

**Effects of Plant Growth Regulators on Plant Phenotypes and Yield  
Components of Grass Seed Crops**

by

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## **Abstract**

Creeping red fescue (*Festuca rubra* L.), meadow brome (*Bromus riparius* Rehmman) and timothy grass (*Phleum pratense* L.) are the most important grass species grown for seed production in the Peace River region of Alberta and British Columbia. Creeping red fescue is a popular turf grass species, whereas meadow brome grass and timothy are commonly used for pasture, hay, and silage production for livestock feed. The seed productivity of these grass crops is limited by lodging under abundant rainfall conditions. Lodging can negatively impact seed yield and quality in forage crops, and make harvesting more difficult, time-consuming, and prone to seed loss through shattering. Plant growth regulators (PGRs) are an effective means of controlling lodging and seed loss, especially when grasses are grown under high nitrogen (N) fertility and non-limiting soil moisture conditions. A study was conducted over two years in 2021 and 2022 to quantify the morphological development of these grasses in response to PGRs at Beaverlodge Research Farm, Agriculture and Agri-Food Canada (AAFC) in northwestern Alberta, Canada. This study aimed to identify beneficial PGRs for regulating plant photosynthesis, growth, lodging tolerance, seed yield, and yield components of these grasses. A field experiment with split-plot design included PGR treatments as main plots and top-dressed spring nitrogen (urea) at 40 kg ha<sup>-1</sup> as subplots on three and four-year-old stands of these grasses for two consecutive years. Three PGRs, including trinexapac-ethyl (TE), chlormequat chloride (CC) and ethephon (ETH), applied at 0.20 kg, 1.12 kg, and 0.60 kg active ingredient (ai.) ha<sup>-1</sup> were used separately on each grass at the two-node stage of crop development (BBCH 31-32) and compared to untreated grasses. The results showed that TE was efficacious in shortening internode and tiller height and reducing lodging in meadow brome grass and timothy. However, PGRs did not alter grass seed attributes such as panicle length, seed weight, and seed number, as well as the total seed yield in all three grasses. TE demonstrated similar levels of marginal economic returns to the check, indicating comparable profitability for

timothy and creeping red fescue. Spring nitrogen was beneficial in increasing biomass and seed yield in timothy and meadow bromegrass without significant lodging. The effects of PGRs were more pronounced in improving grass morphology under normal rainfall conditions. However, under moisture deficit climatic conditions, PGRs had inconsistent and minimal effects on grass plant morphology.

A greenhouse study was also conducted to investigate the effects of PGRs on several variables related to vegetative growth, root-shoot allometry, chlorophyll pigmentation, and photosynthetic efficiency at the juvenile stage of these grass crops. The study found that TE reduced root, shoot, and total biomass up to 40 days in timothy, and up to 55 days in creeping red fescue after application, but no PGRs affected meadow brome biomass at any point of time. The PGRs had no effect on growth variables such as root area, root length, root to shoot ratio, leaf area, tiller number, final biomass, and other growth parameters like relative growth rate, net assimilation rate, leaf weight ratio and specific leaf area. Both TE and CC reduced the plant height and lodging severity of the three grasses. PGRs did not enhance or hinder the quantum yield of photosystem II ( $F_v/F_m$ ) from 4 h through 12 days after PGR application. However, TE and CC increased chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents ( $\mu\text{g g}^{-1}$  FW) in timothy up to 70 days after application. Despite these limited responses, a principal component analysis revealed that the ETH and check treatments exhibited higher total biomass, shoot biomass, root-to-shoot ratio, and plant height, which were associated with higher lodging severity, while TE and CC exhibited higher contents of chlorophylls and carotenoids along with greater NAR in all tested grasses. Overall, TE was more efficient and efficacious in reducing plant height and lodging severity of timothy and meadow bromegrass under field conditions and did the same for all grasses including creeping red fescue, in the greenhouse. Multiple studies of PGRs under a range of environmental scenarios can

improve our understanding of how these compounds influence grass morpho-physiology. Likewise, long-term field studies under different lodging conditions could lead to the development of more efficient PGRs utilization strategies that maximize forage yield and seed harvestability.

Key words: morpho-physiology, lodging, seed yield, spring nitrogen.

## **Preface**

This manuscript is an original work by Bishnu Pandey, with editorial assistance by Dr. Edward Bork, Dr. Nityananda Khanal, and Dr. Malinda Thilakarathna. Dr. Edward Bork provided guidance on study plan, manuscript composition, revision, and development. Dr. Nityananda Khanal contributed through his leadership in acquiring the project fund, managing the project, and providing expertise in experimental process from field to laboratory. Dr. Khanal's technicians assisted with experimental set-up, seed quality determination, harvesting, and threshing. Dr. Malinda Thilakarathna contributed to editing the manuscript. I was responsible for the refinement of the research concept, developing the methods, collecting, and processing data, statistical analysis, and interpreting, and writing the manuscript. The manuscript data have not yet been submitted for publication in a peer-reviewed journal.

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## List of Abbreviations

<b>Abbreviation</b>	<b>Definition</b>
AI	Active Ingredient
ANOVA	Analysis of Variance
BBCH	Biologische Bundesanstalt, Bundessortenamt and Chemical industry
CAD	Canadian Dollar
Car	Carotenoid
CC	Chlormequat Chloride
Chl	Chlorophyll
CRF	Creeping Red Fescue
DM	Dry Matter
ETH	Ethephon
Fm	Maximum Fluorescence
Fo	Minimum Fluorescence
g	Gram
GA	Gibberellic Acid
GDP	Gross Domestic Product
ha	Hectare
kg	Kilogram
LWR	Leaf Weight Ratio
MB	Meadow Brome
N	Nitrogen
NAR	Net Assimilation Rate
NE	Northeast
NPK	Nitrogen Phosphorus and Potassium
NW	Northwest
PAM	Pulse Amplitude Modulation
PCA	Principal Component Analysis
PGR	Plant Growth Regulator
QY	Quantum Yield
RGR	Relative Growth Rate
SL	Specific Leaf Area
TE	Trinexapac-Ethyl
TM	Timothy
USD	United States Dollar

## **Chapter 1. Introduction and review of literature**

### **1.1 Background**

Canada is the leading producer and exporter of quality seeds for a wide range of crops. Canada exports seed to over 100 different countries that is worth about \$646 million annually (Agriculture and Agri-Food Canada 2020). Fertile soil and favorable climatic conditions allow Canada to grow more than 50 different types of crops. Primary crops grown for seed in Canada includes forages and turf grasses, along with small grains, oilseeds, and pulses. Cooler temperatures during the growing season favor a high frequency of seed set, allowing forage and grass seed crops to be successfully grown across the country, but are particularly common in Manitoba, Alberta, and Saskatchewan (Wong 2020). In the case of forage and grass seed, Canada is a world leader in forage seed production, after the USA and New Zealand. During 2019/2020, Canada exported 53,240 tonnes of grass and legume seed worth \$161.67 million, mostly from alfalfa seed (*Medicago sativa* L.), creeping red fescue (*Festuca rubra* L.), perennial ryegrass (*Lolium perenne* L.), and timothy (*Phleum pratense* L.) (Wong 2020).

Grasses have a comparatively large number of flowers; however, only a few flowers typically pollinate, leading to lower-than-expected potential seed yields. Therefore, actual grass seed production remains below the maximum potential production of grass crops due to a lack of pollination, coupled with inadequate fertilization, poor seed filling, and seed losses due to crop lodging and seed shattering (Chastain 2013). These seed losses are influenced by environmental conditions and agronomic factors (Zhong et al. 2017). Though seed production of grass crops has improved in recent years, further increases are needed from current levels to enhance the profitability of grass seed production. Management practices such as the use of plant growth



regulators (PGRs) and adequate nitrogen (N) fertilization offer the potential to ensure proper pollination and seed set, without lodging, thereby boosting seed yield and profitability.

Seed yield in perennial grasses depends on a number of morpho-physiological traits (Ibrahim and Frakes, 1984). The root and shoot growth (Chastain and Young et al. 1998), number of fertile tillers, number of spikelet/tillers, number of seed number/unit area and seed weight are the attributes contributing the most to seed production of grasses (Hebblethwaite et al. 1980; Chen et al. 2019). N is considered the main nutrient necessary for seed production (Rolston et al. 1985). N addition is reported to increase plant biomass (Lee et al. 2017), root growth (Boot and Mensink 1990), shoot growth (Koeritz et al. 2015), seed number and seed weight (Chastain et al. 2014a) in grass crops. The timing of N fertilization is among the most important factors regulating the growth of perennial temperate grasses, especially in the Peace River region of northern Alberta where short growing seasons exist between cold winters (Fairey 2008). N applied in the fall promotes the development of fall tillers, which after vernalization in winter, undergo floral induction (Boelt 1999) while spring applied N is needed for seed production (Boelt and Studer 2010). In cool temperate grasses, N addition over winter may not promote plant growth (Lloyd et al. 2011) due to greater N loss, ammonia volatilization, and denitrification (Malhi 1997). Additionally, freezing damage can reduce rates of N uptake over winter (Malyshev and Henry 2012). Hence, a combination of N application in early fall and early spring might result in higher seed production of temperate grasses in northern Alberta. Despite the benefit of added N, high N with good soil moisture makes grass crops more vulnerable to lodging (Bitarafan et al. 2019; Havstad and Aamlid 2006.)

Lodging is the process whereby shoot biomass of ramets becomes flattened on or near the soil surface. Lodging, especially when it occurs before, during, or shortly after anthesis, greatly

limits grass seed yields (Chastain et al. 2001). Lodging is more common under high soil moisture accompanied by high spring N, where long elevated tillers of grasses cannot support the developing inflorescence and seed, causing it to collapse to the ground (Chastain et al. 2014 b). Lodging reduces capacity to capture and utilize incoming solar radiation, thereby reducing assimilating supply of photosynthates to developing seeds (Clemence and Hebblethwaite 1984). Lodging before and during flowering physically restricts pollination and fertilization in grasses (Boelt and Struder 2010). Seed yield reductions depend on the severity of lodging and have been recorded to be as high as 95% (Chynoweth et al. 2014). These losses are associated with reduced light interception (Rolston et al. 2007) and reduced availability of photosynthates during seed filling. Other adverse effects of lodging include reduced pollination and seed set (Warringa & Marinissen 1996) and difficult in harvesting (Elgersma 1985). Also, lodging cause an increase in the frequency of vegetative tillering (Hampton & Hebblethwaite 1985). Vegetative tillers compete for resources and don't undergo floral induction, thereby decreasing seed production via a reduction in the resources available for seed filling among reproductive ramets.

Keeping in mind the importance of N and the adverse effect of lodging, the use of plant growth regulators (PGRs) could be an option for modifying morpho-physiological responses and improving the associated seed yields in grass crops. PGRs can be used to minimize lodging and seed loss in grass crops (Rolston et al. 2010). PGRs reduce internode elongation and subsequent lodging by inhibiting gibberellins (GA) or promoting the biosynthesis of ethylene. Trinexapac-ethyl (TE) and chlormequat chloride (CC) are gibberellin inhibitors, where CC prevents the cyclases, blocking ent-kaurene forming at early steps of gibberellin (GA) biosynthesis while TE blocks soluble 2-oxoglutarate-dependent dioxygenases involved in late steps of GA biosynthesis (Rademacher 2015). Unlike the other two PGRs, ethephon (ETH) inhibits stem elongation by

releasing ethylene. TE lowers the occurrence of lodging among grass ramets and increases seed yields in red fescue (Bitarafan et al. 2019), tall fescue (*Schedonorus phoenix* S.) and perennial ryegrass (Chastain et al. 2014 a). Elsewhere, TE application reduces stem length, and increases florets per spikelet, while favoring the pollination and fertilization of florets, thereby maximizing economic seed yield in creeping red fescue (Zapiola et al. 2014). In addition, TE has been reported to reduce shoot elongation, and promote many morpho-physiological attributes, including tiller densities, root growth, chlorophyll content, photosynthesis efficiency, and turf quality (Qian et al. 1998; Ervin et al. 2002; Steinke and Stier 2003), and ultimately the seed yield of grass crops. Both CC and ETH are widely used to limit shoot height and lodging, though mostly in cereals (Rajala and Peltonen-Sainio 2002). To date, limited information is available on how CC and ETH impacts grass growth and lodging. The performance of CC and ETH is likely forage species-dependent, so needs to be evaluated.

The use of high N fertilizers is often associated with increased lodging in grass crops; however, limiting N fertilization to minimize lodging may severely reduce the seed production of grass crops. As a result, the use of PGRs inhibiting the production of gibberellins (GA) to reduce shoot elongation and lodging risk may be beneficial to seed production, particularly if it allows for proper N fertilization. Furthermore, PGR usage could be efficacious in improving the performance of grass crops by altering plant morpho-physiological traits that favor (directly or indirectly) increased seed yields. Few studies have been carried out to examine the effects of PGRs on the morphological and physiological responses of grass crops, particularly relative to attributes reflecting seed production. This includes the forage grasses creeping red rescue, meadow bromegrass, and timothy in relation to PGR application. The research proposed here aims to assess the effects of plant growth regulators (TE, CC, ETH) on the seed production

capacity and performance of creeping red fescue, timothy, and meadow brome grass grown in NW Alberta, an important region for forage seed production in Canada.

## **1.2 Literature review**

### **1.2.1 Forage seed production status in Canada**

The agriculture and agrifood sector are major contributors to the Canadian economy, contributing about 6.8% of Canada's gross domestic product (GDP) (Agriculture and Agri-Food Canada 2021). Canada is one of the world's largest food exporters, exporting \$ 82.2 billion CDN worth in agriculture and food products, to over 200 countries (Agriculture and Agri-Food Canada 2021). The diverse climatic conditions and fertile soils found in Canada are favorable for growing more than 50 different crops. The economic contribution from seed industry (direct or indirect) in Canada is worth over \$ 6 billion CDN annually. Excluding commercial grain, the export of seed crops alone (i.e., those intended for propagation, not consumption), had an export value of approximately \$ 646 million (M) CDN, and were exported to more than 100 different countries in 2017/2018. The top seed importers of Canadian seed were the USA (\$403 M), China (\$47 M), Ireland (\$31 M), Japan (\$19 M), and Turkey (\$13 M) (Agriculture and Agri-Food Canada 2020).

Forage and turf grasses comprised a major portion of Canada's total seed export. Most of this seed was exported to the USA, China, Netherlands, other European countries, and Asia, comprising more than 30 countries in the world (Khanal 2022). In 2019/2020, Canada exported forage and grass seed worth \$161.67 M CDN, mostly from alfalfa (*Medicago sativa* L.), creeping red fescue (*Festuca rubra* L.), perennial ryegrass (*Lolium perenne* L.), and timothy (*Phleum pratense* L.) (Wong 2020). Canada exported 53.24 M kg of grass and legume seed in

2019/20, which included 14.78 M kg of alfalfa, 11.61 M kg of creeping red fescue, 9.07 M kg of perennial rye fescue, 3.62 M kg of timothy grass and 0.19 M kg of meadow bromegrass (*Bromus biebersteinii* Roem. Schult.). Seed exports of alfalfa were worth \$ 61.14 M CDN, mostly to the USA, China, and the Netherlands. Creeping red fescue seed sales were worth \$29.45 M CDN, with 92.5% exported to the USA. Perennial rye fescue (*Lolium perenne* L.) seed sales are worth \$22.43 M CDN and are mainly exported to the US, China, and the Netherlands. Timothy (*Phleum pratense* L.) seed sales were worth \$9.08 M CDN, which were exported to Europe (Germany, Netherlands, and Denmark) in 2020 (Wong 2020).

Canada's forage seed production area covers an average of 52,559 ha of land (Wong 2020 b). The three Canadian prairie provinces, Manitoba (46.6%), Alberta (34.7%) and Saskatchewan (15.7%), contributed approximately 97 % of Canada's grass and legume export in 2020 (Wong, 2020). Canadian grass and legume seed production is mainly concentrated in the Canadian prairie provinces, as well as the Peace River region of Alberta and British Columbia, which is also a primary region for forage and turf seed production (Wong 2005). The Peace River region is one of the world's largest areas for forage and turf seed, contributing about 30% of total forage and turf grass seed in Canada (Wong 2017).

Creeping red fescue is a primary grass seed crop in the Peace River region of Alberta and British Columbia (Fairey 2006). About 99 % of creeping red fescue comes from these regions (Yoder 2000). Red fescue was first introduced to Alberta from Czechoslovakia in 1931, with the first Canadian variety released in 1937. Exports of creeping red fescue seeds increased dramatically in the 1940s to create durable and fast lawns that withstood the traffic in World War II. Today, it is widely used for lawns, playgrounds, and golf courses. In 2019/2020, 14.78 M kg

of creeping red fescue worth \$29.45 M CDN was exported from Canada, with 92.5% exported to the USA (Wong 2020) and the rest to European countries and China.

Timothy is another important forage crop in the Peace River region (Yoder, 2004). Timothy is native to Europe and has been used in many cool or temperate regions of Asia and North Africa (Bélanger et al. 2001). It is also grown in Australia and New Zealand (Charlton and Stewart 2000). It was first introduced to North America within the United States (Hoover et al. 1948) and was found growing in New Hampshire in 1711. Timothy grass was widely promoted from 1720 onwards and was named after the farmer Timothy Hanson. Timothy grass spread into Canada during the late 1700s from the United States (Smoliak et al. 1981). It was deliberately grown in Canada by 1800 and is now commonly grown for hay, pasture and silage (Calvin 2004). Timothy hay is currently exported to Asia, especially Japan and South Korea, because of its high-fiber source for dairy cattle. Canada exported 311,948 tons of timothy grass worth \$133.5 M CDN during 2019/2020 (Alberta 2020). Timothy seed sales were worth \$9.08 M CDN and more than 86% of total timothy seed was exported to Europe (Germany, Netherlands, Denmark, UK, and Poland) in 2019/2020 (Wong 2020).

Meadow brome grass is a widely accepted and cultivated species on the Canadian prairie (Loeppky and Coulman 2002). This forage was first introduced to North America from Europe in the late 1960s and Canada in 1980 (Knowles et al. 1993). This long-lived grass quickly regenerates after cutting or grazing, making it popular in pasture and grazing systems (Lardner et al. 2002). Wong (2020) reported the export of 190,000 kg of meadow brome grass seeds worth \$780,000 CDN. More than 80% of meadow brome grass was exported to the USA in 2019/2020.

### 1.2.2 Nitrogen requirements

Nitrogen is the most limiting nutrient in Alberta soil (Malhi 1996), and the main nutrient limiting seed production in grasses (Ralston et al. 1985). N fertilization in cool-season grasses increases biomass yield and increases seed yield (Chastain et al. 2014a). N availability has been reported to increase the proportional abundance of reproductive tillers (Koeritz et al. 2015), increase the number of florets (Cookson et al. 2001), and individual seed weight (Chastain et al. 2014a) in grass crops. Research conducted at the Beaverlodge Research farm in NW Alberta found that under the prevailing moist environment of the region creeping red fescue produced the highest seed yield ( $2136 \text{ kg ha}^{-1}$ ) over two production years when N was applied at  $68 \text{ kg ha}^{-1}$  to the first seed crop, and at  $34 \text{ kg ha}^{-1}$  in the second year (Fairey 2006). A result from Saskatchewan showed N application at  $100 \text{ kg ha}^{-1}$  in meadow brome increased tiller density (Loeppky and Coulman 2001) as well as increased cumulative two-year seed yield (Loeppky and Coulman 2002).

Unlike creeping red fescue and meadow brome, timothy does not require a vernalization period to initiate floral induction but need a long day length (Heide 1982, 1994). Timothy is highly sensitive to N addition and  $60$  to  $100 \text{ kg ha}^{-1}$  is recommended depending on soil moisture, initial soil nutrients and stand age (Calvin 2004). Studies from Norway show that spring application of  $50 \text{ kg N ha}^{-1}$  at the beginning of growth and supplementary N of  $25 \text{ kg ha}^{-1}$  at tiller elongation resulted in the highest seed yield in timothy (Havstad and Aamlid 2006). Additionally, autumn N application usually has no or limited effect on the following year's seed yield in timothy (Stoddart 1961; Nordestgaard 1986).

In most cool-season grasses, inflorescence relies on the establishment of fertile tillers before winter, so N is typically required during the fall before seed production (Nordestgaard

1986). N application in the fall of some cool season grasses like creeping red fescue (*F. rubra* L.) and Kentucky bluegrass (*P. pratensis* L.) increased number of tillers that undergo floral induction and thus, seed production (Boelt, 1997). Nitrogen is required to ensure the presence of ample fertile tillers, inflorescence elongation, and subsequent seed filling (Loeppky and Coulman, 2001). Flowering and seed production in temperate grasses are highly correlated with the presence of well-developed tillers in fall (Schoeberlein 1987; McDonald et al. 1996). As per Boelt (1999), N application in fall promotes the development of fall tillers, which undergo floral induction the following year after vernalization in winter. Similarly, N applied in spring provides nutrients for sexually reproductive tillers from the start of growth through seed set (Boelt and Studer 2010).

A study conducted in the Peace River region of northwestern Canada also suggests the importance of N supplied before the start of growth in the spring for good seed production of creeping red fescue (Fairey and Lefkovitch 2000). Karamanos and Stevenson (2013) reported that N application in spring resulted in more seed yield in forage crops of the Peace region of Alberta. Similarly, Young et al. (1996) reported that spring N fertilization in perennial ryegrass increased seed yields by as much as 40% compared to autumn N fertilization alone. The authors further stated that the application of 60 kg of N ha<sup>-1</sup> at the early stage of development was enough to maximum seed yield; however, if N was not applied before panicle emergence higher N fertilizer application rates were required. When there is a good supply of N during fall and early spring, maximum seed yield is obtained; conversely, any delay in N availability during spring can decrease the seed yield of creeping red fescue (Nordestgaard 1980). Similarly, N in late spring or winter in grasses may not promote plant growth (Lloyd et al. 2011). This may be due to the greater likelihood of N loss in winter due to ammonia volatilization and denitrification



(Malhi 1996). In addition, freezing damage reduces N uptake during winter (Malyshev and Henry 2012). Therefore, as reported by Cedell (1986), N fertilization in early fall and early spring is recommended to optimize seed production in creeping red fescue.

According to Szczepanek et al. (2021), the application of N during autumn will increase grass tiller vegetative growth, including boosting tiller height and density (number of tillers per plant). In contrast, N applied in early spring facilitates an increase in reproductive tillers for seed production. However, Bolt and Studer (2010) cautioned that excess N application can lead to severe lodging, reduced seed set, and increased vegetative tillering. This notion is further supported by Chastain et al. (2014 a) who indicated that high N availability under good soil moisture conditions lead to developing grass plants that cannot support their inflorescence and seed, and therefore experience lodging that reduces seed yield. In this case, lodging of grass crops hinders harvesting, reduces seed quality, and increases seed loss due to shattering (Macháč 2013). In this scenario, PGRs can be good option in controlling the lodging and seed loss in grass seed crops without compromising the N fertilization.

### **1.2.3 Plant growth regulators (PGRs)**

PGRs are natural or synthetic compounds that stimulate or interfere with plant growth and development by affecting metabolic or physiological processes. Generally, these compounds are exogenously applied substances that interact with phytohormones thereby modulating plant physiological processes. Based on their physiological functions, PGRs are grouped into abscisic acid, auxins, gibberellins, cytokinins, ethylene, brassinosteroids, jasmonic acid, salicylic acid, and strigolactone (Santner et al. 2009; Vanstraelen and Benková, 2012). Collectively, these compounds are used to alter plant morphological structures, including potentially increase qualitative and quantitative plant yield (Sabagh et al. 2021) as well as decrease plant

susceptibility to biotic and abiotic stresses (Rademacher 2015). PGRs are used in turfgrass swards to reduce vertical growth and thereby limit mowing intensity or frequency (Fagerness and Yelverton 2000; Glab et al. 2020). In the case of grass and forage seed production, PGRs are used to reduce plant height and thus control lodging (Zapiola et al. 2014; Rolston et al. 2010; Chastain et al. 2014 b). PGR application to grasses and forages inhibit growth by reducing cell elongation and cell division, so-called growth inhibitors or growth retarders. Generally, these growth retarders antagonize gibberellins and auxin hormones that are primarily responsible for cell elongation (Rademacher, 2015). These growth inhibitors are classified into five classes based on their methods of suppression (March et al. 2013; Fidanza et al. 2006)

1. Class A: Trinexapac-ethyl and Prohexadione-calcium belong to this class of inhibitors, which block the final step gibberellin biosynthesis route by inhibiting GA20 hydroxylation to GA1 (Evans et al. 1999; Rademacher 2000; McCann and Huang, 2007). GA1 promotes stem elongation, and inhibition of GA1 leads to the accumulation of GA20, which causes suppression of plant growth (Rademacher 2000; Reasor et al. 2018). These PGRs are absorbed largely through leaf tissue and crowns of grasses, and then transported throughout plants within one hour of absorption (Rademacher 2000). They can remain for several weeks in a plant depending on temperature and growth behaviors (Beasley et al. 2005; Reasor et al. 2018). Their application can lead to slower shoot growth (Pannacci et al. 2004) and reduced biomass production without affecting or even improving turfgrass quality (Pornaro et al. 2017).
2. Class B: Triazole compounds such as flurprimidol and paclobutrazol belong to this class. According to McCullough et al. (2005) these PGRs inhibit the conversion of ent-kaurene into ent-kaurenole by blocking ent-kaurene oxidase enzyme activities, which prevent the

formation of any type of gibberellin. Flurprimidol is used in internode and growth reduction as well as in controlling leaf diseases in grasses through its fungicide action (Bigelow 2012). Paclobutrazol is also used to inhibit the growth of grasses (Miller 2016) by reducing auxins and boosting cytokinins (McElroy and Martins 2013). They are not easily absorbed by leaf tissue or crowns of turf grasses, but instead are translocated in the plant through root absorption. Adequate soil moisture is required for root uptake.

3. Class C: Cell division inhibitors such as mefluidide, maleic, hydrazide and amidochlor falls into this class (Kreuser 2015). They are more phytotoxic, and their growth suppression period is shorter than class A and B PGRs, requiring frequent application (Haguewood et al. 2013).
4. Class D: This class includes herbicides that behave as PGRs when applied at a lower rate. When timed correctly they are capable of indirectly restricting some physiological mechanisms in plants without any visible damage. The commonly used herbicides glyphosate, imazaquin, imazapic, imazethapyr, metsulfuron-methyl and bispyribac-sodium, are in this group. Use of glyphosate and fenoxamic acid inhibits turfgrass growth and seed head development (Kreuser 2015; McCarty 2018).
5. Class E. This group includes phytohormones that have no effect on gibberellin biosynthesis but are capable of inhibiting branch, root, and leaf elongation (Kreuser 2015). Ethephon is the main compound of this group. Once absorbed by leaves, ethephon gets hydrolyzed and converted into ethylene gas and is moved to other parts of the plant (Taiz & Zeiger 2006). Ethylene interfaces with growth processes and is noted to reduce internode lengths in bermudagrass (Howieson and Christians 2005).

