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Evaluation of creeping bentgrass (*Agrostis stolonifera* L.) responses to an amino acid containing co-product

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Evaluation of creeping bentgrass (*Agrostis stolonifera* L.) responses to an amino acid containing co-product

by

Isaac Mertz

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Major: Horticulture

Program of Study Committee:
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Ames, Iowa

2015

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DEDICATION

I would like to dedicate this thesis to my mother Christine, my father Thomas, as well as April Gunderson and John Blattner, without whose unconditional moral and financial support, I would not have been able to complete this work. I would also like to dedicate this thesis to my siblings Harris, Abbey, and Bennett, who have always been formative role models throughout my life.

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ABSTRACT

Tryptophan, an essential amino acid that acts as a building block in protein synthesis, is a biochemical precursor for serotonin, niacin, and auxin in most organisms. When soil moisture is limited, applying biosolids boosted with auxin from tryptophan may increase root production and endogenous hormone levels that can result in plant growth regulation. Tryptophan is produced industrially, which results in a significant amount of byproducts. Tryptophan byproduct (TRP-B) is considered a waste product, but its amino acid and nutrient content make it a possible growth promoter for turfgrasses. The objective of this research was to determine whether applications of TRP-B improve ‘Penn A-4’ creeping bentgrass (*Agrostis stolonifera* L.) performance more than applications of pure tryptophan and/or urea.

Creeping bentgrass plugs taken from sand-based greens at both Virginia Polytechnic Institute and State University, and Iowa State University, were transplanted into pots and allowed to re-establish in growth chambers before being treated. Treatments included TRP-B, urea, and pure tryptophan + urea applied every 14 days at three different rates. Application rates were based on the amount of nitrogen (N) applied (2.45, 12.23, and 24.46 kg N ha⁻¹). At trial’s end (42 days), plant parts were harvested and used for analysis. On average, TRP-B treatments increased leaf total free amino acid contents by 6.2%, rooting biomass by 9.3%, leaf indole-acetic-acid (IAA) concentration by 20.2%, and root IAA concentration by 145.2%, compared to urea only treatments. Tryptophan + urea treatments increased leaf total free amino acid contents by 5%, rooting biomass by 8.3%, leaf IAA concentrations by 32.6%, and root IAA concentrations by 213% on

average, compared to urea only treatments. At 24.46 kg N ha⁻¹, TRP-B increased root biomass by 18.2% and pure tryptophan + urea produced a 16.3% increase compared to urea only. According to the results, creeping bentgrass treated with TRP-B can result in increased performance, and that response is rate-dependent.

CHAPTER 1. GENERAL INTRODUCTION

Background

For golf course superintendents and turfgrass managers, drought and water conservation have become an increasing problem in recent years. For turfgrass to survive and thrive, two things are required. Firstly, there must be sunlight, and secondly, there must be water. At the beginning of the 2015 calendar year, the city of San Diego, CA made history by imposing mandatory water use restrictions on its residents. When water restrictions such as these are enforced, water use on turfgrass is often one of the first categories to be criticized, reduced, and/or cut. According to the Golf Course Superintendents Association of America, from 2003-2005, the average water use for golf course irrigation in the U.S. was approximately 2.08 billion gallons of water per day (Throssell et al., 2009). This number may seem large, but when estimations from the U.S. Geological Survey are also included, which approximates that 408 billion gallons of water per day are withdrawn from underground aquifers, U.S. golf course irrigation only accounts for 0.5% of total daily water usage (Hutson et al., 2004).

While the percentage of total water used by turfgrass irrigation is miniscule in comparison to total water usage, it is more important now than ever for turfgrass managers to be sure they are using this precious resource as efficiently and responsibly as possible. As drought conditions continue to plague the turfgrass community and grow in importance, research directed towards improving the water use efficiency (WUE) and drought tolerance of high value turfgrasses such as creeping bentgrass (*Agrostis stolonifera* L.) is increasing. Historically, when talking about physiological issues such as these, approaches towards correcting or addressing them often include research at the

genetic level. Through the use of cultivar screenings and cross selections, turfgrass breeders have been able to make some progress towards improving turfgrass performance under heat and drought stressed conditions (Huang et al., 2015). The problem with this type of research is that it can take extensive time and resources before a commercial cultivar is produced.

Recently, there has been an increase in research directed towards the use of biologically active substances and their effect on turfgrass performance under drought conditions. Biologically active substances (BAS) is the term used to describe materials other than fertilizer, that when applied to the plant in small concentrations, have the ability to affect plant biochemistry and influence plant growth. While the materials or active substances in these solutions vary drastically from product to product, these solutions are usually grouped into a category known as biostimulants. Previous research has shown that under drought and heat stressed conditions, hormone levels within the plant, and the soil around it, can become unbalanced (Huang and Fry, 1999; Xu and Huang, 2000). This lack of balance between plant hormone levels has been shown to be the cause behind the decline of turfgrass performance under these conditions (Huang and Xu, 2007).

The idea behind using biostimulants to improve turfgrass performance under drought conditions is directed towards addressing this hormonal imbalance. Applications of biostimulants are thought to improve turfgrass performance in one of two ways. Either these products are supplying the plant with these hormones directly, or the substances in them are in turn being taken up by soil bacteria, and these bacteria are supplying the plant with hormones indirectly. The primary objective of the present study was to investigate

and compare the responses of creeping bentgrass treated with a tryptophan-containing byproduct (TRP-B) with those of urea only, and pure tryptophan plus urea, at equal levels of nitrogen. The secondary objective of the present study was to identify a proper rate of TRP-B that a golf course superintendent would use on creeping bentgrass putting greens.

Thesis Organization

This thesis has been constructed using the journal paper format. Chapter 1 contains a general introduction to the thesis. Chapter 2 includes a review of literature and the application of biologically active substances relevant to the present study. Chapter 3 is the paper that will be submitted to *Crop Science*, for which Isaac Mertz served as the primary author, and also the primary researcher at the Iowa State University location. Chapter 4 contains additional results from a preliminary trial. Chapter 5 is comprised of the general conclusions from the journal paper and the preliminary study. References for each chapter's contents are given at the end of each individual chapter. The final remaining section of this paper contains the acknowledgements, and can be found on the concluding page of the thesis.

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CHAPTER 2. LITERATURE REVIEW

Creeping bentgrass (*Agrostis stolonifera* L.) is a cool-season turfgrass species that is predominantly found in high-value sporting environments such as golf course putting greens. For those that are unfamiliar with the game of golf, the putting green is the area of the hole where the cup and flagstick are located, with the goal of every hole being to hit the ball into the cup using the least amount of strokes as possible. The difference between the way the game is played away from, and on the green, is that when hitting into the green, most of the shots will fly through the air and then land softly on the ground. Putting, however, is one of the few times during a round of golf where it is desirable to leave the ball on the surface, and have it roll towards the cup. This reason alone is why the majority of time and resources put into maintaining a golf course are directed towards the putting greens.

Historically, the putter and short game clubs that get used around a putting green complex are referred to as the scoring clubs. It is with these clubs that the majority of strokes during a round are taken with. Having a dense, uniform, and consistent putting surface is ideal. When this fails to occur, the difficulty of maneuvering the golf ball into the cup increases. It is at this point that the membership, and a golf course superintendent's superiors may begin to complain. As a turfgrass manager, it is important to make sure you are providing an optimal environment and using best management practices to ensure you are giving your turf a chance to succeed.

As previously mentioned, creeping bentgrass is a cool-season turfgrass. Here in the United States (U.S.), creeping bentgrass prefers the cool-moist weather that occurs

during the spring and fall in the upper mid-west and northwestern states. In contrast to creeping bentgrass, bermudagrass (*Cynodon dactylon* L.) is a warm-season turfgrass species that prefers the warmer, drier weather of the southern states. Where creeping bentgrass is often maintained as low as 0.32 cm, bermudagrass must be maintained at a higher height of cut (0.38 cm). To the average person this difference may seem insignificant, but when allowed to view the two side-by-side, it is easy to distinguish the difference. People often use the comparison of linoleum to shag carpet when talking about the differences between putting on a bentgrass and bermudagrass green.

Because of this, premium golf courses in the southern states switching from bermudagrass to bentgrass greens has become an increasing trend. While the golfers of these courses may appreciate the new changes, turfgrass managers are able to see the underlying implications. If we are taking a plant that thrives in cool, wet weather, and placing it in an area that is anything but that, are we ultimately putting that plant in a place where it can succeed? As the use of creeping bentgrass continues to expand into geographic regions it is not well adapted to, the amount of stress put on the plant increases. As temperatures increase during the summer months, plant vigor and productivity can begin to decline. This occurrence is often referred to as summer bentgrass decline (Dernoeden, 2000). Symptoms of summer bentgrass decline include root dieback, excessive leaf senescence, and thinning of the turf canopy, all of which can directly decrease the quality of the playing surface.

Where the initial goals and thoughts behind the conversion were to have a dense uniform surface that was superior to the existing bermudagrass, the end result is often problematic, and additional resources, plus money, must be spent in order for the

bentgrass to perform on an acceptable level. Like all plants, turfgrass requires water to survive. Plants take up water from the soil, and through the use of adhesive and cohesive forces, are able to transport that water up to the leaves where photosynthesis takes place. As the plant leaves exchange this water with the atmosphere by evaporation, carbon dioxide moves into the leaves to be used during photosynthesis. As this water evaporates, there is a cooling effect. This is how plants attempt to cope with extreme temperatures and stresses.

For cool-season turfgrasses such as creeping bentgrass, the optimum soil temperature is in the range of 15 to 20°C (Shearman, 1971). When plants are exposed to temperatures that fall below or above this optimum range, the growth rate of the plant decreases. The bigger the deviation from this optimum, the greater chance there is that plant death will occur (Shearman, 1971). Research has shown that as soil temperature increases beyond that optimum level, cytokinin levels can become imbalanced, leading to a reduction of root and shoot growth (Huang and Fry, 1999; Xu and Huang, 2000). Cytokinin, a phytohormone involved in cell division and suppression of leaf senescence, is primarily synthesized in plant roots. Cytokinin produced in the roots is translocated to the shoots, and together with auxin affects the shoot growth characteristics of the plant. In plants subjected to different forms of stress, Merewitz et al. (2010a, 2010b, 2011) found that the conservation of cytokinins within the plant was a critical factor influencing plant responses to specifically environmental stress. This idea was further demonstrated when improvements in creeping bentgrass plant drought tolerance were shown by increasing endogenous plant cytokinin content through transformation with an adenine isopentyl-transferase (IPT) gene (Merewitz et al., 2012). Huang and Xu (2007) were also able to

show a positive correlation between cytokinin levels and creeping bentgrass performance under heat stress.

