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Amino Acid Biostimulants Enhance Drought and Heat Stress Tolerance of Creeping Bentgrass (*Agrostis Stolonifera* L.)

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Abstract

Creeping bentgrass (*Agrostis stolonifera* L.) is an important cool-season turfgrass species widely used for golf course putting greens; however, it experiences a summer stress-induced quality decline in the U.S. transition zone and other regions with similar climates. The objective of this study was to determine the effects of five amino acid biostimulants on creeping bentgrass drought and heat stress tolerance. The five biostimulants, including Superbia, Amino Pro V, Siapton, Benvireo, and Surety, at the rate of 0.22 g of N m^{-2}, were applied biweekly to foliage, and the treatments were arranged in a randomized block design with four replications and were subjected to 56 days of heat and drought stress in growth chamber conditions. The amino acid biostimulants Superbia and Amino Pro V improved the turf quality, photochemical efficiency (PE), normalized difference vegetation index (NDVI), chlorophyll content, antioxidant enzyme superoxide dismutase activity, root growth, and viability and suppressed leaf H_2O_2 levels when compared to a control. Among the treatments, Superbia and Amino Pro V exhibited greater beneficial effects on turf quality and physiological fitness. The results of this study suggest that foliar application of amino acid biostimulants may improve the summer stress tolerance of cool-season turfgrass species in the U.S. transition zone and other regions with similar climates.

Keywords: creeping bentgrass; drought; heat; protein hydrolysate; root viability; superoxide dismutase; water deficit

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1. Introduction

Nitrogen (N) assimilation is one of the most important metabolic processes of higher plants. Nitrogen is the mineral nutrient required by grass plants in the largest amounts (3–5% dry leaf tissues) [1]. Nitrogen fertilization is one of the most important cultural practices in turfgrass management. Nitrogen nutrition is closely associated with turfgrass quality, color, growth, and tolerance to abiotic stress. Nitrate (NO_3^-) and ammonium (NH_4^+) are the common forms of N available for plants. Plants require substantial metabolic energy for the uptake of inorganic N from environments and assimilation into organic N. Nitrate, the most commonly available form of N for grasses, has to be reduced to nitrite (NO_2^-) and then ammonium, which is incorporated into amino acid biosynthesis [1].

Plant biostimulants have been used to improve turfgrass performance and stress tolerance in cool-season and warm-season turfgrass species. Plant biostimulants are defined as "materials that, in minute quantities, promote plant growth" [2]. The 2018 Farm Bill described a plant biostimulant as "a substance or microorganism that, when applied to seeds, plants, or rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield" [3]. Various biostimulants, such as seaweed extracts, humic acids, protein hydrolysates (PHs) or amino acids, beneficial microorganisms, and small organic molecules, have been used for turfgrass management [1]. Amino acids not only are the building blocks of proteins and enzymes but are also involved in transporting N between roots, leaves, fruits, etc. Amino acids are precursors in the synthesis of chlorophyll and many other N-containing compounds [1]. Amino acids also serve as the carbon and N source to produce most 'secondary' or 'natural' products. Amino acids are also associated with antioxidants and hormone metabolism and play an important role in plant tolerance to abiotic stress [4]. Amino acids play an important role in turfgrass growth and physiological fitness. For example, glutamate is a precursor of chlorophyll and is associated with photosynthesis, and glutamine levels are regulated in response to photosynthetic activity. The amino acid tryptophan serves as a primary precursor of the hormone auxin (indole-3-acetic acid, IAA), which is closely related to root initiation.

The energy required during nitrate reduction and amino acid synthesis is provided through photosynthesis and respiration. As photosynthesis declines and carbohydrates for respiration reduce under abiotic stress, the available energy for N metabolism becomes limiting, which may lead to a reduction in the formation of endogenous amino acids and the accumulation of nitrate. Exogenously applying certain amino acids may improve N metabolism and turfgrass performance, especially under abiotic stress environments and/or N deficiency [1].

Summer stress, characterized by high temperatures and droughts, is the major factor causing the quality decline of creeping bentgrass and other cool-season turfgrass species in the U.S. transition. Heat stress plus drought stress may cause more severe oxidative injury to plants than either heat or drought stress alone. Heat and drought stress damages plants by the accumulation of toxic reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide (H_2O_2) in plant cells. Antioxidant defense systems consisting of various metabolites and enzymes, such as superoxide dismutase (SOD), could scavenge the ROS and protect plant functions under abiotic stress [5].

