis of variance according to the general linear model using SAS (version 9.4 for Windows; SAS Institute, Cary, NC, USA). The five Utilize® treatments were compared by using Fisher's protected least significance difference test at $P = 0.05$.

Results

Turf quality and leaf color

All Utilize® treatments improved turf quality when compared with the control at 0, 14, 28, and 42 d. Of the five treatments, Utilize® at 58, 87, and 116 $\mu L \cdot m^{-2}$ improved turf quality relative to the control and the low rate (29 $\mu L \cdot m^{-2}$) treatment (Table 1, Fig. 1). At 14 d after stress initiation, Utilize® at 58, 87, and 116 $\mu L \cdot m^{-2}$ increased turf quality ratings by 110.6%, 12.1%, and 12.1%. When measured at 28 d, Utilize® at 58, 87, and 116 $\mu L \cdot m^{-2}$ increased turf quality ratings by 13.1%, 10.9%, and 13.9%, when compared with the control. Utilize® at 58, 87, and 116 $\mu L \cdot m^{-2}$ increased turf quality ratings by 10.9%, 10.9%, and 10.9%, when compared with the control at 42 d after stress initiation.

All Utilize® treatments improved leaf color relative to the control at 0, 14, 28, and 42 d. At 14 d, Utilize® at 29, 58, 87, and 116 $\mu L \cdot m^{-2}$ increased leaf color ratings by 7.5%, 11.9%, 14.9%, and 13.4% relative to the control. At 28 d, Utilize® at 29, 58, 87, and 116 $\mu L \cdot m^{-2}$ increased leaf color ratings by 7.8%, 12.5%, 14.1%, and 10.9% when compared with the control. Among the treatments, Utilize® at 58 and 87 $\mu L \cdot m^{-2}$ consistently improved leaf color ratings relative to the control and the low rate (29 $\mu L \cdot m^{-2}$) treatment as measured at 42 d.

Leaf Pn and leaf pigments content

Utilize® treatments at 58, 87, and 116 $\mu L \cdot m^{-2}$ promoted leaf chlorophyll content when compared with the control at 28 and 42 d after stress initiation. Utilize® at 58, 87, and 116 $\mu L \cdot m^{-2}$ increased chlorophyll content by 22.9%, 17.1%, and 11.2%, when compared with the control at 42 d after stress initiation (Table 2).

Carotenoid content was reduced in response to stress treatment. Utilize® treatments at 58 and 87 $\mu L \cdot m^{-2}$ increased leaf carotenoid content when compared with the control at 28 and 42 d after stress initiation (Table 2).

Utilize® treatments at 58, 87, and 116 $\mu L \cdot m^{-2}$ enhanced leaf Pn when compared with the control at 0, 14, 28, and 42 d after stress initiation (Table 3). Utilize® applied at the low rate (29 $\mu L \cdot m^{-2}$) increased Pn at 28 d only. Utilize® at 58, 87, and 116 $\mu L \cdot m^{-2}$ increased Pn by 38.8%, 43.4%, and 30.1%, when compared with the control at 42 d after stress initiation.

Leaf NaR activity

Utilize® treatments at 58, 87, and 116 $\mu L \cdot m^{-2}$ increased leaf NaR activity when compared with the control at 14, 28, and 42 d after stress initiation (Table 3). Utilize® applied at the low rate (29 $\mu L \cdot m^{-2}$) also enhanced NaR activity at 14 and 28 d after stress initiation. Utilize® at 58, 87, and 116 $\mu L \cdot m^{-2}$ increased NaR activity by 26.5%, 16.3%, and 16.3%, when compared with the control at 42 d after stress initiation.

Root growth characteristics and viability analysis

Utilize® at 58 and 87 $\mu L \cdot m^{-2}$ improved root biomass, root length, root SA, and root volume when compared with the control (Table 4). Utilize® applied at the high rate (116 $\mu L \cdot m^{-2}$) also increased root SA, and volume relative to the control.

Utilize® at 58 and 87 $\mu L \cdot m^{-2}$ increased root viability when compared with the control (Table 4). Utilize® at 58 and 87 $\mu L \cdot m^{-2}$ increased root viability by 16.3% and 30.9%, respectively, relative to the control.