In this study, two important GA biosynthesis inhibitors i.e., CC and TE and phytohormone releasing Ethelene, i.e., ETH were tested and studied. TE is used in grass seed production for lodging control (Rolston et al. 2010; Zapiola et al. 2014; Chynoweth and Moot 2016) whereas ETH and CC are commonly used in cereal crops to reduce lodging (Rajala and Peltonen-Sainio 2001). In Canada, CC and ETH are registered for use in wheat, whereas TE is registered for use only in turf-type perennial ryegrass.

Chlormequat chloride was first introduced in 1965 and was the first PGR used for controlling lodging within cereal crops (Rademacher 2015). CC is a quaternary ammonium compound that inhibits cyclases involved in the early stages of GA metabolism, which leads to the formation of ent-kaurene (Rademacher, 2000). Use of CC was effective in controlling lodging in wheat through a reduction in plant height and increasing the stem diameter and stem wall thickness (Zhang et al., 2017). However, this response has been reported to be highly cultivar-specific and less responsive in barley (Rajala and Peltonen-Sainio, 2002). In the case of grasses, CC application had minimal and inconsistent effects. Hampton (1986) reported that CC had no effect on tiller length and lodging in perennial ryegrass, but increased seed yield in ryegrass. The use of CC with TE was more effective than only CC in reducing lodging and increasing seed yield in orchardgrass (*Dactylis glomerata* L.) (Rolston et al. 2014), strong creeping red and chewing fescue (Szczepanek et al. 2021).

Trinexapac-ethyl was first released in 1992 and was widely popular among producers by the 2000s. TE is an acylcyclohexanedione that inhibits the acetyl-coenzyme A carboxylase (ACCase) enzyme during the late stages of GA biosynthesis (Rademacher 2000). Mainly, TE interferes with GA1 by reducing the activity of the GA20-3 $\beta$ -hydroxylase enzyme (March et al. 2013). The use of TE at a rate of 0.1 kg ai. ha<sup>-1</sup> reduced GA1 concentrations by up to 47%, but

increased GA20 concentration by up to 146% in Kentucky bluegrass, thereby demonstrating that TE inhibits GA1 biosynthesis by interfering with the conversion of GA20 to GA1 (Tan & Qian 2003). TE is more effective than other PGRs in reducing lodging within cereals (Rajala and Peltonen-Sainio 2002). Likewise, TE has also been reported to control lodging during forage and turfgrass seed production (Zapiola et al. 2006; Rolston et al. 2010). Research conducted by Silberstein et al. (2000) found that the application of TE reduced plant height and lodging but increased seed yield, in creeping red fescue, chewing fescue, tall fescue, and perennial ryegrass. TE also suppressed the shoot growth and biomass of various turf grasses (Pannacci et al. 2004; McCann and Huang 2007). TE is reported to suppress long-term growth and thereby lower biomass by 40% in bermuda grass (*Cynodon dactylon* (L.) Pers) (Fagerness & Yelverton 2000). The first application of TE at a rate of 0.2 kg ai. ha<sup>-1</sup> suppressed the growth of bermudagrass for up to three weeks, whereas the second rate of 0.1 kg ai. ha<sup>-1</sup> 15 days after the first dose suppressed growth for up to five weeks. Additionally, three applications of TE at a rate of 0.2 kg ai. ha<sup>-1</sup> during spring followed by two consecutive doses at 0.1 kg ai. ha<sup>-1</sup> at 14-day intervals suppressed bermudagrass growth for up to 12 weeks (Johnson 1994). Likewise, sequential application of TE in seashore paspalum (*Paspalum vaginatum* Sw.) at a rate of 0.09 kg ai. ha<sup>-1</sup> and 0.38 kg ai. ha<sup>-1</sup> suppressed vegetative growth by 59% and 96% up to 10 weeks after treatment (Ferrell et al. 2003). A high quantity of TE causes injury to the grass and decreases its quality, so a lower rate is recommended for reducing biomass without affecting turfgrass quality (Pornaro et al. 2017).

The compound ETH was the first registered product that was less phytotoxic than other commercially available PGRs in the mid-1940s. ETH is the mostly widely used PGR in agriculture since the early 1990s and has various purposes, such as altering flower induction,

flower densities and fruit thinning, as well as growth retardation (Abeles et al. 1992). ETH affects plant growth by releasing the hormone ethylene. After it is exposed to water or an acidic environment, ETH decomposes into ethylene and by-products (Ma and Smith 1992, Zhang et al. 2010). It is immediately absorbed within the plant after application (Zhang et al. 2010). Its mode of action is fast but may also remain active for 3-4 weeks depending on the quantity used (March et al. 2013). Ethylene has multiple growth and morphological responses in plants, which may include the inhibition of growth of the cell, and alteration of flowering, abscission, as well as the acceleration of senescence and ripening (Rademacher 2015).

Responses to ETH are highly variable, and may depend on plant species, duration and the concentration of ETH applied. In cereals, ETH is commonly used under lodging conditions (Dahnous et al. 1982; Simmons et al. 1988). While the timing of ETH is important, if timed properly, it reduces stem elongation in cereals (Ma and Smith, 1992; Rajala and Peltonen-Sainio, 2002). In spring and winter wheat, it is recommended to apply ETH between GS 37 and 45 (Bayer, 2019). Likewise, in bermudagrass plant growth also is affected by ETH (Shatters et al., 1998). In some grasses, increased internode length and stems have been reported from ETH. In Kentucky bluegrass (cv. 'Sydsport'), ETH treatment increased stem elongation rate (Poovaiah and Leopold 1973). Similarly, Christians (1985) reported that ETH in Kentucky bluegrasses led to internode elongation, more erect growth and then a tendency to lay flat, leading to a stoloniferous-like growth habit; once the effects of ETH wore off, it grew upright again. Similar findings were reported by Dernoeden (1984) in pastures containing a mixture of Kentucky bluegrass and creeping red fescue. Notably, Fry (1991) reported that ETH also reduced vegetative growth and canopy height of centipedes (*Eremochloa ophiuroides* Munro.).

#### **1.2.4 Plant growth regulators in lodging management**

Lodging is the permanent bending of ramets at or near the soil surface before, during, or shortly after anthesis, greatly limiting grass seed production. Lodging in grasses is common under high soil moisture conditions, particularly when coupled with high N availability in spring, when the structure of the grass stem in later stages of development cannot support the increasing weight of the developing inflorescence and seed, falls to the ground (Chastain et al. 2014 a). Lodging reduces the ability of plants to capture and utilize incident solar radiation and leads to a limited supply of assimilates to developing seeds (Clemence and Hebblethwaite 1984). Seed yield declines depend on lodging severity and can be as large as 95% (Chynoweth et al. 2014). These losses are due to reduced light interception (Rolston et al. 2007) and a reduced availability of photosynthates during seed filling (Griffith 2000). In addition to these effects, lodging lead to an increase in the frequency of vegetative tillering (Hampton & Hebblethwaite 1985). Early lodged, vegetative tillers compete heavily for resources, including sunlight, N, and water, but as they do not undergo floral induction, they decrease seed production both directly, but also indirectly by reducing the resources available for seed filling among existing ramets undergoing floral induction.

Lodging reduces seed yield in tall fescue and perennial ryegrass because of reduced light assimilation during seed filling (Griffith, 2000). A similar reduction in seed production occurred in perennial ryegrass and tall fescue due to lodging under N application in spring. Wet conditions favored lodging in creeping red fescue as reported by Chastain (2003). Similarly, lodging lowered the seed yield of timothy grown in high N with high moisture (Havstad and Aamlid 2006).

PGRs have become a widespread and cost-effective method for reducing plant height and lodging incidence in cool-season grasses (Szczepanek et al. 2021, Chastain et al. 2014). Common plant growth regulators like CC and TE act by inhibiting gibberellic acid, which helps in regulating vertical growth, while ETH produces ethylene that inhibits cell division and elongation (Rademacher 2015). ETH restricts auxin biosynthesis, reducing its ability to promote stem elongation (Ma and Smith, 1991).

Ethephon is commonly used for controlling lodging in cereals (Simmons et al. 1988; Ma and Smith 1992; Rajala and Peltonen-Sainio 2002). A three-year multi-location study conducted in western Canada found that ETH was effective in reducing plant height and lodging in malt barley (Tidemann, 2020). However, studies on the use of ETH for preventing lodging in perennial grasses are less available and inconclusive. Aamlid et al. (2007) found that ETH did not control lodging but increased the seed yield in common bent grass (*Agrostis capillaris*). Similarly, the addition of ETH to CC had no additional benefits on lodging resistance, nor to seed yield of creeping red fescue (Szczepanek et al. 2021).

Trinexapac-ethyl is commonly used as a lodging control agent in the production of perennial ryegrass (Rolston et al. 2010), creeping red fescue (Zapiola et al. 2014; Bitarafan et al. 2019), and tall fescue (Rolston et al. 2004). A three-year study on red fescue found that the application of TE between 0.15 kg and 0.3 kg ai ha<sup>-1</sup> led to increased lodging resistance and seed yield in creeping red fescue (Bitarafan et al. 2019). Similarly, Zapiola et al. (2014) observed that TE reduced plant height of creeping red fescue by 32% and decreased the lodging rate by 60%, which in turn, maximized number of seeds and seed yield by favouring pollination and fertilization. Similarly, TE was very effective in controlling lodging without compromising



spring N additions, leading to 859 kg ha<sup>-1</sup> (126% more) and 538 kg ha<sup>-1</sup> (75% more) more seed yield than untreated in tall fescue and perennial ryegrass, respectively (Chastain et al. 2014 a).

Application of CC is also used to reduce plant height and lodging in cereals crops (Rajala & Peltonen-Sainio 2001, Shah et al. 2017, Zhang et al. 2017) as well as in some grass crops. Research conducted in New Zealand found that use of CC at a dose of 0.750 kg CC ha<sup>-1</sup>, both at stem elongation and again 10 days later, led to shortened stems and reduced lodging, but had no effect on seed yield in orchard grass (Rolston et al. 2014). In contrast, Hampton (1986) reported that CC had no effect on tiller length and lodging but led to increased seed yield due to a higher number of fertile tillers in perennial ryegrass. In general, the use of CC in perennial grass crops was inconsistent and species dependent and depended on other PGRs. For instance, CC was more effective in reducing lodging and improving seed yield of orchard grass (Rolston et al., 2014), tall fescue (Hudgins et al. 2017), meadow brome (Yoder et al. 2019), red fescue and chewings fescue (Szczepanek et al. 2021) when used together with TE.

### **1.2.5 Plant growth regulators in grass seed production**

Grass seed is important for use in managing livestock feed or turf, reclamation efforts to control soil erosion, and for recreational and aesthetic purposes on lawns, golf courses, and playgrounds. Seed production in grasses depends on many morphological and physiological traits (Ibrahim and Frakes, 1984), including the number of plants per unit area, number of fertile tillers per unit area, number of spikelets per panicle, number of florets on each spikelet, number of seeds per floret, and individual seed weight. Variation in the expression of these attributes may change seed yield. Seed production of perennial grass seed crops requires good establishment for favorable yield potential and proper utilization of yield potential i.e., actual yield at harvest (Hebblethwaite et al.1980, Chastain and Young III 1998).

Temperate grasses like creeping red fescue and perennial ryegrass need vernalization (i.e., low temperature and short-day length) for inflorescence primordia initiation, whereas high temperature and long day lengths are needed for culm elongation, inflorescence development and flowering (Chastain and Young III 1998). For such grasses, tillers developed in spring or early fall become fertile (productive) after vernalization, which is an important attribute for establishing seeds (Boelt and Studer 2010). Chastain and Grabe (1988) found that the seed yield of creeping red fescue increased as the number of tillers increased in the preceding season. The number of fertile tillers per unit area, spikelet per fertile tiller, and the number of florets per spikelet at anthesis will determine the potential yield, while seed number and seed weight at harvest are more influential on actual seed yield (Hebblethwaite et al. 1980).

Several years of research conducted by Young et al. (1998) found that the number of fertile tillers per unit area, along with the number of florets, were correlated with seed yield of creeping red fescue in Oregon, USA. Ultimately, the number of seeds per unit area was a key attribute responsible for the seed yield of tall fescue, orchard grass, and chewing fescue (Young et al. 1998; Young et al. 1999). Seed number and seed weight determine the actual seed yield of grass, all of which can be limited by lodging. Lodging in grasses before or after anthesis reduced the photosynthate available to support developing seeds (Griffith 2000) and limited pollination and fertilization (Boelt and Studer 2010). Many studies showed that PGRs have been widely used in reducing lodging in forage and turf grass seed production (Zapiola et al. 2006; Rolston et al. 2010; Zimbra et al., 2020; Szczepanek et al. 2021). Most plant growth regulators are used to reduce stem height and lodging by limiting gibberellin synthesis or accelerating ethylene release, thereby increasing grass seed yield (Zhou and Shao 2000, Fang et al. 2000).

Ethephon is commonly used to increase grain yield when lodging occurs (Rajala and Peltonen-Sainio 2002; Simmons et al. 1988). ETH is reported to increase the number of tillers per plant but decrease plant height and kernel weight on malting barley in western Canada (Tidemann et al. 2020). Furthermore, negative (delayed) effects on barley maturation have been reported (Ma and Smith, 1992). For turfgrass seed crops, ETH was not as effective in reducing shoot growth as Type A or B PGRs (Diesburg and Christians, 1989; McCullough et al. 2006; McCullough et al. 2005) and was less able to increase seed yield (Wenhua et al. 2008). Due to excessive phytotoxicity to warm-season grasses, ETH is only labeled for use in cool-season turfgrasses (McCarty et al. 2011).

Trinexapac-ethyl has been reported to reduce lodging and increase seed yield of creeping red fescue (Bitarafan et al. 2019; Zapiola et al. 2014), perennial ryegrass (Rolston et al. 2010), and tall fescue (Rolston et al. 2004). Research conducted under lodging conditions in Oregon, USA found that the application of 0.4 kg ai ha<sup>-1</sup> TE in perennial ryegrass increased seed yield by 43% as compared to untreated plants. Increased seed yield correlated with an increased number of seeds per spikelet and greater seeds per unit area (Chastain et al. 2015a). Another study by Chastain et al. (2015b) found that TE from 0.20 to 0.6 kg ai. ha<sup>-1</sup> reduced lodging from 17% to 39%, and increased seed yield by 40% over the control in tall fescue, primarily by elevating the number of seeds per unit area.

Chlormequat chloride has been used in some forage and grass seed production. Research in Norway found that CC application reduced lodging and increased seed yield by 21% in common bent (Aamlid et al. 2007). Another study in New Zealand reported that application of CC at 0.75 kg ai. ha<sup>-1</sup> at stem elongation, and again after 10 days, prevented lodging but had no effect on the seed yield of orchard grass (Brown and Lill, 1990). Similarly, Rolston et al. (2012)

found that applying CC alone had no advantage on seed yield in perennial ryegrass. However, a combination of CC and TE ( $0.75 + 0.1 \text{ kg ai ha}^{-1}$ , respectively) applied at GS 31–32 and again at GS 37–39 resulted in the highest seed yield in orchard grass (Rolston et al. 2014). Similarly, Szczepanek et al. (2021) reported that the application of CC ( $0.625 \text{ kg ai ha}^{-1}$ ) at BBCH 30–31 + TE ( $0.075 \text{ kg ai ha}^{-1}$ ) at BBCH 37–39 increased seed yield compared to applying CC alone; this result occurred in both creeping red fescue and chewing fescue.

### **1.2.6 Plant growth regulator impacts on grass morpho-physiology**

PGRs modify the growth and development of plants by altering plant structure as well as physiological and biochemical process (Sabagh et al. 2021). PGRs have been widely used in temperate grasses as lodging control agents for seed production (Chastain et al. 2014; Rolston et al. 2010; Chynoweth et al. 2008). These are also used in turf management for reducing mowing frequency and maintaining desirable turf colour (Fagerness and Yelverton 2000; McCarty et al. 2011; Glab et al. 2020). PGRs are additionally used for regulating plant germination and early growth (Small et al. 2019), tiller density (Ervin and Zhang 2008), and rooting (Zhang et al. 2017), while also impacting disease control (Bigelow 2012), chlorophyll content (McCullough et al. 2004), carotenoids (Gliozzeris et al. 2007; Kasem et al. 2015), and photosynthesis rates (Qian and Engelke 1999). PGRs also can influence the tolerance of plants to stresses, including salinity (Arghavani et al. 2012), drought (Brain et al. 2009), mechanical traffic (Mohamadi et al. 2017), freezing (Fagerness et al. 2002), heat (Xu and Huang 2010), and combinations of drought and heat stress (McCann and Huang 2007).

In the case of grass crops, application of ETH reduced many attributes, including root biomass in creeping bentgrass (McCullough et al. 2006), root length in perennial ryegrass (Jiang

and Fry 1998), growth in bermudagrasses (McCullough et al. 2005 b) and colour quality in creeping bentgrass (Kane and Miller 2003). Shatters et al. (1985) observed that ETH affected the morphology of Tifton 85 bermudagrass. This included an increased stem/leaf weight ratio and number of tiller and buds per crown, but reduced plant height, while decreasing the colour quality of the turf. Likewise, McCullough et al. (2005 b) noted that ETH had a negative effect on root mass (-33%), root length (-16%), total shoot (biomass) yield (-22%), and colour quality (-33%) of bermuda grass. However, McCarty et al. (2010) found that ETH had no or little effect on the growth (clipped yield) or root length density of TifEagle bermudagrass. Similarly, Volterra et al. (2015) found that ETH did not affect stolon's length, internode diameter, and internode length, although a higher rate of ETH reduced turf height and the biomass of pot-grown hybrid bermudagrass. Ethylene may induce many morpho-physiological responses in grass crop, but this remains highly dependent on plant species, and the amount and duration of ethylene exposure.

Like wise, there is also limited information available on the effects of CC on grass growth. Volterrani et al. (2015) reported that increasing the rate of CC had no effect on internode length, stolon length, and internode diameter, but that CC decreased leaf length, plant height and biomass within pot-grown hybrid bermudagrass (Patriot). Similarly, Hampton (1986) reported that CC had no effect on tiller length and lodging in perennial ryegrass. Wenhua et al. (2018) also reported that CC did not affect the biomass of alfalfa. Similarly, Hampton (1986) reported that CC had no effect on tiller length and lodging in perennial ryegrass.

After the introduction of TE, it has been commonly used in cereals as well as turf management and grasses during seed production. TE treated plants had slower growth in various turfgrasses (Pannacci et al. 2004; McCullough et al. 2007; McCann and Huang 2007). The

proper and subsequent application of TE suppressed grass biomass for up to 10-12 weeks (Johnson 1994, Ferrell et al. 2003). Even small rates of TE are enough to reduce biomass production without affecting turfgrass quality (Pornaro et al. 2017). Heckman et al. (2001) found that TE increased concentrations of chlorophyll a, chlorophyll b, and total chlorophyll in Kentucky bluegrass, thereby enhancing the visual color and harvesting capacities for photosynthesis.

The application of TE typically inhibits vegetative growth, thereby affecting the root development of grass plants. Marchi et al. (2017) observed that the reduction of root biomass of broad-leaved carpet grass (*Axonopus compressus* (Sw.) P. Beauv) and bahia grass (*Paspalum notatum* Flügge) was directly related to the suppression of shoot biomass of grasses. Likewise, TE application reduced total root and shoot mass in creeping bent grass (McCullough et al. 2006). Similarly, Beasley et al. (2005) found that the application of TE reduced total root length by 30% and root surface area by 21% compared to non-treated plants in Kentucky bluegrass; however, after 7-weeks treatment differences had disappeared under a continuous supply of nutrients and water. Elansary and Salem (2015) found that TE treatment reduced the leaf area, plant height, and biomass of three ornamental shrubs, (*Spirea nipponica* Maxim. ‘Snowmound’, *P. eugenoides* ‘Variegatum’, and *Viburnum nudum* ‘Smooth withrod’) grown under adequate moisture, but these morphologies increased under drought conditions except for plant height. Furthermore, they reported that TE reduced root biomass when grown under moist conditions, whereas it increased root biomass under low moisture conditions, suggesting photosynthate allocation was shifting from above- to below ground, thereby improving the ability of the plant to cope with drought. In the same study, TE application of 0.2 and 0.3 kg ai. ha<sup>-1</sup> improved photosynthetic and transpiration rates, stomatal conductance, as well as relative water content

and leaf water potential in these shrubs under moisture stress but led to no effect on these physiological processes under ample moisture conditions (Elansary and Salem 2015). Similarly, TE increased photochemical efficiency during drought within creeping bentgrass despite no difference in photosynthetic rate, transpiration or stomatal conductance under moist conditions (Brian et al. 2009).

Use of TE can enhance tolerance to environmental stresses like salinity in bermuda grass (Baldwin et al. 2006), drought in perennial ryegrass and *Zoysia* (*Zoysia japonica* S.) (Jiang and Fry 1998), low moisture in Kentucky bluegrass (Xu and Huang 2011), heat stress in Kentucky bluegrass (Heckman et al. 2001), and simultaneously drought and heat in creeping bentgrass (McCann and Huang 2007). The use of TE maintained the growth and quality in drought conditions by accumulating inorganic solute (Ca and K) and soluble sugars (Bian et al. 2009) as well as enhancing root length and root weight density drought avoidance (Serena et al. 2020). Similarly, the application of PGRs has been reported to be a promising way of lowering stress in plants by increasing antioxidants (Zhang and Schmidt 2000). Sheikh-Mohammadi et al. (2017) reported that the application of TE enhanced the relative water content (RWC), decreased the electrolyte leakage (EL), and increased antioxidant enzymes like ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) in tall fescue (*Festuca arundinacea* L.) and crested wheatgrass (*Agropyron desertorum* L.) under traffic (mechanical disturbance stress) conditions. Correspondingly, TE increased the antioxidant activities of SOD, POX, APX, CAT, and glutathione reductase (GR), leading to enhanced chlorophyll content, total non-structural carbohydrates (TNC), rooting and quality in Kentucky blue grass under high salinity conditions (Arghavani et al. 2012).

Specifically, this study aimed to determine the effect PGRs on the morpho physiology and seed yield of creeping red fescue, timothy grass, and meadow brome grass seed crops with following objectives.

- a) Determine the effect of plant growth regulators (PGRs) on plant stature, seed yield components and seed productivity of creeping red fescue, meadow bromegrass, and timothy grass seed crops under low and high N condition.
- b) Examine the effect of PGRs on growth, phenotypic and photosynthetic at juvenile stage of creeping red fescue, meadow bromegrass, and timothy grass seed.

In Chapter 2, a two-year field experiment was conducted to a) study the efficacy of PGRs in controlling lodging. b) quantification of seed yield and seed attributes under N fertilization in spring. While in Chapter 3, a greenhouse was conducted to study the effect PGRs on c) growth parameters like relative growth rate (RGR), net assimilation rate (NAR), leaf weight ratio (LWR) and specific leaf area (SLA). d) morphological variables such as root and shoot biomass, roots, etc.: shoot ratio, root area, root length, plant height, and tiller numbers. e) physiological variables such as chlorophyll content (a & b), carotenoids, quantum efficiency ( $F_v/F_m$ ) during the juvenile stages of these grass crops.



## **Chapter: 2. Plant growth regulators and spring nitrogen on agronomic characteristics of grass seed crops in western Canada**

### **Abstract**

Plant growth regulators (PGRs) have been used as effective tools to minimize plant height and lodging in grasses, but there is limited information on how PGRs function in important forage grasses such as meadow brome, timothy and creeping red fescue. A two-year field study was conducted to determine the effect of PGRs applied with and without spring top dress nitrogen (N) on the agronomic characteristics of forage seed crops at Beaverlodge Research Farm in northwestern Alberta, Canada. A split-plot design included PGR treatment as main plots and top-dressed spring N (urea) at 40 kg ha<sup>-1</sup> as subplots on three- and four-year-old stands of creeping red fescue (*Festuca rubra* L. ssp. *rubra*), timothy (*Phleum pratense* L.), and meadow bromegrass (*Bromus riparius* Rehmman). Three PGRs were applied, including TE, CC and ETH, at 0.20 kg, 1.12 kg, and 0.60 kg ai. ha<sup>-1</sup> respectively, at two-node for crop development (BBCH 31-32). Application of TE was most effective in consistently shortening internode length, tiller height and reducing lodging of bromegrass and timothy, while increasing the internode diameter of creeping red fescue and timothy. None of the PGRs increased grass seed yield attributes such as panicle length, seed weight (g) and seed number (# 1m<sup>-2</sup>), as well as total seed yield (kg ha<sup>-1</sup>) in these grasses, despite a reduction in lodging severity within timothy and meadow brome. However, TE demonstrated comparable levels gross margin when compared to the check of timothy and creeping red fescue. Spring N addition increased the biomass and seed yield in timothy and meadow bromegrass, as well as seed number in all grasses. No interactions were apparent between the PGRs and spring N fertilization on the yield attributes and overall seed yield of grasses. The most pronounced benefits of PGRs in altering plant morphology were

apparent in the second year of experiment when growing condition were more favourable for these grasses, particularly meadow brome and creeping red fescue. This study showed that PGRs did not have a consistent positive effect on the seed yield of perennial forage grasses under dry to average moisture conditions. Future work should address potential PGRs benefits during wet growing conditions, and on other benefits such as sward harvestability due to reductions in lodging.

**Key words:** Grass morphology, lodging, seed yield, yield attributes

## **2.1 Introduction**

Grass seed is an important commodity used to support the establishment of pastures and hayland that provide livestock feed, reclamation efforts of disturbed land, and the planting of perennial cover for recreational and aesthetic purposes on lawns, golf courses and playgrounds. Canadian grass and legume seed production is mainly concentrated in the Canadian prairie provinces, including the Peace River regions of Alberta and British Columbia, which is one of the worlds largest producers of forage and turf seed production (Wong 2017). Despite high foreign market and good price of forage seed, seed acres are continuous dropping in Canada (Wong et al. 2020). Additionally, seed yields of temperate grasses are lower than their potential yields due to a lack of pollination, fertilization, poor seed filling, and seed shattering (Chastain 2013). Seed production of grass crops is also limited not only by genetic factors, but by environmental and agronomic factors (Szczepanek et al. 2019), including lodging. Lodging of grass seed crops is known to reduce the yield (Griffith 2000, Bitarafan et al. 2019) as well as their harvestability (Macháč 2013). Given the decline in forage seed acreage in recent years (Khanal 2022) an increase in the production efficiency of forage grass seed is needed to improve the

profitability of grass seed production, and support producers seeking to diversify their operations.