Exogenous amino acids can be readily absorbed and translocated by plant tissues [6,7]. In a recent study with creeping bentgrass, both ^{15}N-labeled and ^{15}N,^{13}C double-labeled L-glutamate were applied exogenously to creeping bentgrass foliage, and the uptake of glutamate and its integration into γ-aminobutyric acid (GABA) and L-proline, two amino acids with known roles in plant stress adaptation, were observed. The results demonstrate that glutamate is rapidly absorbed into creeping bentgrass foliage and that it is utilized to produce GABA and proline, which are closely associated with plant tolerance to abiotic stress. Glutamate is predominantly taken up intact [8]. Once absorbed, exogenous amino acids have the capacity to function as compatible osmolytes, regulate ion transport, serve as signaling molecules, and modulate the stomatal opening, among other benefits. In addition, exogenous amino acids may improve soil microbial activity and chelate micronutrients once they enter the soil environment.

Foliar application of amino acids may improve plant N metabolism and turfgrass quality. It has been documented that the exogenous application of amino acids can increase endogenous amino acids in plant leaves [9,10]. Zhang et al. (2013) [11] reported that exogenously applying the amino acid-based biostimulant GreenNcrease improved turf quality and chlorophyll content when compared to ammonium sulfate. It also increased shoot density, leaf soluble protein content, and antioxidant superoxide dismutase activity relative to a control and ammonium sulfate under drought stress conditions. In a study on barley (*Hordeum vulgare* L.), the exogenous application of glutamine, glutamate, asparagine, or aspartic acid increased root concentrations of the applied amino acids and those of other amines and amides. The application of glutamine also increased the nitrate concentration of plant roots [10]. The application of tryptophan increased concentrations of the amino acids proline, lysine, histidine, alanine, and leucine in carrot (*Daucus carota* L.) cells [9]. Grass with better N metabolism may exhibit greater photosynthesis and carbohydrate production, resulting in greater root growth and tolerance to abiotic stress. In addition, tryptophan in the products may increase the endogenous production of auxin, which benefits root initiation and growth. Mertz et al. (2019) [12] reported that the application of a tryptophan-containing product or tryptophan plus urea at 24.5 kg N ha^{-1} every two weeks may improve leaf and root auxin contents, root biomass, and subsequent creeping bentgrass quality relative to applications of urea only. Zhang et al. (2009) [13] showed that the application of a tryptophan-dosed organic fertilizer enhanced endogenous levels of IAA and cytokinins, increased leaf antioxidant enzyme activity, and improved root growth in tall fescue under drought stress conditions. Therefore, several studies have provided evidence showing that exogenous amino acids can be readily absorbed and translocated by plant tissues and play an important role in improving turfgrass quality, growth, and physiological fitness, especially under abiotic stress.

Plant and animal-derived protein hydrolysates (PHs) are good sources of amino acids [14]. PHs are mixtures of amino acids, oligopeptides, and polypeptides, resulting from the partial hydrolysis of various protein sources. Various kinds of free amino acids have been identified in PH-based biostimulants. Glutamic acid, aspartic acid, glycine, and proline have been identified in various PHs [14]. Sun et al. (2024) [15] wrote a comprehensive review, concluding that amino acid biostimulants and PHs may increase chlorophyll and carotenoid contents and promoted the root area and length. This can increase mineral nutrients and water use efficiency as well as improve tolerance to stressful environments. However, few studies have been reported on the effects and the mode of action of amino acid biostimulants of both plant- and animal-derived sources on cool-season turfgrass species under drought and heat stress. The objectives of this study were to determine if foliar application of plant- and animal-derived amino acid biostimulants improve plant tolerance to heat and drought stress associated with nitrogen (N) use efficiency and root growth and to elucidate the mode of action of amino acid biostimulants for affecting drought and heat stress tolerance in creeping bentgrass.

