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Seaweed Extract-based Biostimulant Impacts on Nitrate Reductase Activity and Root Viability of Ultradwarf Bermudagrass Subjected to Heat and Drought Stress

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Abstract. **Bermudagrass is a warm-season turfgrass species widely used for sports fields, home lawns, and golf courses. Ultradwarf bermudagrass has been used for golf course greens, but its quality declines with abiotic stresses. This 2-year study was designed to investigate if foliar applications of seaweed extract-based biostimulant Utilize® could improve ultradwarf bermudagrass photosynthetic function, nitrate reductase activity, root growth, and root function while under heat stress and drought stress conditions. Utilize® was applied to ultradwarf bermudagrass canopy at 0, 88, 117, 175, and 234 μL·m^{-2}. Two weeks after the initial application of Utilize®, bermudagrass was subjected to heat (40/36 °C, day/night) and drought stress (40–50% evapotranspiration replacement) for up to 42 days. Heat stress and drought stress caused decline of the turf quality. Foliar application of Utilize® at 117, 175, and 234 μL·m^{-2} biweekly consistently improved turf quality and leaf color ratings and increased leaf chlorophyll and carotenoid concentrations, net photosynthetic rate, nitrate reductase activity, and root growth and viability. On average, Utilize® at 117, 175, and 234 μL·m^{-2} increased turf quality ratings by 9.1%, 12.1%, and 10.6%, respectively, net photosynthetic rates by 32.4%, 45.0%, and 35.0%, respectively, and nitrate reductase activity by 16.7%, 18.8%, and 14.6%, respectively, compared with the control. Utilize® at 117, 175, and 234 μL·m^{-2} increased the root biomass, root length, surface area, and root volume compared with the control. Utilize® at 88, 117, 175, and 234 μL·m^{-2} increased root viability by 46.2%, 73.1%, 88.5%, and 74.4%, respectively, relative to the control. The results of this study suggest that seaweed extract-based biostimulant Utilize® improves nitrogen metabolism, photosynthetic function, root growth, and root viability. Foliar application of Utilize® at rates between 117 and 175 μL·m^{-2} biweekly can be considered an effective approach to improving ultradwarf bermudagrass performance under heat stress and drought stress environments.**

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During the past two decades, ultradwarf bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy] has become the prevalent turfgrass used on golf putting greens in the southeastern United States (Hartwiger, 2009; Inguagiato and Martin, 2015; Unruh and Davis, 2001). Ultradwarf bermudagrasses gained popularity because of its tolerance to heat and traffic and ability to provide excellent golf putting green surfaces (Goatley et al., 2007; Hartwiger, 2009; Inguagiato and Martin, 2015). Ultradwarf bermudagrass putting greens have been widely adopted throughout the warm/humid zone and southern transition zone, and they are now being grown in the upper transition zone including Virginia (Hartwiger, 2009; Richardson and Booth, 2021).

The new hybrid "ultradwarf" bermudagrass cultivar, including Champion, FloraDwarf and TifEagle, are the most notable. These cultivars have greater shoot density and tolerance to low mowing heights (Hanna, 1998). However, ultradwarf bermudagrass experiences quality decline because of disease and heat stress, especially in the southern part of the United States and other regions with similar climates (Unruh and Davis, 2001). Ultradwarf bermudagrass canopy temperatures during the hottest months of 2000 reached 61.1 °C (Unruh and Davis, 2001). Although bermudagrass is a warm-season species, it cannot maintain good quality at this canopy temperature. In some areas where bermudagrass is grown, there is a severe water shortage, and drought is a major factor causing the quality decline of bermudagrass. Drought and heat stress may damage turfgrass photosynthetic function through oxidative injury (Jiang and Huang, 2001; Zhang and Schmidt, 1999a). As a consequence of drought stress and extreme temperatures, turfgrass quality is negatively impacted (Chang et al., 2016; Zhang et al., 2015, 2021). Bermudagrass has developed various physiological defense systems to cope with abiotic stress and protect photosynthetic function, such as hormonal regulation, osmotic adjustment, antioxidant alteration, stomatal control, and saturation levels of cell membrane lipids (Huang et al., 2014).

