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'TifEagle' Bermudagrass' Response to Commercial Bio-stimulants

Research Article

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Abstract

Biostimulant use in the US is currently not regulated; therefore, much speculation exists as to product benefit claims. Three greenhouse trials were conducted with greens grade 'TifEagle' bermudagrass (Cynodon dactylon (L.) Pers. x C. transvaalensis Burtt-Davy) Clemson, SC during 2019 and 2020. Trial one simulated recovery from "divot" injury while trial two simulated recovery following sod harvest and measured grow-in rate using either pure sand or a native soil. Trial three was a rooting experiment performed in lysimeters to ascertain root growth following biostimulant use. Three commercial biostimulants (EarthMAX, Worm Power, and Hydra-Enrich 20) were used for all three studies, each applied individually or, applied with an 18-3-4 fertilizer (named: "Fert") at 0.2 lb N/1,000 ft2 (9.76 kg N/ha). Trial one measured normalized difference vegetation index (NDVI) ratings where EarthMAX (EM) (mean=0.576) had ~6.4%, ~3.3%, and 5.4% less recovery compared to untreated control, Hydra-Enrich 20 (HH) and Worm Power (WP), respectively. Similar results were provided from digitized photographs where EM+F (mean=0.586) treated turf recovery was ~7% less than fertilizer, and ~11% less than HH+F. Without additional fertilizer, EM (Mean=0.415) was significantly lower in coverage versus all other treatments, including untreated (~21%). From image analysis for trial two, the native soil plus fertilizer resulted in HH+F (Mean=0.375) being significantly less than EM+F (~17%), WP (~12%) and fertilizer (~13%). From NDVI ratings in trial three, EM (mean = 0.542) had significantly greater vegetative index than HH (~16%) and WP (~13%). Although, significant differences were technically recorded for the digital measurements, treatment differences were difficult to detect with the naked eye. Overall, positive results from ratings were only seen in turf subjected to extreme stress from the continual removal of roots every four weeks.

Introduction

A biostimulant can be defined as any substance or microorganism applied to plants to enhance nutrition efficiency, abiotic stress tolerance, or crop quality traits, regardless of its nutrient content [1]. 'TifEagle', a bermudagrass (Cynodon dactylon (L.) Pers. x C. transvaalensis Burtt-Davy) cultivar for high-quality golf course greens and for other areas requiring close mowing, is often managed in stressful situations [2]. Considerable research has been performed with biostimulants, and with prudent use, some can aid in stress tolerance, recovery rates, and increased photosynthetic rates, plus increase seed germination and root growth [3]. Biostimulants often contain cytokinin as the main ingredient, formulated from a natural source of seaweed kelp with the purpose of enhancing rooting. Other products may originate from synthetic sources, such as a synthetically produced cytokinin called benzyladenine [3].

Cytokinins are compounds inciting cytokinesis or cell division, in plant roots and shoots. Most cytokinins are from the base adenine, found in nucleic acids. Zeatin is a naturally occurring cytokinin in plants. Cytokinin production is primarily in leaf tips, then distributed through the vascular system [3].

Liu [4] investigated the effects of cytokinins on heat stress in creeping bentgrass (Agrostis stolonifera L. var palustris (Huds.)). Treatments consisting of low (0.01 and 0.1 mmols) cytokinin concentrations were indistinguishable from the untreated while higher cytokinin concentrations did aid bentgrass during heat stress. Cytokinin levels declined when plants were introduced to heat stress for all treatment levels except higher (1 and 10 mmols) cytokinin treatments [4]. This suggests during heat stress situations, cytokinin content declines which may contribute to shoot and root damage often seen. Untreated treatments had ~40 ng g-1 of cytokinin at 56 days after initial treatment (DAIT) while being maintained at a temperature of 20oC day and night. Untreated treatments at 35oC day and night (heat stress temperatures) at 56 DAIT had root cytokinin content near 0 ng g-1, whereas a single application of 10 mmols at 0 DAIT had root cytokinin levels ~10ng g-1. Samples with two applications of 10 mmols each at 0 and 14 DAIT had 20ng g -1 root cytokinin content at 56 DAIT [4]. Higher cytokinin treated plants not only had an increase in cytokinin levels, but also a decrease in heat stress injury [4]. This suggests cytokinins could improve turf quality through stressful situations.

Elliot [5], investigated seaweed-derived biostimulants on Tifdwarf bermudagrass. Significant differences in clipping yields in treated versus untreated were not observed. The scientists speculated biostimulants may be absorbed by plants more readily under stressful situations but noticed no difference in treatments under heavy rainfall conditions [5].