2. Materials and Methods

Mature 'A4' creeping bentgrass plugs (10 cm diameter, 2 cm deep) from field plots (sand-based root zone) were transplanted into pots (15 cm in diameter on top and filled with fine sand mixed with 10% calcined clay) on 30 April 2024. The bentgrass was maintained at a height of 15 mm and fertilized at 1 g of N m^{-2} at transplanting and then 0.5 g of N m^{-2} biweekly before amino acid biostimulant treatments were initiated. A complete fertilizer (28N-8P-18K) with micronutrients was dissolved in water, and the solution was applied to a canopy and rinsed immediately after application. After about 5 weeks of non-stressed growth with optimum temperature, water, fertilizer, and light conditions, the grass was subjected to heat and drought stress treatment by placing the pots in a controlled-environment growth chamber (Environmental Growth Chamber, GC-30, Chagrin Falls, OH, USA) at 35 °C (day, 12 h)/25 °C (night) and with a light intensity of 400 µmol m^{-2} s^{-1}, a 12 h photoperiod, and 65% RH on 6 June 2024. Deficit irrigation was used to induce drought stress by watering the pots five times weekly to compensate for 45–55% of gravimetrically measured evapotranspiration (ET) loss [1]. These conditions simulate summer putting green stress. We evaluated 6 treatments, replicated 4 times. The treatment solutions listed below were applied with a CO_2-pressurred sprayer to the canopy uniformly biweekly, and the same amount of water solution of ammonium nitrate was applied to the control (treatment #1) on 6 June.

The 6 treatments (which provided 0.22 g m^{-2}) are listed as follows: (1) a water treated control; (2) Superbia (10-0-0) at 2.19 g m^{-2}; (3) Amino Pro V (5-0-0) at 4.39 g m^{-2}; (4) Siapton (10-0-0) at 2.19 g m^{-2}; (5) Benvireo (16-0-0) at 1.37 g N m^{-2}, and (6) Surety at (1.5-0.2-0.5) at 14.65 g m^{-2}. Superbia, supplied by Fertinagro Biotech Intl. S.L. (Portage, MI, USA), contains 10% N of animal-derived protein hydrolysates. Amino Pro V, supplied by Harrell's (Lakeland, FL, USA), is made of plant-derived protein hydrolysates with 5% N. Siapton, supplied by Isagro USA (Morrisville, NC, USA), is made of animal-derived protein hydrolysates with 10% N. Surety, supplied by AlgaeEnergy (Cumming, GA, USA), is made of micro algae with 1.5% N, 0.2% phosphorus, and 0.5% potassium.

All treatments were fertilized with the 28N-8P-18K complete fertilizer at 0.27 g N m^{-2} biweekly. The rate of regular N fertilization for treatment #2 to 6 was the same as treatment #1 (fertilized control). The amino acid products provided 0.22 g of N m^{-2} (=4 mg/pot) for treatment #2 to 6, and ammonium nitrate at the same N rate was applied to the control (treatment #1). The fertilizer was dissolved in water, and the solution was sprayed on the canopy and water immediately after application.

The stress period of the trial lasted 8 weeks and for a total of 4 treatment applications. We took the following measurements/data on day 0, 14, 28, 42, and 56. For leaf protein and superoxide dismutase (SOD) activity, fresh leaf samples were collected every two weeks and frozen with liquid and stored at -80 °C before lab analysis.

2.1. Measurements

2.1.1. Turf Quality

Turf quality was rated on a visual scale from 1 to 9, with 1 indicating completely dead or brown leaves, 6 representing minimum acceptability, and 9 indicting turgid and green leaves, with optimum canopy uniformity and density [1,16].

2.1.2. Photochemical Efficiency (PE)

The PE was measured biweekly with a chlorophyll fluorometer (Mini Pam II, photosynthetic analyzer, Heinz Walz Gmbh, Effeltrich, Germany) based on the Fv/Fm, which is the ratio of variable chlorophyll fluorescence (Fv) to maximum chlorophyll fluorescence (Fm). Leaves were dark-adapted for 30 min before each measurement, and three readings were collected from each plot, and the average was used for statistical analysis.

2.1.3. Leaf Chlorophyll Content

The chlorophyll content was measured with a CCM-300 chlorophyll meter (Opti-Sciences, Inc., Hudson, NH, USA) on the youngest fully developed leaf. Three readings were taken from each plot, and the average was used for statistical analysis.