Heat stress and drought stress may damage the photosynthetic function and cause an energy imbalance so that the energy absorbed through the light harvesting complex exceeds what can be dissipated or transduced by photosystem II (Wang et al., 2012; Zhang and Ervin, 2008). Excess energy may be directed to molecular O_2 and results in the accumulation of toxic reactive oxygen species (ROS) (Huang et al., 2014; Wu et al., 2017). The stomatal closure under drought stress may limit CO_2 and O_2 exchange through guard cells, resulting in higher O_2 accumulation and ROS production in cells. Heat-induced and drought-induced oxidative stress have been reported for cool-season turfgrass species (Jiang and Huang, 2001; Man et al., 2011; Wang et al., 2011; Zhang and Ervin, 2004; Zhang et al., 2012) and warm-season turfgrass species (Liu et al., 2020).

Nitrogen is the mineral nutrient required in the largest amount (3–5% dry leaf tissues) by grass plants. Nitrogen nutrition is closely associated with turfgrass quality, color, growth, and tolerance to abiotic stresses. Nitrate (NO_3^-) and ammonium (NH_4^+) are the common forms of nitrogen available for plants. Plants require

substantial metabolic energy for the uptake of inorganic nitrogen and subsequent assimilation into organic nitrogen. Nitrate, which is the most commonly available form of nitrogen for grasses, has to be reduced to nitrite (NO_2^-) and then ammonium, which is then incorporated into amino acid biosynthesis (Wang et al., 2013). Nitrate reductase (NaR) is the key enzyme catalyzing the conversion from nitrate to nitrite. Higher NaR activity is associated with greater nitrogen assimilation, use efficiency, and chlorophyll biosynthesis, especially under abiotic stress and nitrogen deficiency conditions.

Plant biostimulants have been used to improve the turfgrass performance of cool-season and warm-season turfgrass species (Zhang and Schmidt, 1999a, 1999b, 2000; Zhang et al., 2021). Plant biostimulants are defined as "materials that, in minute quantities, promote plant growth" (Zhang and Schmidt, 1999a). The 2018 Farm Bill described a plant biostimulant as "a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield" (Cox, 2019). Various plant biostimulants, such as seaweed extract (SWE), humic and fulvic acids, protein hydrolysates, and other nitrogen-containing compounds, beneficial microorganisms, and small organic molecules, have been used for turfgrass management (du Jardin, 2015; Zhang and Schmidt, 1999a; Zhang et al., 2003, 2021). SWE contains various organic components and trace amounts of inorganic nutrients, but the generation of plant active hormones, especially cytokinins and auxin, are considered the major components responsible for the biostimulant activity (Storer et al., 2016). Although various hormones (cytokinins, auxins, abscisic acid, gibberellins) and other hormone-like compounds (sterols, polyamines) have been identified in some SWE by bioassays and immunological tools (Craigie, 2011), the hormonal effects of extracts of brown seaweed *Ascophyllum nodosum* are explained, to a larger extent, by the downregulation and upregulation of hormone biosynthetic genes in plant tissues and, to a lesser extent, by the hormonal content of SWE (du Jardin, 2015; Wally et al., 2013).

Previous research showed that SWE may protect chlorophyll and photosynthetic function, delay leaf senescence, and promote root growth and viability (Zhang and Ervin, 2008; Zhang et al., 2003). However, few studies have reported the effects of SWE-containing material on photosynthesis, NaR activity, and root function of ultradwarf bermudagrass under heat stress and drought stress conditions. Utilize®, an SWE-based biostimulant, contains 5% nitrogen from urea, is derived from extracts of brown seaweed *Ascophyllum nodosum*, and is formulated exclusively for Helena Agri-Enterprises by Goemar (Saint Malo Cedex, France). The objective of this study was to examine whether foliar application of Utilize® could improves ultradwarf bermudagrass photosynthesis, NaR activity, and root viability under heat stress and drought stress conditions.