Zhang [6] investigated the impact of seaweed extract (SWE) and humic acid (HA) on antioxidant status of tall fescue (Lolium arundinaceum (Schreb.) Darbysh.) and creeping bentgrass subjected to drought stress. The authors noted both SWE and HA stimulated plant shoot and root growth of both grasses in high (non-stressed) and low (stressed) moisture levels. The authors hypothesized SWE and HA contributed to the growth increase; however, they also recognized in a drought induced situation, antioxidants and ascorbic acid increased which could contribute to plant growth. The authors suggested SWE and HA caused the plant antioxidants and ascorbic acid levels to increase [6].

Schmidt [7] stated biostimulants alleviate plant stress symptoms most efficiently when applied before stressful environmental conditions. The author stated when plants are grown in optimal conditions, oxygen accepts electrons from metabolic processes and produces water, sequestering cuticle damaging oxygen radicals from forming. However, under stressful conditions, the oxygen/electron-accepting process can be overwhelmed causing toxic oxygen variants (free radicals), to be produced. These radicals are also referred to as superoxide, singlet oxide, hydrogen peroxide, or hydroxyl radicals. Radicals are believed to cause chlorosis of leaves, significant plant cell organelle damage, plant cell termination, and eventual plant termination [7]. To combat oxygen radicals, plants naturally produce antioxidants which react positively with the various radicals and produce water or molecular oxygen sequestering the radicals [7]. During high stress times, plants produce higher levels of ethylene, which promote leaf senescence and conservation of energy. In addition, photosynthetic processes are halted, respiration is reduced to only the crown and roots, and naturally occurring cytokinins and auxins are reduced [7]. Biostimulants combat this by promoting higher cytokinin and auxin levels, which help plants to produce more antioxidants to combat stressful periods before senescing.

Hamza [8] tested if biostimulants helped initiate germination and growth of perennial ryegrass (Lolium perenne L.) seedlings. Biostimulants did provide higher rates of seedling establishment compared to the untreated. However, biostimulants plus fertilizer or fertilizer alone had higher color ratings than biostimulants alone. Root biomass %, root and shoot dry weight, and seedling shoot height had no significant differences. Authors noted plants used in the studies were grown in optimal conditions with adequate nutrients, water, and temperatures. Authors suggested plants produce sufficient hormones in optimal growing conditions and do not need supplemental hormones/biostimulants applied [8].

An alternative view regarding positive plant response during stressful conditions and biostimulant use is most likely from N in the biostimulants [9]. Biostimulants used in this research contained L-amino acids, which degraded to N, thus, was believed correlated with positive plant responses in induced heat stress situations. Authors recommended to facilities located in the southern US states or transition zone with cool-season turf, to apply biostimulants in spring and summer to avoid summer heat stress symptoms. However, by day 33 of their field study, overall turf health was very low due to an extended period of high temperatures. From this, authors noted extended periods of high temperatures could override heat stress tolerance biostimulants may provide [9].

Karnok [10] explained since biostimulants have yet to be regulated, the term “biostimulant” is extremely broad and can mean any product that stimulates life. Unfortunately, this infers many commercial products provide beneficial results due to the nitrogen or iron within a product and not from products defined as a “biostimulant” [10]. This is deceptive, as many biostimulant products containing seaweed extracts, humic acids, etc., have published benefits without the addition of fertilizers. Karnok [10] offers an explanation why biostimulants often have variable results depending on environmental cues. When environmental conditions become unfavorable for plant growth, many hormones cease in production. When sufficient hormone is added, this may cause a growth regulating effect. From this, it appears optimal timing for adding biostimulants is when plants have or soon will have a hormone imbalance. Additionally, different plants or those grown in varying micro-climates may react differently to similar biostimulants. Finally, it is suggested biostimulants should be viewed as a supplementary product, and not used to replace a research-proven best management practice [10].

As considerable variability has been reported in the literature regarding the benefits of using biostimulants, a major question remains whether these work as often advertised. Due to hundreds of products being marketed as biostimulants are commercially available and the majority have not been independently tested, the purpose of this research was to investigate whether three commercial biostimulants improve shoot lateral spread, root growth and if turfgrass growth after biostimulant applications varies between pure sand or native soil.