2.1.4. Leaf Normalized Difference Vegetation Index (NDVI)

The NDVI was measured with the Geoscout X data logger (Holland Sci., Lincoln, NE, USA) and about 40 readings were collected from each pot, and the average was used for statistical analysis.

2.1.5. Leaf Protein and N Content

Leaf protein was extracted by using a 50 mM sodium phosphate buffer containing 1% and 0.2 mM of EDTA and analyzed using the Bradford method. The leaf N content was calculated by dividing the protein content by 4.43 [1].

2.1.6. Leaf Superoxide Dismutase (SOD) Activity and H_2O_2 Content

SOD activity was determined following the procedure described by Chang et al. (2016) [5]. The leaf H_2O_2 content was determined according to the method by Bernt and Bergmeyer (1974) [17], as described by Chang et al. (2016) [5]. Briefly, frozen leaves (200 mg) were homogenized in 1.5 mL of a 100 mM sodium phosphate buffer (pH 7) with 0.005% (*w*/*v*) o-dianisidine and 40 µg of peroxidase mL^{-1}. The mixture was incubated at 30 °C for 10 min, and 0.17 mL of 1 M perchloric acid was added. The absorbance was measured at 436 nm by using a spectrophotometer, as described previously. The H_2O_2 concentration in the leaf tissue was calculated by using the standard H_2O_2 curve.

2.1.7. Root Growth Characteristics and Viability

At the end of the trial, four root samples (1.9 cm in diameter and 15.24 cm deep) were collected from each plot and washed. The root length, root diameter, root surface area, and root volume were analyzed using WinRhizo technology. Briefly, after fine cleaning of each root sample, the sample from each plot was divided into multiple subsamples. Each subsample was scanned using the WinRhizo software (Version Pro, Regent Instruments, Inc., Quebec, QC, Canada), and all root morphological parameters were generated and analyzed [1]. The root dry weight was determined after the samples were dried at 70 °C for 72 h. Root viability was analyzed according to the method described by Zhang et al. (2022) [1].

2.1.8. Experimental Design and Statistical Analysis

A randomized block design was used with four replicates for each treatment. The data were analyzed with an analysis of variance according to the general linear model using SAS (version 9.4 for Window; SAS Institute, Cary, NC, USA, 2016). The six treatments were compared by using Fisher's protected least significant difference test at p = 0.05.

3. Results

3.1. Turf Quality

Foliar application of the five amino acid products improved turf quality, as measured from day 14 through day 56 after stress initiation (Table 1). As measured at the end of the trial (day 56), Superbia, Amino Pro V, and Surety treatments increased the turf quality rating by 23.8%, 19.6%, and 18.4%, respectively, when compared to the control. On average, all five products had better turf quality ratings relative to the control, with Superbia having the best turf quality among the treatments.

Table 1. Turf quality and photochemical efficiency (PE) responses to amino acid-based products in creeping bentgrass under heat and drought stress.

Treatment	**Rate**	**0**	**14**	**28**	**42**	**56**	
	(g m^{-2})	6 June	20 June	4 July	18 July	1 August	Ave.
				Turf quality (1–9, 9 = best)			
Control	0	7.5 a	6.75 c	6.48 c	6.25 c	5.60 d	6.53 d
Superbia	2.19	7.5 a	7.35 a	7.15 a	7.03 a	6.93 a	7.19 a
Amino Pro V	4.39	7.5 a	7.08 b	7.03 a	6.95 a	6.70 b	7.05 b
Siapton	2.19	7.5 a	7.33 a	6.75 b	6.58 b	6.45 d	6.92 c
Benvireo	1.37	7.5 a	7.35 a	6.68 b	6.85 a	6.53 cd	6.98 bc
Surety	14.65	7.5 a	7.18 ab	7.03 a	6.98 a	6.63 bc	7.06 b

Table 1. *Cont.*

Treatment	Rate	0	14	28	42	56	
		PE (Fv/Fm)					
		6 June	20 June	4 July	18 July	1 August	Ave.
Control	0	0.823 a	0.808 b	0.748 b	0.743 c	0.645 b	0.753 c
Superbia	2.19	0.828 a	0.826 a	0.823 a	0.808 a	0.781 a	0.813 a
Amino Pro V	4.39	0.826 a	0.815 ab	0.814 a	0.800 a	0.777 a	0.806 ab
Siapton	2.19	0.827 a	0.817 ab	0.811 a	0.790 ab	0.765 a	0.802 ab
Benvireo	1.37	0.834 a	0.821 a	0.770 ab	0.761 bc	0.754 a	0.788 b
Surety	14.65	0.838 a	0.823 a	0.796 ab	0.799 a	0.769 a	0.805 ab

