Developing Agronomics of Intermediate Wheatgrass as a Perennial Grain

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Abstract

The forage species intermediate wheatgrass (*Thinopyrum intermedium* L.; IWG) is being developed for use as a perennial grain crop. Field tests have shown that improved IWG populations are suitable for many food applications, creating demand for IWG grain. Producers are eager to learn how IWG can be incorporated into their cropping systems. However, a lack of understanding of IWG morphological characteristics related to grain production has limited the ability for researchers to recommend best management practices for increasing grain yields. The first objective of this research was to quantify the morphological development of an IWG population managed under field conditions as a grain crop. In 2015 and 2016, data on the growth and development of IWG was collected from spring growth through plant senescence, with an emphasis on reproductive development and changes in seed moisture through time. We used a quantitative growth index to report IWG development as a function of growing degree days (GDD). Growth parameters including plant height, first node height, and biomass partitioning, were measured through time and analyzed in response to GDD. This information allows the ability to make growth predictions based on rainfall, temperature, and other management decisions that may impact how an IWG sward develops. By describing IWG development through quantitative modeling, we generated useful information that can be applied to practical management decisions in future production of the perennial grain crop. A production challenge limiting IWG grain production is stem lodging, which hinders pollination, grain fill, and reduces overall grain quantity.

The second objective of this research was to measure the effect of nitrogen fertilizer and plant growth regulators (PGRs) on IWG grain yield and morphology. Trinexapac-ethyl (TE) and prohexadione-calcium (PC) are PGRs widely used for mitigating lodging and improving seed yields in the turf grass seed industry, and have potential for use in IWG. The second study examined the effect of two PGRs (PC and TE), application rate, and nitrogen (N) fertilizer rate (0, 40, 80 kg N ha^{-1}) on IWG lodging, plant height, and grain and biomass yield. Trinexapac-ethyl reduced lodging and increased IWG grain yields by 65 to 80% relative to PC and the non-treated control. Applying TE at the recommended rate for grass seed production or at a rate 1.5x the recommended rate reduced plant height compared to the non-treated control or IWG treated with the 0.5x rate. An N rate of 80 kg ha^{-1} increased IWG lodging in both years, though N rate effects on grain yields were minimal. Pursuit of EPA approval for application of PGRs to IWG is needed for more thorough evaluation of PGR potential for improving the agronomic potential of perennial cereal crops. Results show that applying TE at rates recommended for other grass species has the potential to increase IWG grain yields.

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Chapter 1

Intermediate Wheatgrass as a Perennial Grain Crop: Progress and Production Challenges

A literature review

Introduction

The majority of human calories come from annual grain crops (Awika, 2011). In Minnesota, approximately 60 percent of all farmland, or about 16 million acres, are allocated to corn and soybean production annually (MDA, 2015). Annual cropping systems are often associated with soil disturbance and costly inputs compared to natural perennial land cover (Glover et al., 2010). The displacement of natural systems with row crop agriculture across the landscape has also reduced the ecosystem services and biodiversity supported by native landscapes (Foley et al., 2005). Growing awareness of the consequences of current agricultural practices has prompted Minnesota as well as other states to develop enduring conservation programs. Well known programs include the Conservation Reserve Program (CRP), Conservation Reserve Enhancement Program (CREP); which seek to replace agricultural row crop landscapes with perennial species, promote ecosystem services, and provide sufficient habitat for wildlife. While the adoption of these programs is subsidized by government agencies, the land is temporarily or permanently retired from farming, offering limited economic opportunity for growers and land-owners alike (USDA, 2005).

Perennial crops are under development to provide agroecosystems with year-round ground cover and active roots that sequester carbon, retain moisture, and cycle nutrients in soils (Asbjornsen et al., 2013). Rapid advancements have been made to domesticate the perennial grass intermediate wheatgrass (*Thinopyrum intermedium*; IWG) into the first widely available perennial grain crop (DeHaan et al., 2016). Intermediate wheatgrass is a perennial, cool-season forage grass identified as a candidate for domestication into a perennial grain crop due to its large seed size, compared to other perennial grasses (DeHaan et al., 2005). Introduced to the U.S. in 1932, IWG was implemented as a forage crop in the upper Midwest and northern Great Plains due to its winter hardiness and high forage quality (Hitchcock, 1950; Moore et al., 1995). The species develops deep, fibrous roots that provide ecosystem services such as enhanced soil conservation, reduced nitrogen (N) leaching, and carbon sequestration (Culman et al., 2013), and could mitigate some negative environmental impacts of agriculture if used to replace annual row crops. Efforts to develop IWG into a grain crop by increasing grain size and yield has been a focus for breeders over the last decade (DeHaan et al., 2005).

Plant breeding efforts to develop IWG as a perennial grain were first initiated by the Rodale Research Center in Pennsylvania in 1988 (Wagoner, 1990). Since the Land Institute and University of Minnesota have undertaken this project, four cycles of phenotype recurrent selection have focused on improving of the major determinants of grain yield which include seed size, floret utilization, and inflorescence (spike) weight (DeHaan et al., 2014; Kantar et al., 2016). The best germplasm available harvested mechanically produced approximately 950 kg ha^{-1} de-hulled seed in Minnesota and seed size larger than 15 mg (Zhang et al., 2016). Breeding work has produced improved populations of IWG that produce higher grain yields under field conditions compared to forage varieties (Jungers et al., 2017). The grain is also suitable for many food applications, resulting in the emergence of a commodity market for IWG (Young, 2015; Zhang, 2015).

As a cereal food crop, IWG grain is similar nutritionally to common wheat (*Triticum aestivum* L.), but with slightly higher protein and lower gluten content (Becker et al., 1986). Recognized by the American Food and Drug Administration as a safe food source, advancements have been made to develop the IWG grain enterprise, with much of the emphasis directed toward end-use products. Consequently, market interest in IWG – called Kernza®, a trademark name given by The Land Institute - has swelled, creating demand for IWG grain. With producers eager to learn how IWG can be incorporated into their cropping systems, the University of Minnesota is conducting agronomic research to develop best management practices for an array of cropping systems where IWG could fit and be utilized effectively for both its grain and forage qualities.

As a cool-season forage grass, IWG initiates growth earlier in the spring compared to warm-season perennials while maintaining a relatively high forage quality (Asay, 1995). Early vegetative spring growth of IWG has the potential to be grazed as an additional source of feed for livestock because it is able to recover in time for grain harvest that summer. The fall vegetative regrowth following grain harvests may also be grazed. Feed represents the major cost in most livestock production systems (Ballard, 2004). The utilization of on-farm sources of animal feed is an effective way to maintain an economically viable livestock system. Following grain harvest, fall regrowth of IWG is ideal for grazing because they are relatively high in nutritive value for livestock (Hendrickson et al., 2005). Utilizing IWG in Midwest cropping systems will provide growers with new markets for economic opportunity, and give animals an additional source of feed throughout the year. Being able to accurately time these forage harvest events is crucial for ensuring persistence and productivity of IWG grain production for the following growing seasons. To accurately apply agronomic treatments to IWG, the underlying growth and development characteristics of the crop when grown as a grain crop should be addressed in the field.

Forecasting future growth and development of IWG is critical for optimizing management as a dual-use grain crop. Knowing the rate of development for vegetative, structural, and reproductive plant components throughout the growing season will enable growers to understand the production potential, as well as management restrictions associated with growing perennials for their grain. It is also important to understand how differences in growing environments and climate conditions determine grain and biomass yields. This will allow for growers to exercise the appropriate management strategies to successfully grow IWG on the landscape. Before large-scale adoption of IWG, agronomic research will need to address production challenges of the crop when grown for its grain, forage, or both.

Agronomic challenges and a gap in knowledge surrounding IWG development are currently limiting overall IWG grain production. Such challenges include diminishing grain yields as stands age, and stem lodging. In this thesis, these obstacles are addressed by two research projects. Intermediate wheatgrass grain yields are typically highest during the first or second year of production (Wagoner, 1990), experiencing a dramatic decline in year three. The cause of this decline is currently unknown, but could be related to changes in growth and development of IWG over multiple growing seasons. Describing and quantifying the morphological stages of development for perennial grain crops is important for the development of agronomic practices. With sufficient knowledge about the timing of key morphological events, treatments can be more accurately timed and will be more effective at managing the crop as needed for optimal grain and biomass production across multiple growing seasons.

Lodging can severely reduce grain profitability by compromising the yield and quality of cereal crops (Sterling et al., 2003). It is well understood that nitrogen fertilizer improves grain yields (Koeritz et al., 2013). However, when grown at high densities, grain crops can develop smaller stem diameters compared to those at lower densities, which leads to lodging (Gimplinger et al., 2008; Snider et al., 2012). Plant growth regulators (PGRs) have also been introduced as a viable option to reduce the incidence of lodging in stands of IWG because of their success in ryegrass (*Lolium perenne* L.) production (Chastain et al., 2014; Koeritz et al., 2013). With EPA approval, PGRs could be used at a large scale to manage lodging in fields of IWG. However, it was a goal of this research to identify the potential benefits and uses of PGRs in IWG cropping systems that are managed for high grain yields.