Materials and Methods

General Maintenance Practices

Three greenhouse trials were conducted in Clemson, SC during 2019 and repeated in 2020. Plugs 4.25-inch diameter, 1.5-inch depth (10.8 cm diameter, 3.8 cm depth) were extracted from a ‘TifEagle’ research golf green at Clemson University in Clemson, SC. All plugs were trimmed at 1.5-inch (3.8 cm) depth to remove all soil or roots, and then placed either in a USGA greens grade sand, or a native sandy loam soil. The USGA greens grade sand is classified as consisting of no more than 10% fine gravel (2-3.4 mm) and very coarse sand (1-2 mm), a minimum of 60% coarse sand (0.5-1 mm) and medium sand (0.25-0.5 mm), no more than 20% fine sand (0.15-0.25 mm), and less than or equal to 10% total fines (0.002-0.15 mm) [11]. The native sandy loam soil is classified as a native Toccoa soil (coarse-loamy, mixed, active, nonacid, thermic typic udifluvents) on a floodplain in Clemson, SC [12]. Greenhouse temperature was maintained between 70oF (21.1oC) and 85oF (29.4oC) during all trials. Pots were watered sufficiently to prevent visual drought stress. Treatments are listed in Table 1 and were in a factorial arrangement using a randomized complete block design with comparisons made between treatments, stratifying by environmental conditions. “Environmental conditions” refer to both fertilizer application and soil type (when applicable). EarthMAX (EM) (Harrell’s LLC., Lakeland, FL), Worm Power Turf (WP) (Aqua-Aid Solutions, Rocky Mount, NC), and Hydra-Enrich 20 (HH) (HydraSmart, Roanoke, VA) were applied individually as well as with an 18-3-4 liquid fertilizer [13] at 0.2 lb N/1,000 ft2 (9.76 kg N/ha) for comparison. An untreated control, referred to as “UTC” when no fertilizer was included, and a fertilizer treatment, referred to as “Fert” was also included. Products were applied at label rates at the time of study initiation and included EM at 4 oz/1,000 ft2 (12.74 L/ha), WP at 8 oz/1,000 ft2 (25.48 L/ha), and HH at 2.25 oz/1,000 ft2 (7.17 L/ha). All trials used the same products at the same rates and followed the same biweekly application timeline. Treatments were applied with a handheld spray bottle [14] calibrated to deliver 1 mL of mix per sample and watered in per label recommendations.

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Experimental Design and Data Collection

Destructive harvests were uniformly processed as followed. For roots, plugs were washed gently and thoroughly in a water bath, and any roots below the previously trimmed thatch layer (3.8 cm) were removed and collected. For shoots, any tissue above the crown was removed and collected. Thatch was considered to be the area between the soil surface and green shoot tissue, as described by Weaver et al. [15]. After harvest, samples were dried for 72 hours at 176 oF (80oC) in an Isotemp Lab Oven [16,17], weighed to determine dry weight, and then ashed in a Thermolyne Lab Furnace (Thermo Fisher Scientific Inc., Waltham, MA) at 977 oF (525oC) for 4 hours and then reweighed to determine true biomass weight as the difference between the two.

Roots Trial

The first trial investigated root growth over a 12-week period following biostimulant applications (Table 1). Biostimulant rates and timings followed those recommended on product labels at the time of study. Root growth was determined by extracting a 4.25-inch (10.8 cm) diameter, 1.5-inch (3.8 cm depth) plug from the 'TifEagle' green and placed in an (45.72 cm) deep lysimeter filled with pure USGA sand. To determine root regrowth following biostimulant use, at 4 and 8 WAIT, roots were removed and collected, then plugs were replaced into the lysimeters and allowed four weeks of regrowth. At 12 WAIT, thatch, shoots, and roots were destructively harvested for biomass weight. Weekly ratings included normalized difference vegetation index (NDVI) which quantified treatment effects on turfgrass color and quality. NDVI ratings were recorded weekly throughout the study using a Field Scout TCM 500 NDVI Turf Color Meter [18].

Recovery Trial

The second trial simulated divot recovery on a fairway/par-three tee by investigating how quickly damaged turf would recover following biostimulant applications. Plugs 4.25-inch (10.8 cm) diameter and 1.5-inch (3.8 cm) depth were extracted from the 'TifEagle' green in Clemson, SC. Plugs were placed in 6" (15.24 cm) pots and filled in with USGA greens grade sand. To simulate golf divot recovery, at 4 WAIT, 2-inch (5.08 cm, 3.8 cm depth) diameter samples were removed from the center of the established plugs and back filled with the same sand used to initially establish them (Figure 1). NDVI and photographs were recorded weekly to track recovery of damaged area. At studies end at 12 WAIT, destructive root, shoot and thatch harvest were conducted as previously described.

Table 1: Treatments and rates applied to 'TifEagle' bermudagrass samples in three trials during the summers of 2019 and 2020 in Clemson, SC.