Discussion

The use of seaweed-based bioproducts has been gaining momentum in crop production systems due to their unique bioactive components and effects (Ali et al. 2021). Brown SWE-based biostimulants have been increasingly used to promote turfgrass stress tolerance and quality (du Jardin 2015; Zhang et al. 2022). SWEs have been able to significantly mitigate drought stress in plants, although its mechanism of action is still not clear (Ali et al. 2021). Studies conducted with tomato and bell pepper indicated that SWEs were able to significantly upregulate genes involved in auxin (IAA), gibberellin, and cytokinin (IPT) biosynthesis, which could be correlated to the increased plant growth effects and performance (Ali et al. 2019, 2020). Previous studies have indicated beneficial effects of cytokinin (zeatin riboside) on creeping bentgrass tolerance to heat stress (Wang et al. 2011). Seaweed extracts have been reported to increase endogenous cytokinin content (Werner et al. 2001). In the present study, we used the commercial product that contains SWE and N. Utilize® exhibited high hormonal activity, with cytokinin content being equivalent to 3.33 $\mu g \cdot mL^{-1}$ at 1.0% solution based on the bioassay. In this study, we eliminated possible N effect from the product by equalizing N input for each treatment. Extract of brown seaweed contains 45% to 55% organic matter, mineral nutrients, amino acids, etc. (Fike et al. 2001). However, previous studies have indicated that phytohormones, particularly cytokinins, are the most important components responsible for the beneficial effects of SWE (Zhang et al. 2022). Brown SWEs also contain other components, such as auxins, based on our assays of pure brown SWEs and published literature (Ali et al. 2021). In the present study, we characterized the product and identified the cytokinin level in the product. In addition, the rates were selected based on the label, bioassay results, turfgrass species, and management levels.

Table 2. Leaf chlorophyll and carotenoid content response to Utilize® in creeping bentgrass subjected to heat and mild drought stress.

		Time of stress (d)				
Treatment	Rate ($\mu L \cdot m^{-2}$)	−14	0	14	28	42
		Chlorophyll ($mg \cdot g^{-1}$ FW)				
Control	0	2.52 ab	2.60 b	2.20 a	1.91 b	1.75 c
Utilize®	29	2.58 ab	2.55 b	2.42 a	2.17 ab	1.82 bc
Utilize®	58	2.38 b	2.72 ab	2.68 a	2.44 a	2.15 a
Utilize®	87	2.54 ab	2.79 ab	2.49 a	2.33 a	2.05 a
Utilize®	116	2.81 a	2.90 a	2.40 a	2.30 a	1.96 ab
		Carotenoids ($mg \cdot g^{-1}$ FW)				
Control	0	0.87 a	0.91 a	0.86 a	0.77 b	0.70 c
Utilize®	29	0.90 a	0.92 a	0.95 a	0.87 ab	0.72 c
Utilize®	58	0.83 a	0.95 a	1.04 a	0.98 a	0.85 a
Utilize®	87	0.89 a	1.00 a	0.99 a	0.92 a	0.80 a
Utilize®	116	0.95 a	1.02 a	0.94 a	0.91 a	0.76 bc

Means followed by same letters within same column for each data set are not significantly different at $P = 0.05$.
FW = fresh weight.

Table 3. Leaf photosynthetic rate (Pn) and nitrate reductase (NaR) activity response to Utilize® in creeping bentgrass subjected to heat and mild drought stress.

Treatment	Rate ($\mu L \cdot m^{-2}$)	Time of stress (d)				
		−14	0	14	28	42
		Pn (CO_2 $\mu mol \cdot m^{-2} \cdot s^{-1}$)				
Control	0	4.81 a	4.91 b	3.25 c	3.04 d	2.86 c
Utilize®	29	4.65 ab	5.21 ab	3.48 bc	3.40 c	3.13 c
Utilize®	58	4.68 ab	5.36 a	3.76 b	3.83 b	3.97 ab
Utilize®	87	4.56 b	5.41 a	4.72 a	4.23 a	4.10 a
Utilize®	116	4.56 b	5.05 ab	3.89 b	3.90 ab	3.72 b
		NaR activity (NO_2 $\mu mol \cdot h^{-1} \cdot g^{-1}$ FW)				
Control	0	0.81 a	0.75 a	0.49 d	0.45 d	0.49 c
Utilize®	29	0.79 a	0.79 a	0.57 c	0.50 c	0.63 bc
Utilize®	58	0.78 a	0.80 a	0.70 a	0.60 a	0.62 a
Utilize®	87	0.79 a	0.86 a	0.63 b	0.55 b	0.57 ab
Utilize®	116	0.80 a	0.83 a	0.62 b	0.55 b	0.57 ab

Means followed by same letters within same column for each data set are not significantly different at $P = 0.05$.
FW = fresh weight.