The problem here is that when soil temperatures are above the optimum growth range, lowering them back to optimal levels is extremely difficult. Plants may use water as a way to deal with extreme temperature stresses, but as the soil temperatures approach 30°C, stomata of cool-season plants begin to close. When this occurs, the photosynthesis process is stopped, and the plant loses the ability to cool itself down. At this point, even if excess water is applied, the plant is not able to use it for either photosynthesis, or evaporative cooling.

Historically, turfgrass managers have used a number of approaches to combat this issue. Syringing involves a light application of irrigation, and is often used during the daytime when the air temperatures have peaked. The idea here is to apply just enough irrigation water to cover the leaf surface, and as that water evaporates, there is a slight cooling effect much like when plants are able to take up water and photosynthesize. It should be noted however, that this is considered only as a temporary solution. As soil temperatures decline and the day progresses, the hope is that plant stomata will begin to open and photosynthesis will start up again. Subjecting turfgrass to this type of maintenance regime over extended periods of time is not only detrimental to plant health, it can also put additional strain on a maintenance budget. With prolonged use, syringing can be viewed as an inefficient use of water resources, since water applied to the surface readily evaporates, and rarely infiltrates the soil profile.

Somewhat more permanent solutions involve the use of large fans, and in extreme cases, a sub-air cooling system below each putting green complex. Some superintendents

use fans from early morning to evening, others run fans only during the early morning to mid-morning when dew and surface moisture are greatest (Huang, 2002). A decrease in canopy temperatures (2.2-5.6°C) during peak periods of sunshine and air temperature (11am to 2pm), and a decrease in soil temperatures at a 10-cm depth of 1.1 to 3.3°C have been reported due to the use of fans (Taylor, 1995). Prior research has also shown that pulling air through the green for several hours, through the use of a sub-air system, decreased the temperature by 1.7 to 2.2°C at a 5-cm soil depth (Dodd et al., 1999).

While these may seem like practical solutions to this issue, these management practices must be used for extended periods of time, and continuously reducing soil temperatures over those long periods can become costly. Additionally, these extended periods are occurring during the day when play takes place, and implementing these strategies are not always practical for that reason (Huang, 2002).

The strategies previously mentioned have one thing in common, they are all types of management strategies or cultural practices. An alternative to this involves creeping bentgrass genetics and physiology. As the importance of water conservation continues to increase, turfgrass breeders are starting to direct their concentrations towards producing and developing cultivars that are more drought tolerant, and more efficient users of water (Huang et al., 2015). Plant breeders utilize genetic analysis and the manipulation of plant traits in attempts to develop plant lines better suited for human purposes, or the application they are intended to be used for. Having a plant that is adapted to, or suitable for, the conditions it will be used in is always going to be the best choice. This all comes back to the choice to remove the existing acclimated vegetation (bermudagrass for example) and replacing it. Unfortunately, commercially available cultivars can take a lot

of time and money to produce. As a result, there has been an increasing trend towards using plant growth regulating (PGR) compounds to control and manipulate the plant genetics, and physiology, through exogenous application.

Commercially available plant growth regulators can be separated into two categories based on the way they are intended to affect plant growth. Firstly, there are those chemicals/compounds that are intended to minimize or slow down plant growth. These are often referred to as plant growth suppressants, and historically have been used as a way to control annual bluegrass (*Poa annua* L.) in creeping bentgrass putting greens (Johnson and Murphy, 1996). Secondly, there are those chemicals/compounds that are intended to promote plant growth, and these are often referred to as biostimulants. Compared to fertilizer products, which only contain mineral nutrition, biostimulants may contain plant hormones, organic acids, minerals, or a combination thereof (Schmidt, 1999). The presence of biologically active substances (BAS) such as humic acids, vitamins, and amino acids in certain biostimulants or biosolids have been shown to increase the plant's ability to with-stand environmental stresses and/or positively affect crop growth and quality (Subler et al., 1998).

From an agricultural perspective, it has long been known that the addition of organic materials to the soil can enhance the biological, physical, and chemical properties of the soil. Typically, yield increases from the addition of organic materials into the soil are attributed to increased nutrient availability (Akrivos et al., 2000; Boquet et al., 1999; Bugbee, 2002; Tester, 1989) and/or improved soil physical properties (Klock-Moore, 2000). However, the role of BAS within those organic materials, and their effect on plant growth enhancement, has not been well studied until recently. It is thought that the

presence of BAS may enhance crop production by directly providing PGR's to the plant directly, or through the stimulation of soil microbes responsible for supplying substrates and hormones.

In an early effort, Fletcher and Hofstra (1988) found that an application of biosolids, or other organic amendments, can result in a shift in the balance of plant hormones, which may account for observed plant growth regulating activity following application. This finding was later confirmed by Zhang and Ervin (2004), who observed increased concentrations of some plant hormones (cytokinins, indole-acetic-acid), and decreased concentrations of others (gibberellins, ethylene) following foliar applications of cytokinin-containing seaweed and humic acid extracts. It was further demonstrated that biosolids boosted with auxin, in the form of tryptophan, may enhance plant defense chemical responses during limited soil moisture conditions (Zhang et al., 2007).

Tryptophan is an essential amino acid that acts as a building block in protein synthesis, and is a biochemical precursor for serotonin, niacin, and auxin in most organisms. By adding tryptophan to their experimental biosolids, Zhang and Ervin were able to increase the amount of auxin in their products, and the amount getting to the plant (Zhang et al., 2007). As previously mentioned, it is thought that exogenous applications of these materials are either supplying the plant directly with these compounds, or stimulating the soil microbes that are subsequently responsible for supplying them back to the plant. By using nitrogen isotopes, Stiegler et al. (2013) demonstrated that creeping bentgrass is capable of foliarly absorbing amino acid nitrogen compounds, and the nitrogen uptake from these applications were equivalent to an inorganic nitrogen application commonly used in the industry today.

While research has been done to evaluate the effect of biosolids containing tryptophan, it is to the best of our knowledge that this is the first study to evaluate plant responses to a tryptophan-containing byproduct (TRP-B). The tryptophan-containing byproduct is a result of pure tryptophan production, contains 1.4% pure tryptophan by weight, and has a total nitrogen content of 0.6%. This byproduct is currently considered a waste product, however, its amino acid and nutrient contents make it an intriguing subject for the use as a biostimulant and a potential tool for golf course superintendents dealing with summer bentgrass decline.

Drought continues to be a major limiting factor for creeping bentgrass quality and its use in intensely managed, high value sporting environments. Research specifically aimed at improving bentgrass drought tolerance through breeding is increasing, but the development of commercially available cultivars can be a lengthy process.

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CHAPTER 3. EVALUATION OF CREEPING BENTGRASS (*AGROSTIS STOLONIFERA* L.) RESPONSES TO AN AMINO ACID CONTAINING CO-PRODUCT

Modified from a paper to be submitted to *Crop Science*

Isaac Mertz, Nick Christians, Erik Ervin, and Xunzhong Zhang

Abstract

Tryptophan, an essential amino acid that acts as a building block in protein synthesis, is a biochemical precursor for serotonin, niacin, and auxin in most organisms. When soil moisture is limited, applying biosolids boosted with auxin from tryptophan may increase root production and endogenous hormone levels that can result in plant growth regulation. Tryptophan is produced industrially, which results in a significant amount of byproducts. Tryptophan byproduct (TRP-B) is considered a waste product, but its amino acid and nutrient content make it a possible growth promoter for turfgrasses. The objective of this research was to determine whether applications of TRP-B improve ‘Penn A-4’ creeping bentgrass (*Agrostis stolonifera* L.) performance more than applications of pure tryptophan and/or urea.

Creeping bentgrass plugs taken from sand-based greens at both Virginia Polytechnic Institute and State University, and Iowa State University, were transplanted into pots and allowed to re-establish in growth chambers before being treated. Treatments included TRP-B, urea and pure tryptophan + urea applied every 14 days at three different rates. Application rates were based on the amount of nitrogen (N) applied (2.45, 12.23, and 24.46 kg N ha⁻¹). At trial’s end (42 days), plant parts were harvested and used for

analysis. On average, TRP-B treatments increased leaf total free amino acid contents by 6.2%, rooting biomass by 9.3%, leaf indole-acetic-acid (IAA) concentration by 20.2%, and root IAA concentration by 145.2%, compared to urea only treatments. Tryptophan + urea treatments increased leaf total free amino acid contents by 5%, rooting biomass by 8.3%, leaf IAA concentrations by 32.6%, and root IAA concentrations by 213% on average, compared to urea only treatments. At 24.46 kg N ha⁻¹, TRP-B increased root mass by 18.2% and pure tryptophan + urea produced a 16.3% increase compared to urea only. According to the results, creeping bentgrass treated with TRP-B can result in increased root production, and that response is rate-dependent.

Introduction

Creeping bentgrass (*Agrostis stolonifera* L.) is a cool-season grass that is commonly used for intensely managed, high value sporting environments. This species grows best during the fall and spring when temperatures are cool. In the presence of these favorable environmental conditions, creeping bentgrass forms a dense and uniform playing surface that is very desirable. As temperatures increase during the summer months, plant vigor and productivity can begin to decline. This occurrence is often referred to as summer bentgrass decline (Dernoeden, 2000). Symptoms of summer bentgrass decline include root dieback, excessive leaf senescence, and thinning of the turf canopy, all of which can directly decrease the quality of the playing surface. As soil temperatures increase beyond optimal levels for creeping bentgrass, cytokinin levels can become imbalanced, leading to a reduction of root and shoot growth (Huang and Fry, 1999; Q. Xu and Huang, 2000). Root production in turfgrass is therefore often used as an

indicator of overall plant health as well as a predictor of how the plant will stand up to drought and heat stress.

Cytokinin, a phytohormone involved in cell division and suppression of leaf senescence, is primarily synthesized in plant roots. Cytokinin produced in the roots is translocated to the shoots, and together with auxin affects the shoot growth characteristics of the plant. In plants subjected to different forms of stress, Merewitz et al. (2010a, 2010b, 2011) found that the conservation of cytokinin within the plant was a critical factor influencing plant responses to specifically environmental stress. This was further demonstrated, where improvements in creeping bentgrass plant drought tolerance were shown by increasing endogenous plant cytokinin content (Merewitz et al., 2012). Huang and Xu (2007) were also able to show a positive correlation between cytokinin levels and creeping bentgrass performance under heat stress.