Treatment	Rate (oz/1,000 ft2, unless otherwise specified)
Untreated Control	N/A
EarthMAX	4 (12.74 L/ha)
EarthMAX + Fertilizer†	4 + 0.2 lb N/1,000 ft2 (9.76 kg N/ha)
Worm Power	8 (25.48 L/ha)
Worm Power + Fertilizer	8 + 0.2 lb N/1,000 ft2
Hydra Enrich 20	2.25 (7.17 L/ha)
Hydra Enrich 20 + Fertilizer	2.25 + 0.2 lb N/1,000 ft2
Fertilizer	0.2 lb N/1,000 ft2 (9.76 kg N/ha)

† Gary's Green 18-3-4 + Fe liquid fertilizer.

Soils Trial

A third trial was designed to simulate regrowth of bare areas, such as strips (ribbons) remaining from freshly harvested sod using two different soil mediums. Soils were placed in a perforated soil flat 24" x 12-inch x 3" (60.96 cm x 30.48 cm x 7.62 cm) with a divider to separate them. Two 2-inch (5.08 cm) plugs were extracted from the 'TifEagle' green in Clemson, SC, and placed evenly spaced in each respective soil media for each flat. Four replications were used for each per treatment and per soil media. At 12 WAIT, destructive roots, shoots, and thatch harvest were taken, as described previously. Trials were photographed weekly to ascertain lateral spread rates by stolons, better described as canopy spread. Photographs were analyzed in batch load image processor (BLIP) (Clemson University, Clemson, SC). BLIP is an image analyzer program developed by Clemson Precision Agriculture for many different rating types. Pixels were analyzed for percent color, and then graded accordingly. Pixels graded as white or black were considered as bare plant areas. Pixels graded with red, yellow or blue light values were classified as plant pixels. Values used for analysis were the percentages of plant pixels to total pixels.

Statistics

Data were analyzed in R Version 3.6.1 GUI 1.70 El Capitan build (7684) (The R Foundation, Vienna, Austria) with significant effects and differences for above ground ratings (shoots, NDVI, BLIP) based on $\alpha = 0.05$. For organic matter and soil properties, alpha was set at 0.10 to better avoid Type II errors (concluding that mean responses to all treatments are equal when they are actually different) that might occur due to inherent variability in soil measurements [19,20]. Data were subject to analysis of variance with mean squares separated to assess treatment, environmental condition, and treatment by environmental condition. Data were analyzed for both fertilizer application and soil type (when applicable) with the set of soil and fertilizer conditions referred to as "environmental conditions". This decision was made prior to the initiation of analysis as it is common knowledge fertilizer causes a significant increase in plant growth compared with none. Similarly, a native soil typically provides more optimal growth conditions compared with pure sand. To separate treatments via the addition of fertilizer or soil growth media, the analysis seemed to have clearer outcomes as differences between similar environmental conditions showed true biostimulant effects. Significant interactions were not found pertaining to year; thus, only the main effect of year was included in analysis to offset any year over year differences in growth.

Differences in means are sometimes reported in percent difference in means which is calculated as follows. μ_x is the means value of treatment x averaged over replications and year. μ_y is the means value of treatment y averaged over replications and year.

$$(\mu_x-\mu_y)/(((\mu_x+\mu_y)/2))\times 100=Z$$

Where Z is the percent difference between mean x and mean y.

Results

Recovery Trial

Recovery trial had few significant differences between NDVI and BLIP ratings. NDVI differences only involved within treatments not including fertilizer (Table 2). As demonstrated in Figure 2, with no additional fertilizer, EM had a lower NDVI rating than HH (~3.25%), WP (~5.4%) and UTC (~6.4%). The largest difference was between EM and UTC (Figure 1), but when visually evaluating the two samples side-by-side, (Figure 1) practical differences were difficult to ascertain.

BLIP analysis also revealed similar trends as the recovery study. EM+F (mean = 0.586) was significantly lower than fertilizer (~7%), as was HH+F (~11%) (Figure 3). As indicated in Figure 4, without

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fertilizer, EM (mean = 0.415) was significantly lower compared to UTC (~21%), HH (~17%) and WP (~18%). Additional differences were not detected within the recovery trial. Again, although statistical differences were detected in percentage of green graded pixels to total pixels, visually, differences appeared minor (Figures 3 & Figure 4). Figure 2 indicates visual representation of stolon/rhizome recovery at 12 WAIT, again demonstrating visual differences being minor.

