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Article

Performance of *Panicum virgatum* cultivars in competition with *Bromus inermis* and differing amounts of N fertilizer

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SUMMARY

With growing demands for ethanol, many researchers are turning to *Panicum virgatum* (switchgrass) as a feedstock of cellulosic ethanol. This study was conducted to examine the germination, biomass, nitrogen, survival, and chlorophyll absorbance of two cultivars of switchgrass grown in competition with *Bromus inermis* (smooth brome) and with two different levels of nitrogen fertilizer. I predicted that these factors would affect the aforementioned variables, as nitrogen promotes plant growth, competition from other plant species detracts from nutrient availability, and these cultivars are physiologically different. The experiment was conducted in a greenhouse as 12 treatments replicated 20 times. Results indicated that the Liberty cultivar had lower germination, but higher survivorship than the Cave-in-Rock cultivar and that treatments with higher levels of competition resulted in lower biomass. Additionally, control treatments of both switchgrass cultivars grown in the absence of smooth brome had the highest levels of chlorophyll absorbance, and competition treatments had overall lower levels of nitrogen than control treatments. Both levels of nitrogen fertilizer decreased chlorophyll absorbance. Furthermore, switchgrass cultivars grown in competition with smooth brome had lower levels of nitrogen compared to control treatments. My results also indicate that during establishment, competition from other species has a greater effect than nitrogen fertilizer. Replicating this experiment for a multi-year randomized block setup would be ideal for extending this study.

INTRODUCTION

Panicum virgatum, more commonly known as switchgrass, is a native perennial warm-season grass (NPWSG) native to North America. It has been used in hay systems for livestock, as a soil conservation crop, as well as for wildlife cover. Switchgrass is also considered an ideal herbaceous model for biomass energy (1). As a high yielding native prairie grass, switchgrass has the potential to be a major source of bioenergy due to its low input requirements, as well as its broad range of adaptation (2).

I conducted this study to analyze several crucial aspects of switchgrass management as a cellulosic ethanol crop when establishing the crop in competition with *Bromus inermis*, or smooth brome, a non-native cool season perennial grass selected in previous studies as a vigorous competition model (3). This study also addressed the performance of the upland switchgrass cultivar “Cave in Rock”, which is typically used for wildlife cover and reestablishing grasslands, and the lowland switchgrass cultivar “Liberty”, developed for use as a cellulosic ethanol feedstock for the Great Plains region. Additionally, because nitrogen fertilizer is commonly considered one of the most important inputs for a producer, I varied applied at planting to assess its effects (4-5). Upon the conclusion of the three-month growth period, dry biomass yield, I assessed the number of surviving switchgrass and smooth brome plants, chlorophyll absorbance, plant nitrogen content, and soil nitrate levels.

I hypothesized that treatments receiving urea nitrogen fertilizer would experience increased germination levels, plant survival, nitrogen content, chlorophyll absorbance, and dry biomass yield in both switchgrass cultivars. Conversely, I also hypothesized that smooth brome competition would affect germination levels, plant survival, nitrogen content, chlorophyll absorbance, and dry biomass yield in both switchgrass cultivars, with all of these variables decreasing as the level of competition increased.

RESULTS

Methods Summary

Cubic foot pots were placed in a heated greenhouse kept at and filled with soil. Each pot received one treatment comprised of one of two switchgrass cultivars (Cave in Rock or Liberty), one of two levels of urea nitrogen fertilizer, and one of three smooth brome competition levels (no competition, 33% competition, and 67% competition). With every combination including 1 variable from each field (switchgrass, fertilizer, and competition level), 12 treatments were created, each of which was replicated 20 times for a total of 240 pots. The location of these pots within the greenhouse was randomized to avoid biased management and to mitigate variable climate conditions in the greenhouse. Pots were planted switchgrass to 30 pure live seed (PLS) per pot, with smooth brome competition percentages (0%, 33%, and 67% making up the remaining seedlot. Plants were allowed to grow normally for three months, during which plants were watered once per week. Two stand counts (one taken shortly after germination, and one taken just before harvest) were performed in which emerged switchgrass plants were counted. After the growth period, a SPAD chlorophyll meter was used to measure chlorophyll absorbance of switchgrass leaves, and plants