Turfgrass production systems often involve the use of plant growth regulators (PGR's) to manipulate plant growth. Commercially available PGR's can be separated into two types, those intended to suppress plant growth, and those that are intended to promote plant growth. The plant growth promoters are often referred to as biostimulants and may contain plant hormones, organic acids, minerals, or a combination thereof. Subler et al. (1998) was able to show that the presence of biologically active substances (BAS) such as humic acids, vitamins, and amino acids in certain biostimulants or biosolids enabled crops to with-stand environmental stresses and/or positively affect crop growth and quality.

It has long been known that the addition of organic materials to the soil can enhance the biological, physical, and chemical properties of the soil. Typically, yield

increases from the addition of organic materials into the soil are attributed to increased nutrient availability (Akrivos et al., 2000; Boquet et al., 1999; Bugbee, 2002; Tester, 1989) and/or improved soil physical properties (Klock-Moore, 2000). However, the role of BAS within those organic materials and their effect on plant growth enhancement has not been well studied until recently. It is thought that the presence of BAS may enhance crop production by directly providing PGR's to the plant or through the stimulation of soil microbes responsible for supplying substrates and hormones.

Fletcher and Hofstra (1988) found that an application of biosolids or other organic amendments can result in a shift in the balance of plant hormones, which may account for observed plant growth regulating activity following application. This finding was later confirmed by Zhang and Ervin (2004), who observed increased concentrations of some plant hormones (cytokinins, indole acetic acid) and decreased concentrations of others (gibberellins, ethylene) following foliar applications of cytokinin-containing seaweed and humic acid extracts. Zhang and Ervin later demonstrated that biosolids boosted with auxin, a result of adding pure tryptophan to the biosolids, may enhance plant defense chemical responses during limited soil moisture conditions (Zhang et al., 2007).

While research has been done to evaluate the effect of biosolids containing tryptophan, it is to the best of our knowledge that this is the first study to evaluate plant responses to a tryptophan-containing byproduct (TRP-B). The tryptophan-containing byproduct is a result of pure tryptophan production, contains 1.4% pure tryptophan by weight, and has a total nitrogen content of 0.6%. This byproduct is currently considered a waste product, however, its amino acid and nutrient contents make it an intriguing subject

for the use as a biostimulant and a potential tool for golf course superintendents dealing with summer bentgrass decline.

Drought continues to be a major limiting factor for creeping bentgrass quality and its use in intensely managed, high value sporting environments. Research specifically aimed at improving bentgrass drought tolerance through breeding is increasing, but the development of commercially available cultivars can be a lengthy process. The objectives of our research were to (1) determine whether applications of TRP-B improves creeping bentgrass performance more than that provided by pure tryptophan and/or urea, and (2) identify a proper rate of TRP-B that would match a foliar rate of urea, in terms of kg N ha⁻¹, that a golf course superintendent would use on a creeping bentgrass putting green.

Materials and Methods

A collaborative study was initiated in June 2013 at both Virginia Polytechnic Institute and State University and Iowa State University. Mature 'Penn A4' creeping bentgrass (*Agrostis stolonifera* L.) plugs (11.43-cm dia.) were taken from similar United States Golf Association (USGA) specification putting green field plots at the Virginia Tech Turfgrass Research Center (Blackburgs VA) and the Iowa State Horticulture Research Station (Ames, IA). Following collection, bentgrass plugs were separated from their roots by cutting just below the crown and then transplanted into 12.7-cm diameter plastic pots. The growing medium used for this trial was calcareous sand and was chosen for its similarities to that of the underlying root zone mix found at both sites. The use of a growing medium with a coarse texture and low water holding capacity allowed us to recreate the drought/stressful conditions we sought to study.

Plants were irrigated every other day to 50% of field capacity throughout the study. Field capacity of each pot was determined gravimetrically and prior to first treatment applications. Pots were saturated with distilled water and allowed to drain for 48-h. Next, pots were weighed and this weight was recorded as 100% field capacity in grams. Prior to irrigation, pots were weighed, and the recorded weight for each pot was subtracted from its 100% field capacity weight. By dividing this number in half, we were able to calculate the amount of water in grams required to maintain pot moisture at 50% field capacity. Zhang and Schmidt (1999) had success demonstrating drought stress in Kentucky bluegrass by maintaining the moisture content between 40-50% field capacity versus well-watered conditions that had moisture contents between 80-90% field capacity. By maintaining our moisture content near 50% field capacity, we were able to assess a treatment effect under deficit irrigation conditions.

Following the establishment of pot capacity weights, the plants were placed in a growth chamber and allowed to become acclimated to the conditions for 1-week before being treated. A growth chamber was chosen over a greenhouse setting due to better control of light, wind, air temperature, and humidity. Controlling these factors increases the chances of having equivalent environmental conditions across locations, and this results in a minimal treatment-by-location interaction, compared to a greenhouse setting. Both growth chambers had average day/night temperatures of 26.5/20 °C and an average 14-h photoperiod at 400 $\mu\text{mol m}^{-2}\text{s}^{-1}$ photosynthetically active radiation (PAR).

Treatments and Experimental Design

After 1-week acclimation to growth chamber conditions, plants were exposed to three treatments, with each treatment having three separate application rates. The three

treatments included: 1) TRP-B; 2) Urea; and 3) Pure tryptophan + Urea. TRP-B is what is left over after the production of pure tryptophan. The TRP-B used in this study contained 1.4% pure tryptophan by weight and had a total nitrogen content of 0.6%. Application rates were established based on the amount of nitrogen being applied and those rates in units of kg N ha⁻¹ were: 1) 2.45, 2) 12.23, and 3) 24.46. Treatments were applied every 14 days (applications on days 0, 14, and 28). All treatments were applied foliarly and then watered in to just above 50% pot capacity as described above. Application rates for each treatment type had four replicates (four pots) and were arranged in a completely randomized design within the growth chamber at each respected site. Treatment effects were assessed by measuring turfgrass quality (TQ), photochemical efficiency (PE), total leaf chlorophyll, total leaf free amino acids, and rooting biomass.

Turfgrass Quality

Weekly turfgrass visual quality ratings were taken throughout the experiment at both Iowa State University and Virginia Tech. Quality was rated using a 1 to 9 scale. Turfgrass samples receiving a rating of 1 were deemed as having the lowest quality- complete wilting, and 9 indicating the highest quality. A turfgrass quality rating of 6 was used to indicate the minimum acceptable quality.

Photochemical Efficiency

Photochemical efficiency measurements were taken weekly throughout the experiment. At Iowa State University, photochemical efficiency of each pot was measured using a normalized difference vegetation index (NDVI) meter (Spectrum Technologies Inc.). The NDVI is a unit of measurement designed to account for both red and near infrared reflectance. In the case where a plant is under stress, the amount of red

band reflectance increases and the amount of near infrared band reflectance decreases. The NDVI measurements range from -1 to 1, with higher values indicating greater levels of overall plant health. This is considered a measurement of photochemical efficiency because overall plant health can be directly attributed to rate and amount of photosynthesis within the plant itself.

Photochemical efficiency data at Virginia Tech were taken using a chlorophyll fluorometer (OS-50II, Opti-sciences, Inc., Tyngsboro, MA) and were measured as the potential quantum yield of photosystem II (F_v/F_M). F_v/F_M is the ratio of variable fluorescence to maximal fluorescence, and is a measure of the maximum efficiency of photosystem II, if all of the photosystem II centers were open. This type of chlorophyll fluorescence measurement can also be used as a proxy of plant stress, because environmental stresses can reduce the ability of the plant to metabolize normally. This disruption of metabolic processes can then lead to an imbalance between the absorption of light energy by chlorophyll molecules, and the use of that energy in photosynthetic processes. As this imbalance increases, stress tolerance within the plant and photochemical efficiency decrease, resulting in a decline of turfgrass quality and overall plant health.

Leaf Chlorophyll

Fresh leaf samples were collected at 0, 14, 28, and 42 days of the experiment. These samples were initially frozen with liquid nitrogen and stored at $-80\text{ }^{\circ}\text{C}$ until laboratory analysis for total chlorophyll content. At each sampling date, plant tissues were clipped to a height of 2.54-cm. Leaf chlorophyll was extracted in acetone, in the dark for approximately 2 days, as previously done by Zhang et al. (2010). Following

extraction, samples were analyzed using a spectrophotometer, which measures the absorbance of crushed leaf material at specific wavelengths in order to quantify the amount of chlorophyll a and chlorophyll b within plant material (Zhang et al., 2005). Leaf chlorophyll concentrations were then calculated using the equations of Lichtenthaler (1987).

Leaf Total Free Amino Acids

The frozen leaf samples collected at 0, 14, 28, and 42 days of the experiment were also used for total free leaf amino acid content measurements. Frozen leaf samples were initially ground with a mortar and pestle, in liquid nitrogen. Amino Acids were extracted as previously reported by Zhang et al. (2011). Following extraction, absorbance was measured using a spectrophotometer, and total free amino acid content was calculated based on a glycine standard curve.

Root Biomass

At day 42 of the study, samples were removed from their pots to quantify root biomasses. Upon removal of the pot, plant materials were rinsed free of potting medium using distilled water. Following the removal of rooting medium, plant roots were removed from the above-ground portion of the plant near the crown. Plant roots were dried in an oven at 80 °C overnight before being measured for root biomass production.

Leaf and Root Indole-3-Acetic Acid

Tissue and root samples from Virginia Tech used for prior quantifications were also assessed for leaf and root indole-3-acetic acid content. Samples from Iowa State University were not analyzed for this portion of the study. Indole-3-acetic acid (IAA) is a

naturally occurring plant hormone, and the most common form of auxin. Leaf and root IAA measurements were done as previously described by Zhang et al. (2009).

Statistical Analysis

The statistical design was a completely randomized design with four replications. Data from TQ, total leaf chlorophyll, total leaf free amino acid content, and rooting biomass were analyzed using PROC GLM procedure in Statistical Analysis Software version 9.3 (SAS Institute Inc., 2011) to test for overall treatment effects and interactions. Fisher's protected LSD was used to test for specific differences among treatment levels. All tests were performed at a significance level of 0.05.

Data for PE measurements were analyzed separately since they were unique to their respective sites and were not repeated across location. Data from PE were also analyzed using the PROC GLM procedure of SAS, and Fisher's protected LSD was again used to test for differences among treatment levels. While TQ, total leaf chlorophyll, total leaf free amino acid content, and PE were repeated measurements, only the means are reported in this paper.