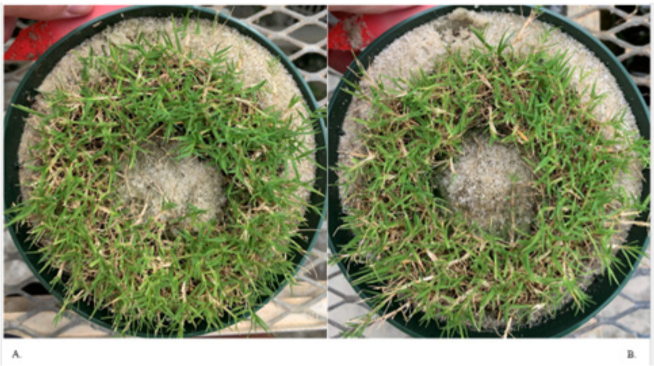

Figure 1: Representative photographs from 'TifEagle' bermudagrass recovery trials to determine differences 12 weeks following commercial biostimulant applications. A represents EarthMAX samples without additional fertilizer; B represents untreated.

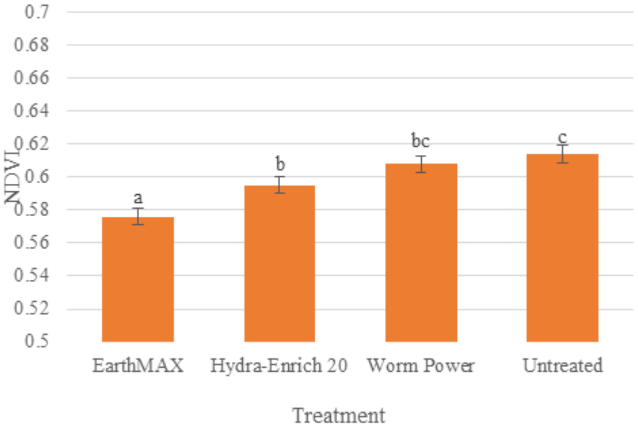

Figure 2: Least squares means of normalized difference vegetation index (NDVI) weekly ratings from 'TifEagle' bermudagrass recovery trial following applications of commercial biostimulants. Means were separated under the environmental condition of no additional fertilizer. Means were averaged over two years. Values used for analysis were rated on a 0-1 scale. EarthMAX has lower NDVI ratings compared to all other treatments and Hydra-Enrich 20 has lower NDVI ratings compared to untreated.

Roots Trial

In the roots trial, the only significant difference detected was within NDVI ratings under no additional fertilizer regimes. EM (mean = 0.542) was significantly greater than all other treatments by an average of 13% (Table 3). HH (mean = 0.469) and WP (mean =0.481) were significantly worse than EM (by 16 and 13%, respectively) (Figure 5).

Soils Trial

For the soils study, all biomass analyses were non-significant. BLIP had the only significance in samples grown in soil (Figure 6). BLIP analyses of treatments shows HH+F had less lateral growth than EM+F (~17%), WP+F (~12%) and fertilizer (~13%) (Table 4). Although certain treatments were significantly lower than others, visual representation of the difference in spreading between HH+F and fertilizer under the environmental condition of added fertilizer appeared to be minor (Figure 7). No other significant differences were detected within the soils trial.

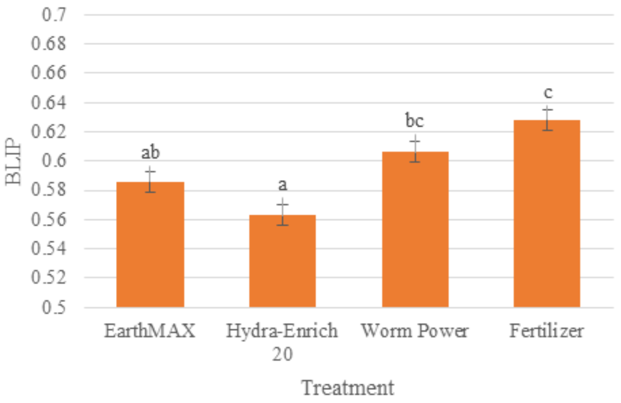

Figure 3: Least squares means of batch load image processor (BLIP) weekly ratings from 'TifEagle' bermudagrass recovery trial following applications of commercial biostimulants. Means were separated under the environmental condition of additional fertilizer. Means were averaged over two years. Values used for analysis were the percentages of plant pixels to total pixels. Hydra-Enrich 20 has lower recovery compared to Worm Power and fertilizer treatments while EarthMAX has lower recovery than fertilizer.