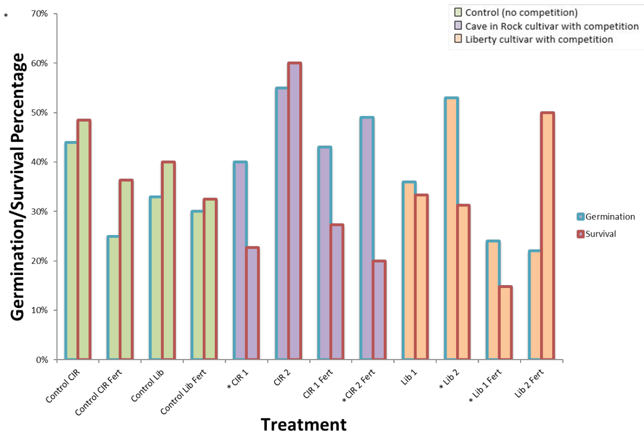

Figure 1. Germination and Survival. Germination data (taken 43 days after planting) by treatment compared to survival (taken 38 days after germination recorded) at conclusion of growth period. Single-factor ANOVA and Tukey-Kramer test were run. Asterisks indicate treatment with statistical significance ($p < 0.05$) between treatment and corresponding control treatment conditions according to Tukey Kramer test.

Treatment Name	Cultivar	Competition	Fertilizer
Control CiR	Cave in Rock	None	None
Control CiR Fert	Cave in Rock	None	1.1g urea N per rep.
Control Lib	Liberty	None	None
Control Lib Fert	Liberty	None	1.1g urea N per rep.
CiR 1	Cave in Rock	33% smooth brome per rep.	None
CiR 2	Cave in Rock	67% smooth brome per rep.	None
CiR 1 Fert	Cave in Rock	33% smooth brome per rep.	1.1g urea N per rep.
CiR 2 Fert	Cave in Rock	67% smooth brome per rep.	1.1g urea N per rep.
Lib 1	Liberty	33% smooth brome per rep.	None
Lib 2	Liberty	67% smooth brome per rep.	None
Lib 1 Fert	Liberty	33% smooth brome per rep.	1.1g urea N per rep.
Lib 2 Fert	Liberty	67% smooth brome per rep.	1.1g urea N per rep

Table 1. Description of Treatments.

were harvested for a dry biomass measurement and for percent nitrogen, measured by Ward Laboratories.

Germination/Survival

The effect of different levels of nitrogen fertilizer and competition from smooth brome on germination and survival of two cultivars of switchgrass was assessed (**Figure 1**). An ANOVA test performed on germination and survival data showed different levels of nitrogen fertilizer resulted in differences in survival rate ($p = 3.88 \times 10^{-6}$). Individual treatment comparisons revealed that all control treatments, which contained no smooth brome competition (Control CiR, Control CiR Fert, Control Lib, and Control Lib Fert, **Table 1**), resulted in statistically increased survival, but not germination, as compared to those with competition ($p < 0.05$, Tukey-Kramer test) (**Figure 1**). The Cave in Rock treatments, with the exception of CiR 2 (Cave in Rock cultivar with 67% smooth brome competition and no fertilizer; **Table 1**), all showed relatively low survivorship. The results also indicate that the Liberty cultivar had better survivorship compared to the Cave in Rock cultivar.

Biomass

Dry biomass is depicted as grams of dry aboveground matter taken from four replicates for each treatment (**Figure 2**). Control treatments (**Table 1**) with nitrogen fertilizer resulted in lower germination than their non-fertilized cultivar counterparts (**Figure 2**). With treatments containing the Cave in Rock cultivar and competition from smooth brome, increased competition resulted in lower biomass yields regardless of fertilizer rate. This trend was also observed in the treatments containing the Liberty cultivar and competition from smooth brome, though with less disparity. A correlation test showed a moderate positive correlation between biomass and chlorophyll absorbance (**Figure 5**).