Results and Discussion

Of the variables measured throughout the experiment, TQ was the only variable with a significant treatment-by-location interaction, and therefore those results were not included in this paper. Due to a lack of a treatment-by-location interaction among the other variables measured, the results from those measurements were pooled across locations and analyzed together.

Photochemical Efficiency

For the photochemical efficiency measurements quantified by an NDVI meter at Iowa State University, there were no differences among treatments, and on average, NDVI measurements were equivalent for the three product types.

On average, $F_v F_M^{-1}$ measurements were 0.688, 0.689, and 0.690 for plants treated with urea only, tryptophan + urea, and TRP-B respectively. Plants treated with applications of TRP-B had mean PE readings that ranged from 0.648 to 0.693 (*Table 1*). The highest rate of TRP-B produced the highest $F_v F_M^{-1}$ measurement of all treatments.

Tryptophan + urea treatments had mean PE measurements of 0.650, 0.686, and 0.686 $F_v F_M^{-1}$ for the low, medium, and high rate, respectively. Urea only treatments had the lowest mean PE reading among the three treatment types, and produced mean PE readings of 0.668, 0.672, and 0.689 $F_v F_M^{-1}$ for the low, medium, and high rate, respectively. These results suggest that TRP-B could potentially be used as a nitrogen source in place of urea, and that those applications will not jeopardize the photochemical efficiency of the plant.

Leaf Chlorophyll

Leaf tissue samples taken on 14-day intervals during the trial were frozen initially and then analyzed for total leaf chlorophyll (mg g^{-1} FW) at the conclusion of the study. On average, total leaf chlorophyll measurements were 2.36, 2.46, and 2.50 mg g^{-1} FW for TRP-B, tryptophan + urea, and urea only, respectively.

Plants treated with TRP-B had mean chlorophyll measurements that ranged from 2.16 to 2.48 mg g^{-1} FW (*Table 1*). These measurements were below those of the other

two treatment types, which is interesting considering the TRP-B treatments produced the highest mean photochemical efficiency readings of the three.

Urea only treatments had the highest mean total leaf chlorophyll measurement among the three treatment types. The low rate of urea produced a mean chlorophyll measurement of 2.08 mg g⁻¹ FW. The medium and high rates of urea resulted in mean chlorophyll measurements of 2.70, and 2.71 mg g⁻¹ FW, respectively, which were the second and third highest mean chlorophyll measurements among all treatment levels. These results support previous research (Bojović and Marković, 2009), which has shown that urea contains nitrogen that is primarily in the quick release form and directly available for plant uptake. Upon application, urea begins to breakdown in the presence of water and urease, an enzyme found in the soil. At this point, urea nitrogen is converted to ammonium, and is available for plant uptake. Compared to an organic nitrogen source, such as recycled sewage materials that have a nitrogen release period upwards of 12 weeks, urea can be broken down and made available for plant uptake in as little as 2-4 days. By being in a quick release form easily taken up by plants, this nitrogen can be utilized faster within the plant during photosynthesis, which could explain our higher measurements for this treatment type. The fact that the tryptophan + urea treatments had chlorophyll contents that were between that of the TRP-B and urea only treatments further supports the idea that the differences in chlorophyll measurements could be a result of the form of nitrogen that was applied.

Plants treated with tryptophan + urea produced mean total chlorophyll measurements of 2.04, 2.48, and 2.86 mg g⁻¹ FW for the low, medium, and high rate, respectively. While the low rate of this treatment type did result in the lowest mean

chlorophyll measurement among all treatments, the high rate of tryptophan + urea also accounted for the highest mean measurement among all treatments. These results suggest that the amount of chlorophyll contained within the leaves of creeping bentgrass is dependent on the form of nitrogen being applied which is consistent with previous research (Bojović and Marković, 2009).

Leaf Total Free Amino Acids

Leaf tissue samples previously described for chlorophyll measurements were also simultaneously measured for leaf amino acid content ($\mu\text{mol g}^{-1}$ FW) at the conclusion of the study. On average, leaf amino acid content measurements were 61.0, 64.0, and 64.8 $\mu\text{mol g}^{-1}$ FW for plants treated with urea only, tryptophan + urea, and TRP-B, respectively (*Figure 1*).

Plants treated with TRP-B had mean amino acid measurements that ranged from 59.8 to 68.3 $\mu\text{mol g}^{-1}$ FW, and accounted for the highest mean amino acid measurement among the three treatment types (*Table 1*). The medium and high rate of TRP-B produced leaf amino acid contents of 66.2 and 68.3 $\mu\text{mol g}^{-1}$ FW, respectively, which were the second and third highest measurements among all treatments. These results make sense, as TRP-B contains an assortment of amino acids, which could have led directly to higher leaf amino acid measurements. This is in line with previous research, where Liu et al. (2002) observed increased cytokinin content in the leaves and roots of creeping bentgrass following exogenous applications of cytokinin.

Tryptophan + urea treatments had mean leaf amino acid measurements of 57.8, 64.5, and 69.8 $\mu\text{mol g}^{-1}$ FW for the low, medium, and high rate, respectively. The high rate of this treatment type which produced the mean amino acid content of 69.8 $\mu\text{mol g}^{-1}$

FW was the highest among all treatments. The fact that pure tryptophan is an amino acid further supports the idea that the increase of leaf amino acid content is related to direct application of the amino acid based products.

Urea only treatments had mean leaf amino acid measurements that ranged from 56.8 to 64.8 $\mu\text{mol g}^{-1}$ FW and had the lowest mean amino acid measurements among the three treatment types. The low rate of urea that produced the mean amino acid content of 56.8 $\mu\text{mol g}^{-1}$ FW was the lowest among all treatments. These results suggest that the use of urea only fertilizers can lead to lower amino acid contents within the leaf tissue of creeping bentgrass compared to that of leaf tissue receiving a tryptophan-based fertilizer.

Root Biomass

On average, root biomass measurements were 0.809, 0.876, and 0.884 grams for plants treated with urea only, tryptophan + urea, and TRP-B, respectively (*Figure 2*). TRP-B treatments had mean root biomasses that ranged from 0.747 to 0.997 grams, with the high rate resulting in the highest root biomass of all treatments (*Table 1*). Surprisingly, the medium rate of TRP-B resulted in the third highest mean root biomass production which was 0.908 grams.

Urea only treatments produced mean root biomasses that ranged from 0.741 to 0.815 grams. The low rate of urea which resulted in the mean biomass production of 0.741 grams was the lowest among all treatments. Interestingly, the medium rate of urea resulted in a higher mean root biomass than that of the high rate of urea. This result differs from the rest of the data that was produced, where a linear increase was seen between the rate of nitrogen that was applied and rooting biomass production. This illustrates the benefit of including a slow-release fertilizer during nitrogen applications.

Since the nitrogen contained in the TRP-B and pure tryptophan + urea applications was of a slower release mechanism than that of urea, the plants were able to maintain a balance between nitrogen uptake and root production. When that slow release nitrogen is not included, and all the of the nitrogen applied was in the form of quick release nitrogen, the plant was not able to maintain this balance and rooting production began to decline at this higher rate.

Tryptophan + urea treatments produced mean root biomasses that ranged from 0.783 to 0.974 grams; with treatment 9 resulting in the second highest root biomass on average among all treatments. Root production is often used as a way to assess the performance of a turfgrass stand. It appears that the TRP-B and tryptophan + urea treatments were able to produce a root biomass that was similar, and sometimes superior, to that of the urea only treatments. These results support the idea of using tryptophan-based products as an alternative or in combination with urea, and creeping bentgrass treated with tryptophan-based biosolids may result in an increase of root production and help better maintain a nitrogen balance within the plant compared to applications of urea only.

Leaf and Root Indole-3-Acetic Acid

Leaf and root IAA content was affected by treatment. On average, leaf IAA measurements were 162.8, 176.8, and 189.3 ng g⁻¹ FW for urea only, TRP-B, and tryptophan + urea, respectively (*Figure 3*). The TRP-B treatments had mean leaf IAA measurements that ranged from 92.7 to 123.5 ng g⁻¹ FW (*Table 2*). Urea only treatments had mean leaf IAA measurements that ranged from 80.7 to 98.3 ng g⁻¹ FW, and were significantly lower than the other two treatment types. Pure tryptophan + urea treatments

on average were the highest for the three product types and ranged from 96.1 to 132.1 ng g⁻¹ FW.

Root IAA measurements on average were 1.15, 2.82, and 3.60 µg g⁻¹ FW for urea only, TRP-B, and tryptophan + urea, respectively (*Figure 4*). TRP-B treatments had mean root IAA measurements that ranged from 2.09 to 3.52 µg g⁻¹ FW (*Table 2*). Like leaf IAA measurements, urea only treatments also had the lowest mean root IAA measurements, which ranged from 1.03 to 1.29 µg g⁻¹ FW. Pure tryptophan + urea only treatments also resulted in the highest mean root IAA measurements compared to the leaf IAA measurements, and they ranged from 1.25 to 4.88 µg g⁻¹ FW.

Conclusions

Plant hormone-boosted biosolids containing tryptophan had a greater positive effect of creeping bentgrass performance under drought stress when compared to that of the urea only treatments. On average, root biomasses were 0.884, 0.810, and 0.876 grams for TRP-B, urea only, and tryptophan + urea, respectively. Since nitrogen levels were equal between rates 1, 2, and 3 for each treatment, tryptophan derived biosolids positive effect on creeping bentgrass performance under drought stress conditions is likely to be hormonally-based. Biostimulants often include a variety of ingredients including plant hormones or substances that affect endogenous hormone levels. For example, humic substances inhibit indole acetic acid (IAA) oxidase, the enzyme responsible for the irreversible degradation of auxin (Schmidt and Zhang, 1998). Auxin is responsible for many aspects of creeping bentgrass root development, including initiation and emergence of lateral roots. Humic substances can also influence metabolic processes such as photosynthesis, respiration, and nutrient uptake (Schmidt and Zhang, 1998).

Tryptophan has been shown to boost auxin levels when added to biosolids (Zhang et al., 2007) making TRP-B an intriguing prospect for the use as a biostimulant/ plant growth promoter. This is further supported by our results, where TRP-B and pure tryptophan treatments resulted in more root production than that of the urea only treatments.