The results of this study indicate that foliar application of Utilize® at 58, 87, and 116 $\mu L \cdot m^{-2}$ consistently increased turf visual quality and leaf color ratings when compared with the control. This is consistent with the results of Zhang and Schmidt (1999) and Ali et al. (2019, 2020, 2021). Deolu-Ajayi et al. (2022) pointed out that foliar application of SWEs enhanced crop productivity under drought stress conditions. Previous studies have shown that cytokinins may improve turf quality and heat stress tolerance by improving N metabolism (Wang et al. 2011). In the present study, we proved that SWE-based commercial product at the label rate (58 $\mu g \cdot mL^{-1}$) or higher enhanced creeping bentgrass tolerance to drought and heat stress.

Abiotic stress such as heat and drought stress may damage plant cells through oxidative injury of photosynthetic apparatus including chlorophyll and other pigments (Deolu-Ajayi et al. 2022; Huang et al. 2014). The data of this study showed that Utilize® at 58, 87, and 116 $\mu L \cdot m^{-2}$ improved chlorophyll and carotenoid content when compared with the control under heat and drought stress (Table 2). This is consistent with previous findings that SWE alleviated chlorophyll decline under abiotic stress in bermudagrass (Zhang et al. 2022). Wang et al. (2011) showed that exogenous cytokinin improved creeping bentgrass N metabolism, which is associated with chlorophyll and carotenoid biosynthesis. The cytokinins in SWEs may improve chlorophyll and carotenoid content by improving N metabolism and suppressing ROS-induced oxidative injury under heat and drought stress.

Heat and drought stress could damage photosynthetic function through overaccumulation of ROS because the photosynthetic apparatus is rich in unsaturated lipids, which are susceptible to ROS-induced damage (Zhang et al. 2015, 2022). Previous studies indicated that SWE-based biostimulants enhanced antioxidant defense and suppressed ROS production under abiotic stress, thus protecting photosynthetic function (Deolu-Ajayi et al. 2022; Zhang and Schmidt 1999). Compounds in SWEs may act as signaling molecules regulating key pathways at the transcriptional and/or posttranscriptional levels (microRNAs), causing differential expression of important genes in plants that contribute to the increased plant growth and abiotic stress tolerance (Deolu-Ajayi et al. 2022). Modulation of hormone pathways due to SWE treatment enhances plant growth and development under abiotic stress conditions. The hormone pathways regulate plant growth and development by modulating several plant activities including nutrient assimilation and both physiological and molecular responses to abiotic stress (Ryu and Cho 2015). In the present study, through bioassay, Utilize® contained an equivalent 3.33 $\mu g \cdot mL^{-1}$ of plant active cytokinins at 1.0% solution. The results of this study indicate that Utilize® treatments at 58, 87, and 166 $\mu L \cdot m^{-2}$ increased Pn under heat and drought stress. This suggests that SWE-based biostimulants can regulate photosynthetic function by reducing or preventing ROS toxicity, and destruction of the photosynthetic apparatus and pigments.

The NaR is an important enzyme in plant N metabolism and in regulating N assimilation from inorganic forms to organic acids (Wang et al. 2011). The data of this study showed that Utilize® treatments at 58, 87, and 116 $\mu L \cdot m^{-2}$ increased NaR activity when compared with the control. This is in an agreement with a previous study by Wang et al. (2011) and Zhang et al. (2022). It has been reported that exogenous cytokinins or cytokinins containing SWE can increase endogenous cytokinin, and also NaR activity (Wang et al. 2011; Zhang and Ervin 2004; Zhang et al. 2022).