Materials and Methods

Plant materials and growth conditions. The experiment was initially conducted from 22 May through 25 Aug. 2020, and it was repeated in the same growth chambers from 16 July through 13 Oct. 2021. Mature 'Champion' ultradwarf plugs (diameter, 10 cm; depth, 5 cm) were collected from Independence Golf Course, Richmond, VA, and planted in plastic pots (diameter, 16 cm; depth, 15 cm) filled with United States Golf Association-specified sand with 10% peat (by volume). Plants were cultured in growth chambers with temperatures (mean ± *SD*) of 30 ± 1.0/24 ± 0.7 °C (day/night), relative humidity (mean ± *SD*) of 65% ± 7%, a 14-h photoperiod, and photosynthetically active radiation (*PAR*) of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (*SD* ±10). Plants were fertilized with 0.73 $g \cdot m^{-2}$ nitrogen from bent special 28–8–18 plus micronutrients every 2 weeks, trimmed to 0.99 cm weekly, and irrigated by hand until water drained from bottom of the pots three times per week.

Heat stress and drought stress treatments. Plants were grown in growth chambers for ≈12 weeks. On 30 June 2020 and 20 Aug. 2021 (day 0), treatments were applied to the grass as follows: control; Utilize® at 88 $\mu L \cdot m^{-2}$; Utilize® at 117 $\mu L \cdot m^{-2}$; Utilize® at 175 $\mu L \cdot m^{-2}$; and Utilize® at 234 $\mu L \cdot m^{-2}$. The treatments were applied with a delivery volume of 82 $mL \cdot m^{-2}$ every 2 weeks for a total of four applications. The rates for this trial were selected based on the label rate of the product, bioassay results, turfgrass species and management level. The label rate of this product for turfgrass is 58.5 $\mu L \cdot m^{-2}$. Because Utilize® contains 5% nitrogen from urea, additional nitrogen from urea was added to all treatments so that the nitrogen rate was normalized across treatments. Therefore, the possible nitrogen effect other than that from the seaweed extract in the product was eliminated. Utilize® exhibited biostimulant activity, generating an equivalent 3.33 $\mu L \cdot m^{-2}$ of plant active cytokinins based on a radish cotyledon expansion bioassay at 1.0% solution that was performed before trial initiation according to the method of Yopp et al. (1986). Fourteen days after the initial application, bermudagrass was subjected to heat stress and drought stress treatments with temperatures (mean ± *SD*) of 40 ± 1.1/36 ± 0.9 °C (day/night) and deficit irrigation to induce drought stress. The bermudagrass was irrigated daily to compensate for 40% to 50% of gravimetrically measured evapotranspiration loss.

Measurements. The heat stress and drought stress lasted for 56 d. Fresh leaf samples were collected on days 0, 14, 28, 42, and 56 to evaluate NaR, chlorophyll, carotenoids, and plant tissues. The NaR activity was analyzed immediately after sampling. A portion of fresh leaf samples were frozen in liquid nitrogen and kept at −80 °C before the final analysis. At the end of the experiments, roots were removed and rinsed free of soil. A small portion of fresh roots (0.2 g) was sampled to perform a root viability assay, and the remaining roots were scanned and analyzed to evaluate root characteristics using WinRhizo Technology. The remainder of the roots were dried in an oven for 48 h and then weighed.

Turf quality. The turf quality was visually rated using a scale of 1 to 9 based on leaf color, uniformity, and density, with 1 indicating completely dead or brown leaves, 6 representing minimum acceptability, and 9 indicating turgid and green leaves with optimum canopy uniformity and density.

Leaf color. The leaf color was visually rated using a scale of 1 to 9, with 1 indicating completely dead or brown leaves and 9 indicating dark green color.

Leaf photosynthetic rate. The leaf photosynthetic rate (Pn) was measured using a portable photosynthetic system (LI-6400XT; LI-COR Corporation, Lincoln, NE). Four uniform leaf blades were sampled from each pot and placed in the gas chamber for quantification using a temperature of 24 °C, CO_2 flow rate of 400 $\mu mol \cdot s^{-1}$, CO_2 concentration of 400 ppm, and *PAR* of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The leaf area (≈2 cm^2) was measured and the actual leaf area was used for each Pn measurement. Three readings of each sample were collected and the average was used for the statistical analysis (Zhang et al., 2017).

Leaf chlorophyll and carotenoid content. Frozen leaf tissues were ground into powder and samples were weighed and placed in test tubes with 3 mL acetone. The samples were incubated in the dark at 4 °C for 48 h. The supernatant was collected and the absorbance was measured with a spectrophotometer; the chlorophyll and carotenoid contents were calculated according to the method described by Zhang et al. (2005).