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

3.2. Photochemical Efficiency (PE)

Foliar application of the five amino acid products improved the leaf PE relative to the control, as measured from day 14 through day 56 after stress initiation, except for Amino Prov and Siapton on day 14, Benvireo and Surety on day 28, and Benvireo on day 42 (Table 1). On average, all five products had greater PE values relative to the control, with Superbia having a greater PE value than Benvireo among the five products. On day 56, Superbia, Amino Pro V, Siapton, Benvireo, and Surety increased the PE by 21.1%, 20.5%, 18.6%, 16.9%, and 19.2%, respectively, when compared to the control.

3.3. Leaf Chlorophyll Content

Foliar application of the five amino acid products improved the leaf chlorophyll content, as measured from day 28 through day 56 after stress initiation, except for Siapton and Benvireo on day 28 and day 56 (Table 2). On day 56, Superbia, Amino Pro V, and Surety increased the leach chlorophyll content by 17.1%, 19.4%, and 14.0%, respectively, when compared to the control. On average, Superbia, Amino Pro V, and Surety had greater chlorophyll contents relative to the control.

Table 2. Leaf chlorophyll content and normalized difference vegetation index (NDVI) responses to amino acid-based products in creeping bentgrass under heat and drought stress.

Treatment	Rate	0	14	28	42	56	
	(g m^{-2})	6 June	20 June	4 July	18 July	1 August	Ave.
		Chl (mg g^{-1} FW)					
Control	0	606.8 a	519.0 a	475.5 b	433.0 b	392.5 c	485.4 b
Superbia	2.19	587.8 a	577.3 a	544.8 a	510.5 a	459.8 a	536.0 a
Amino Pro V	4.39	583.3 a	503.3 a	534.0 a	509.0 a	568.5 a	519.6 a
Siapton	2.19	594.3 a	548.8 a	511.5 ab	492.0 a	411.3 bc	511.6 ab
Benvireo	1.37	570.6 a	560.5 a	510.3 ab	494.5 a	430.5 abc	513.2 ab
Surety	14.65	596.5 a	550.8 a	526.0 ab	502.5 a	447.3 ab	524.6 a
		NDVI					
		6 June	20 June	4 July	18 July	1 August	Ave.
Control	0	0.855 a	0.854 a	0.815 b	0.798 c	0.722 d	0.809 b
Superbia	2.19	0.871 a	0.894 a	0.879 a	0.860 a	0.816 a	0.864 a
Amino Pro V	4.39	0.869 a	0.869 a	0.861 b	0.856 a	0.814 ab	0.854 a
Siapton	2.19	0.812 a	0.806 a	0.874 b	0.800 c	0.780 c	0.814 b
Benvireo	1.37	0.871 a	0.857 a	0.873 b	0.823 bc	0.791 c	0.843 ab
Surety	14.65	0.845 a	0.869 a	0.852 b	0.849 ab	0.796 bc	0.842 ab

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

3.4. NDVI

All five products improved the NDVI relative to the control, as measured on day 56. The Superbia treatment consistently improved the NDVI, as measured from day 28 through day 56 (Table 2). On day 56, Superbia, Amino Pro V, Siapton, Benvireo, and Surety increased the PE by 13.0%, 12.7%, 8.0%, 9.6%, and 10.2%, respectively, when compared to the control.

On average, Superbia and Amino Pro V improved the NDVI relative to the control.

3.5. Leaf Protein Content

The Superbia treatment improved the leaf protein content relative to the control, as measured from day 28 through day 56 (Table 3). On day 56, Superbia and Amino Pro V increased the leaf protein content by 13.0% relative to the control. On average, only Superbia increased the leaf protein content relative to the control.

Table 3. Leaf protein and nitrogen content responses to amino acid biostimulants in creeping bentgrass under heat and drought stress.