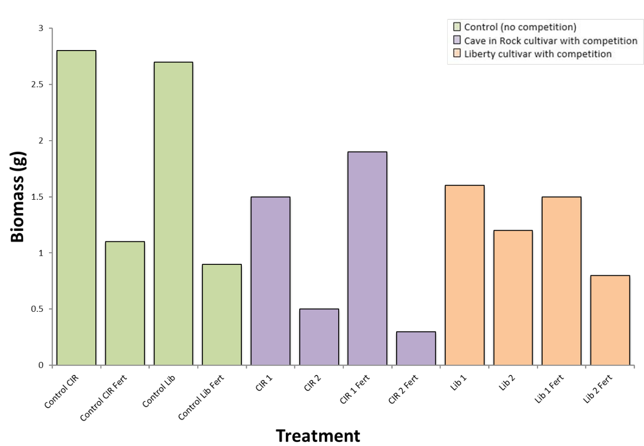

Figure 2. Dry Biomass. Dry biomass of four replicate pots combined per treatment.

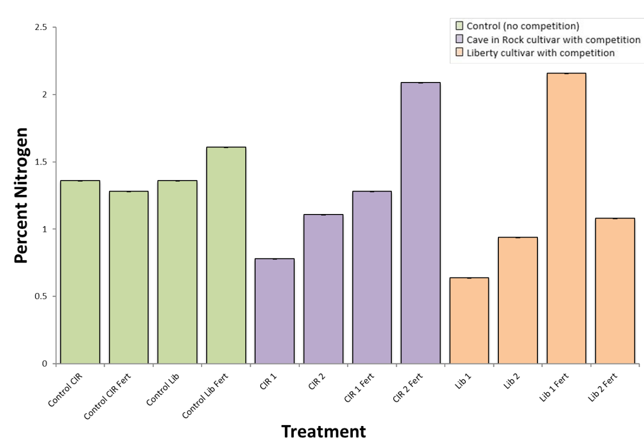

Figure 3. Percent Nitrogen. Percent nitrogen of five replicates per treatment of total elements calculated by Ward Labs using Crude Protein Combustion Method.

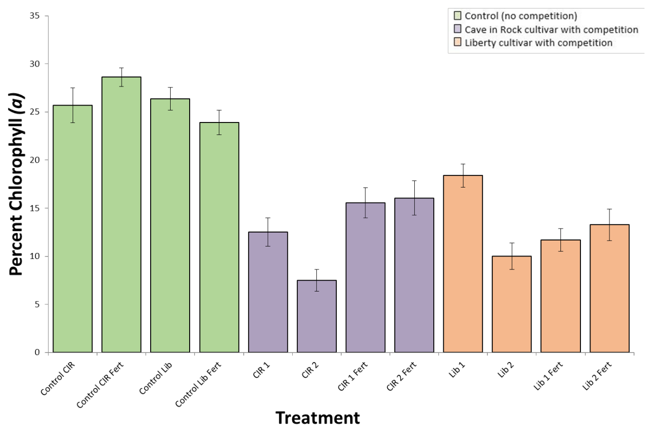

Figure 4. Percent Chlorophyll Absorption. Average percent absorbance of chlorophyll per treatment. Single-factor ANOVA and Tukey-Kramer test were run. Single-factor ANOVA yielded *p*-value of 4.7×10^{-41}. Thin black lines are standard error.