Leaf NaR activity. The in vivo activity of leaf NaR was estimated by using the method of Chanda (2003) with some modifications (Wang et al., 2011). Briefly, ≈100 mg fresh shoot tissue was cut into 0.5-cm lengths. Then, shoot sections were immersed in 10 mL of 0.05 M potassium phosphate buffer (pH 7.5) with 1% n-propanol and 50 mm KNO_3. The samples were vacuum-infiltrated for 4 min to ensure infiltration of incubation buffer; then, they were incubated in the dark for 4 h at 30 °C. At the end of the incubation, 1 mL of solution was transferred to 10-mL glass culture tubes. The nitrite formed was estimated colorimetrically by adding 750 μL of 1% sulfanilamid in 3 M HCl and 750 μL 0.02% N-naphthylethylenediamine hydrochloride. Absorption was determined at 540 nm. For each run, blanks and four nitrite standards (1, 5, 10, and 25 μM KNO_2) were included.

Leaf nitrogen content. Frozen leaf samples (100 mg) were ground in liquid nitrogen and extracted in 1.8 mL of ice-cold 50 mmol sodium phosphate buffer (pH 7.0) containing 0.2 mm EDTA and 1% polyvinylpyrrolidone in an ice-water bath. The homogenate was centrifuged at 12,000 g_n for 20 min at 4 °C. Supernatant was used for the soluble protein

Table 1. Turf quality and leaf color response to Utilize® for ultradwarf bermudagrass subjected to heat stress and mild drought stress.

Treatment	Rate ($\mu L \cdot m^{-2}$)	Time of stress (d)				
		0	14	28	42	56
		Turf quality (1–9, 9 = best)				
Control	0	8.0 a[i]	8.0 b	7.2 d	6.2 d	6.6 b
Utilize®	88	8.0 a	8.0 b	7.4 cd	6.5 c	6.8 b
Utilize®	117	8.0 a	8.3 a	7.6 ab	6.7 b	7.2 a
Utilize®	175	8.0 a	8.3 a	7.8 a	7.1 a	7.4 a
Utilize®	234	8.0 a	8.2 a	7.5 bc	6.8 b	7.3 a
Mean		8.0 z	8.1 z	7.5 y	6.6 x	6.6 x
		Leaf color (1–9, 9 = dark green)				
Control	0	8.0 a	8.0 c	7.3 c	6.4 d	6.7 c
Utilize®	88	8.0 a	8.0 c	7.5 bc	6.7 c	7.0 bc
Utilize®	117	8.0 a	8.3 ab	7.7 ab	6.9 b	7.2 ab
Utilize®	175	8.0 a	8.4 a	7.8 a	7.1 a	7.5 a
Utilize®	234	8.0 a	8.2 b	7.6 b	6.9 b	7.3 ab
Mean		8.0 y	8.2 z	7.6 x	6.8 v	7.1 w

[i] Values followed by the same letters within the same column and means followed by the same letters within the same row are not significantly different at $P = 0.05$.

analysis using the Bradford method (Bradford, 1976; Zhang et al., 2015). The protein content was converted to nitrogen by using a protein-to-nitrogen conversion factor of 4.43 (Yeok and Wee, 1994).

Root growth characteristics. Roots from each pot were cleaned and divided into multiple subsamples. Each subsample was scanned and analyzed using WinRhizo Technology to determine the root length, root surface area, root diameter, and root volume. Root biomass was the total of the subsamples from each pot.

Root viability. Root viability was measured according to the method described by Zhang et al. (2017). Briefly, fresh root samples were collected and rinsed with distilled water and cut into 1-cm sections; any moisture was removed with paper towels. Root samples (50 mg) were incubated in glass test tubes with 5 mL 0.6% (weight/volume) 2,3,5-triphenyltetrazolium chloride in 0.05 M phosphate buffer (pH 7.4), plus 0.05% (volume/volume) wetting agent XT-100. Samples were vacuum-infiltrated and incubated at 30 °C for 24 h. Roots were drained and rinsed twice with distilled water. Formazan was extracted twice with 5 mL boiling 95% (volume/volume) ethanol. Combined extracts were brought to 10 mL. Absorbance was measured at 490 nm, and root viability was expressed as 490 per gram of fresh weight.