Creeping red fescue, timothy, and meadow bromegrass are among the most common forage grasses grown in the Peace River Region of NW Alberta and NE British Columbia for seed production and contribute to the local agricultural economy. Creeping red fescue is a perennial cool-season grass used primarily in lawn and pasture mixes. It has a strongly creeping growth habit and is suitable for soil retention, and therefore makes it an important species for reclamation of disturbed lands. Creeping red fescue was the largest exported turfgrass seed in 2019/2020 with a value of \$29.45 million USD, the largest share of which comes from the Peace River region of Canada (Wong 2020). Similarly, timothy seed is mostly used for hay, pasture or silage (Yoder 2004). Timothy exports are worth \$9.08 million USD in 2020 (Wong 2020). Meadow brome is also perennial forage which quickly regenerates after cutting or grazing, making it popular in hayland and pasture systems (Lardner et al. 2002).

One of the most important inputs for increasing productivity is N. Nitrogen is the most limiting nutrient in Alberta soil (Malhi 1996), with fertilization of N used to increase seed production in grasses (Ralston et al. 1985). Fertilization of N in cool-season grasses increases both sward biomass yield and associated seed yield (Chastain et al. 2015). Added N has been reported to increase fertile tillers (Koeritz et al. 2015), increase florets (Cookson et al. 2001) and seed weight (Chastain et al. 2014) in grass crops. Since N has a positive effect on seed yield potential of grasses, the amount and timing of N addition should be considered (Borm and Berg, 2008),

Within the Peace River region of northern Alberta, N timing and fertilization is crucial for temperate grass development because of the relatively cool and short growing season (Fairey

2008). Addition of N in fall promotes tillers, which after vernalisation in winter, undergo floral induction (Boelt 1999), while N addition in spring provides nutrients for growth and seed production (Boelt 1999). In cool temperate grasses, N addition prior to winter may not promote plant growth (Lloyd et al. 2011) due to greater N loss through ammonia volatilization, and denitrification (Malhi 1997). Additionally, freezing damage can reduce rates of N uptake over winter (Malyshev and Henry 2012). Therefore, proper timing of N application (i.e., early fall and early spring) can produce favorable seed yields in temperate grasses of northern Alberta. However, high N with elevated soil moisture may make the grass sward more vulnerable to the lodging (Bitarafan et al. 2019; Havstad and Aamlid, 2006).

Increased nutrient availability and soil moisture can result in longer tillers that are unable to support their developing inflorescences and seeds, leading to increased lodging of the crop flat to the ground (Chastain et al. 2014). Lodging before and during flowering directly limits pollination and fertilization of grasses (Boelt and Struder 2010). Early lodging also limits the plant's ability to capture and utilize solar radiation, affecting the supply of photosynthate to developing seeds (Clemence and Hebblethwaite 1984, Griffith 2000). Lodging at a later growth stage can inhibit seed filling due to self-shading of the lodged crop (Chastain et al. 2001; Silberstein et al. 2002). Lodging after flowering or at maturity further hinders harvest and increases seed loss due to seed shattering and reduced crop quality (Machá 2013).

Seed yield decreases in grasses depend on lodging severity and can decline up to 95% (Chynoweth et al. 2014). Lodging reduced seed yield in tall fescue (*Festuca arundinacea* S.) and perennial ryegrass (*Lolium perenne* L.) because of reduced light assimilation during seed filling (Griffith, 2000). Another study found that lodging reduced the number of seeds, and consequently seed yield in perennial ryegrass and tall fescue (Chastain et al. 2014). The high N

coupled with wet condition favored lodging and reduced seed yield in timothy (Havstad and Aamlid 2006).

Where lodging risk of grass seed crops is high, the use of plant growth regulators (PGRs) are an option to limit stem elongation and lodging severity in grasses (Griffith 2000; Rolston et al. 2010; Chastain et al. 2014; Chynoweth and Moot 2017). Application of PGRs may be particularly useful for seed production in grasses with increased rates of spring N fertilisation (Young et al. 2007, Szczepanek et al. 2021). PGRs should be applied to grasses when plant growth is from stem elongation (BBCH 31-32) to early panicle initiation (BBCH 50-51) (Griffith 2000). Common plant growth regulators include chlormequat chloride (CC) and trinixapaethyl (TE), which act by inhibiting gibberellic acid biosynthesis that is responsible for stem elongation. TE acts at a later stage, whereas CC acts earlier on during GA biosynthesis (Rademacher 2015). Unlike TE and CC, ETH inhibits cell division and elongation by releasing ethylene; ETH restricts auxin biosynthesis, thereby reducing its ability to promote stem elongation (Ma and Smith, 1991).

Application of TE is commonly used as a lodging control agent in forage and grass seed production of perennial ryegrass (Rolston et al. 2010), creeping red fescue (Zapiola et al. 2014; Bitarafan et al. 2019), and tall fescue (Chaistain et al. 2015; Rolston et al. 2004). TE reduces stem length and lodging, which improves the pollination and fertilization of florets, thereby maximizing seed yield of creeping red fescue (Zapiola et al. 2014). TE and spring N application increase seed yield in perennial ryegrass (Borm and Berg 2008, Young et al. 2007), tall fescue grass (Chaistain et al. 2014), and creeping red fescue (Szczepanek et al. 2021). Both PGRs CC, ETH are mostly used for reducing lodging in annual cereal crops (Rajala and Peltonen-Sainio 2002). There is limited information available however, on the effectiveness of CC and ETH

within grasses for reducing lodging and elevating seed yield. The performance of CC and ETH are grass species-dependent, so need to be verified.

In recent years, the use of PGRs in cereal production have become increasingly popular (Subedi et al. 2020). To date, few studies evaluate the application of PGRs to grass seed crops, including in the Peace River seed growing region of Alberta, Canada. The purpose of this study was to determine: (i) the efficacy of TE, CC, and ETH in controlling lodging in these grasses, (ii) characterize the effects of PGRs and spring N addition on grass morphological properties including internode length, diameter, tiller height, and biomass, and (iii) quantify key grass seed yield components and overall grass seed yield to the combination of spring N and PGR application.

## **2.2 Material and methods**

### **2.2.1 Study area**

The field experiment was conducted from 2020 to 2022 at the Agriculture and Agri-Food Canada Beaverlodge Research Farm (55°12' N, 119°24' W) in the Peace River region of western Canada, situated 45 km west of Grande Prairie. The study site is located on a gentle (2.5% gradient) east-facing slope with a moderately fine textured Dark Gray Luvisol (Polygon\_ID 23564, <https://soil.agric.gov.ab.ca/agrasidviewer/>). The top 15 cm of soil had a pH of 5.93, organic matter (OM) of 4.36 %, and a loamy texture (sand = 31.3%, silt = 46.0%, clay = 22.7%). Soil nutrients were 4.97 mg kg<sup>-1</sup> of NH<sub>4</sub><sup>+</sup>-N, 14.9 mg kg<sup>-1</sup> of NO<sub>3</sub><sup>-</sup> N, 15.4 mg kg<sup>-1</sup> of PO<sub>4</sub>-P, 10.8 mg kg<sup>-1</sup> of SO<sub>4</sub>-S, and 139 mg kg<sup>-1</sup> of K.

Agro-climatic conditions at Beaverlodge include a short growing season with an average of 117 frost free days (20 May - 15 September), day length ranging between 13.2-17 hr (May to

September), warm daytime temperatures (16-22°C), cool night-time temperatures (3–9°C), and an average annual precipitation of 433 mm, of which more than 60 % occurs during the growing season from May to September (Government of Canada, 2013). Mean growing season temperatures (May to August) were 12.8 °C (min temp 6°C and max 19.10°C) in 2021 and 11.9 °C (min temp 5.8°C and max temp 17.91°C) in 2022. Total rainfall in the area during the same period was 167 and 171 mm in 2021 and 2022, respectively (Figure 1). Spring and summer weather (May-July) conditions are crucial for flowering and seed production in cool-season grasses. The 2021 weather was atypical with high erratic rainfall in beginning and end of growing season, and heat wave (up to 42°C) during reproductive stage in the late June amid the dry spell spanning the whole month of June and three quarters of July caused pollen desiccation and premature senescence of crop resulting in very low yield. While in 2022 had favourable growing conditions with normal distribution of precipitation (Figure 1).

### **2.2.2 Experimental design**

Forage grass plots were first established in about 49 m long and 12 m wide strips of each species in 2019. Forage grasses included meadow bromegrass, timothy and creeping red fescue, which were zero-till seeded with a cross-slot seeder (Cross Slot with Varmax, Cross Slot IP Limited, Feilding, New Zealand) with 30 cm row spacing. The forage of each species were divided into four blocks and further each block was divided into four microplot plots, measuring 8 m × 2 m in size (after leaving buffers strips around the plots), PGRs were applied to microplots plots in randomized complete block design and included a non-treated check, TE @ 0.20 kg ai ha<sup>-1</sup>, CC @1.12 kg ai ha<sup>-1</sup> and ETH @ 0.60 kg ai ha<sup>-1</sup>. The PGRs were applied at the beginning of stem elongation stage (BBCH 30-31). These PGRs are registered in Canada for various crops and traded as Moddus (11.3% TE), Manipulator (620 g CC L<sup>-1</sup>) and Ethrel (240 g ET L<sup>-1</sup>).

All the grass plots were evenly fertilized with N, P, K and S at 60, 32, 30 and 14 kg ha<sup>-1</sup>, respectively in the fall (mid-September) in 2019, 2020 and 2021. Plots were further reconfigured in 2020 with subdivision of PGR plots into two levels of spring top-dress N fertilization treatments – with and without top-dress N using a split-plot design. N (urea) (40 N kgha<sup>-1</sup>) was top-dressed by broadcast on the assigned plots in early spring (first week of May) to subplots (4 m × 2 m). For weed control, herbicides (Curtail M) were applied every fall after the establishment year, and large sporadic weeds were manually removed from time to time during growing season.

Overall, there were 8 treatments consisting of four PGRs as whole plots subdivided with two N levels (either 0 or 40 kg ha<sup>-1</sup> N in spring) as split plot treatments. Treatments were assessed separately for each grass due to overarching differences in the growth of each forage. Seed production, lodging and grass morphology were measured in all subplots during 2021 and 2022.

**Table 2.1:** Summary of experimental treatments evaluated on each of three forage grasses during 2020 and 2021 at the Beaverlodge Research Farm, including four PGRs and two N treatments.

SN	Treatments	Abbreviations
1	TE @ 0.20 kg ai ha <sup>-1</sup> without N top-dress in spring	TE-Spring N
2	TE @ 0.20 kg ai ha <sup>-1</sup> with N top-dress in spring	TE+Spring N
3	CC @ 1.12 kg ai ha <sup>-1</sup> without N top-dress in spring	CC-Spring N
4	CC @ 1.12 kg ai ha <sup>-1</sup> with N top-dress in spring	CC+Spring N
5	ETH @ 0.6 kg ai ha <sup>-1</sup> without N top-dress in spring	ETH-Spring N
6	ETH @ 0.6 kg ai ha <sup>-1</sup> with N top-dress in spring	ETH+Spring N
7	Check – No PGR application without N top-dress in spring	Check-Spring N
8	Check – No PGR application with N top-dress in spring	Check+Spring N



### 2.2.3 Grass measurements

Tiller height, internode length and diameter were determined from 5 randomly selected tillers from each subplot after anthesis (flowering) stage (mid-July to early August in maturity order of creeping red fescue, meadow bromegrass and timothy). Tiller height was obtained by measuring the longest length with a ruler from the basal crown to the average tip of the leaves. The three upper-most internode lengths were measured using a ruler at the same time, and culm diameter was taken at the center of each internode using a caliper. Lodging score was assessed at maturity (late July-mid of august) on a five-point scale (Young et al.1999) where 1 = not lodged (fully upright) and 5 = the most severe lodging (horizontal). The lodging tolerance (i.e., the ratio of canopy height to length of tillers) was also estimated before the harvest of grass seed crops.

Grass seed was quantified from a 1 m<sup>2</sup> quadrat randomly placed within each plot when the grasses reached physiological maturity. At harvest, all biomass was first harvested, air dried and weighed to determine DM yield. Next, samples were threshed and sieved to remove the seed from chaff. After cleaning, a thousand kernel seed weights was determined. Finally, seed add counts (# 1m<sup>-2</sup>) from each plot were derived by dividing the harvested clean seed yield by the thousand kernel weight from each plot. For the economic analysis, the input costs among the treatments were calculated based on local purchase receipts and operational costs were calculated based on farm operation custom rate survey from Alberta (Alberta Agriculture and Forestry, 2016). Farm inputs and their application costs among treatments were referred to as partial variable costs (Table A1). The average selling price of seed obtained from the Peace Region Forage Seed Association (Wong, 2019) was used to calculate marginal returns. The

difference between the gross return and total partial variable cost was used to derive the gross margin across treatments.

#### **2.2.4 Statistical analysis**

Analysis of variance (ANOVA) was performed on all response metrics (grass biomass, seed yield, thousand kernel weight, seed counts, plant height, internode lengths and culm diameter) using the lme4 Package (Bates et al. 2022) in RStudio version 1.4.1717 (2021). Year of sampling, PGRs, and spring N fertilizer treatments were fixed effects, and replicate block was a random effect. Each grass species was analyzed separately due to inherent differences in growth form among grasses. Treatment effects were considered significant if  $P < 0.05$  for all main effects and interactions. Post-hoc treatment mean comparisons were conducted using a Tukey test with the lsmeans package for effects that had more than 2 treatments. Correlation coefficients were calculated between the various response variables and seed yield using the correlogram package in R. Finally, linear regression was used to relate final seed yield ( $\text{kg ha}^{-1}$ ) to the lodging tolerance and lodging score of each grass species.

### **2.3 Results**

#### **2.3.1. PGR and N Effects**

Plant height (cm) was affected by the PGRs ( $P \leq 0.001$ ) applied in all grasses tested, with a further interaction of PGR  $\times$  Year for meadow bromegrass (Table 2.1). Among the PGRs, TE application resulted in the shortest plant height for all three grasses, with this effect most apparent in meadow bromegrass (-31% vs check), followed by timothy (-27% vs check), and then creeping red fescue (-7% vs check). ETH and CC did not reduce the height of timothy or

fescue relative to the check, but reduced the height of bromegrass, with CC being more effective than ETH (Table 2.1). The plant height was markedly greater ( $P \leq 0.001$ ) in all three grasses in 2022, while PGRs reduced bromegrass height more apparent in 2022 than 2021 (Figure 2.2). Spring N addition did not affect plant height ( $P \geq 0.2$ ) in any of the grasses (Table 2.1).

Total aboveground grass biomass ( $\text{kg ha}^{-1}$ ) was significantly affected by the PGRs when applied to meadow brome ( $P \leq 0.001$ ) but not timothy and creeping red fescue ( $P \geq 0.2$ ). More specifically, TE application reduced brome biomass by 30%, while CC and ETH reduced brome biomass by 19% and 11%, respectively (Table 2.1). Spring N fertilisation increased the biomass yield of meadow brome and timothy ( $P \leq 0.001$ ) but not creeping red fescue ( $P \geq 0.4$ ) but did not alter PGR responses. Biomass production was higher in the 2022 year of production for meadow brome and timothy ( $P \leq 0.001$ ) (Table 2.2). Spring N application led to particularly large increases in biomass of both meadow brome and timothy during 2022 (Figure 2.3)

The upper three internode lengths (cm) were affected by PGR application ( $P \leq 0.03$ ) but only in meadow brome and timothy ( $P \leq 0.03$ ) and not creeping red fescue ( $P \geq 0.2$ ) (Table 2.3). Internode lengths (cm) were also higher during the 2022 year of production for all grasses, though PGR effects remained similar between years. Overall, internodes were shortest under TE application, and this effect was apparent for all three of the leading internodes in both meadow bromegrass and timothy. In contrast, ETH and CC had negligible effect on the internode length of timothy, but did reduce internode lengths in bromegrass, particularly those of the 3<sup>rd</sup> internode (Table 2.3). Spring N fertilization did not affect internode lengths (cm) in any of the grasses, both alone and in combination with the PGRs ( $P \geq 0.06$ ).

Internode diameter (cm) was significantly affected by PGRs application in timothy and creeping red fescue ( $P \leq 0.05$ ) but not meadow brome ( $P \geq 0.1$ ) (Table 2.2). TE produced the thickest diameter (mm) culm in both timothy and creeping red fescue (Table 2.1). Spring-applied N did not affect the culm diameter of grass crops, although internode diameter was higher in the second year ( $P \leq 0.001$ ). There was also no interaction between PGRs and spring N on internode diameter in any year. Panicle length remained unchanged in all three grasses relative to PGRs ( $P \geq 0.5$ ) and spring N ( $P \geq 0.5$ ) application (Table 2.4).

Lodging in meadow bromegrass and timothy ( $P \leq 0.001$ ), but not creeping red fescue ( $P \geq 0.1$ ), was affected by the PGRs, and was also affected by the PGR  $\times$  Year interaction (Table 2.4). Lodging scores decreased in meadow grass after TE application, and this response was particularly apparent in 2022 (Figure 2.5). Severity of lodging in timothy declined under both TE and CC application, and was lowest under TE (Table 2.4), and these responses were again more apparent in 2022 during which lodging of grasses was overall more common ( $P \leq 0.001$ ). Application of N in spring did not induce changes in lodging in any of these grass crops ( $P \geq 0.2$ ). In general, the check and ETH treated plots for meadow bromegrass, and timothy led to the most lodging in the 2022-year production (Figure 2.4).

Creeping red fescue generally did not experience lodging, as well as lodging tolerance for this grass was not affected by PGRs application ( $P \geq 0.1$ ). But PGR application affected lodging tolerance of timothy and meadow grasses ( $P \leq 0.001$ ). Among PGRs, TE had the highest lodging tolerance among all three grasses. Spring N did not contribute to changes in lodging tolerance across grasses ( $P \geq 0.50$ ). In general, the check and ETH treatments resulted in the lowest lodging tolerance (i.e., most lodging), while the TE treatment resulted in the highest lodging tolerance (%) for meadow brome and timothy in the 2022-year production (Figure 2.5).

Grass seed yield attributes like individual seed weight (g) and seed number (# 1m<sup>-2</sup>) were not affected by the PGR applications ( $P \geq 0.1$ ; Table 2.5). Spring N addition did not affect seed weight in all three grasses ( $P \geq 0.06$ ), but spring N increased the number of seeds per unit area ( $P \leq 0.02$ ) in all grasses tested (Table 2.5). Grass seed weight and seed number were higher in the 2022-year production in both meadow brome and creeping red fescue ( $P \leq 0.001$ ) but not timothy ( $P \geq 0.06$ ). There was no interaction between the PGRs tested and N application on the seed number of any grasses, even though seed numbers (# 1m<sup>-2</sup>) were higher under spring applied N application in 2022-year production for both meadow brome and timothy (Figure 2.6).

Total seed yield of these grasses remained unchanged in relation to PGRs application ( $P \geq 0.1$ ) (Table 2.5). However, spring-applied N led to a significant increase in seed yield for both timothy and meadow bromegrass ( $P \leq 0.001$ ), but not creeping red fescue ( $P \geq 0.09$ ). Increases in seed yield due to N application were more apparent in 2022, when seed yields were generally more favourable for meadow brome and creeping red fescue ( $P \leq 0.001$ ). Importantly, PGRs and N treatment did not interact to effect seed yield (kg ha<sup>-1</sup>) in any of these grasses. Seed yield in meadow bromegrass was overall higher following spring-applied N application in the 2022-year production ( $P \leq 0.001$ ) (Figure 2.7).

### **2.3.2. Relationship between grass seed yield and lodging**

The linear regression of aggregate seed yield with respect to lodging score and lodging tolerance of meadow brome, timothy and creeping red fescue were examined. Grass stands with reduced lodging and higher lodging indices did not contribute to enhanced seed yield of these grasses ( $P \geq 0.05$ ) over the two years of seed production (Tables 2.6 & 2.7). The only exception was meadow bromegrass in the 2022 year of production, during which seed yield was negatively

correlated with lodging score ( $r = -0.39$ ,  $P < 0.0002$ ; Figure 2.8), while seed yield was positively correlated with lodging tolerance in meadow brome ( $r = 0.22$   $P < 0.007$ ; Figure 2.9).

### **2.3.3. Gross return and gross margin.**

The application of PGRs did not impact the gross return of any grass crop ( $P \geq 0.08$ ) but affected the gross margin specifically in timothy and creeping red fescue ( $P \leq 0.003$ ; Table 2.8). In both these grasses, ETH resulted in the lowest gross margin, while the check and TE treatments were comparable in terms of gross margin. The addition of spring N did not affect the gross return and or gross margin in timothy and creeping red fescue; however, returns increased due to added N in meadow brome ( $P \leq 0.001$ ; Table 2.8). In the 2022-year production, both meadow brome and creeping red fescue exhibited higher gross return and margin ( $P \leq 0.001$ ), but this increase was not observed in timothy ( $P \geq 0.1$ ).

## **2.4 Discussion**

### **2.4.1 PGR impacts on grass vegetative morphology and lodging**

Among all PGRs, treatment with TE consistently reduced plant height the most across all three grasses compared to the check. Shorter plant heights of meadow brome and timothy occurred because TE application decreased the length of the three upper-most internodes. In contrast, treatment with CC and ETH had a less consistent effect in reducing internode lengths and associated plant heights. Application of TE suppresses internode elongation and plant heights by limiting GA biosynthesis (Rademacher 2000), with the current results consistent with TE effects on reduced plant height and stem length in intermediate wheatgrass (Frahm et al. 2018), tall fescue (Chastain et al. 2015), creeping red fescue (Szczepanek, 2021), and perennial rye grass (Chastain et al. 2014). Although we did not find reductions in internode length in

creeping red fescue, the modest reduction in plant height for this species suggests that the effects of TE on this species are relatively subtle and distributed throughout the vertical profile of the plant.

Reduced plant height and internode length were expected to reduce biomass production. In this study, the only grass to exhibit reductions in biomass was meadow bromegrass, wherein all PGRs reduced biomass, but particularly TE. Reductions in brome biomass appeared to directly reflect the extent of reduction in plant height associated with each PGR (i.e., TE > CC > ETH > check). TE and CC inhibit GA biosynthesis (Hedden et al. 2000; Rademacher, 2000), thus restricting the cell elongation and leaf blade expansion leading to the reduction in plant height and biomass production (Zhang et al. 2017). Notably, these results contrast earlier studies showing that TE application did not affect the overall biomass of tall fescue (Chastain et al. 2014), intermediate wheatgrass (Frahm et al. 2018), and perennial rye grass (Chynoweth et al. 2014, Borm and van den Berg 2008). In the current study, treatment with TE increased culm diameter in creeping red fescue and timothy, which may have compensated for the loss of biomass from reduced height, leading to relatively constant final biomass under TE application. A similar result was reported by Farhm et al. (2018) for intermediate wheat grass. Of note is that meadow bromegrass did not experience changes in culm diameter from any PGR, which may have ensured a decrease in biomass with reduced height.

Lodging severity was reduced by the PGRs, particularly in meadow bromegrass and timothy, with TE generally leading to the least lodging, followed by CC. In contrast, ETH did not reduce incidences of lodging in any grasses. Reductions in lodging were also more apparent during the normal rainfall conditions of 2022, while 2021 (drought conditions) led to minimal benefits of PGRs, thereby highlighting the greater potential benefits of PGRs when growing

conditions are better for plant growth. Despite this, added spring N was not found to increase the risk of lodging in any grass. Spring N did not increase internode length and plant height, so there was no lodging notice in grasses, as lodging was reported for longer stems and plant height increased lodging in perennial ryegrass (Borm and Berg, 2008) and in intermediate wheatgrass (Jungers et al. 2017).

Reduced lodging of these grasses likely reflects the effects of a combination of the plant morphological changes reported above. Reduced plant heights from PGRs might have directly reduced lodging in meadow brome and timothy, as tiller height is a key trait controlling lodging. Elsewhere, treatment with TE reduced stem length by 28 % and led to control of lodging in perennial ryegrass (Chastain et al. 2015). In the current study, the thicker culm from TE application may further have reduced lodging in timothy and creeping red fescue. PGRs like TE and CC are known to create shorter and thickened internodes that increase lodging resistance in creeping red fescue (Szczepanek et al. 2021), and wheat (Wiersma et al. 2011). Similar findings were reported by Zhang et al. (2017) with the use of TE on annual wheat crops. Unlike TE, application of CC led to inconsistent benefits in reducing lodging, and ETH was relatively ineffective in reducing plant height, internode length and subsequent lodging in these grasses. Previous studies also found that ETH did not reduce lodging or change seed yield in common bent (Aamlid et al. 2007) and creeping red fescue (Szczepanek et al. 2021). Similarly, ETH was reported to be less effective in reducing grass shoot growth (Diesburg and Christians 1989; McCullough et al. 2006; McCullough et al. 2005). While we found at least moderate suppression of lodging by CC, Szczepanek et al (2021) reported that CC also had effect reducing internode length and increasing lodging resistance in creeping red fescue. However, studies reported that combination of CC and TE is more effective in reducing lodging and improving seed yield in



orchard grass (Rolston et al. 2014), tall fescue grass (Hudgins et al. 2017), meadow brome grass (Yoder et al. 2019), strong creeping red fescue and chewing fescue grass (Szczepanek et al. 2021).

#### **2.4.2. Effect of PGRs on grass seed production**

While the PGRs examined here markedly altered plant morphology, particularly during 2022, their application did not change key properties associated with grass seed production, including panicle length, seed weight, seed counts, and most importantly, seed yield. Moreover, this was true for all the grasses, and across all PGR treatments. Additionally, while spring N increased seed yields, especially in 2022, no interactions of N treatment with the PGRs were evident.