Treatment	**Rate**	**0**	**14**	**28**	**42**	**56**	
	(g m^{-2})	6 June	20 June	4 July	18 July	1 August	Ave.
				Leaf protein content (mg g^{-1} FW)			
Control	0	5.02 a	4.05 a	3.96 b	3.64 c	3.41 b	4.01 b
Superbia	2.19	4.84 a	4.87 a	4.84 a	4.17 a	3.87 a	4.56 a
Amino Pro V	4.39	4.77 a	4.63 a	4.52 ab	4.15 ab	3.87 a	4.39 ab
Siapton	2.19	4.77 a	4.70 a	4.28 ab	3.80 bc	3.62 ab	4.23 ab
Benvireo	1.37	5.00 a	4.45 a	4.20 ab	3.70 c	3.59 ab	4.19 ab
Surety	14.65	4.89 a	4.43 a	4.44 ab	3.97 abc	3.70 ab	4.29 ab
				Leaf N content (mg g^{-1} FW)			
		6 June	20 June	4 July	18 July	1 August	Ave.
Control	0	1.13 a	0.91 a	0.89 b	0.82 c	0.77 b	0.91 b
Superbia	2.19	1.09 a	1.10 a	1.09 a	0.94 a	0.87 a	1.02 a
Amino Pro V	4.39	1.08 a	1.04 a	1.02 ab	0.93 ab	0.87 a	0.99 ab
Siapton	2.19	1.08 a	1.06 a	0.97 ab	0.86 bc	0.82 ab	0.96 ab
Benvireo	1.37	1.13 a	1.01 a	0.95 ab	0.83 c	0.81 ab	0.95 ab
Surety	14.65	1.10 a	1.00 a	1.00 ab	0.90 abc	0.84 ab	0.97 ab

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

3.6. Leaf N Content

The Superbia treatment improved the leaf N content relative to the control, as measured from day 28 through day 56 (Table 3). On day 56, Superbia and Amino Pro V increased the leaf N content by 13.0% relative to the control. On average, only Superbia increased the leaf protein content relative to the control.

3.7. Leaf Antioxidant Enzyme SOD Activity

Foliar application of Superbia, Amino Pro V, Benvireo, and Surety improved the leaf SOD activity relative to the control, as measured from day 28 through day 56 after stress initiation (Table 4). On day 56, Superbia, Amino V, Siapton, Benvireo, and Surety increased the leach chlorophyll content by 11.1%, 9.3%, 5.1%, 5.0%, and 8.9%, respectively, when compared to the control. On average, Superbia, Amino Pro V, Benvireo, and Surety treatments had greater SOD activity than the control.

Table 4. Leaf superoxide dismutase (SOD) activity and hydrogen peroxide (H_2O_2) content responses to amino acid biostimulants in creeping bentgrass under heat and drought stress.

Treatment	**Rate**	**0**	**14**	**28**	**42**	**56**	
	(g m^{-2})	6 June	20 June	4 July	18 July	1 August	Ave.
				Leaf SOD activity (Unit g^{-1} FW)			
Control	0	887.3 a	852.4 a	805.0 b	786.4 b	772.3 d	817.0 c
Superbia	2.19	854.2 a	871.2 a	842.2 a	829.8 a	826.4 a	844.8 a
Amino Pro V	4.39	878.6 a	869.8 a	838.6 a	824.9 a	813.0 ab	845.0 a
Siapton	2.19	831.2 a	849.7 a	828.2 ab	823.7 a	781.8 c	822.9 bc
Benvireo	1.37	902.1 a	864.4 a	832.7 a	816.9 a	781.2 c	839.5 ab
Surety	14.65	875.6 a	865.1 a	836.1 a	820.0 a	810.2 b	841.4 a
				Leaf H_2O_2 content (ng g^{-1} FW)			
		6 June	20 June	4 July	18 July	1 August	Ave.
Control	0	22.1 a	27.6 a	42.6 a	48.8 a	56.4 a	39.5 a
Superbia	2.19	22.2 a	25.3 ab	30.1 c	35.0 c	47.0 d	31.9 d
Amino Pro V	4.39	22.1 a	23.8 b	31.2 c	35.7 c	46.8 d	31.9 d
Siapton	2.19	22.0 a	24.7 ab	36.9 b	45.3 b	52.1 bc	36.2 bc
Benvireo	1.37	22.5 a	26.3 ab	39.3 ab	45.9 b	52.6 b	37.3 b
Surety	14.65	22.7 a	26.7 ab	38.4 b	36.7 c	49.5 cd	34.8 c