Chapter 2

Growth and Development of Intermediate Wheatgrass as a Perennial Grain Crop Under Field Conditions

Quantifying the growth and developmental characteristics of IWG through time

Introduction

Methods for describing and quantifying the morphological stages of development for annual grain crops have promoted the agronomic advancements of these crops for decades (Haun, 1973; Ritchie and Alagarswamy, 1989). Also, numerical indices have been developed to quantify morphological development of perennial grasses (Moore et al., 1991; Simon and Park, 1983; West, 1990). One example is the index developed by Moore et al. (1991), which was intended for use in forage and range management but also has useful application regarding perennial grains. The system is based on the physical characteristics of individual tillers, allowing growers to classify the average plant stage in a field with ease (Moore et al., 1995). It is divided into primary- and sub-stages which all correspond to morphological events of perennial grasses in a growing season. Growth stage quantification systems such as this would be useful for optimizing management practices of IWG that rely on an understanding the timing of morphological events. Such management includes planting, pest management, fertilization, and harvest.

Growing degree days (GDD) have also been used to inform crop management decisions (Miller et al., 2001). Determining the relationship between growth and development and GDD accumulation is a common method to model crop production, and is useful for improving the management of row crops. Knowledge of the association between GDD and plant height, as well as the first node (apical meristem) is valuable if IWG is to be managed for both its forage and grain potential. Defoliation at certain growth stages may affect regrowth and persistence of the perennial grass (George and

Reigh, 1987). If it is known when stem elongation occurs in relationship to GDD, forage harvests can be more accurately timed to maximize biomass production of IWG.

In regards to the reproductive components, seed yield of cool season grasses in response to GDD is typically expressed quadratically, with a maximum yield potential achieved around the asynchronous development and maturation of reproductive tillers (Berdahl and Frank, 1998). Knowing when grain mass is expected to peak based on GDD will ensure that grain is not harvested too early (Berdahl and Frank, 1998). Predicting reproductive crop development is also important for creating new cultivars with a desired relative maturity range (Ritchie and Smith, 1991). This mitigates issues regarding harvesting under-ripened grain, as well as harvesting too late and experiencing shattering. Intermediate wheatgrass is a relatively new grain crop for which seed shatter is still a challenge. Therefore, knowledge on the rate IWG grain matures with increasing GDD can be used to predict, and potentially prevent, shattering and associated declines in grain yield.

With the opportunity for producers to manage IWG for both its forage and grain, data on the allocation of aboveground biomass to vegetative and reproductive components and how that distribution changes through time can guide management of IWG as a dual-use crop. Many crop simulation models use biomass allocation fractions for predicting growth and development. Plant growth models come in a wide variety, ranging from simple formulas that capture plant size over time to complex models that predict growth trends in response to short and long term climate change (Fourcaud et al., 2008). Intermediate wheatgrass growth and development has not been reported in detail, and therefore has not been parameterized for many widely available crop simulation models. Biomass allocation, growth rates, and other crop developmental parameters are needed so that cropping systems including intermediate wheatgrass can be simulated and compared to common annual crop rotations.

The objective of this study was to quantify the morphological development of a new grain-type IWG population managed under field conditions. We used a quantitative growth index to report IWG development as a function of GDD. This study reports the allocation of aboveground biomass from the initiation of spring growth through plant senescence, with an emphasis on reproductive development and changes in seed moisture through time. This information will allow for more accurate forecasting of plant productivity, as well as the potential plant restrictions associated with growing perennials for their grain over several seasons.

Materials and Methods

Experimental sites and design

Field experiments were conducted at St. Paul and Rosemount, MN. At St. Paul, an improved grain-type IWG from the fourth cycle of a breeding program at The Land Institute (Salina, KS) was seeded at a rate of 12 kg ha^{-1} in 15 cm rows with 10 rows per plot on 5 September, 2014 for sampling in 2015 and 2016. The soil type is a Waukegan silt loam (fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls). The herbicides Dual Magnum (S-metolachlor 82.4%, Syngenta, Basel, Switzerland) and 2, 4-D, (2, 4-dichlorphenoxyacetic acid) were applied on 3 April 2015 and 4 April 2016 at labeled rates for grass seed production. Ammonium nitrate was applied manually at 40 kg N ha^{-1} on 5 April 2015 and 6 April 2016. At Rosemount, an advanced grain-type of IWG from the University of Minnesota breeding program was sown on 4 September, 2015 at 12 kg ha^{-1} in alternating 41 and 61 cm rows. The soil type is also a Waukegan silt loam (fine-silty over sandy, mixed mesic, Typic Agiudoll). Dual Magnum (S-metolachlor 82.4%, Syngenta, Basel, Switzerland) and 2, 4-D, (2, 4-dichlorphenoxyacetic acid) were applied on 4 April 2016 at labeled rates for grass seed production. Ammonium nitrate was applied manually at 40 kg N ha^{-1} on 6 April 2016.

Data Collection

The field at St. Paul was divided into six replicates, each 5 m long and 1.5 m wide (10 rows). Plots were sampled every three to five days starting at green up in late March and ending at grain harvest in August. For each sampling event, one non-edge row was randomly selected. Within the randomly selected row, a 15 cm section was harvested at the soil level to remove all aboveground plant biomass. In 2015 and 2016, the total number of all stems – reproductive and vegetative – were counted. The total number of spikes were counted for each sample during the reproductive phase. Five stems were randomly selected from each sample and were measured for total plant height, first node height (from soil level to the tallest node on the plant), and staged using the Moore growth index (Moore et al., 1991). After the growth and development measurements were recorded, the entire sample for each plot was separated by hand into leaf, stem, and spike components. Each component was dried at 60°C for 48 hrs and weighed for dry matter mass. Water concentration of seeds was also measured and expressed on a wet weight basis. The mass of 50 seeds extracted from 10 spikes was recorded from wet samples that were then dried at 60°C for 48 hrs and weighed again for dry weights.

The field at Rosemount was divided into 10 plots, each 6 m long and 3 m wide containing 6 rows that alternate with 41 and 61 cm spacing. Plots were sampled every five days starting at green up in late March and ending at grain harvest in August. For each sampling event, one row per plot was randomly selected and 30 cm of planted IWG row was harvested using the same methods as those used at St. Paul. The same data were collected using the same procedures as those described above for St. Paul. For both sites, inflouresence components were measured at time of grain harvest. For one sampling date, an additional 30 cm of planted IWG row was harvested at the soil level. The number of total spikes was recorded for 30 cm planted row, and the total seed yield was obtained to express seed mass per spike.

Weather Data

Daily precipitation and temperatures (°C) were recorded from National Weather Service Reporting Stations located at the experimental sites, recording on a midnight to midnight basis (US climate data, 2017). Growing degree days were calculated as:

$$GDD = [(T_{max} + T_{min})/2] - T_{base}$$

where T_{max} and T_{min}, are maximum and minimum temperatures and T_{base} (the base temperature) is the minimum temperature at which plant growth can occur for IWG. A base temperature of 0 °C was used as a minimum temperature threshold for IWG plant growth. Growing degrees began when the average daily temperature exceeded the base temperature for 5 consecutive days.

Statistical Analysis

The R programming language (R Core Team, 2016) was used to analyze data. Analyses were conducted for each environment separately to avoid confounding effects of stand age and variable weather conditions. Linear regression was used to determine the relationship between GDD and the following response variables: growth index, plant biomass, first node height, plant height, leaf fraction of total biomass, stem fraction of total biomass, and spike fraction of total biomass. For all response variables, linear ($Y = \beta_0 + \beta_1 x$) and quadratic ($Y = \beta_0 + \beta_1 x + \beta_2 x^2$) models were developed using GDD as the continuous predictor variable. A likelihood ratio test was used to determine the optimal model based on the chi-squared statistic with 1 degree of freedom ($\alpha = 0.05$). To assess the inflorescence components of IWG, analysis of variance and Tukey's mean separation ($\alpha = 0.05$) was used to compare these response variables (spikes 30 cm^{-1}, Seed mass $spike^{-1}$, 50 seed weight, Spikes 30 cm $stem^{-1}$) across environments.

Results and Discussion

Weather Data

Weather patterns were consistent with long-term climate trends at each location (U.S. climate data, 2017). Total GDD and the distribution of GDD throughout the growing season were similar for the three environments (Figure 1a). From the start of the growing season until day 220, cumulative precipitation was similar across environments (Figure 1b). In 2016, St. Paul received 8.6 cm of precipitation on day 220, which resulted in greater total season cumulative precipitation for that environment. This event occurred near IWG grain harvest, thus it did not strongly influence the variability in IWG growth and development across environments.