These results overall indicate that while PGR application altered plant morphology and reduced the incidence and severity of lodging, these changes did not translate into improved seed production. This contrasts with earlier studies showing TE was effective in reducing lodging and improving seed yield in creeping red fescue (Bitarafan et al. 2019; Zapiola et al. 2014), perennial ryegrass (Rolston et al. 2010) and tall fescue (Rolston et al. 2004). Use of TE is known to improve seed yield of grasses where lodging incidence was high (Chastain et al 2015, Trethewey et al. 2016); however, in our study the use of PGRs may not have influenced seed production attributes and yield as the lodging risk was nearly absent in the drought year of 2021, and remained relatively low during the 2022-year production. Thus, despite the reduction in lodging our field study did not show changes to seed yield among grasses. These results are similar to a study by Frahm et al. (2018) wherein PGR TE had no effect on improving seed yield in intermediate wheatgrass unless lodging severity was high. Similarly, Rolston et al. (2012) also

found no advantage of CC in perennial ryegrass seeds. PGR CC and ETH reported to be ineffective in increasing yield attribute and seed yield of alfalfa (Wenhua et al. 2008).

We did not detect PGR induced changes to any yield attributing traits in our study. This finding was supported by earlier studies showing the number of florets in perennial ryegrass (Rolston 2010), seed weight in tall fescue (Chastain et al. 2015) and perennial ryegrass (Borm and van den Berg 2008) were not affected by TE application. While other studies, such as Chastain et al. (2015), reported that the application of TE improved seed numbers, which contributed to improved seed yield of perennial ryegrass and tall fescue.

Griffith (2000) claimed that assimilates accumulated in tillers are the supply for seed development of perennial ryegrass, Italian ryegrass, and tall fescue. However, Chynoweth and Moot (2017) suggested that reducing tiller height using PGRs would reduce sink capacity, thereby increasing seed filling and seed yield in perennial ryegrass. In another study by Szczepanek et al. (2019), PGR was found to increase seed yield of creeping red fescue despite of reduced tiller height, suggesting a favorable distribution of assimilates in the plant. In our study, despite reduced tiller height and unchanged biomass, PGRs were not enough to contribute to yield attributes like panicle length, seed weight and seed number and subsequently in seed yield of these grasses. However, TE demonstrated comparable marginal gross profit relative to the check in both timothy and creeping red fescue, despite higher input costs due to PGR application.

While high N fertilization is known to exacerbate lodging in grasses (Jungers et al. 2017; Bolt and Studer 2010), the addition of spring N to our forage grasses did not increase lodging risk despite increasing grass biomass and seed yield in meadow brome and timothy. Spring N fertilization can increase grass biomass, leading to more solar energy capture and photosynthate

allocation to developing seeds (Chastain et al. 2014). Similarly, Young et al. (1996) reported that spring N fertilization in perennial ryegrass increased seed yield by as much as 40% compared to autumn N fertilization alone. A study conducted in colder winters and shorter growing seasons of the Peace River region of northwestern Canada recommended N fertilization be done in spring before beginning of vigorous plant growth for grass seed production (Fairey and Lefkovitch 2000). Karamanos and Stevenson (2013) also reported that spring N fertilization resulted in satisfactory yield of forage crops in the Peace region of Alberta. The N application in fall promotes development of fall tillers, which become reproductive after vernalization in winter. Similarly, N applied in spring provides nutrients for the tillers undergoing floral induction from the start of growth to seed set (Boelt and Studer 2010). Unlike creeping red fescue, timothy does not need vernalization, but requires a long day length to produce a seedhead (Heide 1994) and high fertilization for seed production (McKenzie et al. 2009).

In this study, high residue builds up in creeping red fescue immobilize spring applied N. Creeping red fescue has a creeping growth habit (Zapiola et al. 2006) and quickly fills the space between seed rows, and residue removal with proper utilization of N to increase yields (Fairey et al. 2005). However, in bunch grasses such as timothy and meadow brome, N fertilization in spring was beneficial. The studies on similar types of bunch grasses like perennial ryegrass and tall fescue found that spring applied N increased biomass yield and associated seed yield (Chastain et al. 2014).

In the current study, PGRs and N showed no interaction effect on the seed yield of meadow brome, timothy and creeping red fescue. Chastain et al. (2014) reported that PGRs increased seed yield with spring N application to perennial ryegrass. The combination of spring N and TE increased seed yield, largely attributable to increased seed numbers (Chastain et al.

2014). Our study was consistent with Szczepanek et al. (2021) where combinations of PGRs and N did not affect seed yield in creeping red fescue and chewing fescue. Similarly, separate studies by Frahm et al. (2018) and Zimbric et al. (2021) showed no interactions between N rate and TE for any response variable in intermediate wheatgrass.

In 2022, meadow brome and creeping red fescue had higher seed yield and yield components than in 2021, while timothy had similar seed yield in both years. The wide range of weather conditions over the 2 years of study, including hot, dry conditions in June 2021 with an extreme heat wave, resulted in early maturation of creeping red fescue and meadow brome. However, the longer-duration crop - timothy, appeared to better tolerate the heat wave and remained longer in a vegetative state, potentially accounting for the lack of difference in seed yield between years. More favorable growing conditions in 2022 led to greater lodging severity, particularly in the ETH and check treatments of timothy and meadow brome. Not surprisingly, spring N was more effective in increasing seed number and seed yield in timothy and meadow brome during the wetter conditions of 2022.

Finally, it should be highlighted that while no direct effects of PGR treatment were found on the total seed yield of these grasses, reduced lodging severity was associated with an increase in grass seed yield, particularly for meadow bromegrass in 2022 only (Figures 14 and 15). The reductions in lodging were directly associated with PGR application, particularly TE. Hence, it is reasonable to conclude that the application of PGRs played a significant role in increasing the yield by affecting plant morphological characteristics and reducing lodging in field conditions. Despite the higher variable input costs associated with PGR application, marginal gross profits were comparable between the TE and the check in timothy and creeping red fescue. Additionally, given that the PGRs reduced lodging severity in all grasses, especially TE, PGR

treatments may lead to benefits other than seed yield, such as improved harvestability. Within the research framework of this study, harvestability was not tested, and therefore any losses in potential seed harvest efficiency due to increased lodging will not be reflected in our results.

## **2.5 Conclusion**

Among all PGRs examined, the use of trinexpac-ethyl had the largest and most consistent effects on shortening internode lengths, corresponding plant height, and reducing lodging in the forages examined. PGR effects were also more apparent in meadow bromegrass and timothy than red fescue. While CC and ETH also exhibited impacts on grass morphology, these were less consistent, and did not reduce lodging to the same degree. Despite the effectiveness of PGRs in reducing lodging, they did not increase grass seed yield attributes such as panicle length, thousand kernel weights, and seed numbers (# 1m<sup>-2</sup>), and this led to no changes in overall seed yield of the three grasses. While spring N application did not induce lodging it was beneficial in increasing biomass and seed yield in timothy and meadow brome, as well as seed number (#1m<sup>-2</sup>) in all grass crops. PGRs were particularly useful in reducing lodging during favorable growing conditions, with little to no benefit during dry conditions. Further studies on the benefits of PGRs on grass seed production are warranted, including understanding their secondary benefits, such as reduced lodging on grass seed harvestability.

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**Table 2.2:** Effect of plant growth regulators (PGRs) and nitrogen on plant height, internode diameter and biomass of meadow brome, timothy and creeping red fescue. The values are means of four replicates from two years (n = 8) of seed production.

Treatment	Meadow brome			Timothy			Creeping red fescue		
	Plant height (cm)	Internode diameter (mm)	Biomass (kg ha <sup>-1</sup> )	Plant height (cm)	Internode diameter (mm)	Biomass (kg ha <sup>-1</sup> )	Plant height (cm)	Internode diameter (mm)	Biomass (kg ha <sup>-1</sup> )
<b>Plant growth regulator</b>									
TE	73.6 d	1.88	4598 c	63.9 b	1.71 a	5471	66.9 b	1.21 a	3015
ETH	97.5 b	1.91	5786 b	88.4 a	1.56 b	5825	68.8 ab	1.09 b	3019
CC	80.2 c	2.02	5306 bc	82.9 a	1.50 b	5565	69.3 ab	1.09 b	3125
Check	106.5 a	1.77	6526 a	87.6 a	1.51 b	5838	72.3 a	1.09 b	3212
SEM (±)	1.45	0.074	293	1.53	0.04	225	1.180	0.033	188
<b>Nitrogen</b>									
Fall N	89.1	1.92	5117 a	79.8	1.58	5262 b	69.3	1.13	3051
Fall + Spring N	89.7	1.86	5992 b	81.6	1.56	6088 a	69.3	1.12	3135
SEM (±)	1.03	0.05	249.0	1.08	0.028	173	0.83	0.023	171
<b>Year</b>									
2021	77.9 b	1.62 b	4453 b	66.9 b	1.19 b	5078 b	64.0 b	1.06 b	3203
2022	101.0 a	2.16 a	6655 a	94.6 a	1.95 a	6271 a	74.6 a	1.18 a	2982
SEM (±)	1.03	0.05	249	1.08	0.028	173	0.833	0.023	171
<b>ANOVA (p-value significance tests)</b>									
PGRs	<0.0001	0.12	<0.0001	<0.0001	0.0013	0.49	<0.018	0.041	0.54
N	0.67	0.37	0.00022	0.25	0.55	0.00019	0.25	0.79	0.45
Year	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.00098	0.053
PGRs × N	0.82	0.28	0.86	0.80	0.69	0.55	0.80	0.49	0.47
PGRs × Year	<0.0001	0.49	0.48	0.16	0.25	0.4	0.16	0.09	0.22
N × Year	0.57	0.62	<0.0001	0.64	0.61	0.010	0.64	0.97	0.63
PGRs × N × Year	0.17	0.23	0.071	0.24	0.30	0.77	0.24	0.27	0.081

Note: Abbreviations: PGR: Plant growth regulators; TE: Trinexapac ethyl; CC: Chloromequat chloride; ETH: Etephon; N, nitrogen; SEM: standard error of the mean. Within a grass species and treatment grouping, treatment means followed by different letters indicate significant differences using a Tukey test ( $P \leq 0.05$ )

**Table 2.3:** Effect of plant growth regulators (PGRs) and nitrogen (N) on the length of the three uppermost internodes (cm) of meadow brome, timothy and creeping red fescue. Values are means of four replicates from two years (n = 8) of seed production.

Treatment	Meadow brome			Timothy			Creeping red fescue		
	Internode length (cm)								
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
<b>Plant growth regulators</b>									
TE	23.3 b	21.7 b	16.4 c	23.4 b	17.7 b	12.4 b	35.6	11.6	6.77
ETH	33.9 a	23.5 b	19.0 b	25.8 ab	21.0 a	16.3 a	36.6	11.9	6.96
CC	26.6 ab	29.2 a	17.3 bc	26.8 a	20.5 a	13.3 b	38.4	11.9	7.5
Check	34.5 a	28.6 a	21.5 a	26.2 ab	21.5 a	16.5 a	37.8	13.8	7.82
SEM ( $\pm$ )	0.84	0.717	0.577	0.82	0.7	0.58	1.1	0.59	0.44
<b>Nitrogen</b>									
Fall N	29.5	25.5	18.3	25.7	20.2	14.4	37.3	12.7	7.17
Fall + Spring N	29.7	26	18.8	25.7	20.1	14.8	36.8	12.3	7.36
SEM ( $\pm$ )	0.59	0.51	0.45	0.58	0.53	0.41	0.78	0.42	0.35
<b>Year</b>									
2021	27.3 b	21.2 b	14.8 b	22.3 b	15.6 b	11.4 b	31.1 b	11.9	7.48
2022	31.9 a	30.3 a	22.3 a	28.8 a	24.7 a	17.8 a	43.0 a	13.1	7.05
SEM ( $\pm$ )	0.59	0.51	0.45	0.58	0.53	0.41	0.78	0.42	0.35
<b>ANOVA (p-value significance tests)</b>									
PGRs	<0.0001	<0.0001	<0.0001	0.029	0.00096	<0.0001	0.31	0.061	0.17
N	0.82	0.45	0.25	0.67	0.82	0.50	0.66	0.47	0.62
Year	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.053	0.25
PGRs $\times$ N	0.060	0.54	0.15	0.66	0.61	0.31	0.33	0.39	0.14
PGRs $\times$ Year	0.16	0.06	0.055	0.38	0.20	0.76	0.43	0.46	0.37
N $\times$ Year	0.18	0.06	0.54	0.31	0.70	0.76	0.72	0.61	0.12
PGRs $\times$ N $\times$ Year	0.27	0.17	0.31	0.82	0.69	0.99	0.21	0.28	0.54

Note: Abbreviations: PGR: Plant growth regulators; TE: Trinexapac ethyl; CC: Chlormequat chloride; ETH: Etephon, SEM: standard error of the mean. Within a forage grass, treatment means followed by different letters indicate significant differences using a Tukey test ( $P \leq 0.05$ )

**Table 2.4:** Effect of plant growth regulators (PGRs) and nitrogen on lodging score, lodging tolerance (%) and panicle length (cm) of meadow brome, timothy and creeping red fescue. The values are means of four replicates from two years (n = 8) of seed production.

Treatment	Meadow brome			Timothy			Creeping red fescue		
	Lodging score	Lodging tolerance (%)	Panicle length (cm)	Lodging score	Lodging tolerance (%)	Panicle length (cm)	Lodging score	Lodging tolerance (%)	Panicle length (cm)
<b>Plant growth regulators</b>									
TE	1.50 b	89.1 a	13.1	1.12 b	90.2 a	6.36	1.19	83.9	9.49
ETH	2.31 a	71.0 b	13.4	2.00 a	80.5 b	6.10	1.38	81.3	9.86
CC	1.94 ab	84.2 ab	13.3	1.56 ab	89.8 a	6.43	1.25	82.0	9.31
Check	2.31 a	70.1 b	12.8	2.12 a	78.9 b	6.35	1.5	81.4	9.88
SEM (±)	0.11	2.78	0.334	0.094	2.2	0.28	0.121	<u>2.11</u>	0.29
<b>Nitrogen</b>									
Fall N	1.91	77.1	13.2	1.72	85.4	6.38	1.28	82.6	9.55
Fall + Spring N	2.12	80.1	13.1	1.69	84.3	6.24	1.38	81.7	9.72
SEM (±)	0.078	2.34	0.24	0.076	1.76	0.22	0.08	1.64	0.201
<b>Year</b>									
2021	1.66 a	82.8 a	12.4 b	1.12 b	85.0	6.40	1.12	81.6	8.88 b
2022	2.38 b	74.4 b	14.0 a	2.28 a	84.7	6.22	1.30	82.6	10.39 a
SEM (±)	0.078	2.34	0.24	0.076	1.76	0.22	0.08	1.64	0.201
<b><u>ANOVA (p-value significance tests)</u></b>									
PGRs	<0.0001	<0.0001	0.56	<0.0001	<0.0001	0.79	0.11	0.74	0.43
N	0.052	0.17	0.86	0.69	0.54	0.57	0.24	0.64	0.54
Year	<0.0001	0.00031	<0.0001	<0.0001	0.86	0.45	0.053	0.58	<0.0001
PGRs × N	0.54	0.34	0.3	0.40	0.93	0.38	0.87	0.60	0.54
PGRs × Year	0.0016	0.0036	0.82	<0.0001	0.00025	0.12	0.87	0.26	0.65
N × Year	0.16	0.17	0.66	0.24	0.56	0.73	0.24	0.94	0.52
PGRs × N × Year	0.10	0.50	0.99	0.63	0.19	0.22	0.33	0.37	0.79

Note: Abbreviations: PGR: Plant growth regulators; TE: Trinexapac ethyl; CC: Chlormequat chloride; ETH: Ethephon, SEM: standard error of the mean. Within a forage grass, treatment means followed by different letters indicate significant differences using a Tukey test ( $P \leq 0.05$ )

**Table 2.5:** Effect of plant growth regulators (PGRs) and nitrogen on seed weight(g), seed number (1m<sup>-2</sup>) and seed yield (kg ha<sup>-1</sup>) of meadow brome, timothy and creeping red fescue. The values are means of four replicates from two years (n = 8) of seed production.

Treatment	Meadow brome			Timothy			Creeping red fescue		
	1000 Seed weight (g)	Seed number (# 1m <sup>-2</sup> )	Seed yield (kg ha <sup>-1</sup> )	Seed weight (g)	Seed number (# 1m <sup>-2</sup> )	Seed yield (kg ha <sup>-1</sup> )	Seed weight (g)	Seed number (# 1m <sup>-2</sup> )	Seed yield (kg ha <sup>-1</sup> )
<b>PGRs</b>	z								
TE	4.43	15048	755	0.42	160217	688	1.07	31066	462
ETH	4.46	12145	600	0.41	150078	626	1.08	39714	361
CC	4.42	13369	652	0.40	161075	665	1.09	35305	406
Check	4.52	12609	626	0.41	162426	669	1.11	34830	422
Sem (±)	0.0759	1218	63.9	0.0053	5033	18.2	0.018	2403	30.1
<b>Nitrogen</b>									
Fall N	4.45	11170 b	547 b	0.41	148040 b	614 b	1.07	32348 b	388
Fall + Spring N	4.47	15415 a	769 a	0.41	172358 a	710 a	1.11	38110 a	437
Sem (±)	0.068	1001	53.8	0.0038	3757	12.9	0.013	1732	22.7
<b>Year</b>									
2021	3.98 b	5683 b	242 b	0.41	156084	647	0.895 b	27627 b	258 b
2022	4.93 a	20902 a	1074 a	0.42	164314	677	1.281 a	42832 a	567 a
Sem (±)	0.068	1001	53.8	0.0038	3757	12.9	0.013	1732	22.7
<b>ANOVA (p-value significance tests)</b>									
PGRs	0.47	0.18	0.14	0.13	0.073	0.12	0.41	0.094	0.1
N	0.64	<0.0001	<0.0001	0.82	<0.0001	<0.0001	0.060	<b>0.018</b>	0.09
Year	<0.0001	<0.0001	<0.0001	0.058	0.092	0.10	<0.0001	<0.0001	<0.0001
PGRs × N	0.81	0.91	0.82	0.95	0.053	0.062	0.054	0.082	0.09
PGRs × Year	0.54	0.43	0.48	0.72	0.055	0.096	0.87	0.42	0.26
N × Year	0.97	<b>0.00016</b>	<b>0.00011</b>	0.49	<b>0.029</b>	0.051	0.33	0.084	0.24
PGRs × N × year	0.36	0.73	0.83	0.48	0.96	0.82	0.19	0.43	0.39

Note: Abbreviations: PGR: Plant growth regulators; TE: Trinexapac ethyl; CC: Chlormequat chloride; ETH: Ethephon, SEM: standard error of the mean. Within a forage grass, treatment means followed by different letters indicate significant differences using a Tukey test (P ≤ 0)

**Table 2.6:** Linear regression between lodging score (1-5) and seed yield (kg ha<sup>-1</sup>) within each of meadow brome, timothy and creeping red fescue (n =32 per year)

Year	Meadow brome			Timothy			Creeping red fescue		
	R <sup>2</sup>	P value	Equation	R <sup>2</sup>	P value	Equation	R <sup>2</sup>	P value	Equation
<b>2021</b>	0.001	0.86	254.9-7.6 x	0.009	0.60	678.4-25.7 x	0.00021	0.94	253.5 + 4.3x
<b>2022</b>	0.39	<b>0.00012***</b>	1792.7-302.6x	0.050	0.22	745.7-31.6x	0.070	0.14	675.2-70.4x
<b>Overall</b>	0.05	0.075	343.1+156.3 x	0.005	0.59	675.9-8.39x	0.042	0.10	305.9+ 80.5x

\*\*\*P ≤ 0.001.

**Table 2.7:** Linear regression between lodging tolerance (%) and seed yield (kg ha<sup>-1</sup>) within each of meadow brome, timothy and creeping red fescue (n =32 per year)

Year	Meadow brome			Timothy			Creeping red fescue		
	R <sup>2</sup>	P value	Equation	R <sup>2</sup>	P value	Equation	R <sup>2</sup>	P value	Equation
<b>2021</b>	0.10	0.079	168.4+5.06x	0.007	0.64	572.2+0.9x	0.0058	0.678	333.8-0.9x
<b>2022</b>	0.22	<b>0.007 **</b>	320.9 +10.1x	0.006	0.68	602.1+0.9x	0.020	0.060	339.8-2.8x
<b>Overall</b>	0.00012	0.93	691.22-0.4x	0.006	0.54	589.8+0.9x	0.00013	0.89	451.5-0.5x

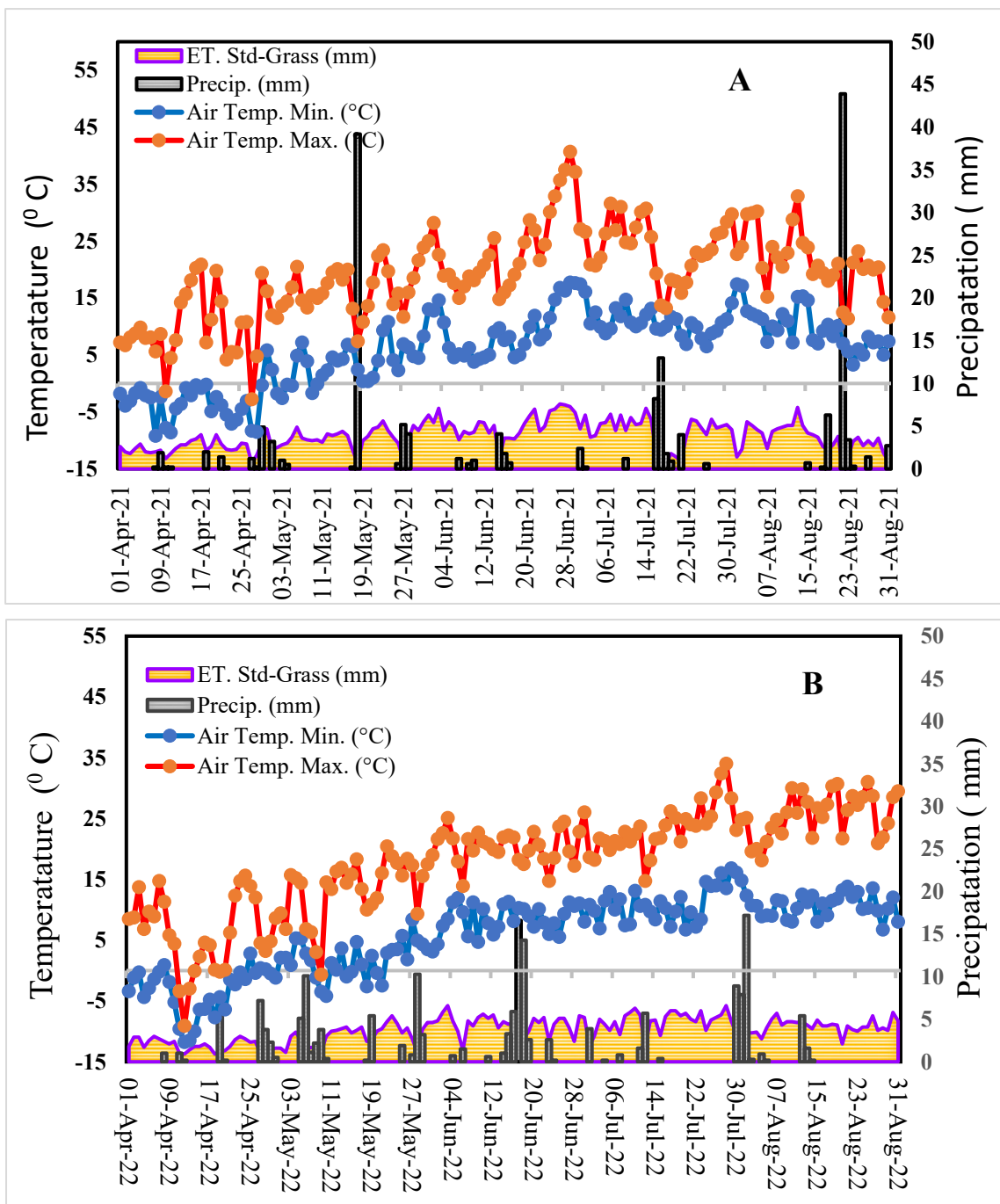
\*\*P ≤ 0.01.

**Table 2.8:** The gross return and gross margin of plant growth regulators (PGRs) and nitrogen on meadow brome, timothy and creeping red fescue. Values are means of four replicates from two years (n = 8) of seed production.