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

3.8. Leaf H_2O_2 Content

Foliar application of Superbia, Amino Pro V, Siapton, and Surety reduced the H_2O_2 content relative to the control, as measured from day 28 through day 56 after stress initiation (Table 4). On day 56, Superbia, Amino V, Siapton, Benvireo, and Surety reduced the leaf H_2O_2 content by 16.7%, 17.0%, 7.6%, 6.7%, and 12.2%, respectively, when compared to the control. On average, all five treatments reduced the H_2O_2 content when compared to the control.

3.9. Root Growth Characteristics and Viability

Foliar application of Superbia and Amino Pro V improved the root biomass, length, surface area, volume, and viability when compared to the control (Table 5). Superbia and Amino Pro V increased the root biomass by 41.1% and 37.4%, respectively, relative to the control. Superbia and Amino Pro V also increased root viability by 21.0% and 19.2%, respectively, when compared to the control.

Table 5. Root length, surface area (SA), diameter, volume, biomass, and viability responses to amino acid-based products in creeping bentgrass under heat and drought stress.

Treatment	**Rate**	**Root Length**	**SA**	**Diam.**	**Vol.**	**Biomass**	**Viability**
	(g m^{-2})	(cm cm^{-3})	(cm^2 cm^{-3})	(mm)	(cm^3 dm^{-3})	(g pot^{-1})	A490 g^{-1} FW
Control	0	21.4 c	0.96 c	0.12 a	3.45 c	0.79 c	0.500 c
Superbia	2.19	30.2 a	1.44 a	0.15 a	5.55 a	1.18 a	0.605 a
Amino Pro V	4.39	29.4 ab	1.36 ab	0.13 a	4.64 ab	1.17 ab	0.596 ab
Siapton	2.19	25.1 abc	1.16 abc	0.15 a	4.28 bc	0.94 bc	0.522 abc
Benvireo	1.37	23.8 bc	1.16 bc	0.16 a	4.36 bc	0.93 bc	0.507 bc
Surety	14.65	27.1 abc	1.25 ab	0.15 a	4.63 ab	0.99 abc	0.507 bc

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

In summary, foliar application of the five amino acid products, especially Superbia and Amino Pro V, improved the turf quality, photochemical efficiency, physiological fitness with higher antioxidant enzyme SOD activity, and root growth in creeping bentgrass subjected to heat and drought stress conditions. Among the five products, Superbia exhibited the greatest positive effects on physiological fitness and root growth in creeping bentgrass under heat and drought stress conditions.

4. Discussion

Creeping bentgrass is one of the most important cool-season turfgrass species for golf courses and some sports fields. However, creeping bentgrass experiences a visual quality decline due to summer stress characterized by droughts and high temperatures during summer months in the U.S. transition zone and other regions with similar climates. In addition, turfgrass practitioners apply quick-release synthesized N fertilizers in order to maintain the quality of creeping bentgrass with a high density and dark green color. The high rate of N input may weaken root growth and also cause possible underground water pollution because of nitrate run off. Grass with an over growth of shoots and weakened root systems are susceptible to heat and drought stress, resulting in a quality decline during summer. The results of this study indicate that foliar application of the five amino acid biostimulants improved the turf quality, PE, chlorophyll content, and NDVI when compared to the control, with Superbia and Amino Pro V exhibiting more consistent positive effects on all the four measurements. The positive effects of the biostimulants on turf quality and PE were observed as early as two weeks after initial applications. Grass treated with amino acid biostimulants may maintain a high level of photosynthetic function (greater PE) and pigment (higher chlorophyll content) and dark green color (higher NDVI), resulting in better turf quality during drought and heat stress. Previous studies also showed that amino acids and PHs improved the leaf chlorophyll content and PE in creeping bentgrass [18]. The amino acids and peptides in the products could be absorbed by plant leaves and roots and quickly incorporated into the N metabolism with less metabolic energy requirements relative to other N forms, such as nitrate. Therefore, foliar application of the amino acid biostimulants could be used to alleviate or present turf quality declines due to heat and drought stress.