Overexpression of endogenous cytokinin has been shown to improve creeping bentgrass performance under various abiotic stresses (Merewitz et al., 2011). Cytokinins have various biochemical roles including suppressing leaf senescence and promoting the initiation of tillers. Maintaining sufficient levels of cytokinins have been found to be an important factor regulating responses of creeping bentgrass to environmental stresses (Y. Xu et al., 2009). In addition, cytokinin synthesis is sensitive to high soil temperatures (Huang, 2001), and could contribute to summer bentgrass decline. Summer bentgrass decline could partially be attributed to an imbalance of auxin and cytokinin due to root decline originating from above optimal soil temperatures. Applications of TRP-B resulted in the highest amount of root biomass production, as well as the highest leaf amino acid content of the three treatment types under drought stress conditions. These results suggest that TRP-B may help maintain that balance of auxin and cytokinin, and could decrease the effect of summer bentgrass decline.

Maintaining auxin and cytokinin production during the summer months through continuous root growth could help alleviate bentgrass decline. If used properly, TRP-B could be an important tool for promoting turfgrass vigor and improving summer stress tolerance of creeping bentgrass. While the potential is promising, additional focus on this topic should be considered. This study investigated three application rates of each

treatment type, with plant response being directly correlated with application rate. Our results indicate that the maximum potential for TRP-B has not been identified, and creeping bentgrass root production could continue to increase, as long as applications of biosolids continue. Therefore, additional research should be done to focus on higher application rates in order to identify any potential phytotoxicity complications. As previous research has shown, plant phytotoxicity in exogenous hormone applications can occur due to hormonal overdose (Liu et al., 2002).

Tables

Table 1. Mean treatment impact of creeping bentgrass under deficit irrigation conditions.

Treatment Number	Treatment	Rate	Photochemical Efficiency		Leaf Chlorophyll and Carotenoids	Leaf Total Free Amino Acids	Root Biomass
			$F_v F_M^{-1}$	NDVI	mg g ⁻¹ FW	μmol g ⁻¹ FW	g
1	TRP-B	2.45	0.648	0.696	2.16	59.8	0.747
2	TRP-B	12.23	0.687	0.703	2.45	66.2	0.908
3	TRP-B	24.46	0.693	0.714	2.48	68.3	0.997
4	Urea	2.45	0.668	0.693	2.08	56.8	0.741
5	Urea	12.23	0.672	0.717	2.7	61.3	0.873
6	Urea	24.46	0.689	0.689	2.71	64.8	0.815
7	Tryptophan+Urea	0.98+1.47	0.650	0.683	2.04	57.8	0.783
8	Tryptophan+Urea	4.04+8.19	0.686	0.706	2.48	64.5	0.870
9	Tryptophan+Urea	8.07+16.39	0.686	0.724	2.86	69.8	0.974
LSD:			0.027	NS	0.31	5.3	0.122
CONTRASTS:							
TRP-B vs Urea			NS	NS	NS	0.018	0.0398
TRP-B vs Tryptophan + Urea			NS	NS	NS	NS	NS
Tryptophan + Urea vs Urea			NS	NS	NS	0.054	0.0652

Table 2. Leaf and root (indole-acetic-acid) IAA responses of creeping bentgrass to treatment application under deficit irrigation conditions.

Treatment Number	Treatment	Rate	Leaf IAA Day-0	Leaf IAA Day-14	Leaf IAA Day-28	Leaf IAA Day-42	Mean Leaf IAA	Root IAA
		kg N ha⁻¹			ng g⁻¹ FW			µg g⁻¹ FW
1	TRP-B	2.45	110.2	134.4	69.0	57.2	92.7	2.09
2	TRP-B	12.23	112.9	140.2	80.2	92.7	106.5	2.84
3	TRP-B	24.46	110.4	142.5	123.6	117.6	123.5	3.52
4	Urea	2.45	106.5	113.5	68.3	34.3	80.7	1.15
5	Urea	12.23	107.6	132.4	73.2	45.8	89.7	1.29
6	Urea	24.46	113.0	146.7	97.5	36.0	98.3	1.03
7	Tryptophan+Urea	0.98+1.47	113.7	141.4	80.6	48.6	96.1	1.25
8	Tryptophan+Urea	4.04+8.19	111.3	151.4	133.1	115.4	127.8	4.67
9	Tryptophan+Urea	8.07+16.39	104.2	160.3	136.3	127.7	132.1	4.88
LSD:			NS	16.7	38	20.1	13.9	1.31
<u>CONTRASTS:</u>								
TRP-B vs Urea			NS	NS	NS	<.0001	0.0001	0.0001
TRP-B vs Tryptophan + Urea			NS	0.0168	0.0235	NS	0.0087	0.0423
Tryptophan + Urea vs Urea			NS	0.0002	0.0019	<.0001	<.0001	<.0001

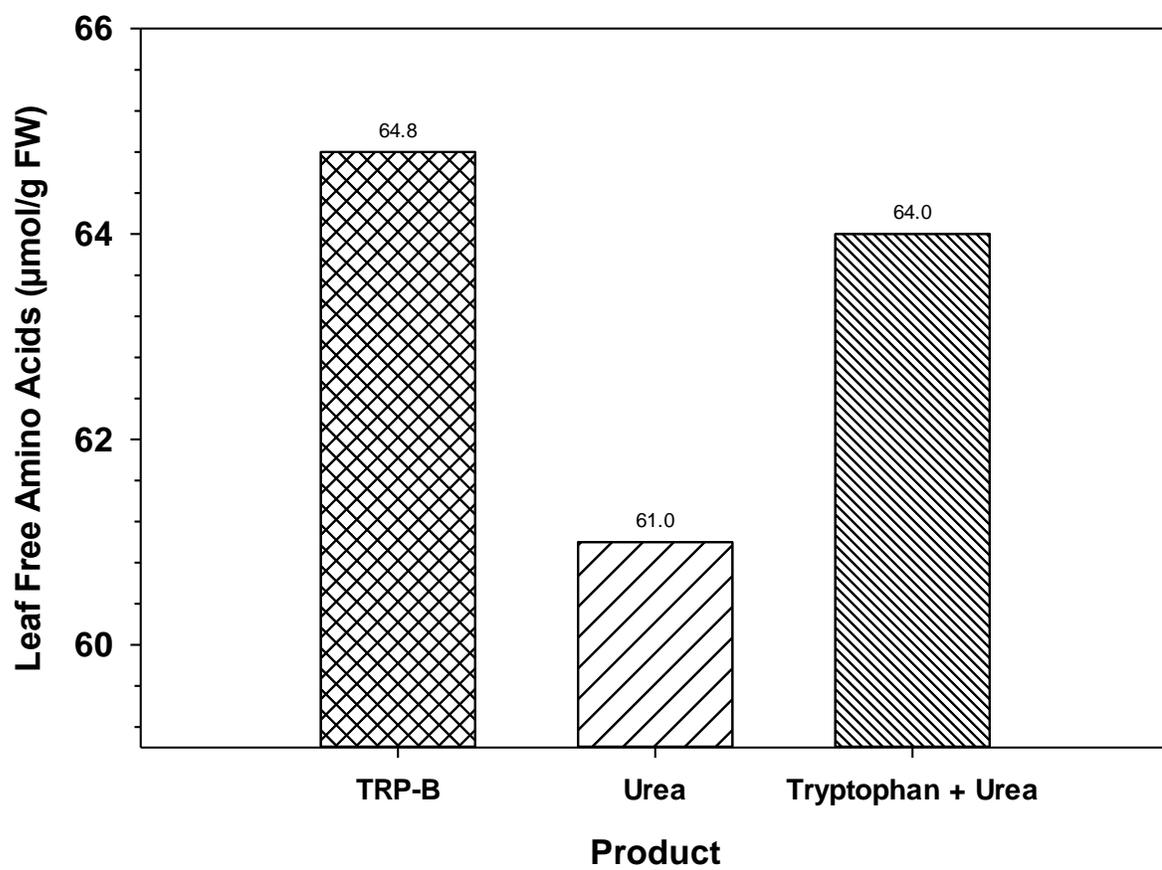
Figures

Figure 1. Leaf total free amino acids response of creeping bentgrass to treatment, by product type.

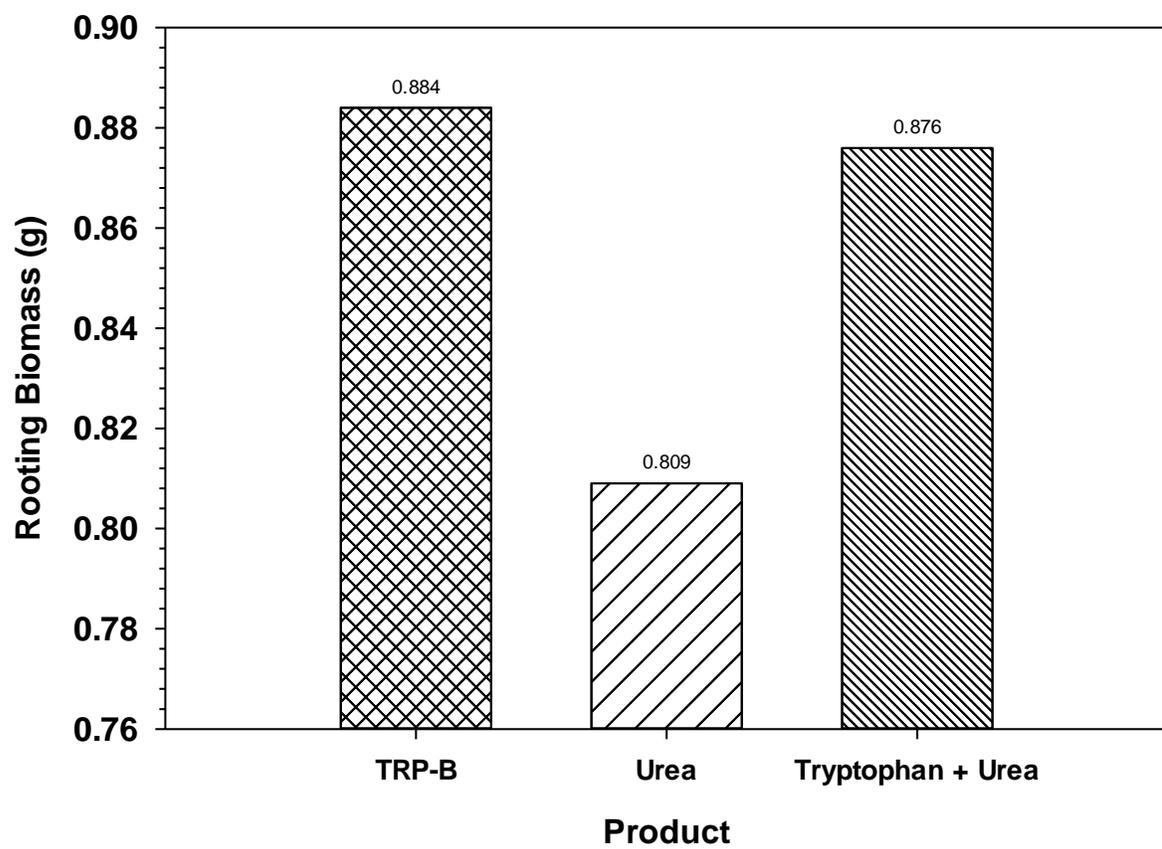


Figure 2. Rooting biomass response of creeping bentgrass to treatment, by product type.