Experimental design and statistical analysis. For each year, a randomized block design was used with four replicates for each treatment. There was no significant year × treatment interaction; therefore, data from 2020 and 2021 were pooled and the averages of each parameter across the two experiments were used for the statistical analysis. Effects of year and treatment and their interactions were analyzed using an analysis of variance according to the general linear model using SAS (version 9.4 for Window; SAS Institute, Cary, NC; SAS Institute, 2016). Comparisons of the five Utilize® treatments were performed using Fisher's protected least significance difference test (significance considered at $P = 0.05$).

Results

Turfgrass quality. Turf quality declined because of heat stress and drought stress (Table 1). Foliar application of Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ improved turf quality relative to the control from day 14 through day 56. Utilize® treatments at 117, 175, and 234 $\mu L \cdot m^{-2}$ increased turf quality ratings by 9.1%, 12.1%, and 10.6% compared with the control at 56 d after the initial application.

Leaf color. Heat stress and drought stress reduced leaf color ratings. All Utilize® treatments except for the low rate (88 $\mu L \cdot m^{-2}$) improved leaf color relative to the control at 14, 28, 42, and 56 d. Among the treatments, Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ consistently improved leaf color ratings relative to the control.

Leaf chlorophyll. Heat stress and drought stress caused a decline in the leaf chlorophyll content (Table 2). Application of Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ increased leaf chlorophyll content relative to the control when measured at 28, 42 and 56 d after the initial application. Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ increased the chlorophyll content by 29.1%, 37.6%, and 32.6% compared with the control at 56 d after the initial treatment.

Leaf carotenoids. Similar to chlorophyll, the carotenoid content declined in response to stress treatments. Application of Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ increased the leaf carotenoid content relative to the control

Table 2. Leaf chlorophyll and carotenoid contents responses to Utilize® for ultradwarf bermudagrass subjected to heat stress and mild drought stress.

Treatment	Rate ($\mu L \cdot m^{-2}$)	Time of stress (d)				
		0	14	28	42	56
		Chlorophyll ($mg \cdot g^{-1}$ fresh weight)				
Control	0	2.59 a[i]	2.60 b	2.07 b	1.95 b	1.41 c
Utilize®	88	2.57 a	2.59 b	2.30 ab	2.04 b	1.63 bc
Utilize®	117	2.41 a	2.76 ab	2.40 a	2.33 a	1.82 ab
Utilize®	175	2.49 a	2.91 a	2.48 a	2.43 a	1.94 a
Utilize®	234	2.60 a	2.70 ab	2.31 a	2.37 a	1.87 ab
Mean		2.53 y	2.71 z	2.31 x	2.22 x	1.73 w
		Carotenoids ($mg \cdot g^{-1}$ fresh weight)				
Control	0	0.90 a	0.88 b	0.75 b	0.73 b	0.54 c
Utilize®	88	0.88 a	0.88 b	0.83 a	0.74 b	0.62 bc
Utilize®	117	0.81 a	0.95 ab	0.86 a	0.84 a	0.68 ab
Utilize®	175	0.84 a	0.97 a	0.89 a	0.88 a	0.71 a
Utilize®	234	0.88 a	0.91 ab	0.83 a	0.85 a	0.69 ab
Mean		0.86 y	0.92 z	0.83 xy	0.81 x	0.65 w

[i] Values followed by the same letters within the same column or means followed by the same letters within the same row are not significantly different at $P = 0.05$.

Table 3. Leaf photosynthetic rate (Pn) and nitrate reductase (NaR) activity responses to Utilize® for ultradwarf bermudagrass subjected to heat stress and mild drought stress.

Treatment	Rate ($\mu L \cdot m^{-2}$)	Time of stress (d)				
		0	14	28	42	56
		Pn ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)				
Control	0	4.81 a[i]	6.77 ab	7.75 c	4.81 d	3.40 c
Utilize®	88	4.65 ab	6.77 ab	8.12 ab	5.18 cd	4.00 b
Utilize®	117	4.68 ab	6.94 a	8.18 b	5.66 ab	4.50 ab
Utilize®	175	4.56 b	6.72 b	8.31 a	5.95 a	4.93 a
Utilize®	234	4.56 b	6.75 ab	8.04 b	5.29 bc	4.59 a
Mean		6.79 y	8.08 z	5.38 x	4.28 w	3.68 v
		NaR activity ($\mu mol\ NO_2\ h^{-1} \cdot g^{-1}$ fresh weight)				
Control	0	0.55 a	0.59 a	0.53 c	0.49 b	0.48 c
Utilize®	88	0.54 a	0.62 a	0.52 c	0.52 ab	0.53 b
Utilize®	117	0.55 a	0.67 a	0.59 ab	0.55 a	0.56 a
Utilize®	175	0.56 a	0.66 a	0.64 a	0.56 a	0.57 a
Utilize®	234	0.56 a	0.63 a	0.56 bc	0.53 ab	0.55 ab
Mean		0.55 xy	0.63 z	0.57 y	0.53 x	0.54 xy