Growth Index

The relationship between GDD and growth index was positive for all environments (Figure 2). At St. Paul in 2015, a linear model best explained the relationship between GDD and growth index, while the best model was quadratic at St. Paul and Rosemount in 2016. Sampling was initiated later in the season in 2015 compared to 2016, which likely resulted in the different response functions for the two years. Although there were fewer data points collected from St. Paul in 2015, the linear model was still accurate and explained 94% of the variation in growth index with GDD during the sampling period (Table 1). Alternatively, the quadratic models used to relate growth index to GDD were adequate for St. Paul and Rosemount in 2016, both with coefficient of determination values of 98%.

The growth index developed by Moore et al. (1991) used here is based on key morphological events, some of which are important for managing a cool season perennial grass as a grain crop. The equations in Table 1 can be used to predict timing of morphological events useful for perennial grain production, such as anthesis and grain maturity, can be predicted using GDD (Table 2). Dates for morphological events are predicted to be similar for all measured environments with the exception of stem elongation at St. Paul in 2015, where sampling was initiated later in the year. Extending the linear model to predict morphological events during early or late times of the growing season may not be as accurate as environments where a quadratic model was most suitable.

Growth & Development

Quadratic models best predicted plant height, height of the first node, and plant biomass as a function of GDD across all environments (Figure 3, Table 1). For St. Paul in 2015, the quadratic model explained 71% of the variation in plant height with GDD during the sampling period. Similarly, the quadratic models used to relate plant height to GDD were adequate for St. Paul and Rosemount in 2016, both with coefficient of determination values of 94 and 92%, respectively. Consistent with trends explained by Moore et al. (1991), elongation ceased at the onset of spike emergence from the flag leaf. During the first half of the growing season, shorter average plant heights were observed for Rosemount compared to both St. Paul 2015 and 2016. Plants oriented at a wider spacing at the Rosemount site, it is possible that differences in light interception, as well as early season weed pressure may have delayed early season growth. Plants at Rosemount had comparable heights to both years at St. Paul at grain harvest (Figure 3a).

The quadratic model explained 59% of the variation in first node height with GDD during the sampling period at St. Paul in 2015. Quadratic models used to relate first node height to GDD were adequate for St. Paul and Rosemount in 2016, both with coefficient of determination values of 86 and 83%, respectively (Figure 3b). Tracking the height of the first node helps avoid removal of the apical meristem responsible for spike development later in the season. Bredja et al. (1994) found that avoiding removal of the apical meristem in early season harvests of switchgrass (*Panicum virgatum* L.) improved forage quality and biomass production later in the growing season. In order to effectively manage IWG as a dual-crop, spring forage harvests should be taken early May just prior to stem elongation to avoid grain yield losses and maximize forage quality (Table 2).

There was more variability in total biomass among plants at each sampling event compared to plant height and node height, which likely contributed to the lower coefficient of determination for the GDD model for the biomass response variable (Figure 3c). The equations developed for estimating IWG growth from GDD can be used to predict optimum timing for agronomic events, similar to the utility of estimating growth index from GDD. For example, producers can estimate GDD requirements for IWG to reach peak biomass, and use such data to predict the day at which this growth event might occur. Setting the derivative of the plant biomass response equation to 0 and solving for the X value provides a GDD estimate for when the biomass response curve plateaus. At St Paul in 2016, biomass yield plateaued at 3130 GDD, which occurred on day 195 (July 14, 2016).

Aboveground Biomass Allocation

The allometric models describing IWG aboveground biomass partitioning were similar across all three environments. The increase over time in allocation of resources to reproductive components was a result of counteracting curvilinear responses between both vegetative (leaf) and structural (stem) plant components (Figure 4). The fraction of total biomass allocated to leaves declined quadratically while the stem fraction increased quadratically with increasing GDD for all environments. These relative changes in leaf and stem biomass over time are consistent with those observed by Sanderson and Wolf (1995) who tracked switchgrass dry matter partitioning of stems and leaves as a function of GDD. When spikes started to emerge after boot stage, the relative mass of leaves was still greater than the relative mass of stems. Spike mass increased quadratically with GDD, and when spike mass increased, the relative mass of leaves and stems was nearly equal. The amount of biomass accumulated by reproductive plant components did not exceed 25% of the total aboveground biomass for all three environments. Since most of the dry weight of plants consists of carbon compounds, harvested grain yield is closely linked to the partitioning of photoassimilate between harvested and non-harvested portions of the crop (Gifford et al., 1984). Increasing the partitioning of photoassimilate to harvested plant components will require a better understanding optimum harvest timing, and reducing the potential grain loss by shatter before non-harvested plant components are able to senesce.

The fraction of total aboveground biomass allocated to stems, leaves, and spikes also provides important information for agronomic decision making. Across all three environments, the dry weight of stem and leaf components were equivalent during most of grain development until harvest. Estimating the relative mass of leaves vs. stems at various time points throughout the growing season can be used to predict relative differences in forage quality through time. Moore et al. (1995) reported a linear decline in forage quality through time in the spring from five different IWG populations including cultivars developed for grazing. Moreover, Berdahl et al. (1994) reported higher neutral detergent fiber (NDF) and lower nitrogen (N) content in stems vs. leaves. Our result showing that the ratio of stem to leaf mass increases through time explains the findings by Moore et al. (1995) and Berdahl et al. (1994). This supports the well-established trend that forage quality is reduced with plant maturity (Buxton, 1996).

This study provides some of the necessary growth and development information for IWG so that this crop can be parameterized in various crop simulation models. Growth response curve equations from this study can be used to estimate these parameters. Biomass allocation fractions are also common parameters for simulating soil nutrient cycling in agroecosystems. Our results can be used as baseline metrics for parameterizing crop simulation models, but more data are likely needed to enhance the precision and accuracy of these models to predict the environmental and economic outcomes of managing IWG for grain.

Reproductive Morphology

In this study there was particular interest in regards to IWG reproductive component development. In 2016, grain moisture was tracked shortly after anthesis through grain development, and decreased quadratically with GDD at St. Paul (Figure 5, $P < 0.001$, Adj. $R^2 = 0.93$). The number of spikes per 30 cm of row and seed mass per spike were greater in Rosemount in 2016 compared to St. Paul in both years (Table 3, $P <$

0.001 and P = 0.006); however, these measurements were similar across years within the St. Paul location (spikes per 30 cm row, P = 0.591; seed mass per spike, P = 0.935). Additionally, spikes stem^{-1} did not significantly change from 2015 to 2016 in St. Paul (Table 3, P = 0.543). The 50 seed weight was greatest in Rosemount in 2016 followed by St. Paul in 2016 and then St. Paul in 2015. The difference in yield components measured at Rosemount vs. St. Paul could be related to genetics and agronomics. The same seeding rate of 12 kg ha^{-1} was used at both locations, yet the row spacing was substantially wider at Rosemount resulting in more seed sown per unit of row compared to St. Paul. This could explain the higher spike density observed at Rosemount. Increasing seed mass and density per unit area is a top priority for trait selection for breeding IWG (Cox et al., 2010; Kantar et al., 2016), and the population used at Rosemount was more advanced compared to that used at St. Paul.

The lack of variability in spike density and seed mass spike^{-1} at St. Paul from 2015 to 2016 suggests that these yield components may be resistant to changes potentially induced by climate and stand age. Hendrickson et al. (2005) found that the net change in tiller number (difference between recruitment and mortality) varied by year, and indicated that water stress may have limited tiller recruitment. In our study, precipitation conditions were relatively similar across years at St. Paul, which may have limited the effect of year variability in spike density. Seed mass spike^{-1} also did not change from 2015 to 2016 despite there being differences in 50 seed weight. There was relatively more variability in seed mass spike^{-1} within each environment compared to 50 seed weight, which could have limited our ability to statistically detect any real changes in seed mass spike^{-1} through time. The increase in 50 seed weight from 2015 to 2016 at St. Paul could have occurred because the cumulative precipitation at St. Paul in 2016 surpassed precipitation in 2015 during grain filling (Figure 1b).

Conclusion

A baseline for quantifying the growth and morphological development of IWG has been presented. Knowledge regarding IWG morphology will be useful in developing management practices of a dual-use cropping system; as well as the precise timing of grain harvest in order to prevent under-ripe seeds or yield losses via seed shatter. This study provides some of the necessary information so that this crop can be parameterized in various crop simulation models. The results can be used alongside local climate data to project the outcomes of managing IWG for grain. The potential applications for the presented data will help streamline IWG into cropping systems across a wide-range of environments.