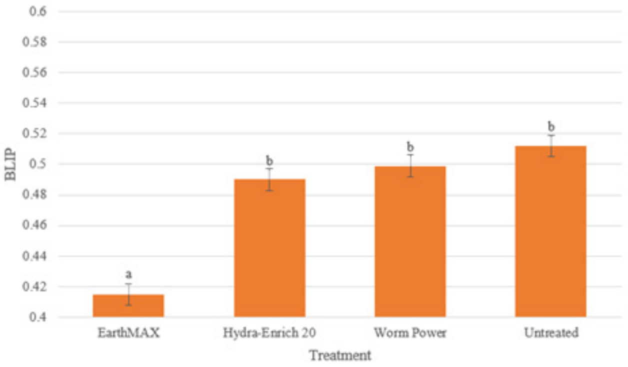

Figure 4: Least squares means of batch load image processor (BLIP) weekly ratings from 'TifEagle' bermudagrass recovery trial following applications of commercial biostimulants. Means were separated under the environmental condition of no additional fertilizer. Means were averaged over two years. Values used for analysis were the percentages of plant pixels to total pixels. EarthMAX has lower recovery compared to all other treatments.

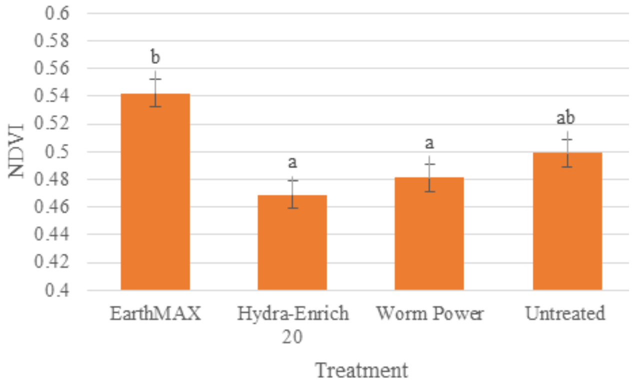

Figure 5: Least squares means of normalized difference vegetation index (NDVI) weekly ratings from 'TifEagle' bermudagrass roots trial following applications of commercial biostimulants. Means were separated under the environmental condition of no additional fertilizer. Means were averaged over two years. Values used for analysis were rated on a 0-1 scale. EarthMAX has higher NDVI ratings than Hydra-Enrich 20 and Worm Power.

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Table 2: Least squares means of normalized difference vegetation index (NDVI), biomass analysis of shoots, roots, and thatch, and batch load image processor (BLIP) for 'TifEagle' bermudagrass recovery trial following applications of commercial biostimulants. All ratings were taken weekly. Means were separated under the environmental condition of the addition or withholding of fertilizer. Means were averaged over two years. Means followed by the same letter code indicate that the treatment means were not significantly different at the level α=0.05 for above ground ratings and at α=0.10 for below ground ratings. Note the letter code convention applies only within environmental conditions and rating type. If column begins with "ns" then all means are equal at α=0.05 for above ground ratings and at α=0.10 for below ground ratings.

Treatment†, ‡	**NDVI§**	**Biomass Analysis (g)**			**BLIP (%)**
Additional Fertilizer		Roots	Shoots	Thatch	
EarthMAX	0.677ns	1.47ns	4.04ns	15.8ns	0.586a
Hydra-Enrich 20	0.664	1.59	4.01	16.3	0.563ab
Worm Power	0.668	1.63	3.24	16.3	0.606bc
Fert	0.67	1.5	3.75	15.5	0.628c
No Additional Fertilizer					
EarthMAX	0.576a	1.17ns	2.69ns	14.9ns	0.415a
Hydra-Enrich 20	0.595b	1.39	2.89	15.7	0.490b
Worm Power	0.608bc	1.29	2.52	14.1	0.499b
Untreated (UTC)	0.614c	1.49	2.53	15.2	0.512b

†EM = EarthMAX at 4 oz/1,000 ft2 (12.74 L/ha), WP = Worm Power at 8 oz/1,000 ft2 (25.48 L/ha), and HH = Hydra Enrich 20 at 2.25 oz/1,000 ft2 (7.17 L/ha), UTC = Untreated (no biostimulant) Fert= Fertilizer (Gary's Green 18-3-4) at 0.2 lb N/1,000 ft2 (9.76 kg N/ha).

‡All treatments were applied biweekly.

§ ¬Rating types.

Table 3: Means of normalized difference vegetation index (NDVI), biomass analysis of shoots, roots (4, 8, and 12 weeks after initial treatment (WAIT)), and thatch taken for 'TifEagle' bermudagrass roots trial following applications of commercial biostimulants. All ratings were taken weekly. Means were separated under the environmental condition of the addition or withholding of fertilizer. Means were averaged over two years. Means followed by the same letter code indicate treatment means were not significantly different at α=0.05 for above ground ratings and at α=0.10 for below ground ratings. Note the letter code convention applies only within environmental conditions and rating type. If column begins with "ns" then all means are equal at α=0.05 for above ground ratings and at α=0.10 for below ground ratings.

