# **Evaluation of Creeping Bentgrass Responses to Fertilization with the Branched-Chain Amino Acids**

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#### Introduction

There has been a recent increase in amino acid-based products used for fertilization in turfgrass management. Amino acids are simple organic compounds that contain both a carboxyl group (COOH) and an amino group (NH<sub>2</sub>). Research has shown when foliarly applied, some of these organic compounds can enter the plant through leaf tissue, making these an interesting source of nitrogen for plant growth. Fertilization with amino acids also has been shown to increase plant performance more than that of equivalent applications of mineral nutrition only.

However, not all amino acids are able to enter the plant in this way, and plant catabolism of amino acids is not entirely understood. Preliminary research indicated when foliarly applied together in combination, the branchedchain amino acids (BCAA) leucine, isoleucine and valine can lead to increases of shoot production in creeping bentgrass (Agrostis stolonifera L.) putting greens. This project involved foliar application to creeping bentgrass of BCAA in three combinations, with and without the addition of urea. The primary objective was to evaluate and compare responses of the BCAA applications to applications of both an equivalent mineral nutrition and a commercially available amino acid complex.

## **Materials and Methods**

A two-year study was initiated in July 2017 and repeated in July 2018 at the Iowa State University (ISU) Horticulture Research Station (Ames, IA). A Penn A4 creeping bentgrass putting green established on a United States Golf Association (USGA) specification root-zone was chosen for the field plots. Prior to treatment initiation in both years, the entire experimental area was treated with 11.84-kg phosphorus (P) per hectare to account for a deficiency. The experiment was organized as a randomized complete block design (RCBD) with four total replications. The layout of the RCBD was not randomized across years.

The treatments listed in Table 1 were applied on a 14-day interval, with all treatments other than the untreated control being based on an equal nitrogen (N) rate of 3.4-kg N per hectare. This low N application rate was intended to simulate a spoon-feeding application commonly used when growing creeping bentgrass on a sand-based root-zone. All treatments were applied foliarly using a carbon dioxide powered backpack sprayer (1220 L ha<sup>-1</sup> at 275 kPa w/ TeeJet XR 8002VS nozzles), with irrigation being withheld until 24-hours post treatment application. In both years, a total of six treatment applications occurred throughout the trial.

The height of cut for the creeping bentgrass putting green was maintained at 0.32-cm throughout the trial in both years. Data collected included STIMP meter, soil moisture, and normalized difference vegetation index (NDVI) meter readings, all done twice weekly throughout the trials. A light-box was used to record digital images of each experimental unit twice weekly in order to quantify and track turfgrass percent cover and dark green color index (DGCI) values. In addition to these measurements, turfgrass clippings were collected and weighed four times each year. Shoot density samples were collected weekly and counted at the end of the trial each year.

## **Results and Discussion**

Unless otherwise indicated, all data were pooled across years due to a non-significant Ftest for treatment x year interactions. At the end of the trial in both years (84 days post treatment initiation), plants that received applications of leucine, isoleucine, and valine in a 4:1:1 ratio exhibited on average a 48 percent, 15 percent, and 6 percent increase in shoot density, respectively, compared with the untreated control, positive control (urea only), and a commercially available amino acid product known to increase shoot density (Figure 5). There were no differences among ratios of BCAA, and substituting half of the applied nitrogen in the form of urea had no impact on shoot density counts.

This concept illustrates the benefit of including some urea with a BCAA mixture, as the urea may help those compounds enter the plant more efficiently. On average, BCAA treatments had significantly higher creeping bentgrass shoot density counts compared with applications of urea only, and were statistically equivalent to the commercially available amino acid product, regardless of ratio. Plots treated with urea only significantly increased shoot density compared with the untreated control, and also were statistically equivalent to the commercially available amino acid product (Figure 5). Measurements for NDVI were significantly different among treatments for several of the rating days (Figure 4). However, there were no differences among the different BCAA ratios, positive control, or commercially available amino acid complex, on average, and only the untreated control was significantly less than the rest throughout the trial.

Treatment also had a significant effect on clipping yield for two of the four dates clippings were collected (Figure 2). However, those differences were only among the fertilized vs. unfertilized plots. Treatment significantly affected DIA data for DGCI and color ratings only (Figures 1 and 3), percent cover was not affected by treatment. Stimp meter readings also were not affected by treatment, in either year.

Shoot density of creeping bentgrass is of the upmost importance, and can be directly related to playing surface quality. Due to the increases in shoot density observed, these results show the potential increased benefits of including an organic source of nitrogen in a fertilizer program.

While the potential benefits are indicated in this study, further research needs to be done. Future studies will focus on the use of isotopic forms of the BCAA, as well as the effect on growth under less optimal conditions. This work hopefully will lead to a better understanding of BCAA catabolism by plants, furthering knowledge of the turfgrass plant.

		Rate	Rate
Treatment	Product	(kg product/ha)	(kg N/ha)
1	Control	-	-
2	Urea	7.4	3.4
3	Commercial AA	44.5-L	3.4
4	BCAA (4 : 1 : 1)*	20:5:5	3.4
5	BCAA (4:1:1)**	10:2.5:2.5	3.4
6	BCAA (8:1:1)*	24:3:3	3.4
7	BCAA (8:1:1)**	12:1.5:1.5	3.4
8	BCAA (12:1:1)*	15:7.5:7.5	3.4
9	BCAA (12:1:1)**	25.8 : 2.15 : 2.15	3.4

Table 1. Treatment	list for various	s fertility treatm	ents tested.
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\*BCAA treatments containing L-leucine:L-isoleucine:L-valine.