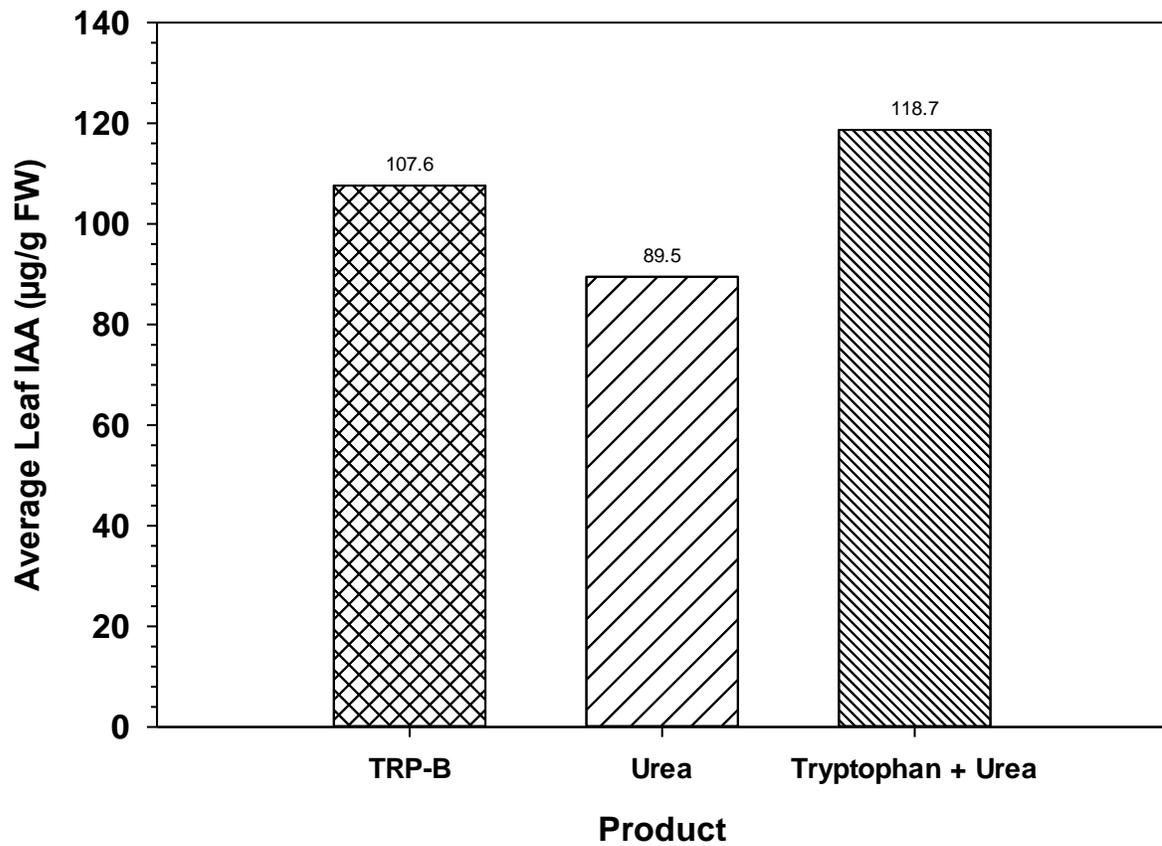


Figure 3. Leaf IAA responses of creeping bentgrass to treatment, by product type.

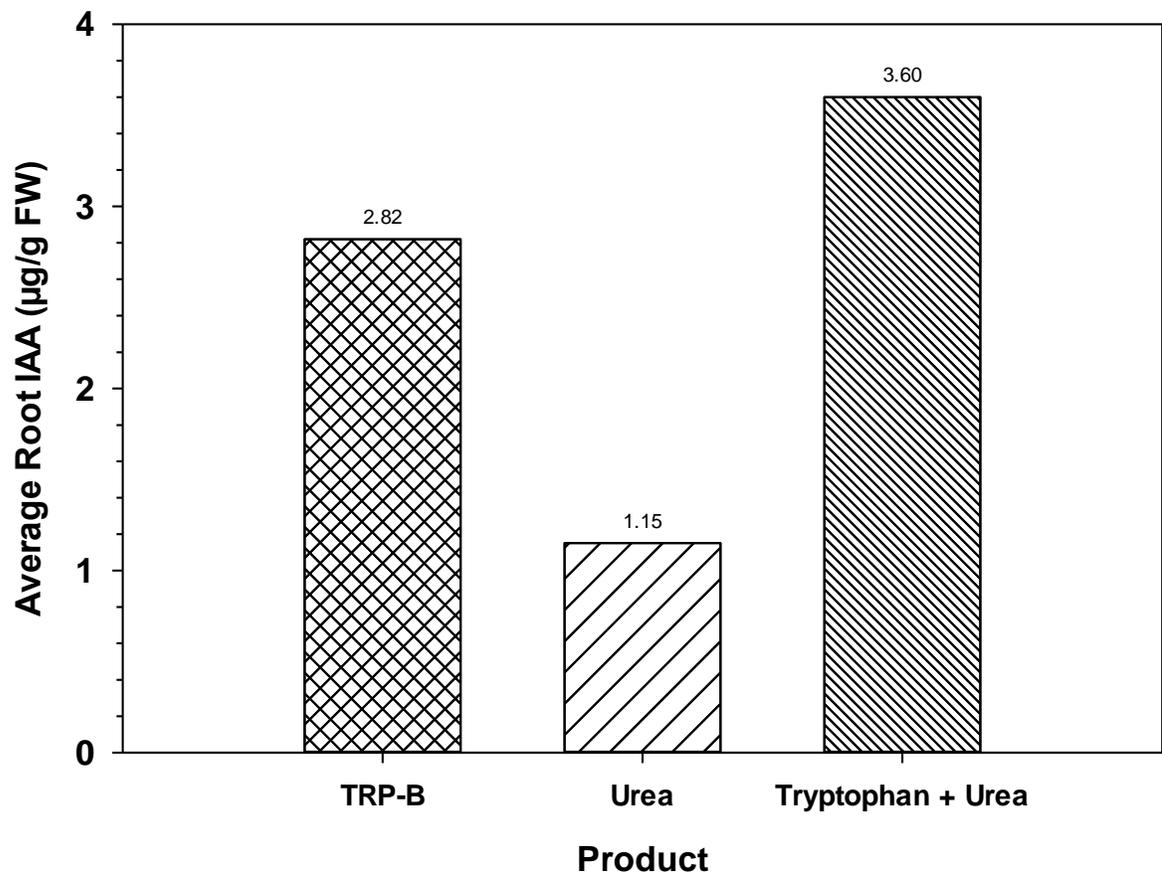


Figure 4. Root IAA responses of creeping bentgrass to treatment, by product type.

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CHAPTER 4. ADDITIONAL RESULTS

Preliminary Study

Objectives

The objective of this research was to evaluate the establishment and rooting responses of perennial ryegrass (*Lolium perenne* L.) to applications of tryptophan, an IBA standard, and a tryptophan byproduct (TRP-B) at the time of seeding.

Materials and Methods

Rooting tubes constructed out of 3.2-cm diameter polyvinyl chloride (PVC) pipe and a 2.6-cm diameter polyethylene insert were used to investigate the responses of perennial ryegrass to applications of tryptophan, an IBA standard, and TRP-B. Each polyethylene insert was filled to a height of 60 cm with a calcareous sand root-zone mixture with a pH of 8.2. The sand was 8.2% very coarse, 35.3% coarse, 44.3% medium, 11.9% fine, 0.1% very fine, and 0.2% silt/clay by volume. The polyethylene inserts were placed into 60-cm long pieces of PVC pipe which were secured to a greenhouse rack at an orientation of roughly 45°.

Supplemental radiation was provided when day-time irradiance dropped below 200 $\mu\text{mol m}^{-2}\text{s}^{-1}$ to ensure a consistency of 16 hours of light per day. Peak daytime irradiance ranged from 350 to 385 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (mean = 330 $\mu\text{mol m}^{-2}\text{s}^{-1}$). Air temperature ranged from 22.3 to 23.6°C (mean = 22.8°C). Relative humidity ranged from 24.3 to 44.7% (mean = 35.6%).

Following the set-up, each tube was saturated with water and inserts were slit near the bottom. The tubes were allowed to finish draining to allow the root-zone medium to settle prior to establishment. Sixteen seeds of '5-Iron' perennial ryegrass blend (88.2%

PLS) were placed into each tube, followed by the application of a modified Hoagland's solution. The modified Hoagland's solution was nitrogen-free, applied at a rate of 48.82-kg phosphorus (P) ha⁻¹ (24-ppm P, 63-ppm K, 19-ppm Mg, and 1.2-ppm Fe).

Treatments that were evaluated included: tryptophan (1, 10, 100, and 1000-mg 180 cm⁻²), an IBA standard (0.56-mg 180 cm⁻²), TRP-B (equivalent to 1, 10, 100, and 1000-mg tryptophan 180 cm⁻²), and an untreated control. Due to the varying nitrogen concentrations in each treatment, a supplemental urea application was included to balance out the differences among treatments. This application was treatment dependent, based on the nitrogen content of the highest application rate of TRP-B, ensuring each treatment received an equal amount of nitrogen.

Six replications of each treatment were evaluated in a completely randomized design. Tubes were mist watered with 2 mL distilled water, four times daily, until seed germination occurred. Following germination, watering was slowly transitioned to 15 mL per tube once a week. On day twenty-six following seeding, a slight phosphorus deficiency was observed, and a second application of nitrogen-free Hoagland's solution was used at a rate of 24.46-kg P ha⁻¹. Thirty-nine days after seeding, watering was stopped, and the samples were introduced to minor drought stress for the remaining seven days of the study.