[i] Values followed by the same letters within the same column or means followed by the same letters within the same row are not significantly different at $P = 0.05$.

when measured at 28, 42, and 56 d after the initial application.

Leaf Pn. Heat stress and drought stress reduced the Pn. Application of Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ increased the leaf Pn relative to the control when measured at 28, 42, and 56 d after the initial application (Table 3). Utilize® applied at the low rate (88 $\mu L \cdot m^{-2}$) also increased the Pn at day 42. Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ increased the Pn by 32.4%, 45.0%, and 35.0% compared with the control 56 d after the initial application.

Leaf NaR activity. The NaR activity declined in response to the heat stress and drought stress treatments. The application of Utilize® at 117 and 175 $\mu L \cdot m^{-2}$ increased the leaf NaR activity relative to the control when measured at 14, 28, 42, and 56 d after the initial application. Utilize® applied at the low rate (88 $\mu L \cdot m^{-2}$) and high rate (474 $\mu L \cdot m^{-2}$) also increased the NaR activity at 56 d after stress initiation. Utilize® at 117 and 175 $\mu L \cdot m^{-2}$ increased the NaR activity by 16.7% and 18.8%, respectively, compared with the control at 56 d after the initial application.

Root growth characteristics. Utilize® at all rates improved root biomass relative to the control, with Utilize® at 117 $\mu L \cdot m^{-2}$ producing the greatest root biomass (Table 4). Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ increased the root length, root surface area, and root volume compared with the control (Table 4).

Root viability. Utilize® at all rates improved root viability when compared with the control, with Utilize® at 175 $\mu L \cdot m^{-2}$ having the greatest root viability (Table 4). Utilize® at 88, 117, 175, and 234 $\mu L \cdot m^{-2}$ increased root viability by 46.2%, 73.1%, 88.5%, and 74.4%, respectively, relative to the control.

Leaf nitrogen content. No significant differences in leaf tissue nitrogen levels were found at the initial sampling date or final sampling date (data not shown).

Discussion

The use of Ultradwarf bermudagrass for golf course putting greens in the southeastern United States and other regions with similar climates has been increasing. Ultradwarf Bermudagrass experiences a decline in quality during the summer months because of the various abiotic stresses such as heat and drought (McCarty and Canegallo, 2005). Seaweed extract-based biostimulants have been widely used to improve turfgrass stress tolerance and quality (du Jardin, 2015; Zhang and Schmidt, 1997, 1999a). The results of this study indicate that foliar application of seaweed extract-based biostimulant Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ consistently improved turf visual quality and leaf color relative to the control. This is consistent with the results of Zhang and Schmidt (1999b) and Zhang and Ervin (2008) in regard to cool-season turfgrass species. No study has reported the effects of SWE-based biostimulants on bermudagrass heat and drought tolerance. Previous studies have shown that seaweed extract-based biostimulants or cytokinins may improve turf quality and stress tolerance by improving hormone and nitrogen metabolism (Wang et al., 2011; Zhang et al., 2010).

Heat stress and drought stress may damage plants through oxidative injury, resulting in destruction of chlorophyll and other pigments (Huang et al., 2014). The results of this study showed that Utilize® at 117, 175 and 234 $\mu L \cdot m^{-2}$ improved the chlorophyll and carotenoid contents relative to the control under heat stress and drought stress (Table 2). Similar results were obtained for cool-season turfgrass species by previous studies (Zhang and Ervin, 2004). Nitrogen is an essential element in chlorophyll and carotenoid biosynthesis. Utilize® contains 5% nitrogen as urea. However, the possible nitrogen effect from the product was eliminated by equalizing the nitrogen input across treatments. Plant active cytokinins generated from Utilize®, which had equivalents of 0.14 and 0.20 μM in the application solutions of 117 and 175 $\mu L \cdot m^{-2}$, respectively, may directly protect chlorophyll and carotenoids and promote biosynthesis of endogenous cytokinins, which protect chlorophyll and delay leaf senescence under abiotic stress.