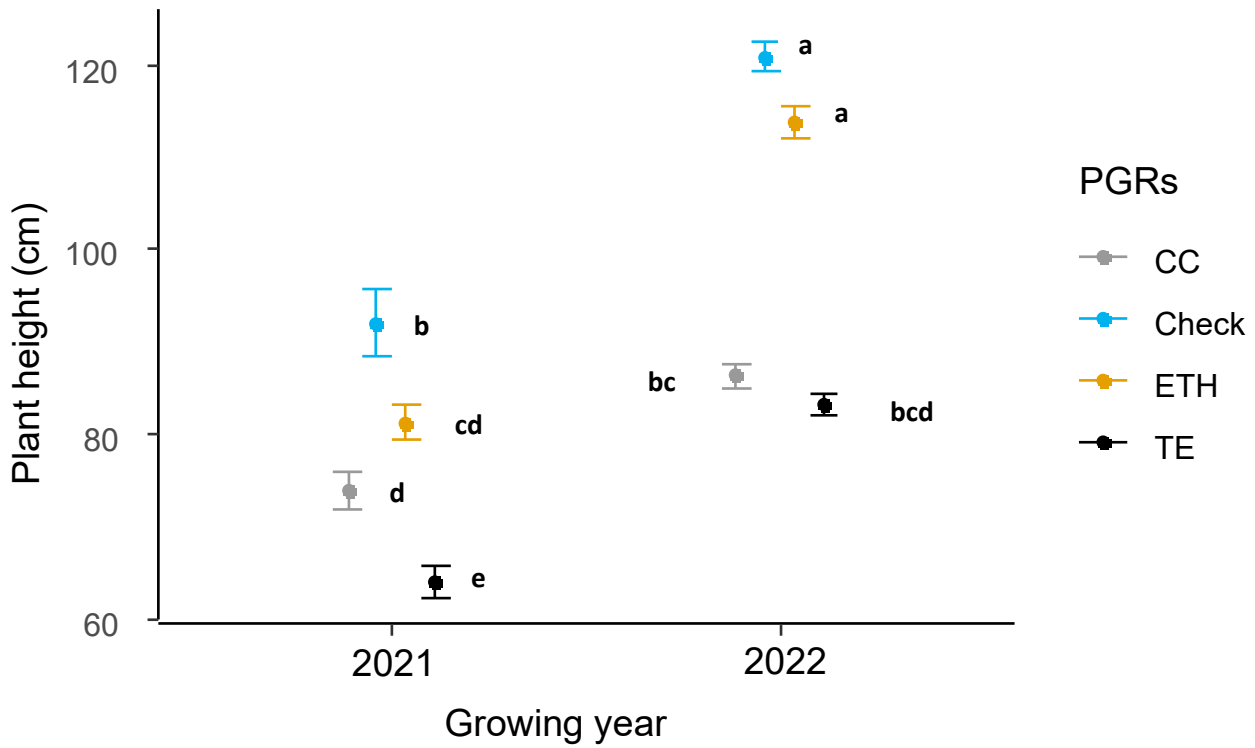
Treatment	Meadow brome		Timothy		Creeping red fescue	
	Gross return (CAN \$ ha <sup>-1</sup> )	Gross margin (CAN \$ ha <sup>-1</sup> )	Gross return (CAN \$ ha <sup>-1</sup> )	Gross margin (CAN \$ ha <sup>-1</sup> )	Gross return (CAN \$ ha <sup>-1</sup> )	Gross margin (CAN \$ ha <sup>-1</sup> )
<b>Plant growth regulators</b>						
TE	2413	2247	941	775 ab	622	456 ab
ETH	1918	1751	856	688 b	486	318 b
CC	2083	1922	909	747 b	546	384 ab
Check	2002	1940	914	851 a	568	505 a
SEM (±)	204	204	24.9	24.9	40.5	40.5
<b>Nitrogen</b>						
Fall N	1750 b	1674 b	839 b	762	522	446
Fall + Spring N	2458 a	2256 a	971 a	769	588	386
SEM (±)	172	172	17.6	17.6	30.5	30.7
<b>Year</b>						
2021	775 b	652 b	884	762	347 b	225 b
2022	3434 a	3277 a	928	769	763 a	607 a
SEM (±)	172	172	17.6	17.6	30.5	30.5
<b>ANOVA (p-value significance tests)</b>						
PGRs	0.14	0.17	0.11	<b>0.0032</b>	<b>0.010</b>	<b>0.0059</b>
N	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.79	0.087	0.12
Year	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.10	0.77	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
PGRs × N	0.82	0.82	0.062	0.061	0.088	0.088
PGRs × Year	<b>0.48</b>	0.48	0.10	0.10	0.26	0.26
N × Year	0.051	0.052	0.053	0.053	0.24	0.052
PGRs × N × Year	0.83	0.83	0.82	0.82	0.39	0.37

INote: Abbreviations: PGR: Plant growth regulators; TE: Trinexapac ethyl; CC: Chlormequat chloride; ETH: Ethephon, SEM: standard error of the mean. Within a forage grass, treatment means followed by different letters indicate significant differences using a Tukey test (P ≤ 0.05)

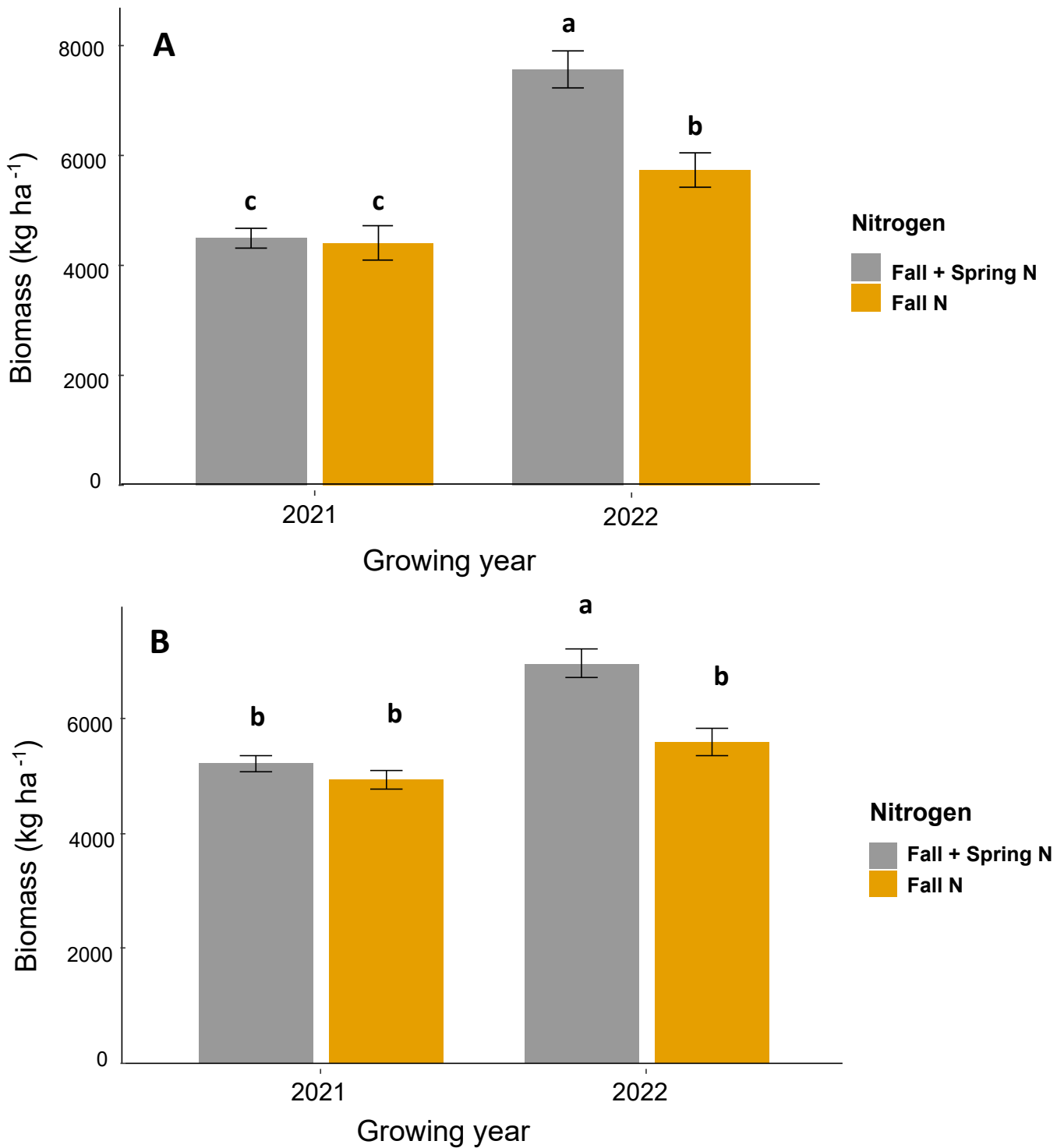




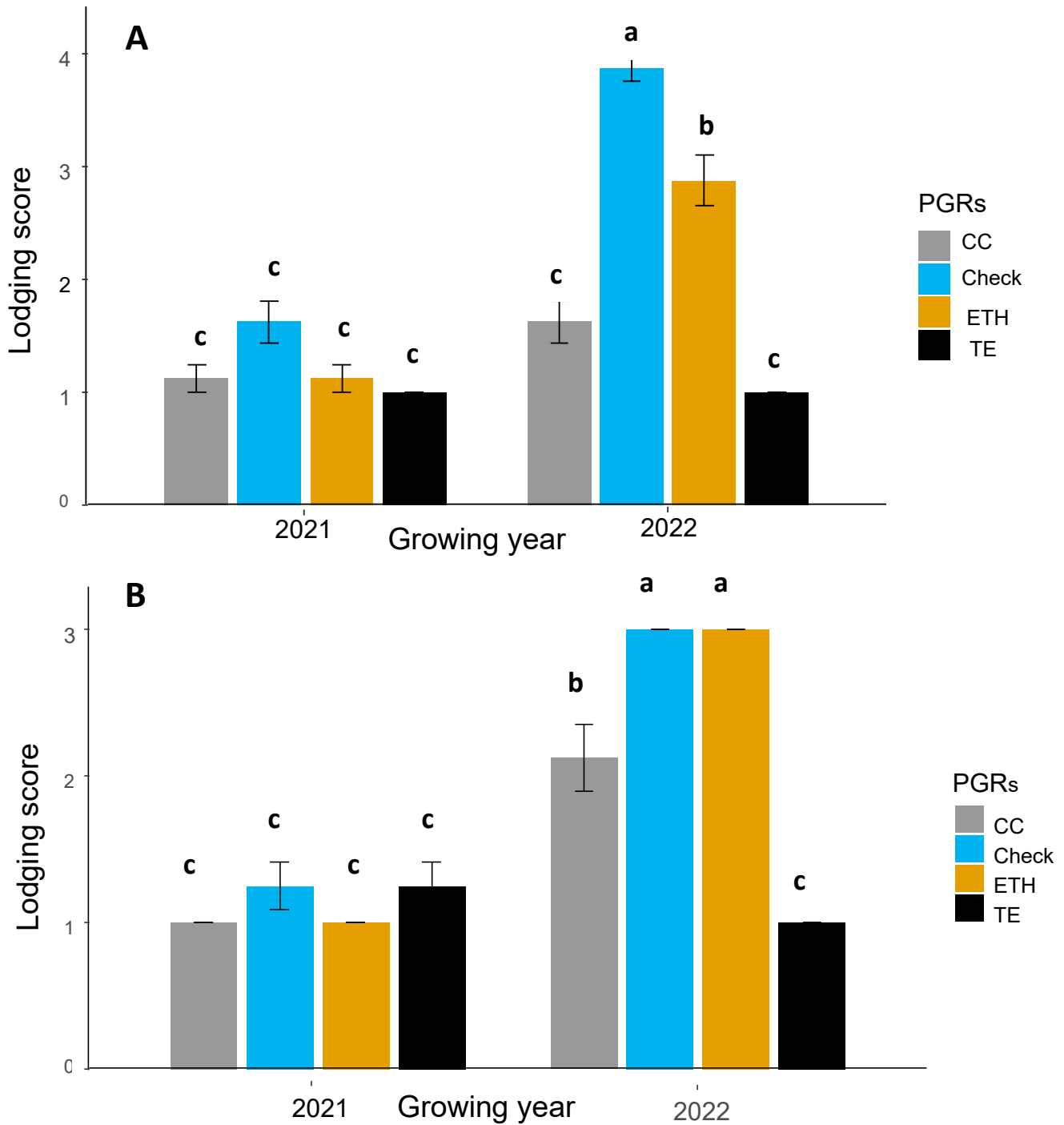
**Figure 2.1:** Summary of the daily growing season (1 April to 31 August) temperatures (minimum and maximum, °C), precipitation (mm) and evapotranspiration - ET (mm) during each of 2021 (A) and 2022 (B) at the AAFC Beaverlodge Research Farm, Alberta.



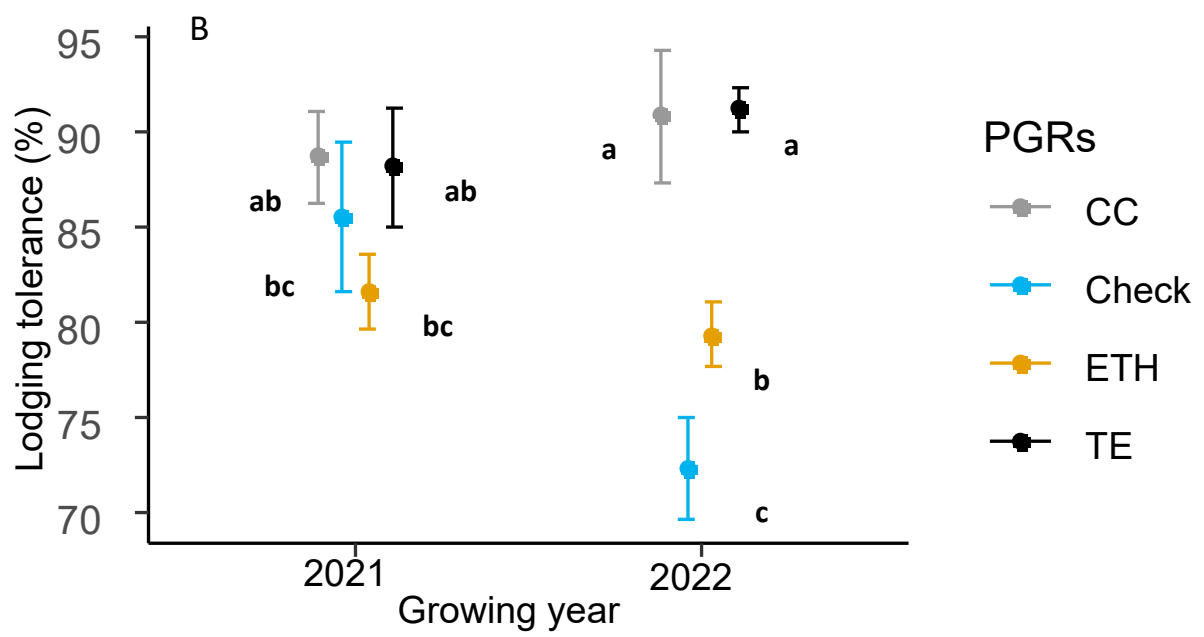
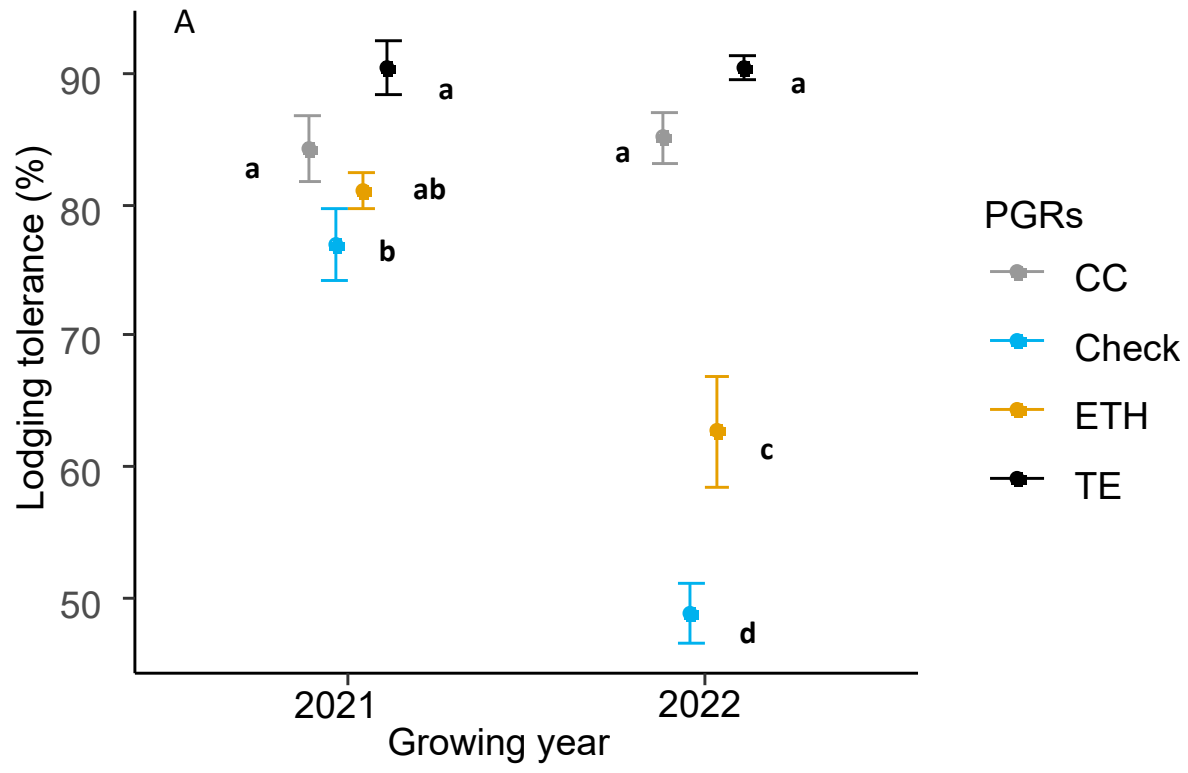
**Figure 2.2:** Effect of plant growth regulators (PGRs) on plant height of meadow brome in 2021 and 2022. The line bars are mean and error bars  $\pm 1$  SE of four replicates. The different letters indicate significant differences between treatments at  $p \leq 0.05$ .



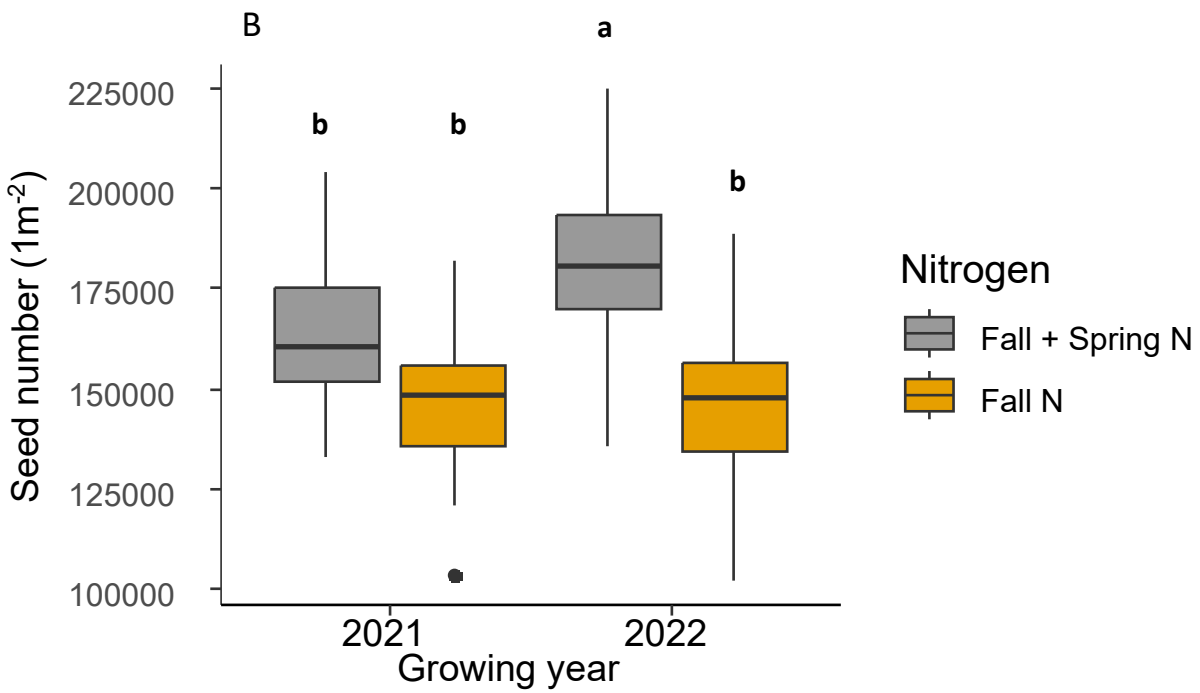
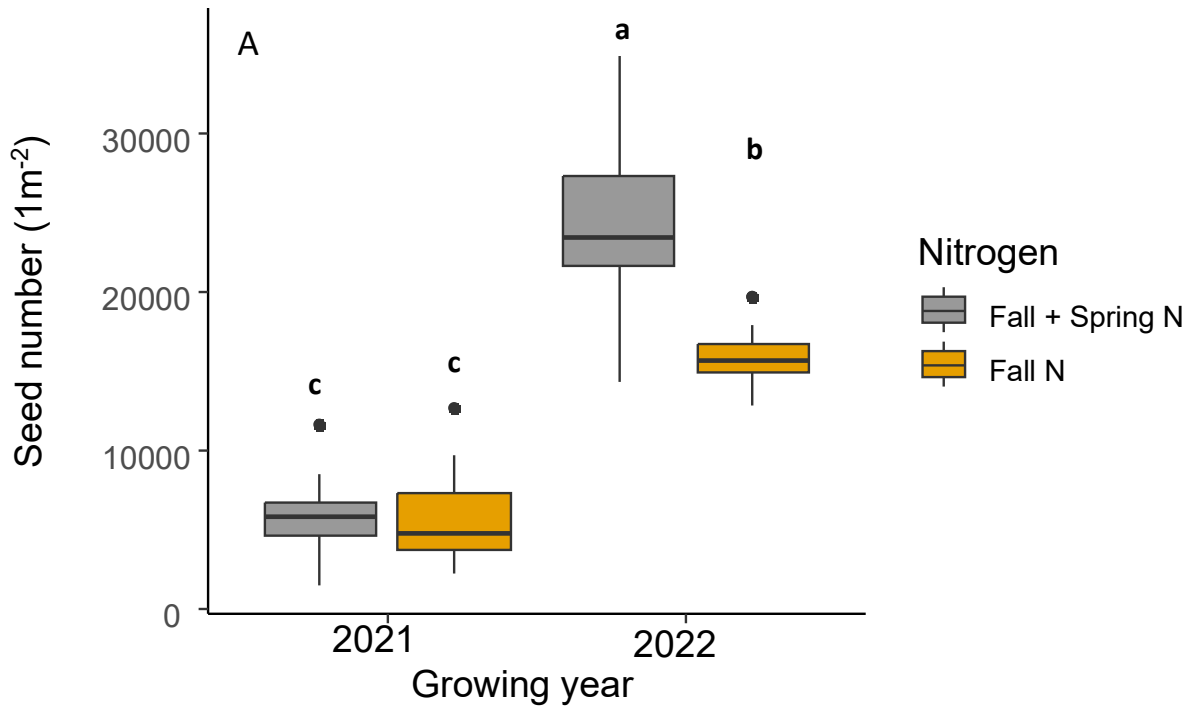
**Figure 2.3:** Effect of the timing of nitrogen application on biomass yield (kg ha<sup>-1</sup>) of meadow brome (A) and Timothy (B) in 2021 and 2022. The bars are mean and error bars  $\pm 1$  SE of four replicates. The different letters indicate significant differences between treatments at  $p \leq 0.05$ .



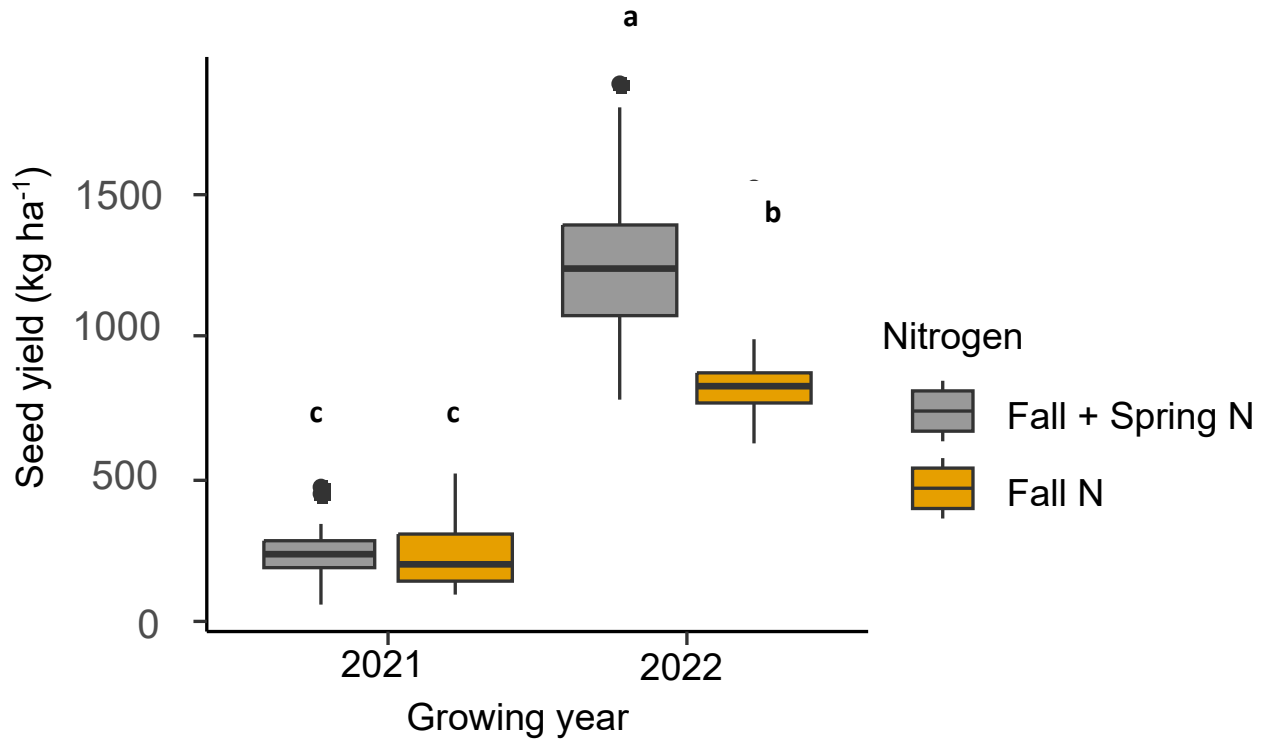
**Figure 2.4:** Effect of plant growth regulators on lodging score of meadow bromegrass (A) and timothy (B) in 2021 and 2022. Lodging score scale (1-5), 1 = not lodged (fully upright) and 5 = most severe lodging (horizontal). Bars are means and error bars  $\pm$  1 SE of four replicates. The different letters indicate significant differences between treatments at  $p \leq 0.05$ .