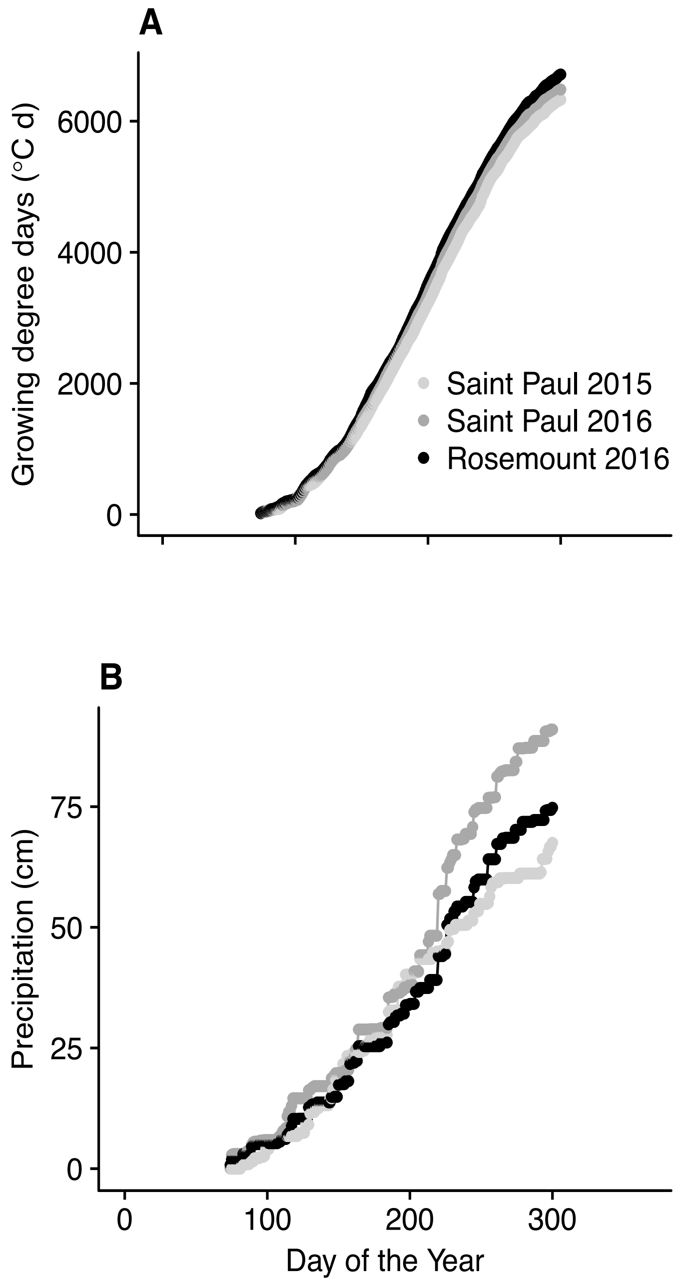

Figure 1. Accumulated growing degree days (GDD) for each environment calculated using a base temperature of 0°C starting March 14/15 for 2015 and 2016 respectively. Annual precipitation (cm) also shown for each environment. The weather stations that provided all GDD and precipitation data were located at the GPS coordinates 44.98472° N, 93.17722° W for St. Paul, and 44.7174° N, 93.0991° W for Rosemount, MN.

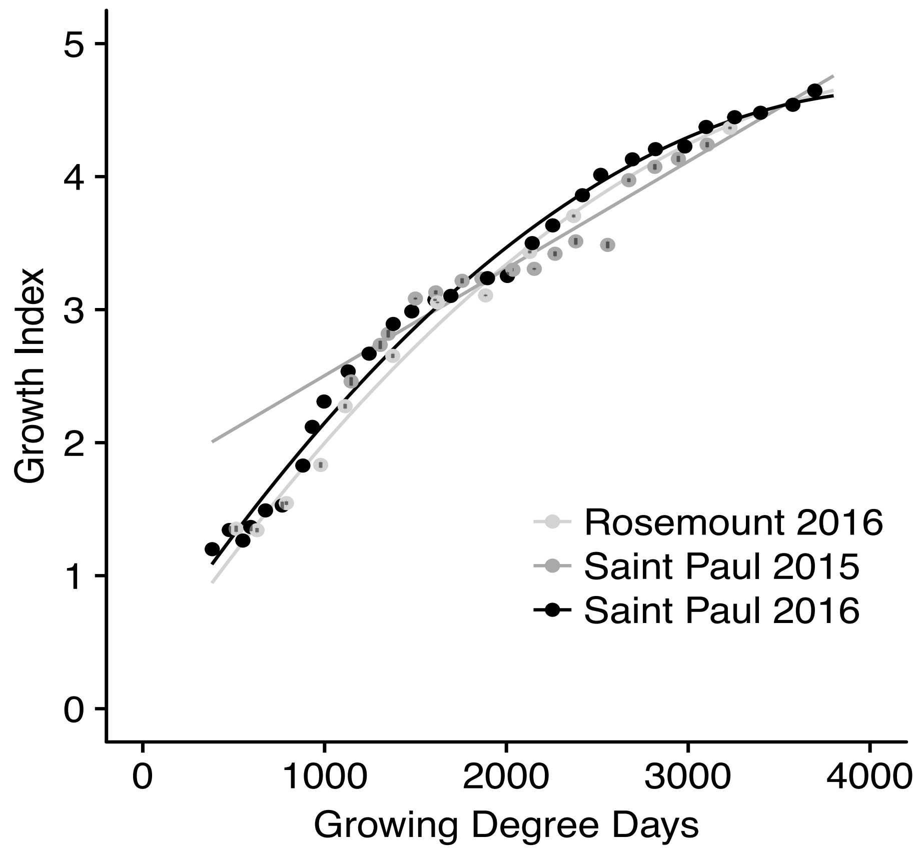

Figure 2. Intermediate wheatgrass (IWG) morphological development illustrated by the Moore et al. (1991) growth index in response to growing degree days (GDD) across three separate environments. Fitted linear or quadratic curves for separate environments. Fitted data were the means calculated for each environment from early spring to grain harvest as a function of Growing Degree Days (GDD).

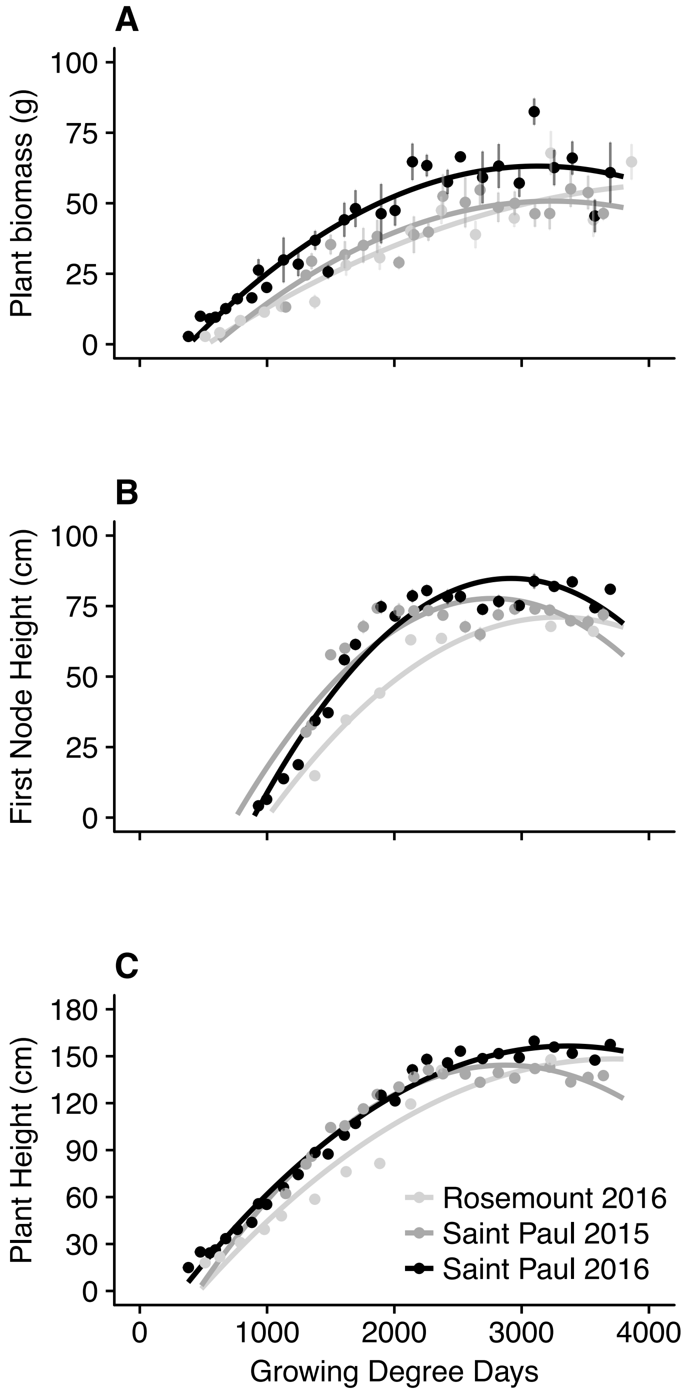

Figure 3. Fitted quadratic curves across environments for (a) total aboveground dry matter (DM), (b) first (tallest) stem node, (c) total plant height (cm). Fitted data were the means calculated for each environment from early spring to grain harvest as a function of Growing Degree Days (GDD).