Heat stress and drought stress could damage photosynthetic function through the overaccumulation of ROS because the photosynthetic apparatus is rich in unsaturated lipids that can sustain ROS damage (Zhang et al., 2012). Previous studies indicated that seaweed extract-based biostimulants improved the antioxidant defense and suppressed ROS production under abiotic stress, thus protecting the photosynthetic function (Zhang and Schmidt, 1999b). Utilize® comprising 1.0% solution generated an

Table 4. Root biomass, length, surface area (SA), diameter, volume, and viability responses to Utilize® for ultradwarf bermudagrass subjected to heat stress 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 fresh weight)
Control	0	0.279 c[i]	6.21 b	0.493 b	0.241 a	3.05 b	0.78 c
Utilize®	88	0.376 b	8.21 ab	0.618 ab	0.231 a	3.65 ab	1.14 b
Utilize®	117	0.470 a	9.98 a	0.760 a	0.230 a	4.40 a	1.35 ab
Utilize®	175	0.381 b	8.55 a	0.667 a	0.235 a	4.02 ab	1.47 a
Utilize®	234	0.381 b	9.37 a	0.707 a	0.227 a	4.06 a	1.36 ab

[i] Values followed by the same letters within the same column for each data set are not significantly different at $P = 0.05$.

equivalent 3.33 $\mu g \cdot mL^{-1}$ of plant active cytokinins. These plant active cytokinins generated from Utilize® may directly improve plant tolerance to ROS toxicity and promote further biosynthesis of cytokinins in the plants. The results of this study indicate that Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ improved Pn under heat stress and drought stress. This suggests that seaweed-based biostimulants improved the photosynthetic function by suppressing ROS toxicity and protecting the photosynthetic apparatus and light-gathering pigments.

NaR is a key enzyme in plant nitrogen metabolism and has an important role in regulating nitrogen assimilation from inorganic to organic forms (Wang et al., 2011). The results of this study showed that foliar application of Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ increased NaR activity compared with the control. This is consistent with the results of Wang et al. (2011), who studied creeping bentgrass. It has been documented that exogenous cytokinins can increase endogenous cytokinin, and cytokinins can increase NaR activity. It is possible that Utilize® may increase NaR activity through its generation of plant active cytokinins.

The results of this study showed that Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ increased root biomass, root length, surface area, root volume, and viability of ultradwarf bermudagrass subjected to heat stress and drought stress conditions relative to the control. This is consistent with the results of Zhang and Schmidt (1999b) and Xu et al. (2016), who studied creeping bentgrass. Under heat stress and drought stress, root growth and viability of ultradwarf bermudagrass may decline because of the limitations of endogenous hormone (cytokinin and auxin) biosynthesis and nitrogen assimilation. The proper application of seaweed-based biostimulants could provide hormonal effects that delay plant senescence, improve root function, and promote cytokinin and auxin biosynthesis.

In this study, we did not observe the response of the plant leaf nitrogen content to foliar applications of Utilize®. The nitrogen content was calculated based on the soluble protein content based on fresh tissue; it was not directly determined by an analysis of the plant nitrogen content based on dry weight. This may not have been accurate. In addition, the nitrogen content in other tissues such as roots was not analyzed. This suggests that Utilize® could improve NaR activity and may or may not affect plant leaf nitrogen levels when bermudagrass is grown under normal fertilization conditions.

In summary, the results of this study indicate that foliar application of Utilize® at 117, 175, and 234 $\mu L \cdot m^{-2}$ (which are in the recommended range for field application) improved turf quality, leaf color, chlorophyll and carotenoid contents, photosynthetic rate, NaR activity, root growth, and root viability of ultradwarf bermudagrass under heat stress and drought stress conditions. Because there were few differences among higher rates (117, 175, and 234 $\mu L \cdot m^{-2}$), the foliar application of Utilize® at 117 $\mu L \cdot m^{-2}$ biweekly could be considered an effective approach to improving bermudagrass performance in heat stress and drought stress environments.

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