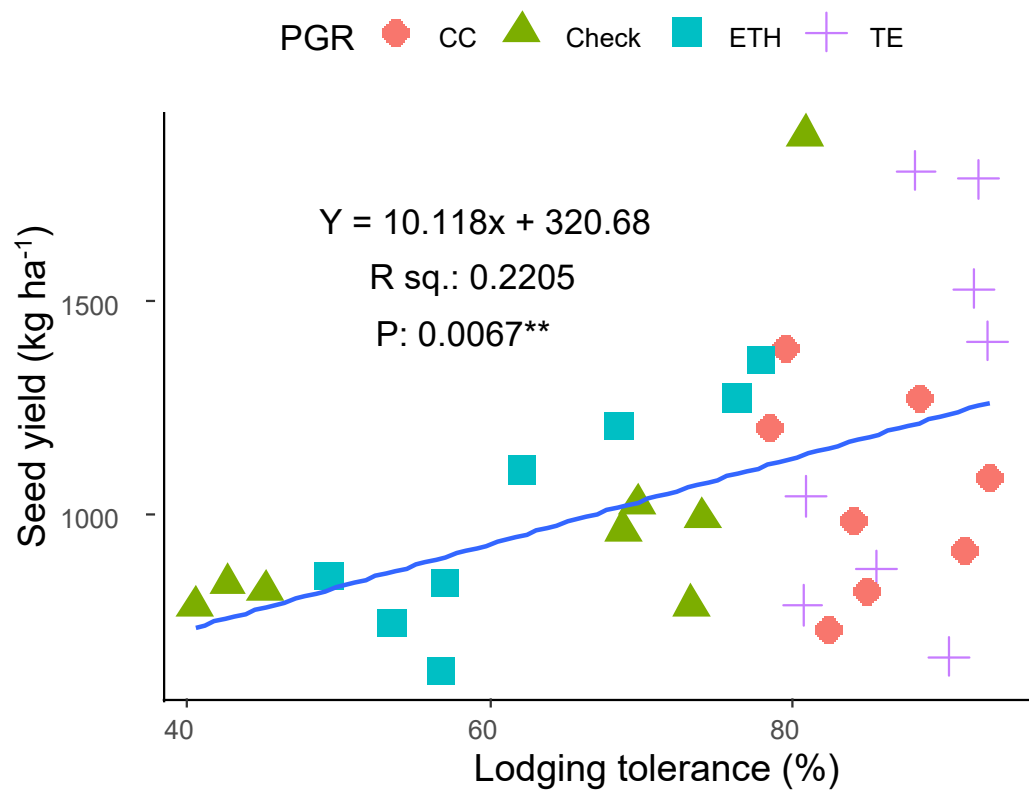
**Figure 2.5:** Effect of plant growth regulators on lodging tolerance in meadow bromegrass (A) and timothy (B) in 2021 and 2022. Line bar are means and error bars ± 1 SE of four replicates. The different letters indicate significant differences between treatments at  $p \leq 0.05$ .



**Figure 2.6:** Effect of nitrogen on seed number (1 m<sup>-2</sup>) of meadow brome (A) and timothy (B) in 2021 and 2022. The box plots are mean of four replicates. The different letters indicate significant differences between treatments at  $p \leq 0.05$ .

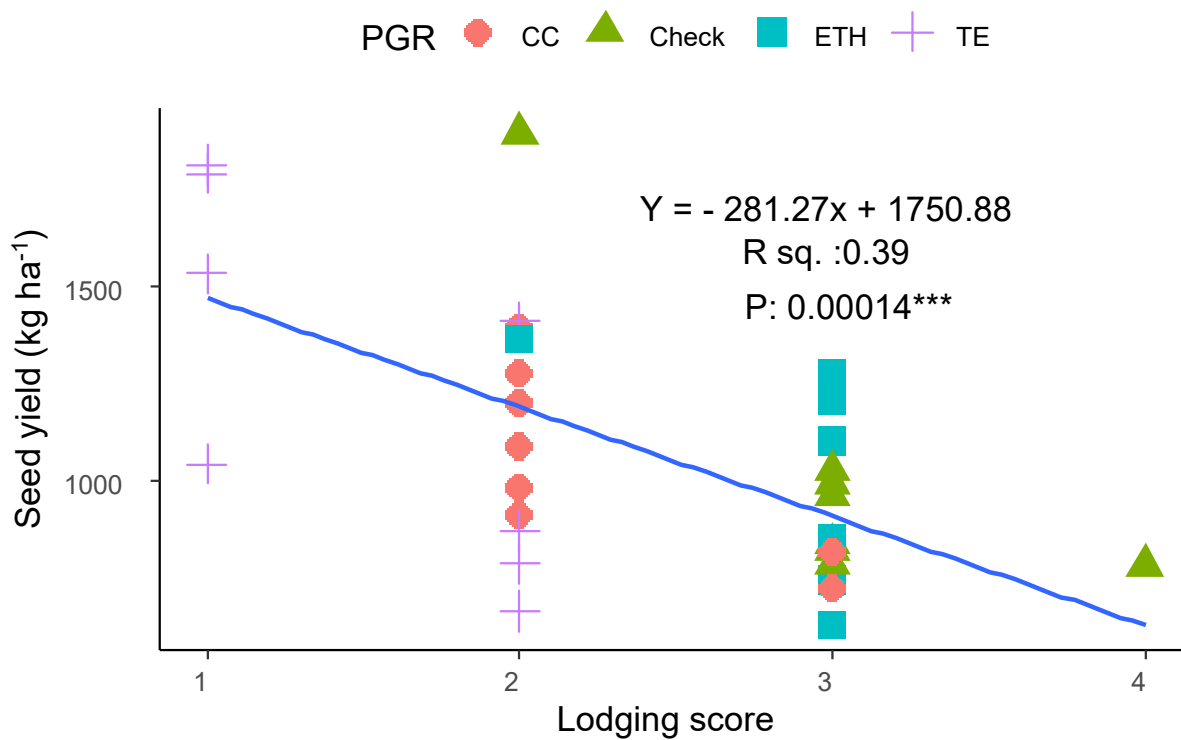


**Figure 2.7:** Effect of nitrogen on seed yield (kg/ha) of meadow brome in 2021 and 2022. The box plots are the mean of four replicates. The different letters indicate significant differences between treatments at  $p \leq 0.05$ .



**Figure 2.8:** Relationship of seed yield to lodging tolerance (%) of meadow bromegrass in 2022 growing season.





**Figure 2.9:** Relationship of lodging score and seed yield of meadow bromegrass in 2022 growing season. Lodging score scale (1-5), 1 = not lodged (fully upright) and 5 = most severe lodging (horizontal).

### Chapter 3. Plant growth regulator effects on grass seedling morpho-physiology

#### Abstract

The growth retardant group of plant growth regulators (PGRs) reduce plant height and lodging, thereby preventing yield losses in grass seed crops. A greenhouse experiment was conducted to study the effect of PGRs on vegetative growth, root-shoot allometry and photosynthetic characteristics creeping red fescue (*Festuca rubra* L.), timothy (*Phleum pratense* L.), and meadow bromegrass (*Bromus riparius* Rehmman). Three types of PGRs, trinexapac-ethyl (TE), chlormequat chloride (CC), and ethephon (ETH) were applied as foliar spray at 0.20 kg, 1.12 kg, and 0.60 kg ai ha<sup>-1</sup>, respectively, to growing seedlings of each grass species at the 5-6 leaf stage of development. The measured variables included root and shoot biomass, root-to-shoot ratio, root area, root length, leaf area, tiller number, plant height, lodging severity, chlorophyll concentration, carotenoid concentration, and the maximum quantum yield of photosystems II. Analysis of variance and principal component analysis were used to differentiate treatments based on their morph-physiological characters on each grass species. The TE-treated plants showed a reduction in root, shoot, and total biomass for up to 40 days in timothy and 55 days in creeping red fescue but did not reduce biomass in meadow bromegrass at any time point after PGR application. None of the PGRs (TE, CC, and ETH) had effects on the root area, root length, leaf area, tiller number, root-to-shoot ratio, and many other growth variables (RGR, NAR, LWR and SLA). However, both TE and CC reduced the plant height and lodging severity of all three forage grasses. The PGRs tested had no effect on the quantum yield of photosystem II ( $F_v/F_m$ ) at any time after treatment, but PGR TE and CC increased concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in timothy even after 70 days after PGR application. PC analysis showed that ETH and check treatments

exhibited higher total biomass, shoot biomass and plant height, and lodging severity, while TE and CC exhibited shorter plant height and lower lodging. Furthermore, TE and CC treatments resulted in higher chlorophyll and carotenoid concentrations as well as higher maximum quantum yields (QY) and net assimilation rate (NAR) for all tested crops. TE consistently reduced plant height and lodging severity without affecting biomass production, while maintaining higher concentrations of chlorophyll and carotenoids in all grasses.

**Keywords:** biomass, chlorophyll, grass morphology, lodging severity, quantum yield

### 3.1 Introduction

Plant growth regulators (PGRs) are naturally occurring or synthetic chemicals that promote or inhibit plant growth and development by affecting metabolic or physiological processes. Generally, these are exogenously applied substances that interact with phytohormones to modulate plant physiological processes. Based on their specific functions, PGRs are grouped into auxins, abscisic acid, cytokinins, ethylene, jasmonic acid, salicylic acid, brassinosteroids, and strigolactone (Santner et al. 2009; Vanstraelen and Benková 2012). These compounds are used worldwide in various crops to improve germination, plant growth, and yield under both normal and stress conditions (Rademacher 2015; Small & Degenhardt 2018). PGRs have mostly been used in cereal and horticultural crops, and more recently have been used in turf grasses and other forage seed crops.

The PGRs that specifically inhibit cell elongation leading to shorter plant height without hampering productivity are known as growth retardants. Growth retardants work by inhibiting the production of gibberellic acid (GA) or promoting the biosynthesis of ethylene. TE, CC, and

ETH are commonly used retardants in cereal, forage and turf grasses. CC is a quaternary ammonium compound that inhibits cyclo-oxygenase and ent-kaurene synthase during the early steps of GA metabolism (Rademacher, 2015). In contrast, TE is an acylcyclohexanedione compound that inhibits 3-hydroxylation during the final stage of GA biosynthesis pathways (Rademacher 2000). Unlike the aforementioned PGRs, ETH affects plant growth by releasing the hormone ethylene, which in turn, inhibits cell growth and stem elongation (Rajala et al. 2002).

Growth retardants are commonly used in turf grass systems to reduce the need for mowing (Czeluscinski et al. 2017) and maintain leaf colour (Głąb et al. 2020). Both CC and TE have had consistent effects in reducing plant height and lodging occurrence of forage seed crops (Zapiola et al. 2014; Chastain et al. 2014; Bitarafa et al. 2019). However, their effect on the morphological responses of grasses, such as tiller number, root and shoot biomass, root length, and root area, were not consistent. Rajala and Peltonen-Sainio (2002) reported that TE applied at an early stage (ZGS 39, Zadoks et al., 1974) reduced shoot length and increased the number of tillers but did not affect the biomass of cereal crops. In contrast, other studies have found that TE reduced the biomass of grass crops (Fagerness and Yelverton 2000; Lickfeldt et al. 2000). Effects of PGRs on root growth are also inconsistent among grass species. Application of TE increased root biomass in Bermuda grass (*Cynodon dactylon* L.) (McCarty et al. 2011; McCullough et al. 2005) but did not affect root biomass in Kentucky bluegrass (*Poa pratensis* L.) (Ervin and Koski, 2001) or bentgrass (*Agrostis stolonifera* L.) (Fagerness and Yelverton 2001). Most findings of PGR effects on root growth are based on biomass rather than root architecture. Beasley et al. (2005) reported that PGRs reduced root length and surface area in the cool-season species Kentucky bluegrass. Several studies suggest that PGRs have no effect on

root architecture in turfgrasses (Serena et al. 2020) nor on the root development of St. Augustine grass (*Stenotaphrum secundatum* (Walter) Kuntze) (Hargey et al. 2016).

While the application of ETH reduces plant height (Shatters et al. 1998), it also reduced root biomass in creeping bentgrass (McCullough et al. 2006) as well as both root biomass and root length in bermudagrass (McCullough et al. 2004). However, other studies using ETH have shown that it is not as effective in reducing internode length and shoot growth as compared to gibberellin biosynthesis inhibitors (McCullough et al. 2006; Volterrani et al. 2015). Iqbal et al. (2017) found that ETH accelerated leaf senescence by releasing ethylene, which caused chlorophyll breakdown and an associated colour fading of leaves. In contrast, TE and CC inhibit leaf elongation, which increases the density of mesophyll cells and their chlorophyll content, resulting in darker green colouration of leaves. Both TE and CC are known to increase chlorophyll content (McCullough et al. 2004) and carotenoid content (Gliozzeris et al. 2007; Kasem et al. 2015).

PGRs can retard the growth of plants by modifying the size and number of organs, despite photosynthesis remaining unaffected in grass crops (Ervin and Koski 1998; Quin et al. 1998). Treatment with PGRs can change important growth variables like relative growth rate (RGR), net assimilation rate (NAR), leaf weight ratio (LWR) and specific leaf area (SLA) through alterations to the morphology and physiology of grasses. PGRs increase chlorophyll content and the photosynthetic efficiency of leaves by regulating endogenous hormonal activity (Zhang and Schmid 2000). Some studies have found that TE application increased chlorophyll (McCullough et al. 2006; Heckman et al. 2000; Amiri-Khah et al. 2015), thereby enhancing photosynthesis (Xu and Huang 2010). TE application led to increased photosynthetic efficiency in creeping bent grasses (*Agrostis stoloniferous* var. *palustris*) (Zhang and Schmidt 2000) and

zoysiagrass (*Zoysia matrella* L.) (Qian and Engelke 1999), while others report no effect on photosynthetic efficiency, for example in Kentucky bluegrass (Beasley and Bruce 2007).

In Canada, both CC and ETH are registered for use on wheat, whereas TE is registered only for use on turf-type perennial ryegrass. Limited information and inconsistent results are therefore available on the effect of these plant growth regulators on forage grass crops, including in northern temperate growing conditions. Creeping red fescue, meadow bromegrass and timothy are popular grass seed crops in the Peace River region of western Canada. Where grown for seed, lodging of grass crops under favorable growing conditions is known to occur (Pavlista et al. 2010), which in turn, compromises forage seed yields, harvest efficiency, and quality (Zhang et al. 2017). In these situations, PGRs may be a useful tool to reduce lodging and associated economic losses (Trethewey et al. 2016). Despite the extensive studies done on the reproductive responses of various grasses to PGRs, there is a lack of understanding about the specific morpho-physiological responses of key grass crops to PGR application, including at the early stage of development. It was hypothesized that plant growth retardants will alter juvenile grass growth, phenotypic and photosynthetic characteristics of perennial grasses. This study quantified the morpho-physiological responses of creeping red fescue, timothy, and meadow bromegrass to different PGRs applied to juvenile plants. We specifically compared the efficacy of several growth retardant PGRs, including TE, CC, and ETH, for altering root and shoot biomass, root: shoot ratio, root area, maximum? root length and plant height, internode length, lodging severity, chlorophyll content, photosynthetic efficiency and other growth parameters on greenhouse-grown seedlings.

## **3.2 Materials and methods**

### **3.2.1 Plant material and growing conditions**

This study was performed from October 2021 to May 2022 under greenhouse conditions at the Agriculture and Agri-Food Canada's Beaverlodge Research Farm (55°12'01.5"N 119°23'53.9"W), which is situated 40.7 km west of Grande Prairie, Alberta. Timothy, creeping red fescue and meadow brome grass were examined as major seed crops in the area. All three grasses were seeded on November 1, 2021, in a seedling tray with Pro-Mix BX Mycorrhizae growing media (Premier Tech, Québec, Canada) and maintained using 16h light and 8h dark periods, with 18°C temperature and 162  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of photosynthetically active radiation. Two weeks after seeding, uniform and healthy grass seedlings that emerged were individually transplanted into two-gallon plastic pots with 100% sand media; there were a total of 96 pots of each grass species. Plants were fertigated with 0.5 g of Miracle Pro (24:8:16 NPK) in 100 ml of water twice a week throughout the growing period. As the plant water need increased, a thin film of water was supplemented every day to prevent desiccation.

### **3.2.2 Treatments and experimental design**

The experiment consisted of four treatments: (1) check (no PGR treatment), (2) TE applied at 0.20 kg ai ha<sup>-1</sup>, (3) CC applied at 1.12 kg ai ha<sup>-1</sup>, and (4) ETH applied at 0.60 kg ai ha<sup>-1</sup>. The four treatments were applied separately onto three grasses - creeping red fescue, timothy, and meadow brome grass. The experiment was arranged in a randomized complete block design with four replications, leading to an initial sample size of 8 pots for each species × treatment combination. Plots with multiple seedlings were thinned to maintain a single seedling in each pot. Plant growth regulators were applied to growing seedlings at 5-6 fully visible leaf stages,

consistent with a 15-16 growth stage based on the Zadok's decimal code framework (Gustavsson 2011).

### **3.2.3 Biometric measurements**

Grass plant height, tiller number, biomass, leaf area, maximum root length, and root area were recorded on days 20, 45, 55 and 70 after plant growth regulators were applied. To obtain destructive measurements such as biomass and root attributes, one sample pot per treatment per replicate was randomly selected at each sampling point in time. After tillers were counted and plant height was assessed, all sand was washed from each sample plant, and leaves and roots were carefully separated. Leaf area and root properties were measured on fresh samples on thin film of water transparent tray using an EPSON Expression 12000XL with Scanner Epson A3 Transparency Unit aided with WinFOLIA and WinRHIZO software (Regent Instruments Inc., Quebec City, QC, Canada). Roots and shoots of individual sample plants were weighed for fresh biomass and put in paper bags for drying. Root and shoot samples were dried at 55°C for 72 hours and weighed. The root-to-shoot ratio was determined using dry weights.

The internode length and stem diameter of timothy and meadow brome grass were determined 70 days after the plant growth regulator application. Creeping red fescue stems did not produce distinct nodes and internodes, hence no measurements were available for these variables. The length of each internode was measured using a ruler. Stem diameter was taken at the center of each internode using a caliper (0.1 mm). Similarly, lodging severity was assessed 70 days after PGR application on a five-point scale, where 1 denoted fully upright tiller with no signs of lodging, and 5 denoted the most severely lodged tillers as represented by a horizontal position (Young III et al. 1999).

Additional measurements were taken to delve further into the effect of plant growth regulators on grass responses; the relative growth rate (RGR) and net assimilation rate (NAR)



were measured between 20 days and 70 days, and the leaf weight ratio (LWR) and specific leaf area (SLA) were measured 70 days after PGR application. RGR ( $\text{g g}^{-1} \text{day}^{-1}$ ), NAR ( $\text{g cm}^{-2} \text{day}^{-1}$ ), SLA ( $\text{cm}^{-2} \text{g}^{-1}$ ) and LWR were calculated using formula published by Sudhakar et al. (2016), as follows:

$$\text{Relative growth rate (RGR)} = [\log_e W_2 - \log_e W_1] / [t_2 - t_1]$$

$$\text{Net assimilation rate (NAR)} = [(W_2 - W_1) \times (t_2 - t_1)] / [(\log_e A_2 - \log_e A_1) / (A_2 - A_1)]$$

$$\text{Specific leaf area (SL)} = [(\text{leaf area} / \text{leaf dry weight})]$$

$$\text{Leaf weight ratio (LWR)} = [(\text{leaf weight} / \text{dry weight of plant})]$$

Where  $W_2$  and  $W_1$  are plant dry weight at time  $t_2$  and  $t_1$ , and  $\log_e A_2$  and  $\log_e A_1$  are natural logs of leaf area  $A_2$  and  $A_1$  measured at time  $t_2$  and  $t_1$ .

### 3.2.4 Physiological measurements

The youngest (uppermost, but fully developed) leaf was used for chlorophyll fluorescence measurement using a MINI PAM (pulse amplitude modulation), portable chlorophyll fluorometer (Heinz Walz GmbH, Germany). Four plants were used for each replication and placed in a dark room for 30 min to bring the electron transport chain into an oxidized state before measuring chlorophyll fluorescence parameters. After dark adaptation, minimum fluorescence ( $F_o$ ) and maximum fluorescence ( $F_m$ ) were measured using the minimal light intensity ( $<1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and saturation pulses ( $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$  for 3 s) at 1 Hz respectively. Variable fluorescence ( $F_v$ ) was calculated as  $F_m - F_o$  and maximum quantum efficiency of photosystem II (hereafter maximum quantum yield, QY), was calculated as  $F_v / F_m$ , where  $F_v / F_m = (F_m - F_o) / F_m$ . The maximum quantum efficiency ( $F_v / F_m$ ) was taken at 4, 24, and 48 hr, as well as 12 days, after the PGR treatment.

Leaves were sampled for the determination of chlorophyll and carotenoid concentration at 70 days after PGR application using spectrophotometry as per Lichtenthaler (1987). About 0.2 g

of fresh plant tissue was mixed with 15 mL of 80% acetone, and the absorbance (optical density) was read with a spectrophotometer at wavelengths of 663, 645, and 470 nm, against an acetone 80% blank. The concentration of chlorophyll (Chl) and carotenoid (Car) were each calculated using the following equations (1-4):

$$\text{Chlorophyll } a \text{ (}\mu\text{g/mL extract)} = 12.25 * A_{663} - 2.79 * A_{645} \quad (1)$$

$$\text{Chlorophyll } b \text{ (}\mu\text{g/mL extract)} = 21.50 * A_{645} - 5.1 * A_{663} \quad (2)$$

$$\text{Total Chlorophyll (}\mu\text{g/mL extract)} = \text{Chl } a + \text{Chl } b \quad (3)$$

$$\text{Carotenoids (}\mu\text{g/mL extract)} = (1000 * A_{470} - 1.8 * \text{Chl } a - 85.02 * \text{Chl } b) / 198 \quad (4)$$

Then, the ratio of chl *a* to chl *b* (i.e., Chl *a/b*) and the ratio of chlorophyll to carotenoid were calculated as follows:

$$\text{Chl } a/b = (\text{Chl } a / \text{Chl } b) \quad (5)$$

$$\text{Chl: Caro} = \text{Total chlorophylls} / \text{Carotenoids} \quad (6)$$

Where A663, A645, A663 and A470 are the wavelengths at which absorbance values were determined; Chl *a* and Chl *b* are chlorophyll *a* and chlorophyll *b*, respectively. The values preceding the above variables in the equations are coefficients standardized to determine the respective pigment concentrations.

### 3.2.5 Statistical analysis

Data were analyzed using a linear mixed effect model with the lme4 package, specifying the PGR treatment as a fixed effect and replicate as random. Each species was analyzed separately due to inherent differences in growth form and associated morphology among grasses. Where statistical significances occurred at  $P < 0.05$ , mean comparisons were conducted using a

Tukey test with the lsmeans package (Lenth 2018). All analyses were performed using RStudio version 1.4.1717 (2021). Finally, a principal component analysis (PCA) was conducted to evaluate the relationship between plant growth regulators and the collective morpho-physiological characteristics of individual grasses using Factoextra and ggplot 2 packages in R. The length of each arrow indicates the strength of the relationship between that variable and the principal component. If the ellipses for different PGRs overlap, it indicates that these variables have the same morphophysiological contribution to grass crops.

### 3.3 Results

The PGRs tested here had differential effects on the morphology, physiology, and chemistry of three grass species. Despite a tendency for the TE treatment to have lower root, shoot, and total biomass in meadow bromegrass relative to all other treatments at the final sampling period, there were no significant effects of PGRs on the root, shoot, or total biomass of meadow bromegrass at any point in time, including from 20 through 70 days after treatment ( $P \geq 0.05$ , Figure 3.1). In contrast, applying plant growth regulators to timothy influenced root, shoot and total biomass ( $P < 0.05$ ), but only at 20 and 40 days after PGR application, and not thereafter (Figure 3.2), treatment with TE and CC led to the lowest biomass of timothy relative to the check (Figure 3.2), with TE the greatest reduction evident 40 days after PGR application (Figure 3.2). Similarly, the PGRs tested affected root, shoot and total biomass of creeping red fescue ( $P < 0.05$ ), with the TE and CC treatments generally decreasing grass biomass at most time periods, but particularly from 20 through 55 days after PGR application (Figure 3.3). Finally, none of the PGRs influenced the root to shoot ratio, and this response was consistent for all three grasses tested at 20, 40, 55, and 70 days post-application ( $P \geq 0.05$ ; Table 3.1).

The PGR treatments had no effect on the root length, root area, leaf area and tiller number of meadow bromegrass, timothy or creeping red fescue when grown under greenhouse conditions ( $P > 0.15$ , Table 3.2). However, PGR application affected plant height and lodging severity in both meadow bromegrass and timothy ( $P < 0.05$ ; Table 3.2). In both these grasses, TE application led to shorter plants relative to the untreated checks, and this was further associated with reduced lodging. In addition, CC application also led to reduced lodging in meadow bromegrass as compared to the ETH treatment and the check (Table 3.2). Meanwhile, there was no effect of PGRs on the plant height of creeping red fescue, although both TE and CC application once again reduced lodging severity in this grass (Table 3.2).

The application of PGRs did not change the growth parameters RGR, NAR, LWR and SLA among the tested grasses ( $P > 0.07$ , Table 3.3). Similarly, maximum quantum yield (QY) was not affected by any of the PGR treatments ( $P \geq 0.10$ ) at any point of time after the application of growth regulators (Table 3.4). But pigment concentrations after 70 Days of PGR application found that chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid were affected by plant growth regulators in timothy ( $P < 0.05$ ; Table 3.5); while both TE and CC application increased these pigment concentrations relative to the check, the ETH treatment led to an intermediate response that remained non-significant (Table 3.5). Within meadow bromegrass, only total chlorophyll content increased due to TE treatment ( $P < 0.05$ ), and this increase was relative to the ETH treatment rather than the check (Table 3.5). Pigment concentrations in creeping red fescue did not respond to the PGRs ( $P \geq 0.15$ ) (Table 3.5). However, the ratio of Chl (*a/b*) and chlorophylls to carotenoid (Chl:Caro) were not affected by any of the PGRs in any grass ( $P \geq 0.25$ ) (Table 3.5).

Except for a few morphological variables such as plant height and lodging, and plant chemistry traits such as chlorophyll and carotenoid contents, most of the grass attributes

quantified were not affected by the PGRs. As a result, principal component (PC) analysis was performed for each grass to further evaluate variation in each grass' response and identify relationships between the measured traits and PGRs. PC analysis of 21 response parameters of meadow bromegrass in relation to the PGR treatments (TE, CC, ETH & check) resulted in PC1 (23.6 %) and PC2 (16.5%) accounting for 40.1 % of the variation across all traits quantified (Figure 3.4). Likewise, PC analysis of 21 trait variables for timothy in relation to the PGRs similarly showed that PC1 (30.1.%) and PC2 (19.1 %) explained 49.2% of the total variation among attributes (Figure 3.5). In the case of creeping red fescue, 47.6 % of the variation among 19 grass traits was explained by PC1 (26.3 %) and PC2 (21.3.%) (Figure 3.6)

Overall, the resulting joint biplots from the various PCAs showed a high degree of overlap between the check and ETH treatments, and between the TE and CC treatments, for each of the grasses tested. Plant growth parameters such as total biomass, shoot biomass, leaf area, lodging, and leaf weight ratio generally showed greater dispersion in relation to PC1 for all grasses, while the photosynthetic relates such as chlorophyll content, carotenoid content, net assimilation rate and relative growth rate showed greater dispersion in relation to PC2. Higher total biomass, shoot biomass, plant height, internode length and lodging severity were attributed to the check and ETH treatments. In contrast, higher contents of chlorophyll and carotenoid, NAR, RGR with thicker stem diameter were accounted for by the TE and CC treatments within meadow bromegrass and timothy. The check and ETH therefore exhibited greater biomass accumulation that was more prone to lodging, while plants treated with TE and CC experienced reduced plant height resulting in lower biomass with lower lodging severity, while also demonstrating higher levels of photosynthetic pigmentation (chlorophyll and carotenoid contents) with elevated net assimilation rate in all three grasses.