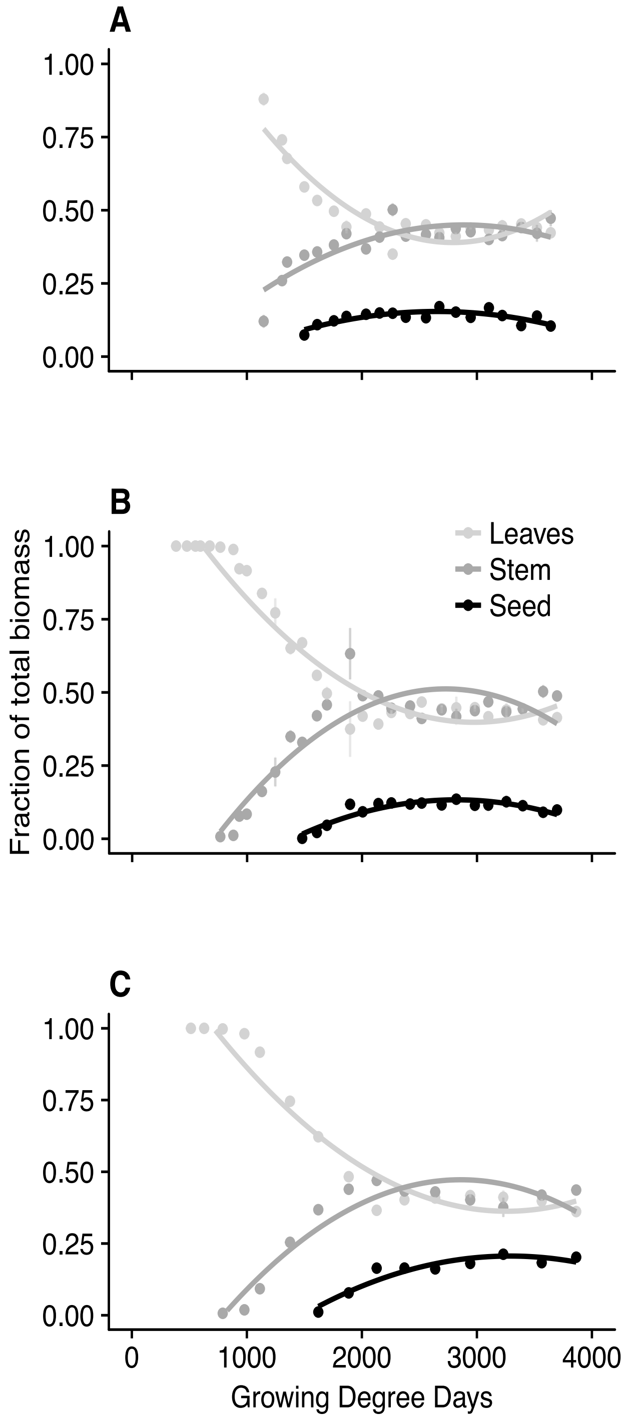

Figure 4. Relative aboveground dry matter distribution of IWG plants during the growing season. Presented partitioning patterns of IWG grown across three environments (a) St. Paul 2015, (b) St. Paul 2016, and (c) Rosemount 2016.

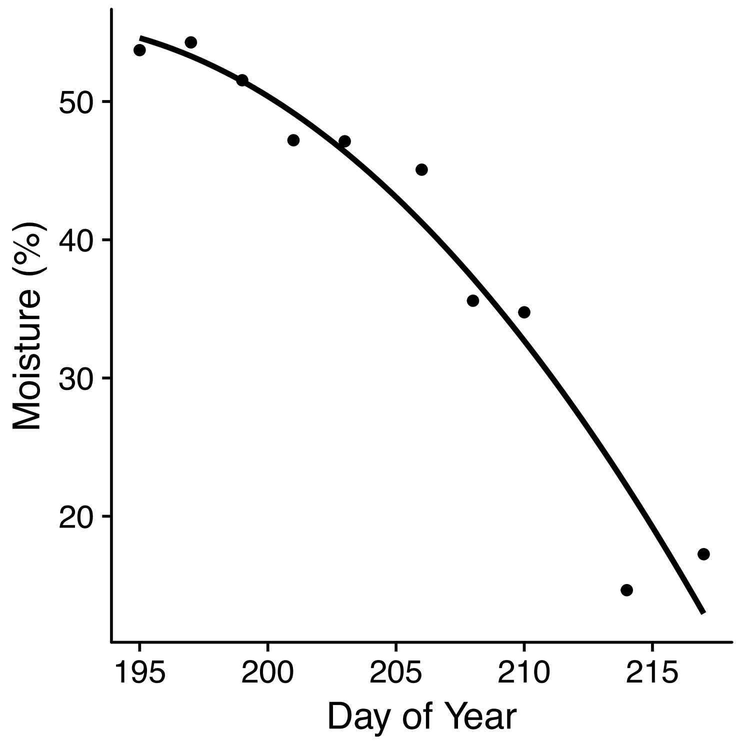

Figure 5. Changes in the moisture content (%, wet basis) of IWG grain during maturation drying and seed development in St. Paul, MN.

Table 1. Best-supported predictor model and parameter estimates for total aboveground dry matter (DM), first (tallest) stem node, total plant height (cm) for all three locations. *P* value comparing linear (LR) and quadratic (QD) fits for annual means for each environment. Adjusted R^2 reporting wellness of fit for model.

Location	Parameter	Model	β_0	β_1	β_2	**P*-value	Adj. R^2
St. Paul 2015	Plant height	QD	-5.94×10^{1}	1.42×10^{-1}	-2.47×10^{-5}	<.001	0.71
	Node height	QD	-6.88×10^{1}	1.06×10^{-1}	-1.91×10^{-5}	<.001	0.59
	Total Biomass	QD	-2.52×10^{1}	4.69×10^{-2}	-7.25×10^{-6}	<.001	0.38
	Growth Index	LR	1.699	8.05×10^{-4}	na	<.001	0.94
St. Paul 2016	Plant height	QD	-3.54×10^{1}	1.14×10^{-1}	-1.70×10^{-5}	<.001	0.94
	Node height	QD	-9.11×10^{1}	1.21×10^{-1}	-2.07×10^{-5}	<.001	0.86
	Total Biomass	QD	-1.91×10^{1}	5.25×10^{-2}	-8.39×10^{-6}	<.001	0.70
	Growth Index	QD	3.40×10^{-1}	2.05×10^{-3}	-2.45×10^{-7}	<.001	0.98
Rosemount 2016	Plant height	QD	-4.57×10^{1}	1.04×10^{-1}	-1.40×10^{-5}	<.001	0.92
	Node height	QD	-7.58×10^{1}	8.94×10^{-2}	-1.36×10^{-5}	<.001	0.83
	Total Biomass	QD	-1.65×10^{1}	3.31×10^{-2}	-3.71×10^{-6}	<.001	0.66
	Growth Index	QD	2.14×10^{-1}	2.00×10^{-3}	-2.20×10^{-7}	<.001	0.98

* *P* values significant at $\alpha = 0.05$

Table 2. Dates of critical IWG growth stages using predictor models generated in Table 1. Reporting Growing Degree Day (GDD) and the associated date and day of the year (DOY).

	Stem Elongation		Flag Leaf		Anthesis		Soft Dough		†Grain Maturity	
	GDD	Date (DOY)	GDD	Date	GDD	Date	GDD	Date	GDD	Date
St. Paul 2015	374	April 17 (108)	1618	June 9 (159)	2489	July 3 (185)	3235	July 22 (204)	3733	August 3 (216)
St. Paul 2016	908	May 15 (134)	1606	June 6 (155)	2237	June 23 (172)	3026	July 13 (192)	3887	August 3 (212)
Rosemount 2016	1004	May 16 (135)	1717	June 6 (155)	2351	June 24 (173)	3100	July 12 (191)	4026	August 2 (211)

† Grain Maturity refers to approximately 20% moisture content

Table 3. Mean and standard errors [mean (SE)] of seed yield components of IWG for all three environments.