Treatment†, ‡	**NDVI§**	**Biomass Analysis (g)**				
Additional Fertilizer			Roots		Shoots	Thatch
-	-	4 WAIT	8 WAIT	12 WAIT		
EarthMAX	0.534ns	0.055ns	0.107ns	0.091ns	1.020ns	19.3ns
Hydra-Enrich 20	0.537	0.095	0.122	0.089	0.734	18.2
Worm Power	0.543	0.11	0.134	0.114	0.712	17.6
Untreated Fert	0.545	0.1	0.148	0.099	0.854	17.9
No Additional Fertilizer						
EarthMAX	0.542b	0.110ns	0.096ns	0.049ns	0.826ns	18.5ns
Hydra-Enrich 20	0.469a	0.055	0.105	0.035	0.392	16.7
Worm Power	0.481a	0.075	0.11	0.038	0.282	18.3
Untreated (UTC)	0.499ab	0.099	0.096	0.081	0.464	17.6

†EM = EarthMAX at 4 oz/1,000 ft2 (12.74 L/ha), WP = Worm Power at 8 oz/1,000 ft2 (25.48 L/ha), and HH = Hydra Enrich 20 at 2.25 oz/1,000 ft2 (7.17 L/ha), UTC = Untreated (no biostimulant) Fert= Fertilizer (Gary's Green 18-3-4) at 0.2 lb N/1,000 ft2 (9.76 kg N/ha).

‡All treatments were applied biweekly.

§ ¬Rating types.

Table 4: Means of batch load image processor (BLIP), and biomass analysis of shoots, roots, and thatch taken for 'TifEagle' bermudagrass soils trial following applications of commercial biostimulants. All ratings were taken weekly. Means were separated under the environmental condition of the addition or withholding of fertilizer, as well as being grown in sand or native Toccoa sandy-loam soil (soil). Means were averaged over two years. Means followed by the same letter code indicate treatment means were not significantly different at α=0.05 for above ground ratings and at α=0.10 for below ground ratings. Note the letter code convention applies only within environmental conditions and rating type. If column begins with "ns" then all means are equal at α=0.05 for above ground ratings and at α=0.10 for below ground ratings.

Treatment†, ‡ and Soil Type	**BLIP (%)§**	**Biomass Analysis (g)**		
Sand	Roots		Shoots	Thatch
Additional Fertilizer				
EarthMAX	0.2134ns	1.25ns	4.18ns	12.47ns
Hydra-Enrich 20	0.2329	1.314	4.64	12.29
Worm Power	0.2088	0.966	4.14	11.47
Untreated Fert	0.2008	1.038	3.91	12.54
No Additional Fertilizer				
EarthMAX	0.1079ns	0.456ns	1.48ns	11.02ns
Hydra-Enrich 20	0.1169	0.449	1.49	11.39
Worm Power	0.1058	0.401	1.31	11.24
Untreated (UTC)	0.0906	0.339	1.13	11.13
Soil				
Additional Fertilizer				
EarthMAX	0.4462b	2.663ns	10.10ns	10.19ns
Hydra-Enrich 20	0.3749a	1.907	8.64	11.19
Worm Power	0.4239b	2.768	10.08	10.43
Untreated Fert	0.4252b	2.761	10.22	10.3
No Additional Fertilizer				
EarthMAX	0.3675ns	2.513ns	7.74ns	10.11ns
Hydra-Enrich 20	0.3845	2.014	8.4	10.04
Worm Power	0.3371	1.896	6.84	9.65
Untreated (UTC)	0.3806	2.509	9.57	10.27

†EM = EarthMAX at 4 oz/1,000 ft2 (12.74 L/ha), WP = Worm Power at 8 oz/1,000 ft2 (25.48 L/ha), and HH = Hydra Enrich 20 at 2.25 oz/1,000 ft2 (7.17 L/ha), UTC = Untreated (no biostimulant) Fert= Fertilizer (Gary's Green 18-3-4) at 0.2 lb N/1,000 ft2 (9.76 kg N/ha).

‡All treatments were applied biweekly.

§Rating types.