### 3.4. Discussion

Plant growth regulators such as TE, CC, and to a lesser extent ETH, influenced plant morphology, particularly through a reduction in maximum grass height. Despite this, PGR application rarely influenced biomass production in the three grasses tested. But the application of TE resisted the early biomass accumulation in timothy and creeping red fescue. Similar results were reported by Jiang and Fry (1998) who observed that TE reduced the biomass of perennial ryegrass for up to four weeks after application in greenhouse conditions. Sequential application of TE can reduce grass growth up to 12 weeks after application in bermudagrass (Johnson 1994) and seashore paspalum (*Paspalum vaginatum* Sw.) (Ferrel et al. 2003). In this study, CC also reduced timothy biomass (shoots and roots) up to 20 days after treatment. Both TE and CC inhibit GA biosynthesis (Hedden and Phillips 2000; Rademacher 2000), thus restricting cell elongation and leaf blade expansion, in turn leading to a reduction in plant height and biomass production (Zhang et al. 2007). Unlike the lasting effects of TE and CC through the interference of gibberellin biosynthesis, ETH is absorbed by shoots and leads to ethylene release within hours (Gelernter and Stowell 2001) and has no longer-lasting effect (Kleczewski and Whaley 2018). The latter may account for why ETH generally did not affect the biomass of tested grasses. In contrast, TE acts on the final steps of the gibberellic acid biosynthetic pathway, resulting in a prolonged slowdown in growth within grasses (McCann and Huang 2007), thereby accounting for the extended biomass reduction from 20 through 70 days after treatment.

The PGRs tested here generally had mirroring effects on grass root and shoot growth, resulting in unaltered root-to-shoot ratios for all three grasses over time. These results correspond with a study by Rajala and Peltonen-Sainio (2001) in which PGRs initially reduced root and shoot growth; however, in the long-term growth recovered and differences among treatments disappeared in spring-planted cereals. Another study also suggested that PGRs did not

affect root and shoot biomass, resulting in no difference in root-to-shoot ratios of creeping red fescue (Szczepanek et al. 2019). Notably, the above findings are inconsistent with studies by Peltonen and Peltonen-Sainio (1997), Naylor (1988) and Enam and Cartwright (1990), all of which reported that gibberellin synthesis inhibitors suppressed shoot growth resulting in an increased root-to-shoot ratio of cereals when grown under both field and greenhouse conditions.

Importantly, reduced plant height resulting from TE and CC application were associated with decreased lodging severity in timothy and meadow brome grass. While creeping red fescue did not exhibit reduced height, its lodging severity also declined from TE and CC treatment. This finding is consistent with earlier studies showing a reduction in plant height decreased lodging risk in grasses and cereal crops (Kashiwagi and Ishimaru 2014; Frahm et al. 2018). The application of TE has previously been known to reduce plant height in cool season grass crops (Evans et al. 1994). Similarly, Zimbric et al. (2020) reported that foliar application of TE reduced stem height compared to untreated plants of intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey). Similar results of TE application were observed in perennial ryegrass (Rolston et al. 2010) and annual ryegrass (*Lolium multiflorum* L.) (Trethewey et al. 2016). CC also reduced plant height and lodging in alfalfa (*Medicago sativa* L.) (Chen et al. 2016). In our PC analysis of grass trait responses, TE, and CC application to meadow brome grass and timothy could be seen to lead to shorter plants with reduced internode lengths, reduced biomass, and a bigger diameter stem, all of which contributed to reduced lodging severity. In the case of creeping red fescue, lower aboveground biomass was simultaneous with lower lodging severity. Shorter plants are known to have higher lodging resistance due to a lower center of gravity and reduced aboveground biomass (Wu et al. 2022). Some studies report that plant height and basal internode length of wheat contribute to lodging resistance (Kelbert et al. 2004), while other studies report a shorter basal internode length, and

increased stem diameter and stem thickness of wheat, all contribute to lodging resistance (Yao et al. 2011; Subedi et al. 2022). Both TE and CC application are known to reduce grass internode length and increase stem diameter of wheat (Zhang et al. 2017) as well as shortened internode and thickened the diameter of the creeping red fescue (Szczepanek et al. 2021). TE and CC inhibit GA biosynthesis (Rademacher 2000) which restrict stem elongation and plant height, consequently, control lodging in grass crops (Griffith 2000; Chynoweth et al. 2016). Unlike TE and CC, ETH impedes plant growth through the production of ethylene (Rademacher 2000), which inhibits auxin biosynthesis that is responsible for stem elongation (Ma and Smith 1991). ETH is reported to reduce internode elongation (Rajala et al. 2002), and therefore plant height and lodging (Tripathi et al. 2004). In the current study, however, ETH treatment produced taller plants than those treated with TE and CC and had more severe lodging in all three experimental grasses. The mode of action in ETH is considered to be relatively fast, starting immediately after foliar application (March et al. 2013). As a result, its effect may have disappeared, particularly with application occurring to plants at such a young vegetative stage. Some studies have found that ETH elongated internode length and increased plant height in Kentucky bluegrass (*Poa pratensis* L.) (Christians 1985; Diesburg & Christians 1993), a finding not corroborated here.

While reductions in plant height from TE and CC were expected to reduce biomass, the only reductions in biomass occurred early on, with little to no differences in biomass after 70 days. Moreover, there were no changes to the leaf area and tiller number of any grasses in response to any PGRs in the current study. These results are consistent with Rolston et al. (2010), in which tiller production of perennial ryegrass was not affected by PGR application. Similarly, the use of TE, CC, or combinations of these did not affect the biomass and tiller numbers of perennial ryegrass (Anderson and Maliszewski 2021) and in orchard grass (Anderson et al. 2019). Since there were generally no long-term differences in root biomass, root length or



total root surface area among grasses, we conclude that the PGRs examined have little to no long-term impact on rooting traits within the grasses tested. Fagerness and Yelverton (2001) reported no difference in root growth between creeping bentgrass (*Agrostis stolonifera*) plants either treated with PGRs or untreated. Beasley et al. (2005) also found that PGRs had no effect on root length and root surface area in Kentucky blue grass (*Poa pratensis*). In contrast to our findings, the use of TE and CC have been shown to have beneficial effects on root growth, although these effects depended on PGR dose and plant species (Rajala and Peltonen-Sainio 2001). Given that the final plant dry matter and leaf area of these grasses were not affected by any of the PGRs, it is not surprising that the associated growth traits of RGR, NAR, LWR, and SLA also remained unchanged.

Although it was expected that growth retardants would directly inhibit the photosynthetic activities of treated plants, there was no difference in photosynthetic efficiency between PGR and control plants between 4 hours through 12 days post-application. A plant with high quantum yield ( $Q_y = F_v/F_m$ ) typically exhibits high photosynthetic efficiency under normal or stress conditions (Zhang and Schmidt 2000). The lack of distinct QY responses in our primary analysis reinforces that tested grasses remained in relatively non-stressed (greenhouse) conditions, even after PGR application. Our results are consistent with Stienke and Stier (2003) and Beasley and Braham (2007) wherein PGRs had no effects on quantum yield of turf grasses. Despite this, we also observed a general association between increased QY, and the application of TE and CC based on the PCA, which also coincided with an increase in chlorophyll and carotenoid.

Increases in the chlorophyll and carotenoid content of grasses treated with TE and CC suggest that along with morphological changes, select PGRs altered the chemistry of these forage grasses. Increased chlorophyll a, chlorophyll b and total chlorophyll content can increase the greenness of vegetative material (Heckman et al. 2001). Inhibition of gibberellin biosynthesis

by PGRs is known to increase cytokinin levels (Grossmann 1991), resulting in higher chlorophyll concentrations and delayed senescence in Kentucky bluegrass (Ervin and Koski 2001). The application of TE can increase chlorophyll (a & b) content, total chlorophyll (McCullough et al. 2006; Amiri-Khah et al. 2015) and carotenoid content (Schiavon et al. 2014) in grass crops. Similarly, application of CC also can increase chlorophyll and carotenoids concentration in *Viola × Wittrockiana* ‘Wesel Ice’ (Glozieris et al. 2007) and ryegrass (Kasem et al. 2014).

While PGRs can also enhance the photosynthetic activity in plants (Heckman et al. 2001), we did not find the latter based on the lack of changes in physiological indicators (Tables 3.3, 3.4), leading us to conclude that the PGRs tested here solely altered the morphology and chemistry of target grasses, rather than their overall physiological function. Of note is that higher carotenoid concentrations serve as antioxidant agents and may enable plants to better resist environmental stresses (Zin hang et al. 2005). Further, despite the changes in chlorophyll, the ratio of Chl (*a/b*) in this study also indicated that once again the PGRs had no influence on photosynthetic capacity of these grasses. While Chl *a* and Chl *b* are essential pigments for photosynthesis, the ratio of Chl (*a/b*) directly correlates with the photosynthetic capacity of plants (Li et al.2018). Plants having higher photosynthesis possess higher Chl (*a/b*) and lower ratios of chlorophyll to carotenoid (Lichtenthaler and Babani 2022), neither of which were observed here. Higher carotenoids (i.e., lower Car:Chl ratio) are also needed for photoprotection of photosystems (Ma et al. 2015). In correspondence with this study, ETH generally led to reduced chlorophyll, and in particular carotenoids (for timothy) relative to the TE and CC treatments. The ETH treatment is likely to enhance senescence through the release of ethylene, which would cause chlorophyll break down and discoloration (Iqbal et al. 2017). Similarly,

Shatters et al. (1998) also reported colour fading and discolouration in bermudagrasses after ETH application.

The strategic application of PGRs has widespread potential to manipulate the morphology, chemistry, and potentially the physiological function of crop plants. Dwarfing effects of PGRs can help maintain plants in an upright non-lodged condition and this property may be utilized to directly increase forage seed harvestability and quality, as well as develop an accelerated breeding strategy for these plants under lodging prone conditions. In winter wheat, priming and foliar treatment with gibberellic acid, kinetin, and 6-benzyl adenine were more effective replacement of the vernalization requirement than the priming with cold stratification (Sayed Shourbalal et al. 2019). Vernalization requirement for reproductive induction in many perennial forage crops slows down the reproductive cycles which leads to the slower breeding cycles (Boelt and Studer 2010; Jung and Muller 2009). The growth manipulation and replacement of vernalization with PGRs can help accelerate breeding cycles for crop improvement (Bhatta et al.2021; Ribalta et al 2014). Further, studies are needed with diverse group of PGRs, and varying light (intensity and photoperiod) and temperature conditions towards the mitigation of the vernalization requirement for accelerating breeding cycles in forage seed crops.

### **3.5 Conclusion**

This study showed that the application of select PGRs, particularly TE and CC, inhibited the early growth of forage grasses with little to no impact on final biomass or photosynthetic quantum yield. The effects of PGRs on these grasses were particularly evident in their impacts on aboveground plant morphology (height) and chemistry (chlorophyll and carotenoid concentration) rather than physiological functional traits, and this in turn, led to a reduced risk of lodging among all three forage grasses. Minor differences were also apparent among forage

grasses, with the greatest impacts of PGRs on key morphological and chemical attributes evident in timothy and meadow bromegrass, rather than creeping red fescue. None of the PGRs (TE, CC, and ETH) had modulating effects on root area, root length, root biomass, tillers number, leaf area, final dry mass and root-to-shoot ratio. Similarly, the growth variables RGR, NAR, LWR and SLA all remained unaffected by the PGRs. Despite the lack of direct effects, assessment of the plant trait data with PCA revealed that the ETH and check treatments exhibited more similar plant traits than the TE and CC treated plants, with the latter having a shift in plant traits that generally favored reduced lodging severity. Further multi-year studies on established forage swards are needed to better understand the complex effects of PGRs and their interactions with environmental conditions (soils, moisture, nutrient supply) on grass root and shoot architectures, morpho-physiological and reproductive traits, thereby enabling PGRs to be optimally used to maximize commercial grass seed production.

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**Table 3.1:** Effect of plant growth regulators (PGRs) on mean root-to-shoot ratios (RS) of meadow brome, timothy and creeping red fescue, as sampled at 20, 45, 55 and 70 days after PGR application. Values are means of four replicates

Grass Crop	Treatment	Root: Shoot ratio			
		20 days	40 days	55 days	70 days
<b>Meadow brome</b>	<b>TE</b>	0.61	0.75	0.46	0.50
	<b>ETH</b>	0.54	0.66	0.51	0.54
	<b>CC</b>	0.67	0.73	0.44	0.50
	<b>Check</b>	0.59	0.66	0.50	0.60
	<b>SEM (±)</b>	0.07	0.09	0.06	0.89
	<b>Pr(&gt;F)</b>	0.50	0.75	0.79	0.11
<b>Timothy</b>	<b>TE</b>	0.47	0.64	0.31	0.41
	<b>ETH</b>	0.56	0.53	0.65	0.63
	<b>CC</b>	0.36	0.65	0.54	0.51
	<b>Check</b>	0.56	0.68	0.45	0.54
	<b>SEM (±)</b>	0.06	0.11	0.18	0.13
	<b>Pr(&gt;F)</b>	0.08	0.77	0.07	0.63
<b>Creeping red fescue</b>	<b>TE</b>	0.57	0.66	0.55	0.53
	<b>ETH</b>	0.77	0.64	0.39	0.60
	<b>CC</b>	0.92	0.64	0.61	0.50
	<b>Check</b>	0.62	0.79	0.66	0.69
	<b>SEM (±)</b>	0.11	0.08	0.66	0.76
	<b>Pr(&gt;F)</b>	0.61	0.75	0.46	0.50

Note: Abbreviations: TE: Trinexapac-ethyl; CC: Chlormequat chloride; ETH: Ethephon; SEM: standard error of the mean.

**Table 3.2:** Effect of plant growth regulators on mean grass plant height, tiller number, leaf area, root length, root area, and lodging score for each of meadow brome grass, timothy and creeping red fescue, as sampled 70 days after PGR application. Values are means of four replicates. Lodging score is from 1-5, with 1 representing no lodging and 5 severe lodging.

Grass Crop	Treatment	Tillers no. plant <sup>-1</sup> )	Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )	Root length (cm)	Root area (cm <sup>2</sup> plant <sup>-1</sup> )	Plant height (cm)	Lodging score
<b>Meadow brome</b>	<b>TE</b>	23.2	1714.0	32.0	1814.0	68.2 b	1.50 c
	<b>ETH</b>	27.8	2062.0	30.4	1900.0	81.0 ab	3.25 ab
	<b>CC</b>	25.0	1453.0	30.4	1687.0	72.2 ab	1.75 bc
	<b>Check</b>	23.5	2194.0	30.8	1918.0	83.2 a	3.50 a
	<b>SEM (±)</b>	3.0	253.0	152.0	116.0	3.6	0.4
	<b>Pr(&gt;F)</b>	0.71	0.19	0.39	0.39	0.026*	0.0067**
<b>Timothy</b>	<b>TE</b>	33.5	2110.0	31.7	1500.0	63.8 b	1.50 b
	<b>ETH</b>	39.8	3298.0	32.0	1725.0	89.8 ab	3.75 a
	<b>CC</b>	38.2	2637.0	30.4	1883.0	79.1 ab	2.50 ab
	<b>Check</b>	32.2	3061.0	30.0	1753.0	100.2 a	3.50 a
	<b>SEM (±)</b>	4.2	432.0	1.55	152.0	7.7	0.3
	<b>Pr(&gt;F)</b>	0.60	0.30	0.75	0.36	0.023*	0.0023**
<b>Creeping red fescue</b>	<b>TE</b>	92.8	2110.0	27.0	1394.0	34.8	1.50 b
	<b>ETH</b>	110.0	3298.0	28.0	1474.0	41.5	4.00 a
	<b>CC</b>	103.2	2637.0	28.4	1459.0	35.8	1.75 b
	<b>Check</b>	110.2	3061.0	28.7	1699.0	38.5	4.00 a
	<b>SEM (±)</b>	14.3	432.0	0.7	103.0	2.4	0.5
	<b>Pr(&gt;F)</b>	0.60	0.28	0.36	0.22	0.20	0.0039**

Note: Abbreviations: PGR: Plant growth regulators; TE: Trinexapac-ethyl; CC: Chloromequat chloride; ETH: Ethepon, SEM: standard error of the mean. Within a forage grass, column means followed by different letters indicate significant differences using a Tukey test ( $P \leq 0.05$ )

**Table 3.3:** Plant growth regulator effects on grass mean relative growth rate (RGR), net assimilation rate (NAR), specific leaf area (SLA) and leaf weight ratio (LWR), for each of meadow brome grass, timothy and creeping red fescue. Values are means of four replicates.

<b>Grass Crop</b>	<b>Treatment</b>	RGR (mg g <sup>-1</sup> day <sup>-1</sup> )	NAR (mg cm <sup>-2</sup> day <sup>-1</sup> )	SLA (cm <sup>2</sup> g <sup>-1</sup> )	LWR
<b>Meadow brome</b>	<b>TE</b>	31.2	0.66	125	0.35
	<b>ETH</b>	28.0	0.54	146	0.35
	<b>CC</b>	30.3	0.75	103	0.33
	<b>Check</b>	29.9	0.58	122	0.39
	<b>SEM (±)</b>	2.51	0.075	14.4	0.048
	<b>Pr(&gt;F)</b>	0.84	0.27	0.27	0.69
<b>Timothy</b>	<b>TE</b>	23.4	0.55	212	0.14
	<b>ETH</b>	23.4	0.51	203	0.21
	<b>CC</b>	23.9	0.66	192	0.19
	<b>Check</b>	17.8	0.43	178	0.21
	<b>SEM (±)</b>	1.44	0.061	8.38	0.022
	<b>Pr(&gt;F)</b>	0.083	0.089	0.091	0.14
<b>Creeping red fescue</b>	<b>TE</b>	18.4	0.27	178	0.39
	<b>ETH</b>	11.8	0.21	194	0.42
	<b>CC</b>	13.4	0.27	201	0.42
	<b>Check</b>	12.9	0.20	238	0.34
	<b>SEM (±)</b>	1.58	0.044	45.4	0.031
	<b>Pr(&gt;F)</b>	0.082	0.55	0.81	0.15

Note: Abbreviations: PGR: Plant growth regulators; TE: Trinexapac-ethyl; CC: Chlormequat chloride; ETH: Etephon, SEM: standard error of the mean.

**Table 3.4:** Plant growth regulator effects on the maximum quantum yield of photosystem II (Fv/Fm) at 4, 24, and 48 hr, as well as 12 days, after the PGR treatment. Values are means of four replicates.

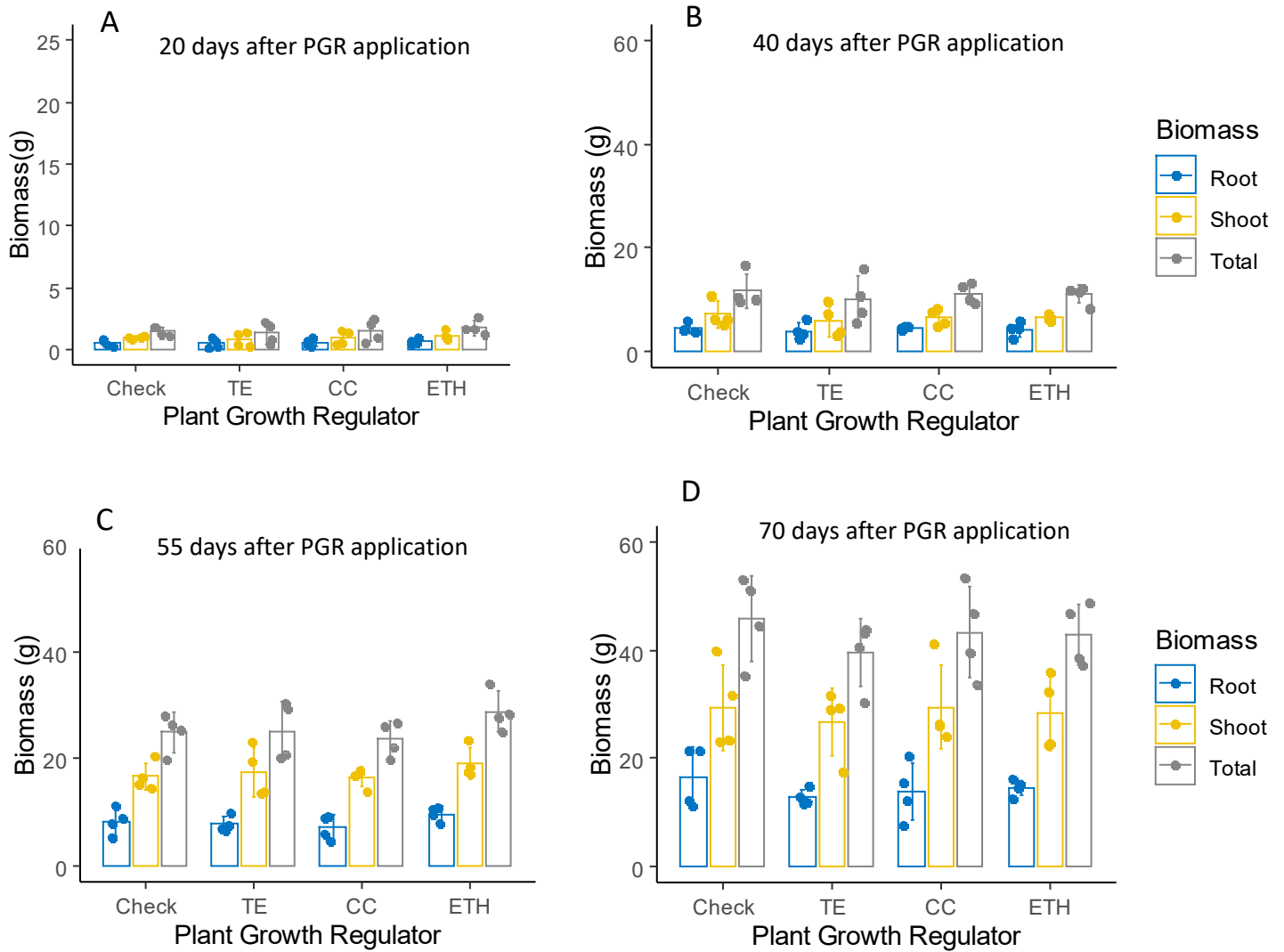
Grass Crop	Treatments	Maximum quantum yield (Fv/Fm)			
		4 hrs	24 hrs	48 hrs	12 days
Meadow brome	TE	0.72	0.74	0.73	0.74
	ETH	0.74	0.74	0.73	0.74
	CC	0.74	0.74	0.74	0.72
	Check	0.73	0.73	0.73	0.73
	SEM ( $\pm$ )	0.0089	0.0039	0.0079	0.0094
	Pr(>F)	0.45	0.15	0.75	0.25
Timothy	TE	0.73	0.72	0.73	0.75
	ETH	0.74	0.72	0.74	0.71
	CC	0.73	0.73	0.73	0.74
	Check	0.72	0.73	0.73	0.73
	SEM ( $\pm$ )	0.01	0.01	0.73	0.012
	Pr(>F)	0.33	0.73	0.95	0.17
Creeping red fescue	TE	0.73	0.73	0.73	0.76
	ETH	0.70	0.71	0.75	0.74
	CC	0.74	0.73	0.74	0.76
	Check	0.72	0.72	0.74	0.75
	SEM ( $\pm$ )	0.013	0.0077	0.0091	0.0067
	Pr(>F)	0.11	0.19	0.48	0.10

Note: Abbreviations: PGR: Plant growth regulators; TE: Trinexapac-ethyl; CC: Chlormequat chloride; ETH: Etephon, SEM: standard error of the mean.

**Table 3.5:** Effect of plant growth regulators on chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid concentration for each of meadow brome, timothy and creeping red fescue, as sampled 70 days after PGR application. Values are means of four replicates.