	Spikes 30 cm^{-1}	Seed mass $spike^{-1}$ (g)	50 seed weight (g)	Spikes $stem^{-1}$
Rosemount 2016	††72.49[a] (4.45)	19.97[a] (1.62)	0.49[a] (0.02)	NA
St. Paul 2015	53.53[b] (1.84)	13.05[b] (1.55)	0.31c[c] (0.01)	0.57[a] (0.06)
St. Paul 2016	57.92[b] (1.82)	13.97[b] (1.46)	0.42 [b] (0.02)	0.50[a] (0.07)

†Means within a section of column followed by different letters are significantly different by Tukey's *t* test ($P \leq 0.05$).

Chapter 3

Yield and Lodging Responses of Intermediate Wheatgrass to Plant Growth Regulator Application

An evaluation of the use of plant growth regulators for improving the agronomic potential of perennial cereal crops.

Introduction

A primary challenge limiting IWG grain production is stem lodging, which results from the bending of lower culm internodes (Sterling et al., 2003). Lodging of cereal crops has shown to limit grain yield, and reduce grain quality (Sterling et al., 2003). Several studies addressing lodging in wheat cultivars suggest that lodging can reduce grain yields by 20 to 80% (Tripathi et al., 2004; Berry et al., 2004). Management decisions including planting date, seeding rate, and soil fertility are known to impact lodging risk (Berry et al., 2004). Crops grown at higher densities may develop smaller stem diameters compared to those grown at lower densities (Snider et al., 2012), resulting in greater susceptibility to lodging from environmental disturbances like rain and wind (Sterling, 2003). The temporal range of lodging events fluctuates seasonally, and occurs most often between the anthesis and grain development stages (Berry et al., 2004). The timing of lodging events during a growing season can affect yield differently based on the developmental status of the crop. When lodging occurs prior to anthesis, it hinders pollen distribution (Hebblethwaite et al., 1978) and subsequent seed production and grain yield. Lodging after anthesis can reduce IWG grain quality because flattened stands can harbor bacterial and fungal growth, which can produce mycotoxins and compromise grain quality (Langseth and Stabbetorph, 1996; Scudamore and Wilkin, 2000; Nakajima et al., 2008). Lodged plants can also impact grain harvestability because field equipment cannot access plants laying on the ground (Tripathi et al., 2004). Preventing IWG lodging is necessary to ensure high grain yield and quality.

Positive correlations between lodging and N fertilizer rates have been observed in many grass crops (Koeritz et al., 2013; Chastain et al., 2014), as well as in IWG. Jungers et al. (2017) reported increased lodging and lower grain yields for IWG fertilized with 160 and 200 kg N ha^{-1}, compared to more moderate rates of 60 to 80 kg N ha^{-1}. While the N requirements of a high-yielding IWG crop are not well understood, reducing N fertilizer rates to decrease lodging risk could result in crop N deficiency and grain yields below theoretical potential. Employing agronomic techniques to prevent lodging that do not alter fertility could increase IWG grain yields.

Plant growth regulators (PGRs) have been used as a management tool to reduce plant height and subsequent stem lodging in wheat (Berry et al., 2004) and cool-season grasses (Koeritz et al., 2013; Chastain et al., 2014). Trinexapac-ethyl [4-(cylopropyl-a-hydroxymethylene)-3,5-dioxo-cyclohexanecarboxylic acid ethylester] (TE) and prohexadione-calcium (calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate) (PC) are two PGRs commonly used in perennial ryegrass seed production systems. Both chemicals are acycyclohexanedione compounds, and are applied during particular plant growth stages to mimic 2-oxoglutaric acid, which blocks β3-hydroxylation in the gibberellic acid (GA) biosynthesis pathway, resulting in GA inhibition (Hedden and Phillips, 2000; Rademacher, 2000). Both TE and PC have been used successfully to reduce lodging in turf seed and cereal crop production, making them viable prospects for use in IWG cropping systems. Decreased GA biosynthesis in grasses treated with TE has been observed to shorten plant height and reduce lodging, important factors that influence seed yields in other grass species (Chastain et al., 2003). Koeritz et al. (2013) found that with proper N fertilizer and PGR application timing, perennial ryegrass seed yields increased by 14 to 36%. They also observed decreased plants heights in perennial ryegrass treated with PC, along with reduced lodging and improved grain yields and harvest index.

Application rate is also an important factor in PGR effectiveness. Matysiak (2006) studied two TE rates on winter wheat (75 and 125 g ai ha^{-1}), and found that the higher rate was more effective in shortening plants and promoting grain yield. Kim et al. (2007) found greater yield for rice after PC was applied at two times the recommended rate. To effectively manage a perennial grain crop, alternative management techniques in addition to N fertilizer and herbicide treatments must be investigated to evaluate their potential to fit into IWG cropping systems. In this study, two experiments were conducted to evaluate PGR application to IWG over multiple years of the perennial cereal stand. The objectives of this study were to 1) evaluate PGR type (PC vs. TE) and N fertilizer rates (Experiment 1) and 2) PGR type and rate (Experiment 2), on IWG plant height, lodging, and grain and biomass yield.

Materials and Methods

Experimental design and crop management

Experiment 1 was initiated in September 2014, when an improved line of IWG from The Land Institute (Salina, KS) was seeded at a rate of 12 kg ha^{-1} in 15 cm rows at the University of Minnesota Agriculture Experiment Station in St. Paul, MN. The soil type is a Waukegan silt loam (fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls). The experiment was laid out in a randomized complete block design with a split-plot restriction on randomization with four replicates, with N fertilizer rate as the whole plot treatment, and PGR type [Palisade 2EC® (trinexapac-ethyl 25.5%, Syngenta, Basel, Switzerland) and Apogee® (prohexadione calcium 27.5%, BASF corporation, North Carolina, USA)] as the split-plot treatment. The N treatments were applied as ammonium nitrate fertilizer on 5 April 2015 and 6 April 2016 at rates of 0, 40, or 80 kg N ha^{-1}.Weeds were controlled with S-metolachlor and 2,4-D in April of each year.

The PGR treatments were applied at recommended labeled rates for grass seed production (TE at 437 g ai ha^{-1}; PC at 154 g ai ha^{-1}). The water conditioning agent N-Pak® (ammonium sulfate 34.0%, Winfield, St. Paul, Minnesota, USA) was used at 2.5% v/v in all PC mixtures to ensure adequate solubility, and the non-ionic surfactant Preference® (Alkylphenol ethoxylate, sodium salts of soya fatty acids, isopropyl alcohol 89.5%, Winfield, St. Paul, Minnesota, USA) was added to PC mixtures at 0.25% v/v, according to label recommendations.

Growth regulators were applied when the majority of plants reached the early onset of stem elongation (E0-E1; Moore et al., 1991), which occurred at 898 (13 May) and 933 (16 May) GDD in the 2015 and 2016, respectively. Growth regulators were applied using a CO_2-powered 11002 TurboTeeJet (TeeJet, Springfield, IL) nozzles at 186 kpa using a hand-held sprayer. Plot dimensions were 1.5 x 4.5 m, with a 1.5 m untreated border to minimize edge effects and spray drift.

Experiment 2 was initiated in September 2014 in a second-year stand of improved grain type IWG from The Land Institute seeded in 15 cm rows. The experiment was conducted in Rosemount, MN on a Waukegan silt loam. Treatments were applied in 2015 and 2016 in a completely randomized design with four replicates, with PGR as the whole plot treatment and PGR rate as the split plot treatment. The PGR rate treatments were chosen to reflect "low," "medium," and "high" rates at 50, 100, and 150% of the recommended labeled rates for grass seed production (Table 1). PGRs were applied when plants reached early onset of stem elongation (Moore et al., 1991), at 935 (13 May) and 1011 (16 May) GDD in spring of 2015 and 2016, respectively with the same methods as used for Experiment 1 (described above). Plots were 3 x 9 m with no untreated border on plot edges, but plots were sampled in the center to avoid possible edge and drift effects.

Data Collection

Lodging was rated visually prior to harvest on a 0 to 10 scale, with 0 representing no lodging and all plants upright, and 10 representing complete lodging where all plant stems were parallel to the ground. Plant height was measured from 10 random plants within a plot immediately prior to harvest. Grain and straw biomass yield was determined by harvesting a 0.5-m^2 area within each plot when a majority of the IWG had reached physiological maturity (hard endosperm/S4 stage) (Moore et al., 1991). Spikes were clipped from the straw and both fractions were dried for 72 h at 60° C until constant weight before being weighed for dry matter determination. Grain was threshed from the spikes using a laboratory thresher (Wintersteiger LD 350, Ried, Austria) followed by sieving to remove chaff. Following harvest, all biomass was cut to a 5-cm stubble height and straw was removed from the field. Harvest index (HI) was calculated by dividing the seed mass by total aboveground biomass.