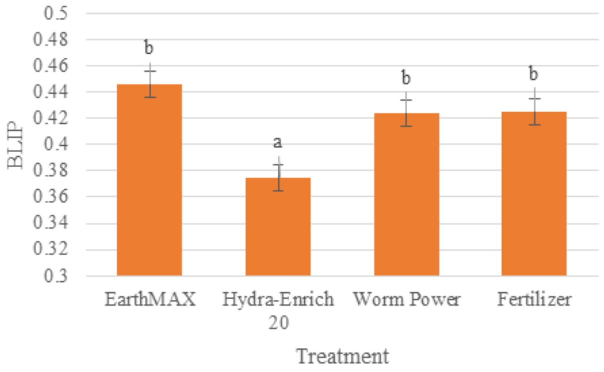

Figure 6: Least squares means of batch load image processor (BLIP) from 'TifEagle' bermudagrass soils trial following applications of commercial biostimulants. Photographs were taken weekly. Means were separated under the environmental condition of the addition of fertilizer, as well as being grown in native Toccoa sandy-loam soil. Means were averaged over two years. Values used for analysis were the percentages of plant pixels to total pixels. Hydra-Enrich 20 has lower spreading rates than all other treatments.

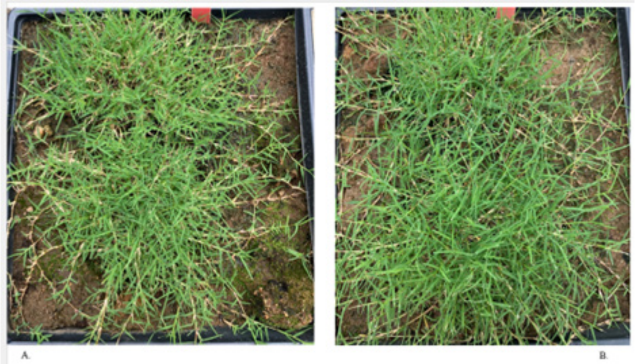

Figure 7: Representative photographs from 'TifEagle' bermudagrass soil trials to determine differences 12 weeks following commercial biostimulant applications. A represents Hydra-Enrich 20 samples with additional fertilizer, grown in native Toccoa soil; B represents fertilized saples grown in native Toccoa soil.

Discussion

While few significant differences occurred between treatments, those noted agreed with previous literature. The recovery and soil trials were grown in optimal water and temperature conditions. As Karnok (2000) mentioned, plants grown in stressful conditions often have a more efficient uptake and utilization of biostimulants versus plants exposed to stressful conditions where natural plant hormone production is halted. In this study, significantly negative responses were seen from EM treatments not including fertilizer. This may be due to growth regulating effect from additional plant hormones being added to a plant with normal plant hormone levels [10].

Within the soils trial; however, negative plant growth also happened with biostimulant treatments plus additional fertilizer. This is also likely as these plants were not stressed and continued to have ample amounts of plant hormones being naturally produced, so the addition of biostimulants caused a negative growth regulating effect [10]. This research emphasizes the necessity of researching each biostimulant product prior to application, especially under the environmental conditions expected.

The roots trial also agrees with [6,10] research. Roots in this study were destructively harvested every four weeks throughout the trial. Severing roots every four weeks and then placing plugs back in pure sand in the heat of the summer induced plant stress from constantly regenerating new roots. This scenario was the only one throughout all three trials where a biostimulant had a significantly greater response than the untreated. This reinforces the theory stressed plants are likely to produce a positive response from exogenously applied biostimulants versus plants grown in optimal conditions [6,10].

Although the NDVI means showed positive results, by the 12th week of the root trials, any plugs with remaining green tissue still showed significant injury, presumably from heat/abiotic stress of removing the roots every four weeks. This aligns with previous research where biostimulants applied prior to severe heat stress may alleviate plant stress symptoms for a short period of time; however, additional biostimulant use may amplify plants response to the stress [9].

Conclusion

This research agrees with previous research that indiscriminate biostimulants use may negatively affect the growth of turfgrasses. Two out of three greenhouse trials indicated EarthMAX and Hydra Enrich 20 produced lower rating values than the untreated (Tables 2 - 4). However, even though positive and negative impacts were detected, these were quite small as indicated by Figure 2 & Figure 7. Positive results from biostimulant use were only recorded for the roots trial where plants were subjected to extreme stressful conditions by the removal of roots every four weeks. However, stress-relief provided by biostimulants were undetectable by the 12th eek. Future research should include different turfgrass types and biostimulant rates to determine efficacy of biostimulant for stress alleviation; and, to determine how biostimulants affect established microbial communities. A biostimulant product that increases turfgrass growth and color, alleviates stress, increases beneficial microbial communities and provides overall plant health would be beneficial to highly maintained turfgrass stands.

Core Ideas

1. Biostimulants are not regulated and encompass many different types of active ingredients.
2. Published research on biostimulants is limited with variable results.
3. In these studies, significant differences were recorded; however, were difficult to distinguish with the naked eye.

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