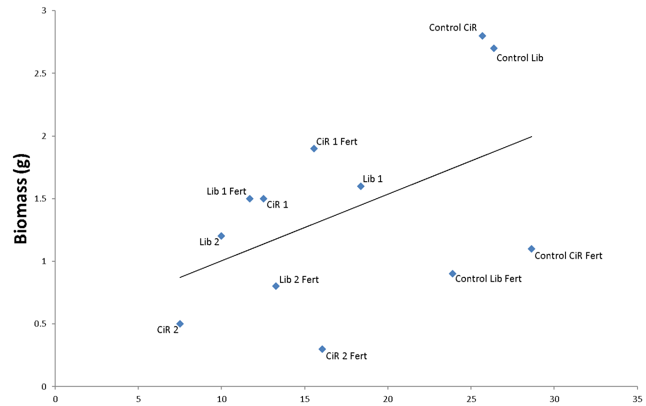

Figure 5. Correlation Coefficient Test of Dry Biomass and Chlorophyll Absorbance. Pearson correlation coefficient test indicates moderate positive correlation (R=0.48) between chlorophyll absorbance and dry biomass. Each data point depicts the relationship between chlorophyll and dry biomass per treatment.

Nitrogen

Depicts the nitrogen content refers to five replicates from each treatment (**Figure 3**). These results indicate that all treatments contained similar levels of nitrogen. Control treatments contained higher levels of nitrogen, but all treatments were within two percent of total nitrogen.

Chlorophyll

We next examined the chlorophyll absorbance between the different treatments (**Figure 4**). An ANOVA test performed on chlorophyll data yielded a *p*-value of 4.7×10^{-41}, which indicated statistical significance. This ANOVA was followed by a Tukey-Kramer test in order to determine statistical significance for individual treatment comparisons. All experimental manipulations (CiR 1, CiR 2, CiR 1 Fert, CiR 2 Fert, Lib 1, Lib 2, Lib 1 Fert, and Lib 2 Fert) all had statistically lower chlorophyll absorbance levels compared to their corresponding control (**Figure 4**). Treatments containing the Cave in Rock cultivar and competition from smooth brome showed a statistical difference between fertilized and unfertilized treatments, with the unfertilized, more-competitive treatment ranking statistically lower than all other treatments containing the Cave in Rock cultivar and smooth brome competition. The chlorophyll absorbance of the treatments containing the Liberty cultivar and competition from smooth brome was statistically equivalent, with the exception of the unfertilized, less-competitive treatment, which showed a statistically greater absorbance in chlorophyll compared to the other three treatments containing the Liberty cultivar and competition from smooth brome. A correlation test showed a moderate positive correlation between chlorophyll and biomass (**Figure 5**).

DISCUSSION

This study was conducted with the intent of determining the effect of different levels of competition of smooth brome with differing levels of nitrogen fertilizer on two cultivars of switchgrass. Treatments without competition yielded statistically higher survivorship when compared to treatments with competition. Additionally, treatments that contained greater levels of urea nitrogen fertilizer and competition resulted in lower dry biomass yield, while total nitrogen levels were similar for all treatments. It should also be noted that the application of urea nitrogen fertilizer at planting resulted in lower biomass for treatments containing no competition as well as for those treatments which contained competition. Finally, switchgrass grown in the absence of smooth brome competition showed statistically greater levels of chlorophyll absorbance. The data from this study suggests that during stand establishment, the presence of competition has a negative effect on both cultivars of switchgrass. Furthermore, these results suggest that application of urea nitrogen fertilizer at planting did little to affect switchgrass growth, but likely aided the smooth brome competitor and impeded the young switchgrass plants in their ability to produce biomass, which in addition to the quality of major cell wall components such as lignin and cellulose, is a major factor in cellulosic ethanol yield (6). This claim is supported by past studies on switchgrass, which recommend foregoing nitrogen application during switchgrass establishment, as the crop is unable to effectively utilize the nitrogen and outperform competitor species until it has matured for one year (2).

The data collected in this study suggests that that managing competitor species during the establishment period is of the utmost importance to the producer, as nitrogen fertilizer applied once the stand is mature enough to benefit from it is likely to aid and propagate any remaining competitor species like smooth brome. Contrarily, a legume species, when grown in concert with a well-established stand of switchgrass, has been shown to increase switchgrass biomass, as it is able to access nitrogen fixed by the legume species (7). Replicating this experiment in a multi-year random block design study would prove beneficial for gauging the effects of competition and nitrogen fertilizer application at all times in the life cycle of switchgrass produced for cellulosic ethanol, rather than just the establishment period.