On day forty-six following seeding, the polyethylene inserts were removed from the PVC tubes and placed on a wire-mesh screen. The insert was carefully removed by cutting down the top seam, leaving the intact root-zone and above-ground plant material behind. The plant parts were rinsed free of sand over the mesh screen using deionized water, and allowed to air-dry over-night. Plant roots were removed from above-ground

leaf tissue, oven dried overnight at 70°C, before being weighed separately. Due to inconsistent germination of the ryegrass seed, the number of live plants per tube was recorded and used during the statistical analysis as a covariate.

Statistical Analysis

Data were analyzed with the PROC GLM procedure in Statistical Analysis Software version 9.3 (SAS Institute Inc., 2011). Data that were analyzed included shoot biomass, shoot height, rooting biomass, and root length. Statistical significance was evaluated based on $P \leq 0.05$ using an analysis of covariance with the number of live plants per tube being the covariate.

Results

Of the four variables analyzed, tissue weight ($P < .0001$), root weight ($P < .0001$), and root length ($P = .0036$) were influenced by treatment (*Table 3*). Shoot height was the only variable that was not affected by treatment.

Compared to the control, which still received an equal amount of nitrogen as the other treatments, tryptophan, TRP-B, and the IBA standard on average, increased rooting biomass by 17.7, 54.8, and 74.2%, respectively (*Figure 5*). The IBA standard produced the largest root biomass among all treatments (0.108-g), and was equivalent to the 1000-mg tryptophan (0.106-g) and 100-mg TRP-B (0.103-g) rates. Tryptophan at the 100-mg rate was the only treatment that resulted in less rooting biomass than the control on average.

On average, tryptophan, the IBA standard, and TRP-B increased shoot biomass by 20, 27.5, and 62.5%, respectively (*Figure 6*). The TRP-B 10, 100, and 1000-mg rates resulted in three of the four highest shoot biomass measurements, which were greater

than the other treatment products (*Table 3*). Only tryptophan at the 1000-mg rate (0.063-g) was on an equivalent level. TRP-B at the 1-mg rate (0.038-g) was the only treatment that resulted in less shoot biomass production than the control (0.04-g).

In contrast to the root and shoot biomass measurements, treatment application decreased the rooting depth on average. Compared to the control, the IBA standard, tryptophan, and TRP-B decreased rooting depth by -0.2, -1.7, and -5.5%, respectively (*Figure 7*). The 100-mg tryptophan rate resulted in the deepest rooting depth (59.7-cm), and this was equivalent to the control, IBA standard, TRP-B at 1, 10, and 100-mg, as well as the 1 and 10-mg rates of tryptophan (*Table 3*). For tryptophan and TRP-B, there was a correlation between the decrease in rooting, and the rate that was applied. For tryptophan, rooting length increased with application up until the 100-mg rate, with the 1000-mg rate resulting in a decline. For TRP-B, rooting depth peaked at the 10-mg rate, with the 100 and 1000-mg rates resulting in a decline. The 1000-mg rate of TRP-B resulted in the shortest rooting depth (50.8-cm) of all treatments.

Conclusions

Tryptophan is a known precursor for auxin in most organisms, and has been shown in the past to be able to boost auxin concentrations of biostimulant products (Zhang et al., 2007). Through exogenous tryptophan application, we may be affecting the endogenous hormone levels within the plant, and this may be what is accounting for the differences in root and shoot biomass measurements. This idea is further supported by previous research, where the application of biostimulants containing biologically active substances has been shown to influence endogenous hormone levels (Fletcher and Hofstra, 1988; Zhang and Ervin, 2004). It has also been shown that foliar absorption of

amino acid nitrogen compounds can occur (Stiegler et al., 2013), therefore, plants could also be absorbing those plant growth regulating compounds, which could help explain the differences in growth across treatments.

Auxin and cytokinin are both plant hormones, and the concentration ratio at which these two hormones are found within the plant is what primarily controls the root and shoot growth characteristics of the plant (Su et al., 2011). As previously mentioned, tryptophan is a known precursor for auxin in most organisms. The TRP-B contains both nitrogen and traces of pure tryptophan, which makes it an intriguing prospect for the use as a biostimulant/plant growth promoter of turfgrass. However, further research should be done.

Tryptophan and TRP-B at the 1000-mg rate resulted in significant decreases in rooting compared to the other treatments. It was also observed throughout the trial that the TRP-B 1000-mg rate samples drastically affected the soil physical properties, and these samples consistently took more time for the treatment and irrigation applications to move through the soil profile.

According to the results, tryptophan and TRP-B appear to improve perennial ryegrass performance compared to equivalent applications of only mineral nutrition (the control), and those results are rate dependent. At this time, additional greenhouse research should be done in order to dial in the proper application rate, as well as investigate the changes in soil properties over time following application.

Tables

Table 3. Mean treatment impact of perennial ryegrass performance.

Treatment Number	Treatment	Root Biomass	Shoot Weight	Root Length	Shoot Height	Live Plants per Tube
		g	g	cm	cm	#
1	Control	0.062	0.040	58.9	5.6	5
2	Tryptophan 1-mg	0.075	0.043	58.4	5.7	7
3	Tryptophan 10-mg	0.064	0.046	58.6	5.3	5
4	Tryptophan 100-mg	0.052	0.042	59.7	5.9	6
5	Tryptophan 1000-mg	0.106	0.063	54.3	5.6	4
6	IBA Standard	0.108	0.051	58.8	5.0	6
7	TRP-B (equivalent to 1-mg Tryptophan)	0.085	0.038	57.4	4.5	5
8	TRP-B (equivalent to 10-mg Tryptophan)	0.102	0.059	58.2	5.3	7
9	TRP-B (equivalent to 100-mg Tryptophan)	0.103	0.075	56.4	6.3	5
10	TRP-B (equivalent to 1000-mg Tryptophan)	0.093	0.090	50.8	5.4	6
LSD:		0.039	0.023	4.7	NS	NS
CONTRASTS:						
TRP-B vs Control		NS	0.0587	0.035	NS	NS
TRP-B vs Tryptophan		0.0845	0.0184	0.0616	NS	NS
TRP-B vs IBA Standard		NS	0.0407	NS	NS	NS
Tryptophan vs Control		NS	NS	NS	NS	NS
Tryptophan vs IBA Standard		NS	NS	NS	NS	NS
IBA Standard vs Control		NS	NS	NS	NS	NS

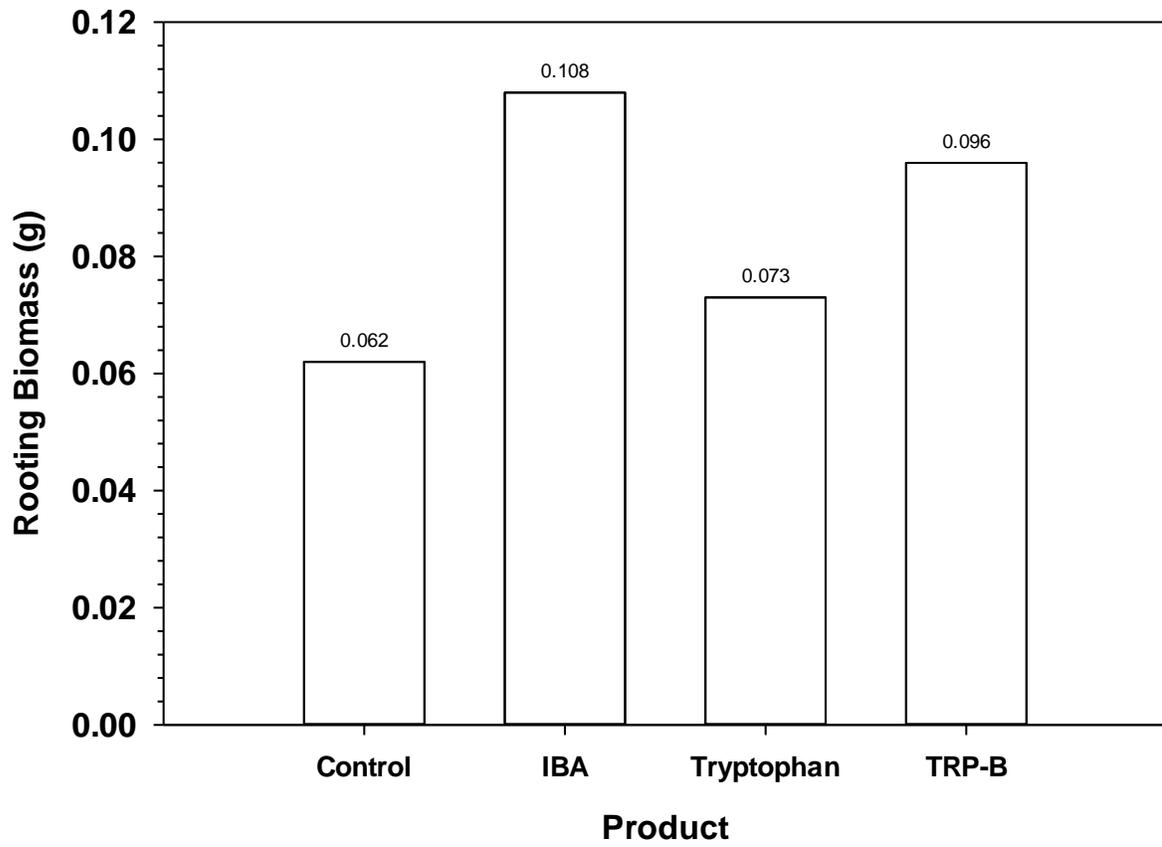
Figures

Figure 5. Rooting biomass responses of perennial ryegrass to treatment application, by product type.