The results of this study showed that Utilize® application at 58 and 87 $\mu L \cdot m^{-2}$ increased root biomass, root length, SA, root volume, and viability relative to the control in creeping bentgrass subjected to heat and drought stress conditions. This is consistent with previous results by Alkaabi et al. (2022), Rayorath et al. (2008), Zhang et al. (2022), and Xu et al. (2016). Root growth and viability are important indicators of plant survival of abiotic stresses. Heat and drought stress may reduce root growth and viability of creeping bentgrass due to the limitation of endogenous hormones (cytokinin and auxin) and N assimilation. Recent studies showed that SWEs can modulate the expression of genes responsible for the endogenous biosynthesis of growth hormones including auxin, cytokinin, and gibberellin (Ali et al. 2019). Auxin is closely associated with root growth. Proper application of seaweed-based biostimulants could provide plants with active cytokinin and delay plant senescence. In addition, seaweed-based biostimulants could improve root growth and viability by providing auxin directly and/or promoting endogenous auxin biosynthesis.

In summary, the results of this study indicate that foliar application of Utilize® at 58, 87, and 166 $\mu L \cdot m^{-2}$ improved turf quality, leaf color, chlorophyll and carotenoid content, photosynthetic rate, NaR activity, root growth, and viability in creeping bentgrass under heat and drought stress conditions. Because no consistent differences among the three rates (58, 87, and 116 $\mu L \cdot m^{-2}$) were evident, Utilize® treatment at 58 $\mu L \cdot m^{-2}$ biweekly can be considered an effective approach for improving creeping bentgrass performance during summer stress. Foliar application of the *A. nodosum* extract–based biostimulant Utilize® at the proper rate could improve turf quality, root growth, and physiological fitness in creeping bentgrass under heat and drought stress environments. In this study, mowing height (5.0 cm) was a little greater than those of putting greens (4.7 cm) or fairways/tees (4.0 cm); additional study in the field of creeping bentgrass and fairways/tees is warranted to confirm the positive results of Utilize®.

Table 4. Root biomass, length, surface area (SA), diameter, volume, and viability response to Utilize® in creeping bentgrass subjected to heat and mild drought stress.

Treatment	Rate ($\mu L \cdot m^{-2}$)	Biomass (g/pot)	Length ($cm \cdot cm^{-3}$)	SA ($cm^2 \cdot cm^{-3}$)	Diam (mm)	Volume ($cm^3 \cdot dm^{-3}$)	Viability (A490/g FW)
Control	0	1.08 c	32.4 c	2.49 c	0.231 bc	14.2 c	1.23 c
Utilize®	29	1.21 bc	36.5 bc	2.78 bc	0.227 c	15.8 bc	1.31 bc
Utilize®	58	1.32 ab	41.7 ab	3.15 b	0.223 c	17.7 b	1.43 ab
Utilize®	87	1.46 a	43.5 a	3.68 a	0.254 a	23.6 a	1.61 a
Utilize®	116	1.36 ab	36.4 bc	2.97 b	0.244 ab	18.1 b	1.19 c

Means followed by same letters within same column for each data set are not significantly different at $P = 0.05$.
FW = fresh weight.

References Cited

Ali OA, Ramsubhag A, Jayaraman J. 2019. Biostimulatory activities of *Ascophyllum nodosum* extract in tomato and sweet pepper crops in a