Statistical Analysis

The R programming language (R Core Team, 2016) was used to analyze data. Analysis of variance was performed using the nlme package (Pinheiro et al., 2017) to assess the effect of PGR type and N fertilization rate (experiment 1) and PGR type and rate (experiment 2) on IWG lodging, plant height, grain and biomass yields. Sites were analyzed separately due to soil fertility and stand age differences of the IWG populations. Year, PGR type, N fertilizer rate, and PGR rate were treated as fixed effects, and block was treated as a random effect. For experiment 2, PGR rate was nested within PGR type to account for the non-treated control plots. Treatment effects were considered significant if $P < 0.05$. Treatment means separations were performed using the Tukey-Kramer honestly significant difference (HSD) test in R with the agricolae package (Mendiburu, 2016). Pearson correlation coefficients were calculated for all variables.

Results

Experiment 1: PGR type and N rate

Intermediate wheatgrass grain yield was influenced by the main effects of PGR type and N rate (Table 2). Across years, TE increased IWG grain yields by 64 and 78% relative to PC and the non-treated control, respectively ($P < 0.001$; Table 3). Intermediate wheatgrass that received 40 kg N ha^{-1} had 23 and 38% greater grain yields compared to 0 and 80 kg N ha^{-1}, respectively (P = 0.018; Table 3). Biomass yields were similar among PGR types and N rates in both years (Table 2). However, 2015 biomass yields were 14% greater than 2016 yields across all treatments (P = 0.011; Table 3). IWG harvest index was affected by the main effect of PGR type, but not N rate (Table 2). In both years, TE increased the harvest index of IWG by 63 and 77% relative to PC and the non-treated control (P < 0.001; Table 3).

There was an interaction of PGR type and year on plant height (P = 0.010; Table 2). In 2015, plant heights were similar among PGR types. In 2016, TE decreased IWG plant heights by 11% relative to PC and the non-treated control in 2016 (Table 3). Decreased plant heights in TE-treated IWG increased grain yields by approximately 32 kg ha^{-1} for every 1 cm reduction in stem length. Lodging of IWG was affected by the main effects PGR type, N rate, and year, with no interactions (Table 2). Across all treatments, IWG lodging was 85% greater in 2015 compared to 2016 (P < 0.001; Table 3). In 2015, IWG lodging was greater in the 80 kg N ha^{-1} than the 40 kg N ha^{-1} rate, and was similar to the non-fertilized control. In 2016, IWG lodging was greater in the 80 kg N ha^{-1} rate than the non-fertilized control, and was similar to the 40 kg N ha^{-1} rate (Table 3).

Across both years, IWG grain yields were positively correlated with IWG biomass yields (r = 0.42; P < 0.001) and negatively correlated with plant height (r = -0.26; P = 0.027) and lodging (r = -0.23; P = 0.048). Across both years, harvest index was negatively correlated with lodging (r = -0.33; P = 0.005), as well as plant height (r = -0.25; P < 0.001).

Experiment 2: PGR type and application rate

Neither IWG grain nor biomass yield was affected by the main effects of PGR type, and rate (Table 2). However, 2016 biomass yields were 27% greater than 2015 yields across all treatments (P = 0.0002; Table 4). Among all years and treatments, grain yields averaged 130 kg ha^{-1} and biomass yields averaged 7.8 Mg ha^{-1}, which was similar to the yields observed in Experiment 1 (Tables 3 and 4).

There was an interaction between PGR type and year on plant height (P = 0.025; Table 2). In both years, plant heights were similar in PC and non-treated IWG, but lower in IWG treated with TE, though the magnitude of difference between treatments was greater in 2016 (Figure 1). There was also an interaction between PGR type and rate (P = 0.001) on IWG plant height. In both years, the medium and high TE rates reduced IWG plant heights by 17% compared to all PC rates, and by 20% compared to the non-treated control (Figure 1). Lodging varied by PGR type and was similar in PC and non-treated IWG, but was lower in IWG treated with TE by 66% in 2015 and 92% in 2016 (P = 0.002; Table 4). A negative correlation was observed between grain yield and lodging, where less lodging resulted in higher grain yield potential (r = -0.30; P = 0.027). There were no correlations among IWG grain yield, biomass yield, and plant height, nor a correlation between plant height and lodging.

Discussion

The PGR TE consistently reduced plant height of IWG compared to the non-treated control, indicating that the chemical is effective at inhibiting internode elongation in IWG to a similar extent as has been observed in other crops (Lickfeldt et al., 2001; Rolston et al., 2010). Conversely, IWG plant height was similar between PC-treated and non-treated controls in both experiments and all years. Although PC has been shown to similarly reduce plant height in turf grasses compared to TE, the absorption pathways and rates vary, which can lead to differences in efficacy (Rademacher, 2014). Variations in PGR absorption could explain the differences we observed in the effect of PC and TE on plant height.

Reduced IWG plant heights from TE application were associated with decreased lodging and increased grain yield. We found significant correlations between these response variables, suggesting that they are related. Many studies have found that reducing plant height prevents lodging in annual and perennial grass and cereal crops (Vogel et al., 1963; Johnson et al., 1986; Miralles et al., 1998; Kashiwagi and Ishimaru, 2004). Likewise, a reduction in lodging often translates to greater seed yields due to a number of factors, including increased grain quality and greater mechanical harvesting efficiency (Kono, 1995). Decreased internode length in grasses has also been associated with greater investment in seed vs. vegetative production, often leading to a greater harvest index in grass crop stands with lower plant heights (Johnson et al., 1986; Shekoofa and Emam, 2008). The significant increase in grain yields alongside lower rates of lodging in IWG treated with TE in Experiment 1, compared to the control, suggest that these relationships hold true in a perennial cereal crop as well. Genetic factors controlling height also play a role in grain yield, as GA-sensitive dwarfing genes that confer shorter height in wheat have been correlated with increased grain yield (Rebetzke and Richards, 2000). Breeding efforts are underway to increase IWG grain production by selecting for decreased plant height to mitigate lodging risk and increase yields. Additionally, agronomic intervention through the use of PGRs to regulate plant height may be necessary to control lodging and increase grain yields.

Reductions in plant height as a result of TE application were expected to decrease biomass yields; however, this was not the case in Experiment 1, where IWG biomass yields were similar among PGR treatments. It appeared that IWG treated with TE had thicker stems relative the PC and non-treated control treatments, which may have increased stem biomass at the base and offset the loss of biomass from reduced height. Other studies have reported positive effects of TE application on stem diameter in wheat, which conferred greater straw strength and resistance to lodging (Nolte, 2007; Wiersma et al., 2011). Improved lodging resistance from TE application without sacrificing biomass yields is promising for the economic feasibility of IWG systems, where forage harvest in addition to grain harvest has been proposed as a means of offsetting low IWG grain yields relative to other cereal crops. Increased lodging resistance would also likely increase forage quality and biomass yields.

Contrary to our expectations, N fertilization did not affect plant height or total biomass. However, IWG lodging did increase with N fertilization rate. The relationship between excess N uptake and lodging in cereal crops is well documented (Ohm, 1976;

Knapp and Harms, 1988; Gibson et al., 2007), and application of PGRs has enabled the use of greater N fertilizer rates for increasing grain yields (Nafziger et al., 1986; Ramburan and Greenfield, 2007). In this study, greater lodging in the 80 kg N ha^{-1} treatment negatively impacted IWG grain yields only in one of two years. High background soil available N levels at the St. Paul location may have resulted in the lack of N fertilizer rate effects on grain yields, limiting the ability to investigate PGR benefits for grain yields at greater N rates. Given IWG's high N uptake potential (Jungers unpublished), further investigation of PGR and N fertilizer interaction effects on IWG grain yield is needed.

The lack of PGR effects on grain and biomass yields in Experiment 2 at the Rosemount location was likely due to the experiment being conducted in an old (>3 years) IWG stand with low inherent grain production potential. Significant grain yield declines after year 2 of IWG stands have been reported (Vico et al., 2016; Jungers et al., 2017), but the causes of this phenomenon are not well understood. The lack of flowering observed in >3-year-old IWG stands, relative to younger stands, suggests that physiological reproductive processes go uninitiated or are suppressed in older plants. Gibberellic Acid is known to be involved in floral initiation pathways. Though its role in the flowering process differs greatly among species, GA has been observed to inhibit flowering in perennial plants (Mutasa-Göttgens and Hedden 2009). Therefore, it is possible that intervening in the GA biosynthesis pathway through application of PGRs to IWG could affect seed production, though the direction of the effect is unknown and could change as stands age. Despite the lack of similar TE effects on grain yield at

Rosemount compared to the St. Paul location, TE application reduced IWG height at Rosemount, confirming that TE has a beneficial effect on IWG lodging resistance across stands of different ages.