METHODS

Preparation

Pots were filled with one cubic foot of Lockton series soil (8), saturated with water, assigned a number between 1 and 240, and placed in a location that corresponded with a randomly generated number in a heated greenhouse. Pots were then left overnight to drain. Using damp soil, pots were refilled the following day to create a smooth, level, compact surface for planting. Seed treatments were created using Liberty seed from UNL's USDA Agricultural Research Service, Cave in Rock seed from Stock Seed Farm, urea nitrogen fertilizer sourced from Anderson Farms, and smooth brome seed from Deer Creek Seed Inc.

Generating Treatments

Each treatment was one combination of the aforementioned variables. Each contained one of two switchgrass cultivars (Cave in Rock or Liberty), one of two levels of urea nitrogen fertilizer (0g or 1.1g), and one of three smooth brome competition levels (no competition, 33% competition, and 67% competition). With every combination including 1 variable from each field (switchgrass, fertilizer, and competition level), 12 treatments were created, each of which was replicated 20 times for a total of 240 pots. Treatments were assembled using a sorting knife by counting switchgrass seeds to 30 pure live seeds (PLS) per treatment, with the smooth brome competition percentages (0%, 33%, and 67%) making up the remaining seedlot. This meant that for treatments containing no competition, those pots received either 33 Cave in Rock seeds per pot, or 40 Liberty seeds per pot. Similarly, treatments that contained 33% competition from smooth brome received 22 Cave in Rock seeds per pot or 27 Liberty seeds per pot, with the smooth brome seeds added to 30 seeds total per pot. Finally, treatments that contained 67% competition from smooth brome received 10 Cave in Rock seeds per pot or 16 Liberty seeds per pot (**Table 1**). PLS was only calculated for the switchgrass, not for the smooth brome. Treatments requiring fertilizer received 1.1g of urea nitrogen fertilizer dispersed with the seeds at planting.

Planting and Maintenance

To avoid bias, each greenhouse location's randomly assigned treatment was recorded onto a master sheet, which was hidden from the researcher until data was evaluated. Pots were planted in a broadcast manner with thin layer of soil applied evenly over the scattered seeds. After the seeds were planted and covered with soil, pots were re-wetted with a seedling wand, taking care not to disturb the seeds. The internal temperature of the greenhouse was kept at 16°C using propane heaters.

Data Collection

Germination data was taken 43 days after planting to ensure easy distinction between brome and switchgrass species. After germination data was taken, pots were watered for three seconds each using an irrigation wand once per week. After the three-month growth period, a SPAD chlorophyll meter manufactured by Konica Minolta was used to measure chlorophyll absorbance, then plants were harvested at soil-level, dried, and weighed for a dry biomass yield. Soil samples were collected from each treatment as well as from soil in which plants were not grown and was dried and packaged for an S1 soil analysis by Ward Laboratories. Additionally, dried harvested plants were sent to Ward Laboratories for nitrogen analysis.

Statistical Analysis

After data was collected, a series of statistical tests were performed using Microsoft Excel 16.0. For germination/survival and chlorophyll results, a single-factor ANOVA was run, with a Tukey-Kramer test performed after that in order to determine statistical significance between individual treatment combinations (e.g. Control CiR (Cave in Rock cultivar) and Control CiR + Fert, Control CiR and Control Lib. (Liberty cultivar), etc.), so every combination was analyzed. The combinations that differed significantly ($p < 0.05$) were then used to draw conclusions. Biomass data was collected by compiling the harvested grass from 4 replicates per treatment into 1 sample, and then weighed on a scale with a linearity of +/- 0.01 which produced 12 individual data points (1 for each treatment). A similar process was used in obtaining percent nitrogen results. 5 replicates per treatment were harvested and compiled into 1 sample (12 data points total) which was measured using Ward Laboratory's Crude Protein Combustion Method which has a nitrogen detection limit of 0.005% and is generally reproducible within +/- 5.0%.

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