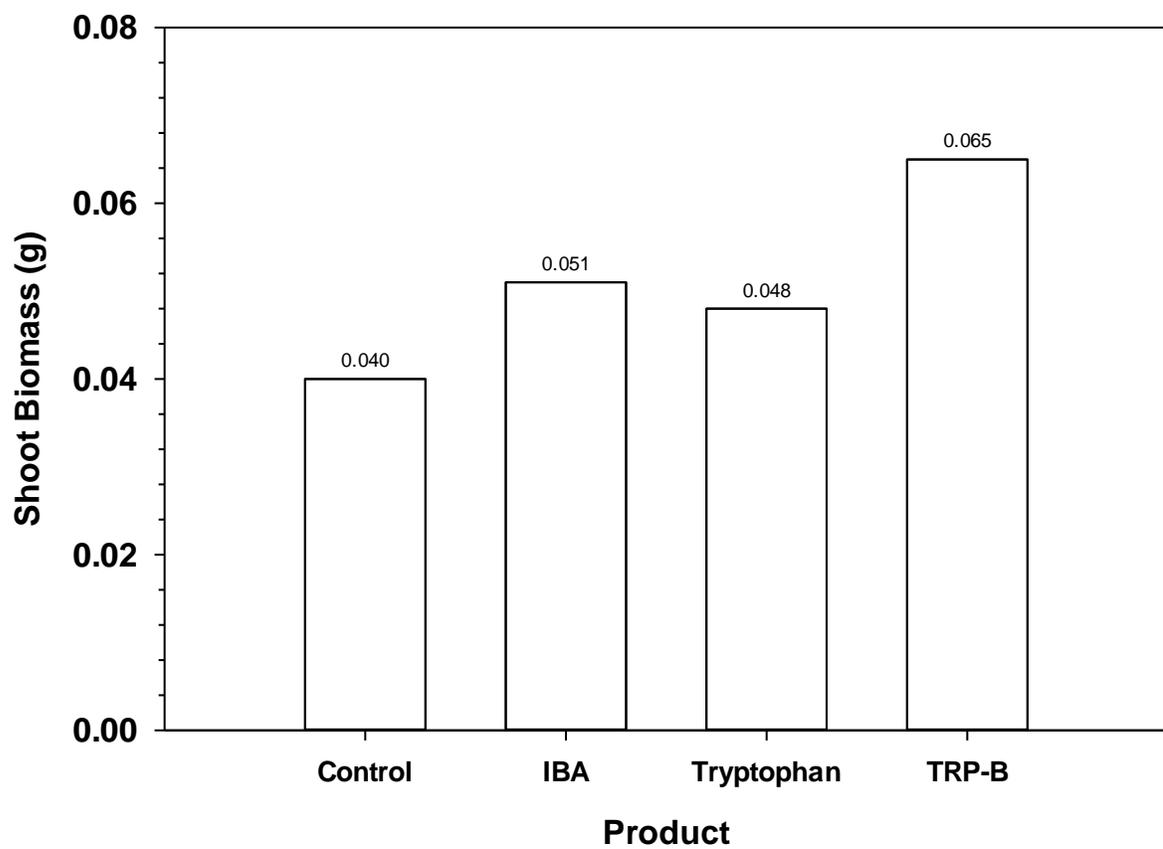


Figure 6. Shoot biomass responses of perennial ryegrass to treatment application, by product type.

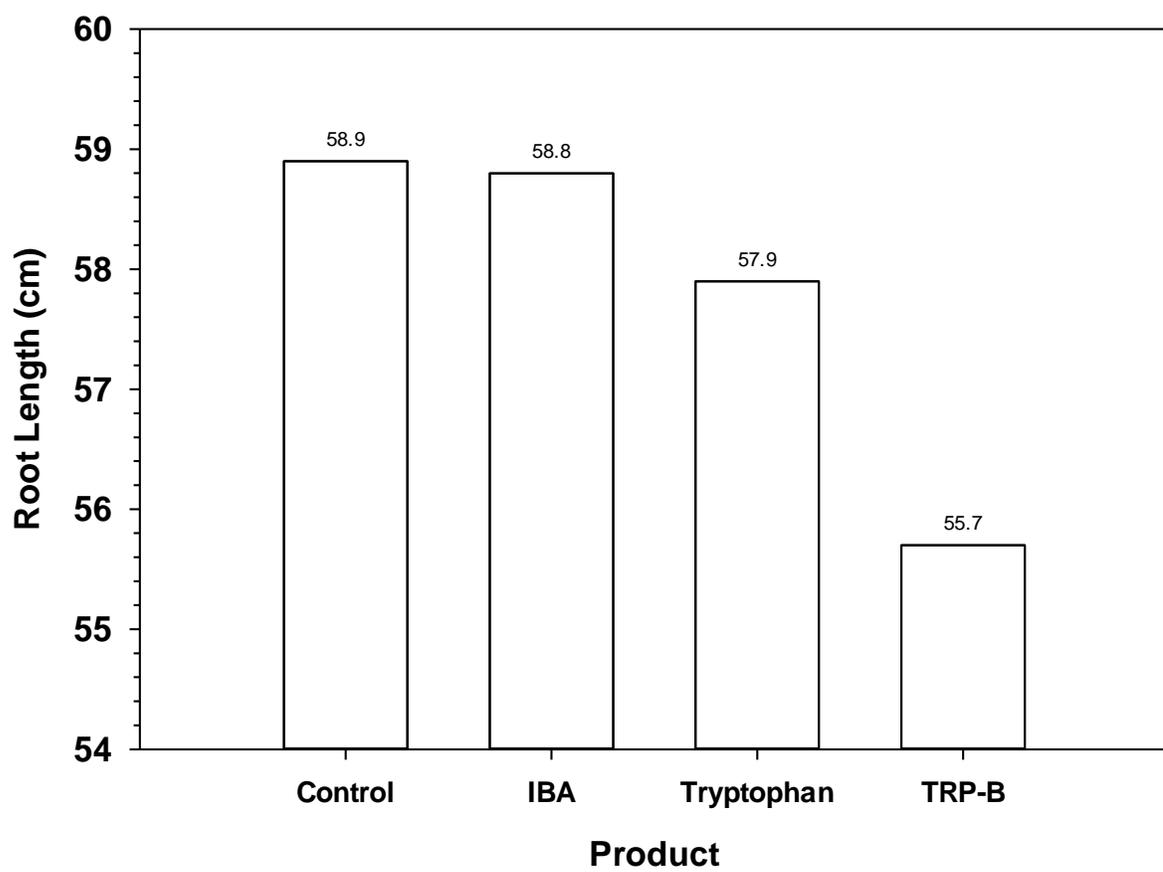


Figure 7. Treatment effect on rooting depth of perennial ryegrass, by product type.

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CHAPTER 5. GENERAL CONCLUSIONS

General Discussion

The goal of the present study was to evaluate and compare the responses of creeping bentgrass to applications of a tryptophan-containing byproduct (TRP-B) with those of pure tryptophan and/or urea under drought conditions. By using a growth chamber, environmental conditions were able to be controlled, and the trial was able to be run across two locations simultaneously with a minimal treatment-by-location interaction. Each treatment type (TRP-B, urea only, and pure tryptophan + urea) was applied at three different rates, and these rates were based on the amount of nitrogen being applied. Each treatment type was applied at 2.45, 12.23, and 24.46 kg N/ha, and replicated six times.

Plants treated with biostimulants containing biosolids with traces of tryptophan had a greater positive effect on creeping bentgrass performance under drought stress, when compared to that of the urea only treatments. On average, root biomasses were 0.884, 0.810, and 0.876 grams for TRP-B, urea only, and tryptophan + urea, respectively. As previously stated, the three rates used for each product type were N based, so the low, medium, and high rate for each product type contained equal amounts of nitrogen. Since the nitrogen content across the three rates were equal, regardless of product type, and plants treated with TRP-B or tryptophan in combination with urea outperformed the urea only treatments, the tryptophan derived biosolid's positive effect on creeping bentgrass under drought stress conditions is likely to be hormonally-based.

Compared to fertilizer products which only contain mineral nutrition, biostimulants often contain a variety of ingredients including plant hormones, organic acids, minerals, or a combination thereof (Schmidt, 1999). These biologically active

substances are likely what enables biostimulants to affect plant endogenous hormone levels following application. For example, humic substances have been shown to inhibit indole-acetic-acid (IAA) oxidase, the enzyme responsible for the irreversible degradation of auxin (Schmidt and Zhang, 1998). Auxin is responsible for many aspects of creeping bentgrass root development, including initiation and emergence of lateral roots. Humic substances can also influence metabolic processes such as photosynthesis, respiration, and nutrient uptake (Schmidt and Zhang, 1998).

Tryptophan has been shown to boost auxin levels when added to biosolids (Zhang et al., 2007), making TRP-B an intriguing prospect for the use as a biostimulant/plant growth promoter. This is further supported by our results, where TRP-B and pure tryptophan + urea treatments increased creeping bentgrass performance under drought more than that of the urea only treatments. Root production is often used as an indicator of overall plant health, as well as a predictor of how the plant will withstand heat and drought stress. Cytokinin, along with auxin, are the two plant hormones most responsible for the shoot and root growth characteristics of the plant. As soil temperatures increase beyond optimal levels for creeping bentgrass, the cytokinin levels within the plant can become imbalanced, leading to a reduction of root and shoot growth (Huang and Fry, 1999; Xu and Huang, 2000). This reduction in growth is often referred to as summer bentgrass decline (SBD) (Dernoeden, 2000). It was later found that specifically the conservation of cytokinins within the plant was a critical factor influencing plant responses to environmental stress (Merewitz et al., 2010a, 2010b).

In plant roots, auxin induces meristematic cell division, while cytokinins promote the cell to switch from the meristematic to differentiated state by inhibiting

auxin signaling, which results in the redistribution of auxin and cell division (Su et al., 2011). This means that when the auxin to cytokinins ratio is high, root regeneration is induced, whereas when it is low, shoot induction is promoted (Su et al., 2011). As shoot induction is promoted, that increased above ground growth occurs at the expense of the root system. This idea further illustrates the importance cytokinins, and the balance between cytokinins and auxin, play in enabling a plant to withstand environmental stress.

Applications of TRP-B and tryptophan + urea resulted in a higher amount of root biomass, leaf amino acid content, and root IAA content than that of the urea only treatment under drought stressed conditions. These results suggest that TRP-B may help maintain that balance of auxin and cytokinins, and could decrease the effect of summer bentgrass decline.

Maintaining auxin and cytokinin production during the summer months through continuous root growth could help alleviate bentgrass decline. If used properly, TRP-B could be an important tool for promoting turfgrass vigor, and improving summer stress tolerance of creeping bentgrass.

Recommendations for Future Research

While the potential is promising, additional focus on this topic should be considered. This study investigated three application rates of each product type, with plant response being directly correlated with application rate. Our results indicate that creeping bentgrass root production could increase indefinitely, as long as applications of the treatments were to continue. Therefore, additional research should be done to focus on higher application rates in order to identify any potential phytotoxicity complications. As previous research has shown, plant phytotoxicity in exogenous hormone applications can occur due to hormonal overdose (Liu et al., 2002).

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