tropical environment. PLoS One. 14:e0216710. https://doi.org/10.1371/journal.pone.0216710.
Ali O, Ramsubhag A, Jayaraman J. 2020. Phytoelicitor activity of *Sargassum vulgare* and *Acanthophora spicifera* extract and their properties for use in vegetable crops for sustainable crop production. J Appl Phycol. 33:639–651. https://doi.org/10.1007/s10811-020-02309-8.
Ali O, Ramsubhag A, Jayaraman J. 2021. Biostimulant properties of seaweed extracts in plants: Implications towards sustainable crop production. Plants. 10:531. https://doi.org/10.3390/plants10030531.
Al-Juthery HWA, Drebee HA, Al-Khafaji BMK, Hadi RF. 2020. Plant biostimulants, seaweed extract as a model (article review). Earth and Environ Sci. 553:012015. https://doi.org/10.1088/1755-1315/553/1/012015.
Alkaabi AK, Ramadan GA, Taj Elddin AM, El-Tarabily KA, AbuQamar SF. 2022. The multifarious endophytic actinobacterial isolate, *Streptomyces tubercidicus* UAE1, combined with the seaweed biostimulant further promotes growth of *Avicennia marina*. Front Plant Sci. 9:1–20. https://doi.org/10.3389/frnars.2022.896461.
Chanda S. 2003. Factors affecting nitrate reductase activity in some monocot and dicot species. Plant Biol. 46:41–45.
Chang Z, Liu Y, Dong H, Teng K, Han L, Zhang X. 2016. Effects of cytokinin and nitrogen on drought tolerance of creeping bentgrass. PLoS ONE. 2016;11(4):e0154005. http://dx.doi.org/10.1371/journal.pone.0154005.
Cox T. 2019. What the U.S. Farm Bill really means for biostimulants? https://www.agribusinessglobal.com/plant-health/biostimulants/what-the-u-s-farm-bill-really-meansfor-biostimulants/. [accessed 1 Mar 2019].
Craigie JS. 2011. Seaweed extract stimuli in plant science and agriculture. J Appl Phycol. 23:371–393. https://doi.org/10.1007/s10811-010-9560-4.
Deolu-Ajayi AO, van der Meer IM, van der Werf A, Karlova R. 2022. The power of seaweeds as plant biostimulants to boost crop production under abiotic stress. Plant Cell Environ. 45:2537–2553. https://doi.org/10.1111/pce.14391.
du Jardin P. 2015. Plant biostimulants: Definition, concept, main categories, and regulation. Scientia Hortic. 196:3–14. https://doi.org/10.1016/j.scienta.2015.09.021.
Fike JH, Allen VG, Schmidt RE, Zhang X, Fontenot JP, Bagley CP, Ivy RL, Evans RR, Coelho R, Wester D. 2001. Tasco-Forage: I; Influence of a seaweed extract on antioxidant activity in tall fescue and in ruminants. J Anim Sci. 79:1011–1021. https://doi.org/10.2527/2001.7941011x.
Huang B, DaCosta M, Jiang Y. 2014. Research advances in mechanisms of grass tolerance to abiotic stresses: From physiology to molecular biology. Crit Rev Plant Sci. 33:141–189. https://doi.org/10.1080/07352689.2014.870411.
Jiang Y, Huang B. 2001. Drought and heat stress injury to two cool-season grasses in relation to antioxidant metabolism and lipid peroxidation. Crop Sci. 41:436–442. https://doi.org/10.2135/cropsci2001.412436x.
Man D, Bao YX, Han LB, Zhang X. 2011. Drought tolerance associated with proline and hormone metabolism in two tall fescue cultivars. HortScience. 46:1027–1032. https://doi.org/10.21273/HORTSCI.46.7.1027.
Merewitz EB, Liu S. 2019. Improvement in heat tolerance of creeping bentgrass with melatonin, rutin, and silicon. J Am Soc Hortic Sci. 144:141–148. https://doi.org/10.21273/JASHS04643-19.
Morris K. 2022. A guide to NTEP turfgrass quality ratings. National Turfgrass Evaluation Program, Beltsville MD. https://www.ntep.org/reports/ratings.htm#:∼:text=How%20is%20Turfgrass%20Quality%20Evaluated,an%20absolutely%20outstanding%20treatment%20plot. [accessed 1 May 2020].
Rayorath P, Khan W, Palanisamy R, Mackinnon SL, Stefanova R, Hankins SD, Crichley AT, Prithiviraj B. 2008. Extracts of the brown seaweed *Ascophyllum nodosum* induce gibberellic acid (GA_3)-independent amylase activity in barley. J Plant Growth Regul. 32:123–128. https://doi.org/10.1007/s00344-008-9063-6.