The results of this study indicate that the amino acid biostimulants Superbia and Amino Pro V improved the leaf protein and N content when compared to the control, as measured on day 42 and day 56. The other three products also tended to increase the leaf protein and N content, though not statistically significantly at a 5% probability level. Previous studies showed that the application of the amino acid biostimulant Greenacres increased the leaf protein content in creeping bentgrass [11]. The application of protein hydrolysis increased the leaf N content by 21.5% relative to a control in tomato plants [19]. A presumed mechanism involved in the stimulation of N assimilation in response to PHs is the increase in the activity of two key enzymes, nitrate reductase and glutamine synthetase [20]. A more vigorous root system may enhance the efficiency of nutrients and water uptake [21] and thus increase the leaf N content. The results of the present study suggest that the selected amino acid biostimulants may increase N uptake and assimilation, resulting in a higher leaf N content relative to the control, possibly due to an enhancement of the activities of key enzymes in N metabolism and also root growth and viability.

Plants require N to synthesize proteins, enzymes, chlorophylls, hormones, and other important macromolecules for growth and development. Under abiotic stress, antioxidant enzymes and other N-containing compounds play an important role for plants to adapt and survive stressful environments. The results of the present study indicate that the amino acid biostimulants enhanced the SOD activity of important antioxidant enzymes, suppressed ROS H_2O_2 levels, and protected chlorophyll and photosynthetic activity. This is consistent with the results obtained by Osman et al. (2021) [22] in pea (*Pisum sativum* L.) and Tallarita et al. (2023) [23] in tomato (*Solanum lycopersicum* L.) plants. The application of selected amino acids also improved antioxidant activity in creeping bentgrass [12] and tall fescue (*Festuca arundinacea* L.) [13]. This suggests that amino acid biostimulants could enhance drought and heat tolerance by improving the antioxidant capacity.

The results of this study indicate that the application of Superbia and Amino Pro V increased the root biomass, length, surface area volume, and viability relative to the control. The rest of the amino acid biostimulants, Siapton, Benvireo, and Surety, also tended to improve root growth and viability. Colla et al. (2014) [19] found that PH treatments increased the root dry weight, length, and surface area relative to a control in tomato plants. Lucini et al. (2015) [24] reported that plant-derived PH treatments increased the root biomass relative to a control in lettuce (*Lactuca sativa* L.). Mertz et al. (2017) [18] found that a tryptophan-containing biostimulant increased the root biomass in creeping bentgrass under drought stress. Root size and viability are closely associated with the plant uptake of nutrients and water, and a greater root system with higher viability could allow plants to effectively uptake nutrients and water and sustain stressful environments. In turfgrass management, turf practitioners may use a high rate of a quick-release N fertilizer to meet the needs for plant metabolism. However, a high rate of N fertilization may promote shoot growth and weaken root growth, especially during drought and heat stress conditions. The incorporation of amino acid biostimulants into the fertilization program may improve the balance of root and shoot growth and summer stress tolerance [25,26].

The results of this study show that both plant- and animal-derived amino acid biostimulants, when applied at the same N rates, exhibited similar beneficial effects on drought and heat stress tolerance of creeping bentgrass. Both the Superbia, animal-derived, and Amino Pro V, plant-derived, biostimulants contain 17 free amino acids and exhibit similar positive effects on turfgrass performance and physiological fitness in creeping bentgrass under heat and drought stress. This suggests that amino acid biostimulants could improve abiotic stress tolerance regardless of amino acid sources. In addition, amino acid biostimulants may enhance plant defensive metabolism and thus tolerance to abiotic stress, as they are not regular fertilizers which are solely associated with plant growth and/or mineral nutrition.

5. Conclusions

Foliar application of five amino acid biostimulants improved heat and drought stress tolerance and turf visual quality by enhancing N metabolism, antioxidant defense, and photosynthetic efficiency as well as improving root growth and viability. Among the five products, animal-derived Superbia and plant-derived Amino Pro V had greater beneficial effects on turf quality and physiological responses in creeping bentgrass under drought and heat stress conditions. Turf practitioners may improve summer stress tolerance and turf quality by incorporating amino acid biostimulants in the nutrient management program. Future research is needed to obtain the optimum combinations of amino acid biostimulants in field environments.

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