\*\*BCAA treatments containing L-leucine:L-isoleucine:L-valine:and 50% of N coming from Urea.

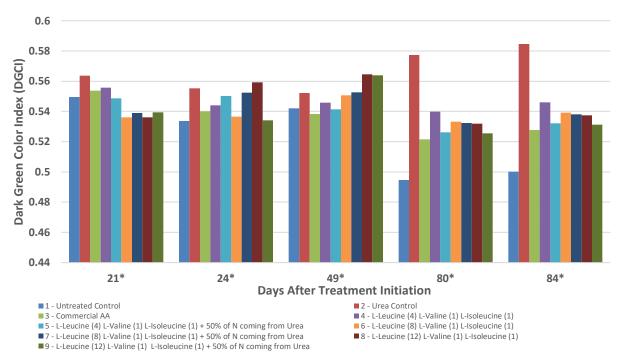


Figure 1. Average of DGCI values by treatment. Days with "\*" indicates a significant difference among treatments at  $\alpha = 0.05$ .

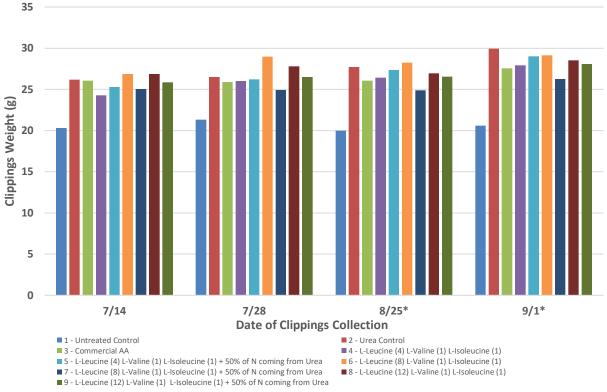
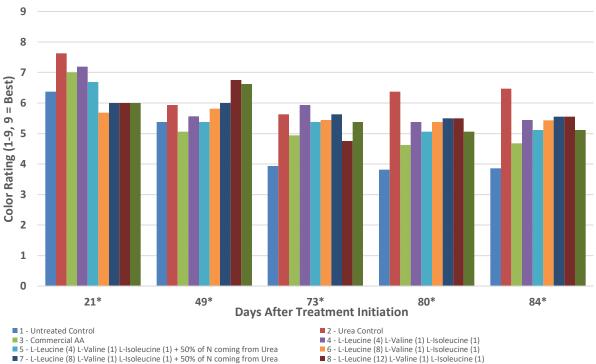


Figure 2. Average of clippings (g) by treatment. Bars within a day represent different treatments. Days with "\*" indicates a significant difference among treatments at  $\alpha = 0.05$ .



■ 7 - L-Leucine (8) L-Valine (1) L-Isoleucine (1) + 50% of N coming from Urea

9 - L-Leucine (12) L-Valine (1) L-Isoleucine (1) + 50% of N coming from Urea

Figure 3. Average of color ratings by treatment. Days with "\*" indicates a significant difference among treatments at  $\alpha = 0.05$ .

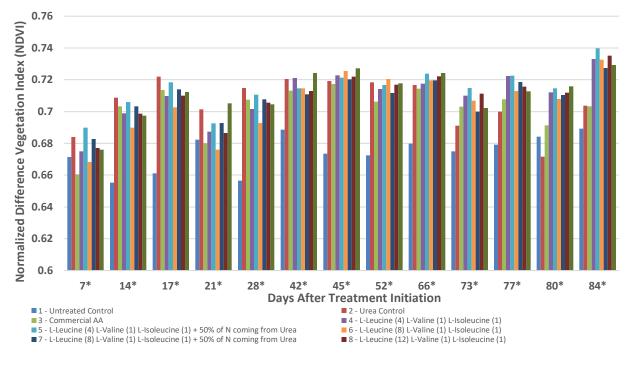


Figure 4. Average of NDVI measurements by treatment. Days with "\*" indicates a significant difference among treatments at  $\alpha = 0.05$ .

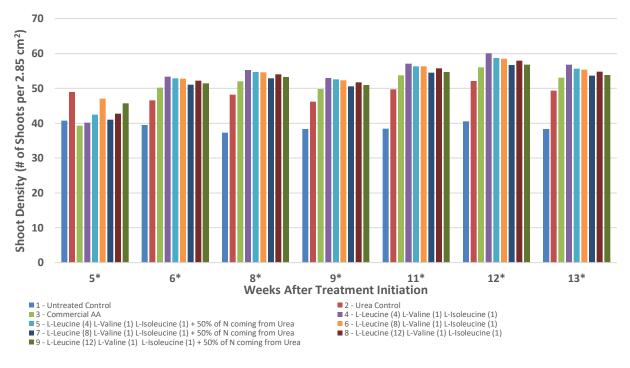


Figure 5. Average number of shoots per 2.85-cm<sup>2</sup>, by treatment. Days with "\*" indicates a significant difference among treatments at  $\alpha = 0.05$ .