Ryu H, Cho YG. 2015. Plant hormones in salt stress tolerance. J Plant Biol. 58:147–155. https://doi.org/10.1007/s12374-015-0103-z.
Sakakibara H, Takei K, Hirose N. 2006. Interactions between nitrogen and cytokinin in the regulation of metabolism and development. Trends Plant Sci. 11:440–448. https://doi.org/10.1016/j.tplants.2006.07.004.
SAS Institute. 2016. SAS for window (ver. 9.4). SAS Institute, Cary, NC, USA.
Storer K, Kendall S, White C, Roques S, Berrey P. 2016. A review of the function, efficacy and value of biostimulant products available for UK cereals and oilseeds. Research review No. 89. Agriculture and Horticulture Development Board (AHDB). https://ahdb.org.uk/a-review-of-the-function-efficacy-and-value-of-biostimulant-products-available-for-uk-cereals-and-oilseeds. [accessed 3 Mar 2020].
Wally OSD, Critchley AT, Hiltz D, Craigie JS, Han X, Zaharia LI, Abrams SR, Prithiviraj B. 2013. Regulation of phytohormone biosynthesis and accumulation in *Arabidopsis* following treatment with commercial extract from marine macroalga ascophyllum nodosum. J Plant Growth Regul. 32:340–341. https://doi.org/10.1007/s00344-012-9301-9.
Wang K, Okumoto S, Zhang X, Ervin E. 2011. Circadian patterns of the major nitrogen metabolism–related enzymes and metabolites in creeping bentgrass and the influence of cytokinin and nitrate. Crop Sci. 51:2145–2154. (IF 1.88) https://doi.org/10.2135/cropsci2011.01.0024.
Wang K, Zhang X, Ervin EH. 2013. Effects of nitrate and cytokinin on creeping bentgrass under supraoptimal temperatures. J Plant Nutr. 36: 1549–1564. https://doi.org/10.1080/01904167.2013.799184.
Wang K, Zhang X, Goatley M, Ervin EH. 2012. Antioxidative responses in roots and shoots of creeping bentgrass under high temperature: Effects of nitrogen and cytokinin. J Plant Physiol. 169:492–500. https://doi.org/10.1016/j.jplph.2011.12.007.
Werner T, Motyka V, Strnad M, Schmulling T. 2001. Regulation of plant growth by cytokinin. Proc Natl Acad Sci USA. 98:10487–10492. https://doi.org/10.1073/pnas.171304098.
Wu W, Zhang Q, Ervin EH, Yang Z, Zhang X. 2017. Physiological mechanisms of enhancing salt stress tolerance of perennial ryegrass by 24-epibrassinolide. Front Plant Sci. 8:1–11. https://doi.org/10.3389/fpls.2017.01017.
Xu Y, Burgess P, Zhang X, Huang B. 2016. Enhancing cytokinin synthesis by overexpressing ipt alleviated drought inhibition of root growth through activating ROS-scavenging systems in *Agrostis stolonifera*. J Expt Bot. 67:1979–1992. https://doi.org/10.1093/jxp/erw019.
Yopp JH, Aung LH, Steffens GL. 1986. Chapter 4. Cytokinin bioassays, p 63–84. In: Yopp JH, Aung LH, Steffens GL (eds.). Bioassays and other special techniques for plant hormones and plant growth regulators. Plant Growth Regulator Society of America, Lake Afred, FL.
Zhang X, Ervin EH. 2004. Cytokinin-containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. Crop Sci. 44:1737–1745. https://doi.org/10.2135/cropsci2004.1737.
Zhang X, Ervin EH, Liu Y, Hu G, Shang C, Fukao T, Alpuerto J. 2015. Differential responses of antioxidants, abscisic acid, and auxin to deficit irrigation in two perennial ryegrass cultivars contrasting in drought tolerance. J Am Soc Hortic Sci. 140: 562–572. https://doi.org/10.21273/JASHS.140.6.562.
Zhang X, Schmidt RE. 1997. The impact of growth regulators on the a-tocopherol status in water-stressed *Poa practensis* L. Int Turfgrass Soc Res J. 8:1364–1371.
Zhang X, Schmidt RE. 1999. Biostimulating turfgrass. Grounds Maintenance. 34(11):14–32.
Zhang X, Taylor Z, Goatley M, Booth J, Brown I, Kpsiarski K. 2022. Seaweed extract-based biostimulant impacts on nitrate reductase activity and root viability of ultradwarf bermudagrass subjected to heat and drought stress. HortScience. 57:1328–1333. https://doi.org/10.21273/HORTSCI16746-22.