The effect of TE on IWG plant height was dependent on application rate. Applying TE at the recommended labeled rate for grass seed production (437 g ai ha^{-1}) or at a rate 150% higher than the recommended rate (656 g ai ha^{-1}) was necessary to reduce plant height compared to the non-treated control or IWG treated with a reduced rate (219 g ai ha^{-1}). However, increasing the application rate to 150% that of the recommended rate did not decrease plant heights further. With known variability in PGR absorption, split application methods may improve PGR efficacy rather than increasing the PGR rate of a single application. Our results provide a baseline for additional studies on TE application rates for IWG grain production, which are necessary to improve IWG economics.

Conclusion

The PGR TE consistently reduced plant height, which translated to a reduction in lodging and increased grain yield in one of two experiments. Trinexapac-ethyl Trinexapac-ethyl was effective when applied at rates labeled for grass seed production, but increasing the application rate did not affect plant height, lodging, or grain yield. The effects of TE were independent of N fertilizer rates. Pending the addition of IWG to the list of EPA-approved crops for receiving TE treatments, its application to IWG is recommended to improve the agronomic potential of this crop.

Table 1: PGR application rates used in Experiment 2 at St. Paul, MN and Rosemount, MN, in 2015 and 2016.

PGR Chemical Name	PGR Trade Name	PGR Rate (g ai ha^{-1})	
Trinexapac-ethyl (TE)	Palisade® 2EC	"Low"	219
		"Medium"	437
		"High"	656
			g ai ha^{-1}
Prohexadione Calcium (PC)	Apogee®	"Low"	77
		"Medium"	154
		"High"	232

Table 2. Analysis of variance for grain yield, biomass, harvest index, plant height, and lodging calculated for Experiment 1 (St. Paul, MN) and Experiment 2 (Rosemount, MN), in 2015 and 2016.

Experiment	**Treatment**	**Grain Yield**	**Biomass**	**Harvest Index**	**Plant Height**	**Lodging**
1	PGR type	**	NS	**	**	**
	N rate	*	NS	NS	NS	*
	Year	NS	*	NS	**	**
	PGR type × N rate	NS	NS	NS	NS	NS
	PGR type × Year	NS	NS	NS	*	NS
	N rate × Year	NS	NS	NS	NS	NS
	PGR type × N rate × Year	NS	NS	NS	NS	NS
2	PGR type	NS	NS	NS	**	*
	Year	NS	**	NS	**	NS
	PGR type × PGR rate†	NS	NS	NS	**	NS
	PGR type × Year	NS	NS	NS	*	NS
	PGR rate × Year	NS	NS	NS	NS	NS
	PGR type × PGR rate × Year	NS	NS	NS	NS	NS

* Significantly different at α = 0.05; ** Significantly different at α = 0.01; NS = not different at α = 0.05;
†PGR rate effects only shown nested within PGR type

Table 3. Main effects of plant growth regulator (PGR) type and nitrogen (N) fertilizer rate on intermediate wheatgrass (IWG) grain yield, biomass yield, harvest index, plant height, and lodging, for Experiment 1, conducted at St. Paul, MN in 2015 and 2016.

	Grain Yield	**Biomass Yield**	**Harvest Index**	**Plant Height**	**‡Lodging**
	kg ha^{-1}	Mg ha^{-1}	%	cm	
			2015		
PGR Type					
None	†493 a	10.0 a	4.7 a	†136 a	8.0 a
PC	468 a	9.8 a	4.5 a	134 a	8.0 a
TE	816 b	9.2 a	8.2 b	130 a	6.5 b
N Rate					
0	562 a	9.5 a	5.7 a	137 a	7.2 ab
40	647 a	10.0 a	6.1 a	134 a	7.0 a
80	565 a	9.6 a	5.6 a	130 a	8.2 b
			2016		
PGR Type					
None	369 a	8.5 a	4.0 a	148 a	5.2 a
PC	469 a	8.4 a	5.2 a	149 a	4.9 a
TE	721 b	8.7 a	7.5 b	133 b	1.8 b
N Rate					
0	485 ab	7.9 a	5.7 a	143 a	3.1 a
40	646 b	9.5 a	6.2 a	146 a	4.3 ab
80	428 a	8.1 a	4.7 a	142 a	4.6 b

†Means within a column followed by different letters are significantly different by Tukey's *HSD* test ($P \leq 0.05$).
‡ Lodging scale: 0 = plant fully erect to 10 = plant flat on the ground.

Table 4. Main effects of plant growth regulator (PGR) type and application rate on intermediate wheatgrass (IWG) grain yield, biomass yield, harvest index (HI), plant height, and lodging, for Experiment 2, conducted at Rosemount, MN in 2015 and 2016.

	Grain Yield	**Biomass Yield**	**Harvest Index**	**Plant Height**	**‡Lodging**
	kg ha^{-1}	Mg ha^{-1}	%	cm	
			2015		
PGR Type					
None	†125 a	7.7a	1.3a	141 a	1.3 a
PC	123 a	6.5a	1.5a	136 a	1.3 a
TE	152 a	6.9a	1.8a	122 b	0.4 a
PGR Rate					
Low	125 a	6.4a	1.5a	138a	0.4 a
Medium	138 a	7.3a	1.6a	125a	0.6 a
High	150 a	6.5a	1.8a	125a	1.6 a
			2016		
PGR Type					
None	131 a	8.8 a	1.2 a	134 a	0.8 ab
PC	106 a	9.6 a	1.1 a	135 a	2.8 a
TE	135 a	7.8 a	1.6 a	109 b	0.1 b
PGR Rate					
Low	129 a	10.2 a	1.2 a	132 a	2.1 a
Medium	131 a	7.9 a	1.5 a	118 a	1.1 a
High	96 a	8.0 a	1.2 a	115 a	1.1 a

†Means within a section of column followed by different letters are significantly different by Tukey's *t* test ($P \leq 0.05$).
‡ Lodging scale: 0 = plant fully erect to 10 = plant flat on the ground.

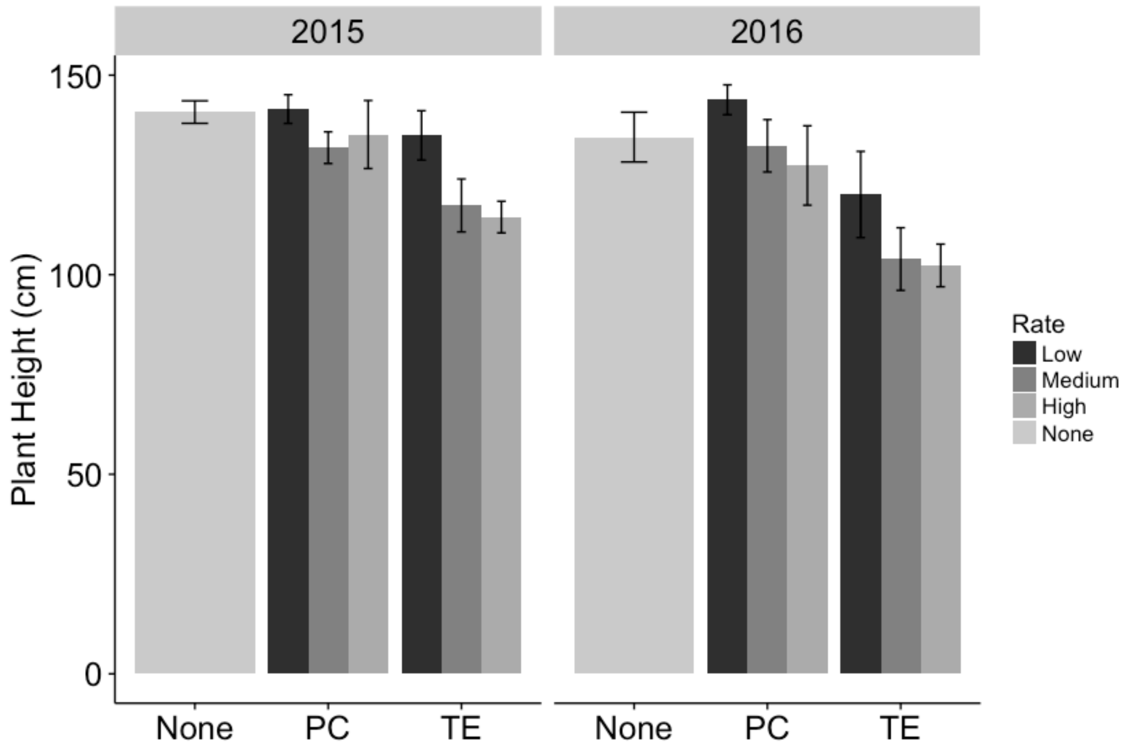

Figure 1. Intermediate wheatgrass (IWG) plant heights among PGR types and application rates in Experiment 2, conducted at Rosemount, MN in 2015 and 2016. “None” = Non-treated control; “PC” = prohexadione-calcium (Apogee®); “TE” = trinexapac-ethyl (Palisade 2EC®). Error bars represent the standard error (SE) of the mean plant heights for the respective treatments.

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