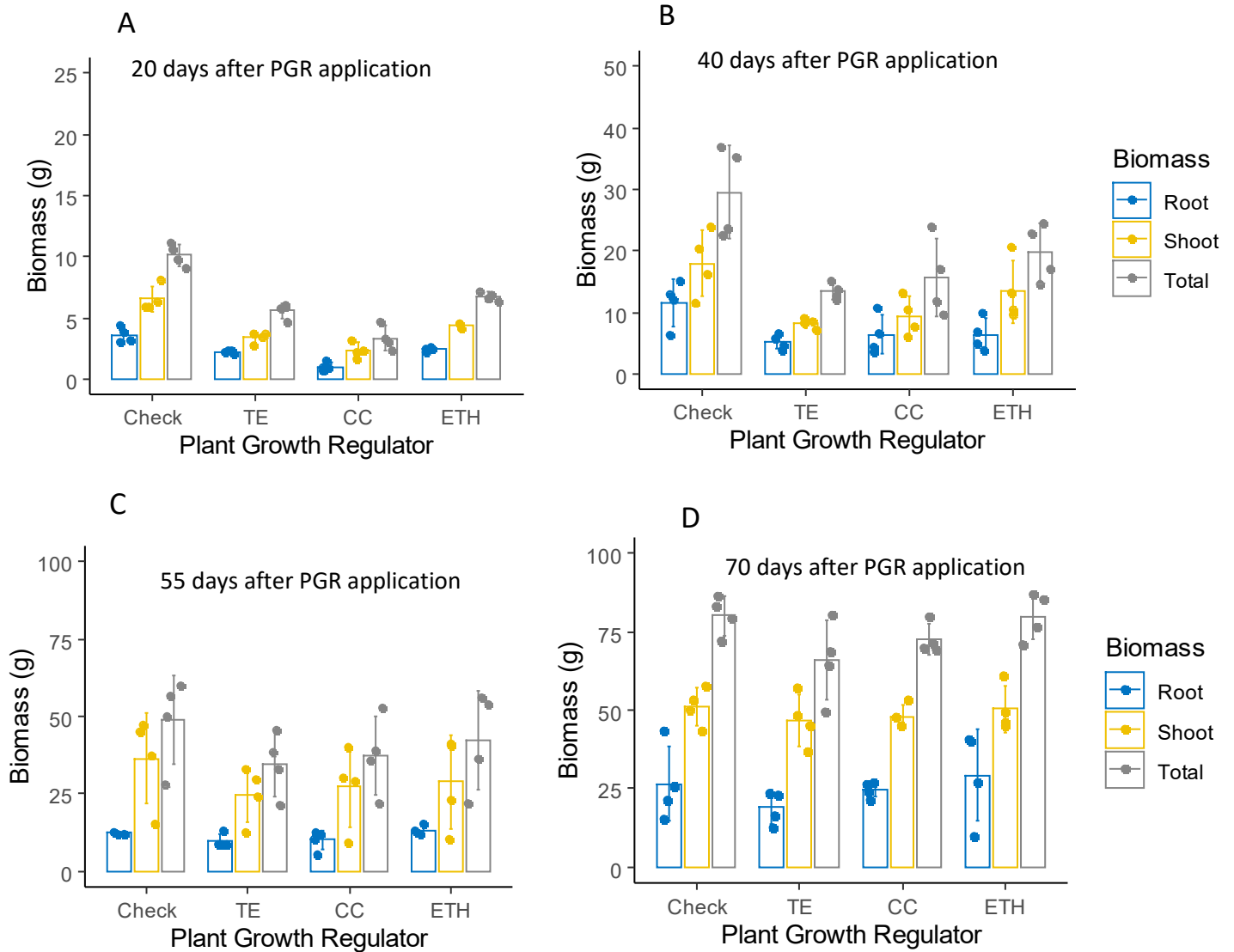
<b>Crop</b>	<b>Treatment</b>	<b>Chl a (<math>\mu\text{g g}^{-1}</math> FW)</b>	<b>Chl b (<math>\mu\text{g g}^{-1}</math> FW)</b>	<b>Total Chl (<math>\mu\text{g g}^{-1}</math> FW)</b>	<b>Caro (<math>\mu\text{g g}^{-1}</math> FW)</b>	<b>Chl (a/b)</b>	<b>Chl: Caro</b>
<b>Meadow brome</b>	<b>TE</b>	1070	546	1616 a	239	2.17	7.16
	<b>ETH</b>	819	414	1229 b	194	2.11	6.46
	<b>CC</b>	1002	528	1530 ab	237	1.91	6.49
	<b>Check</b>	851	414	1265 b	184	2.11	6.86
	<b>SEM (<math>\pm</math>)</b>	69	64.7	96.3	19.5	0.287	0.66
	<b>Pr(&gt;F)</b>	0.052	0.281	0.0181 *	0.1499	0.92	0.84
<b>Timothy</b>	<b>TE</b>	1189 a	517 a	1706 a	342 ab	2.32	4.96
	<b>ETH</b>	881ab	372 ab	1253 ab	274 bc	2.37	4.50
	<b>CC</b>	1113a	504 a	1617 a	376 a	2.20	4.26
	<b>Check</b>	666 b	287 b	953 b	212 c	2.34	4.45
	<b>SEM (<math>\pm</math>)</b>	143	65.5	207	28.9	0.091	0.36
	<b>Pr(&gt;F)</b>	0.017 *	0.033 *	0.02*	0.0022**	0.55	0.25
<b>Creeping red fescue</b>	<b>TE</b>	973	569	1542	265	1.73	6.29
	<b>ETH</b>	779	508	1287	175	2.20	7.78
	<b>CC</b>	948	612	1561	204	2.20	7.67
	<b>Check</b>	969	553	1522	189	1.95	8.42
	<b>SEM (<math>\pm</math>)</b>	94.9	151	231	25	0.54	1.62
	<b>Pr(&gt;F)</b>	0.44	0.96	0.79	0.11	0.87	0.82

Note: Abbreviations: PGR: Plant growth regulators; TE: Trinexapac-ethyl; CC: Chlormequat chloride; ETH: Ethepon, Chl a: chlorophyll a, Chl b: chlorophyll b, Total Chl: total chlorophyll, Caro: carotenoid, measured unit  $\mu\text{g}$ : microgram, g FW: gram fresh weight, SEM: standard error of the mean. Mean values in columns within each grass crop followed by different letters indicate significant differences using a Tukey test ( $P \leq 0.05$ ).

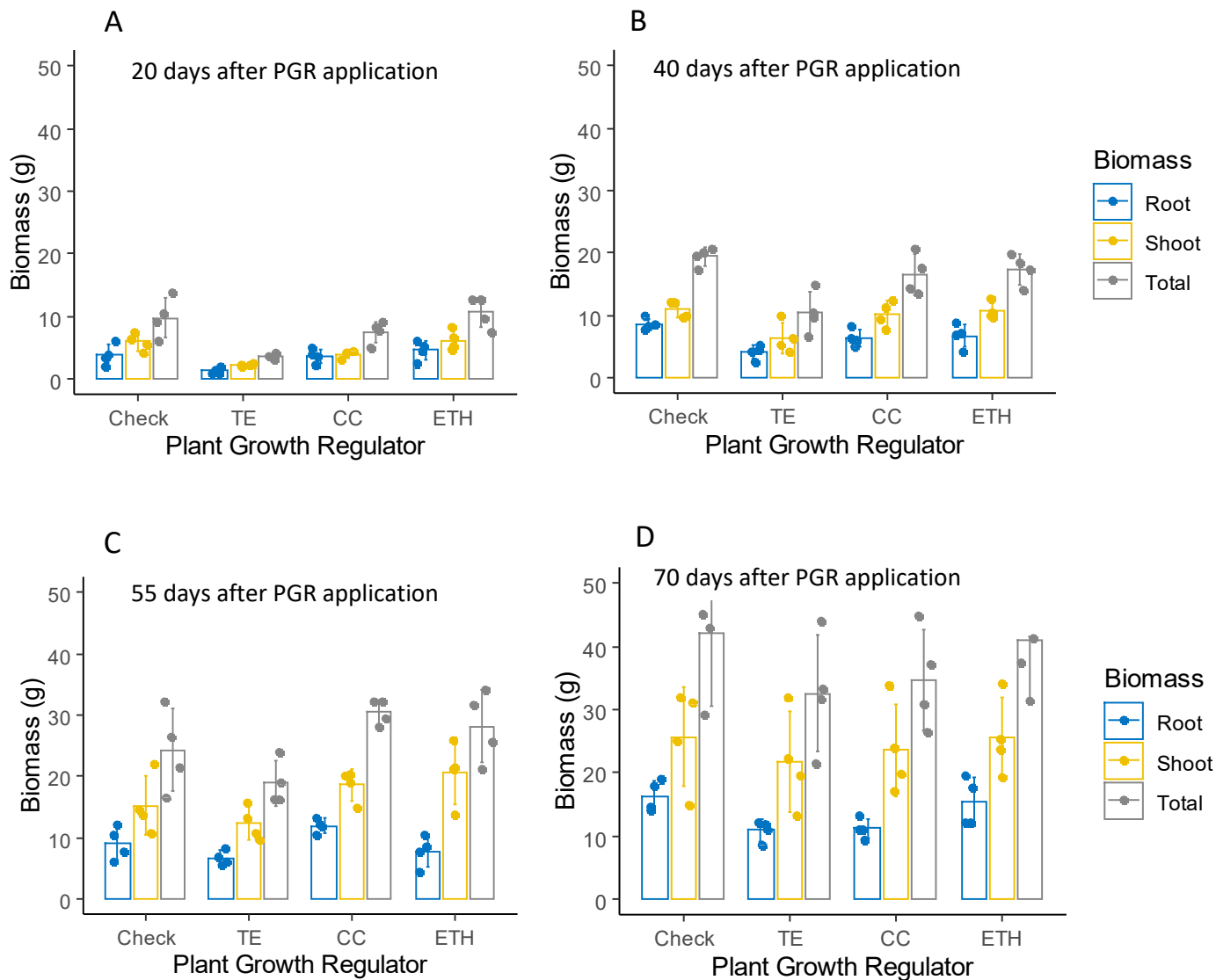


**Figure 3.1:** Effect of plant growth regulators (PGRs) on root, shoot and total biomass of meadow brome grass as sampled at A) 20, B) 40, C) 55 and D) 70 days after PGR application. The graph bars are means and error bars are  $\pm 1$  SE of four replicates. Measured of biomass (root, shoot and total) unit is gram (g) from one pot.

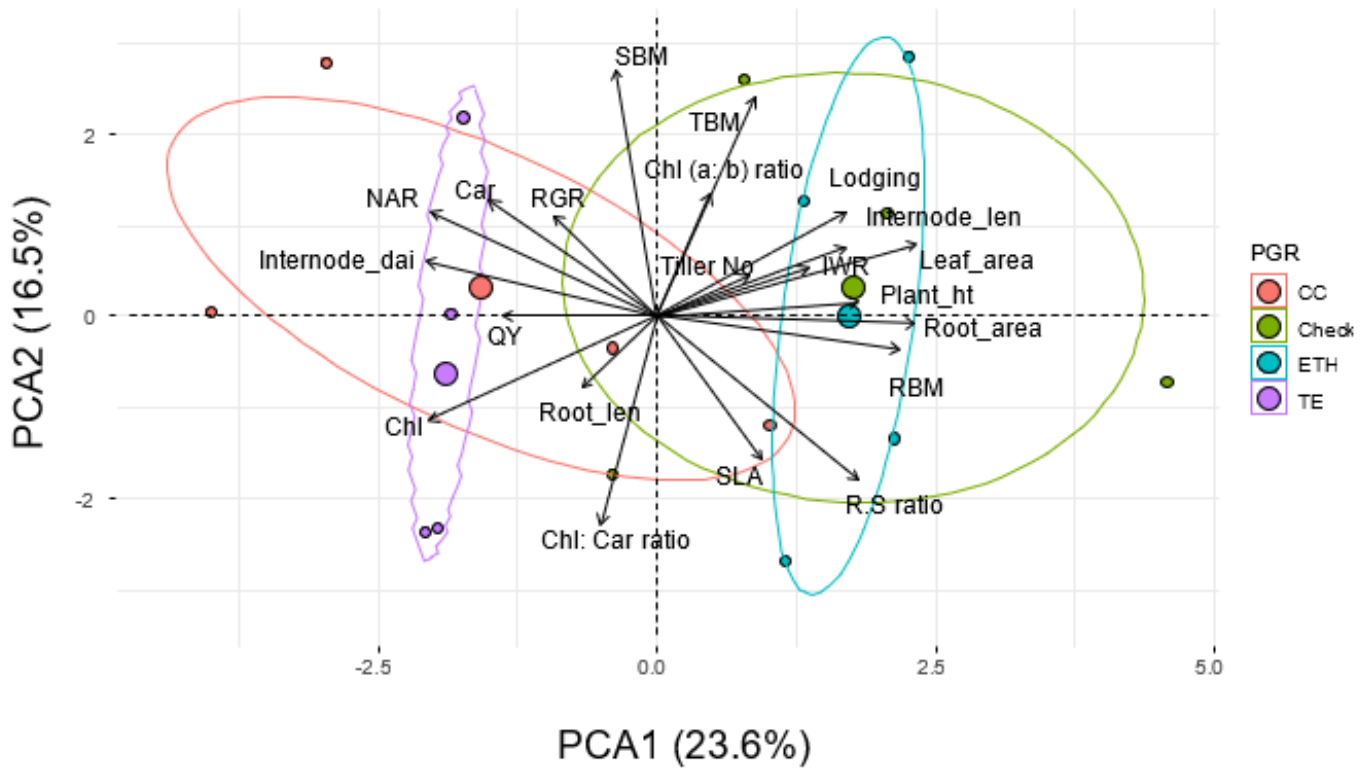




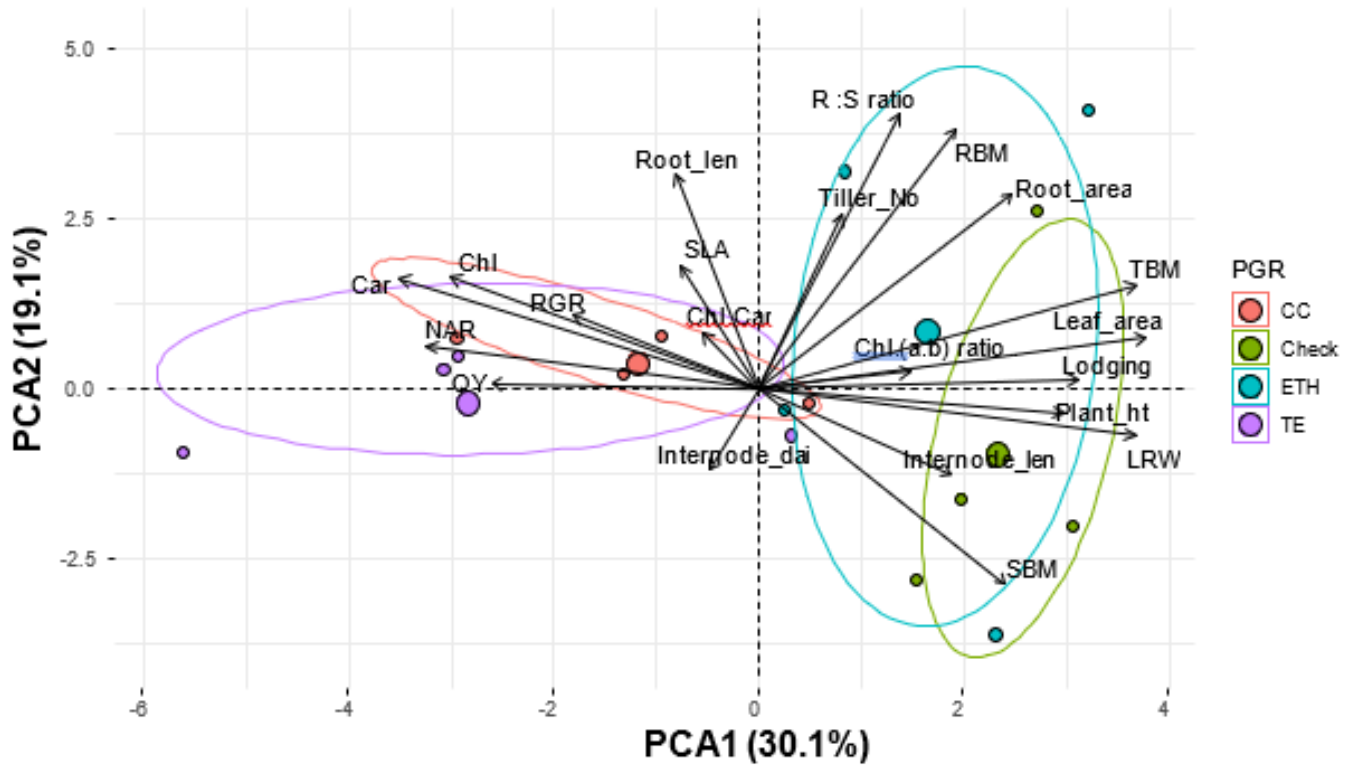
**Figure 3.2:** Effect of plant growth regulators (PGRs) on the root, shoot and total biomass of timothy as sampled at A) 20, B) 40, C) 55 and D) 70 days after PGR application. The bars are means and error bars are  $\pm 1$  SE of four replicates. Measured of biomass (root, shoot and total) unit is gram (g) from one pot.



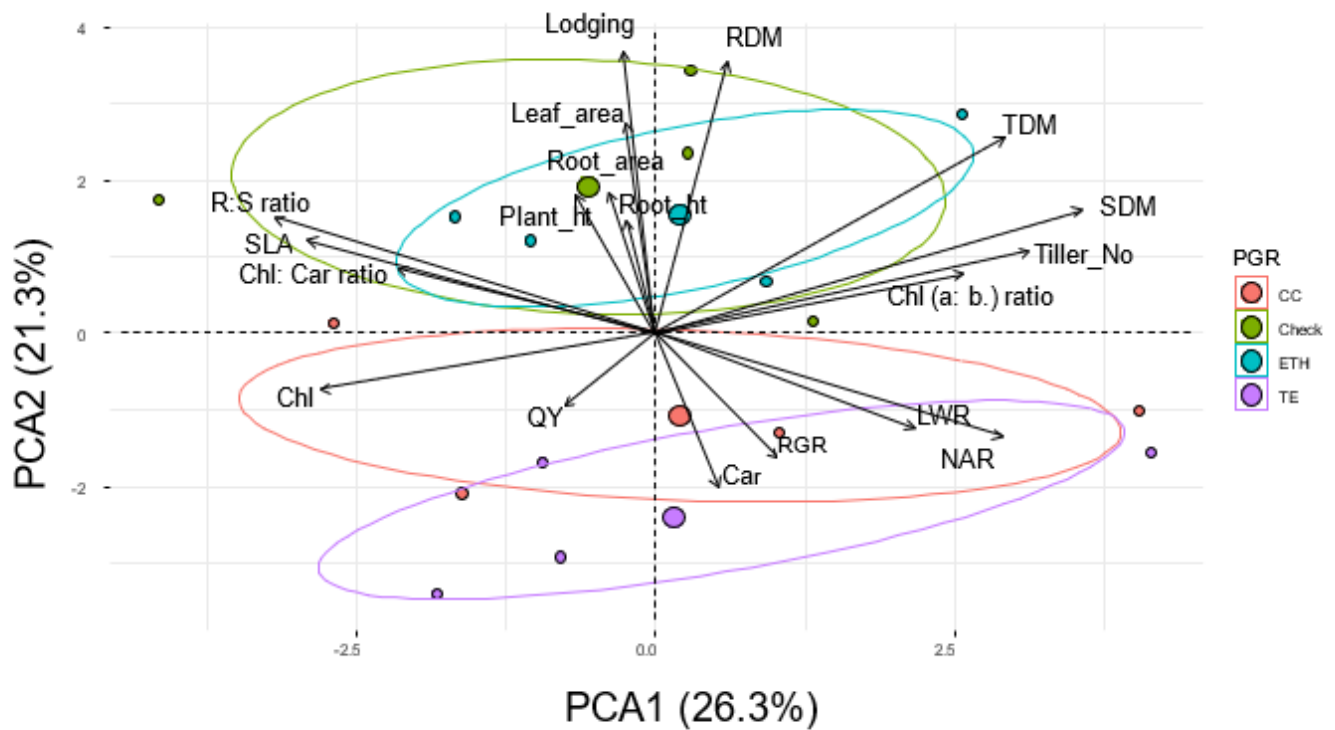
**Figure 3.3:** Effect of plant growth regulators (PGRs) on the root, shoot and total biomass of creeping red fescue as sampled at A) 20, B) 40, C) 55 and D) 70 days after PGR application. The graph bars are means and error bars are  $\pm 1$  SE of four replicates. Measured of biomass (root, shoot and total) unit is gram (g) from one pot.



**Figure 3.4:** Biplot graphs of 21 variables with treatment contribution of meadow brome in response to three plant growth regulators and an untreated control. SBM, shoot biomass; TBM, total biomass; RBM, root biomass; Root\_area, Root area; Leaf\_area, leaf area; Root\_len, max root length; Internode\_len, internode length; Internode\_dai; internode diameter; Tiller\_No, tiller number; Plant\_ht, plant height; R.:S ratio, root to shoot ratio; Chl, total chlorophyll content; Car, Carotenoid; Chl (a: b) ratio, Chlorophyll (a: b) ratio; Chl: car ratio, Chlorophyll: carotenoid ratio; SLA, Specific leaf area; NAR, Net assimilation rate; IWR, leaf weight ratio (LWR), RGR, relative growth rate; QY, maximum quantum yield, Lodging, lodging score from 1 (no lodging) to 5 (high lodging). All variables are the mean of four replications, and all biometric variables (biomass, root area, root length, plant height, tiller number) were measured per plot.



**Figure 3.5:** Biplot graphs of 21 variables with treatment contribution of timothy in response to three plant growth regulators and an untreated control. SBM, shoot biomass; TBM, total biomass; RBM, root biomass; Root\_area, Root area ; Leaf\_area, leaf area; Root\_len, max root length ; Internode\_len, internode length; Internode\_dia; internode diameter; Tiller\_No, tiller number ; Plant\_ht, plant height; R:S ratio, root to shoot ratio; Chl, total chlorophyll content; Car; Carotenoid, Chl (a: b) ratio, Chlorophyll (a: b) ratio; Chl: car ratio, Chlorophyll: carotenoid ratio; SLA, Specific leaf area; NAR, Net assimilation rate; IWR, leaf weight ratio (LWR), RGR, relative growth rate; QY, maximum quantum yield, Lodging, lodging score from 1 (no lodging) to 5 (high lodging). The all variables are mean of four replications, all biometric variable (biomass, root area, root length, plant height, tiller number) were measured per plots.



**Figure 3.6:** Biplot graphs of 19 variables with treatment contribution of timothy in response to three plant growth regulators and an untreated control. SBM, shoot biomass; TBM, total biomass; RBM, root biomass; Root\_area, Root area ; Leaf\_area, leaf area; Root\_len, max root length ; Tiller\_No, tiller number ; Plant\_ht, plant height; R:S ratio, root to shoot ratio; Chl, total chlorophyll content; Car; Carotenoid: Chl (a: b) ratio, Chlorophyll (a: b) ratio; Chl: car ratio, Chlorophyll: carotenoid ratio; SLA, Specific leaf area; NAR, Net assimilation rate; IWR, leaf weight ratio (LWR), RGR, relative growth rate; QY, maximum quantum yield, Lodging, lodging score from 1 (no lodging) to 5 (high lodging). The all variables are mean of four replications, all biometric variable (biomass, root area, root length, plant height, tiller number) were measured per plots.

## **Chapter 4. Synthesis.**

### **4.1 Introduction**

Timothy, meadow brome and creeping red fescue are popular forage grasses grown in the Peace River region of Alberta and British Columbia for seed production. Creeping red fescue is a turf-grass for lawns and playground while timothy and meadow brome grass are commonly used for grazing, hay, and silage production for livestock feed. Grass seed production is a significant agricultural activity in the area, and the region is one of the leading producers of grass seed in Canada (Wong, 2017). Despite strong foreign markets and favorable prices of forage seeds (Wong 2020), there has been a decline in forage seed acreage over recent years (Khanal 2022). In addition, there are lodging problems when there is abundant rainfall. Lodging was more severe under higher fertility and higher precipitation growing conditions (Berry et al. 2004). Besides cultivar choice, environment and agronomical factors affect seed quality and production (Szczepanek et al. 2019). Lodging in grasses is an important factor that negatively affects seed yield in grass crops (Griffith 2000).

Lodging in grasses is most likely to occur when fertile tillers cannot support their weight, and this inhibits pollination, seed development, and consequently, seed yield (Chastain et al. 2014). In addition, lodging makes harvesting more difficult and time-consuming (Elgersma 1985) and increases seed loss due to shattering (Machac 2013). In addition, plants subject to early lodging produced more vegetative tillers, which will compete for resources, sunlight, N, and water, thus reducing the resources available at a later date for seed filling (Rolston et al., 2007). Lodging is exacerbated when grasses are grown with high N and high moisture (Harvstad and Aamlid 2006). Reducing N fertilizer can limit plant lodging (Wiersma et al. 1986); however, reduction of N also reduces yield potential (Tidemann 2020). As a result, there is a need for an

alternative for managing lodging in grasses without compromising N fertilization. To counteract lodging and seed loss, the use of plant growth regulators is a potential alternative for treating grasses intended for seed production (Chynoweth and Moot 2017), particularly under high N fertilisation (Young III et al. 2007; Szczepanek et al. 2021).

## **4.2 Key findings**

In this study, gibberellin inhibitors like CC and TE, or phytohormone-like ETH were examined to assess various morpho-physiological grass responses and their ability to regulate lodging and seed production of creeping red fescue, timothy, and meadow bromegrass. Collectively, the research findings facilitate the selection of consistent and efficient use of PGRs to control lodging in grass crops. This information can serve as a baseline study for the registration of these products by the Canadian government, as well as solve the lodging problem faced by grass seed producers in the Peace River region of Canada. In addition, this work may assist with breeding strategy development for grass crops under lodging-prone conditions, in turn increasing lodging resistance and seed yields. This strategy could, for example, select and breed grass varieties that have stronger stems, shorter height, and greater root depth, which can help reduce lodging and improve seed yield and harvestability.

A two-year field study was conducted at Beaverlodge Research Farm to determine the effects of PGRs with and without spring top-dressed N application on the agronomic characteristics of forage seed crops. In this study, three PGRs were applied, including TE, CC and ETH at the two-node stage of crop development (BBCH 31-32). Morphology variables like plant height, internode length, culm diameter, dry biomass, lodging score, lodging severity, along with seed attributes (panicle length, seed number per unit area, 1000 seed weight and final seed yield, were assessed in response to PGR and N application to these grasses.

Among the three PGRs examined, the use of TE consistently reduced lodging by shortening internode length, reducing plant height and increasing the culm diameter. In contrast, CC and ETH were less effective in reducing lodging in these grasses. TE had previously been reported to reduce internode length (cm) and plant height, increasing lodging resistance in perennial grasses (Sibbertain et al. 2002). In addition, TE thickened stem diameter (Farhm et al. 2018), which prevented lodging (Zhang et al. 2017), while CC negligible effect on plant height and lodging control (Rolston et al. 2014, Szczepanek et al. 2021). Similarly, ETH was not effective in reducing the lodging of common bent grass (Aamalid et al. 2007). Although all PGRs reduced lodging to some degree, this change did not affect seed yield attributes nor total seed yield of the grasses. This result was similar to the study by Frahm et al. (2018) where TE did not improve wheatgrass seed yield unless lodging severity was high. Similarly, Rolston et al. (2012) found no advantage of CC in stands of perennial ryegrass. The PGRs CC and ETH were both reported to be ineffective in improving yield attributes and seed yield in alfalfa (Wenhua et al. 2008). Notably, our findings contrast with earlier investigations showing that PGR effectively reduces lodging, and this improves seed yield of creeping red fescue (Bitarafan et al. 2019; Zapiola et al. 2014), perennial ryegrass (Rolston et al. 2010) and tall fescue (Rolston et al. 2004). Similarly, the application PGRs did not result in an increase in the gross return of any grass crop. However, in the case of timothy and creeping red fescue, the use of PGRs, particularly TE contributed to comparable gross margins as in when PGRs were not applied.

Although high N fertilizers are known to exacerbate grass lodging (Jungers et al. 2017; Bolt and Studer 2010) N application in our study increased the biomass and seed yield of meadow brome and timothy. N fertilization in spring increased biomass leading to more solar energy capture and photosynthate allocation to developing seeds and higher seed yield (Young



III et al. 1996; Chastain et al. 2014). The normal growing condition in 2022 contributed to more favorable seed attributes and seed yield of meadow brome and creeping red fescue and led to a greater role of PGRs in regulating plant growth, particularly in comparison to the dry hot weather of 2021. Thus, under drought and non-lodging conditions, the benefits of using PGRs appear to be minimal. Strydhorst et al. (2018) recommended against the use of PGRs under drought or adverse growing conditions.

A greenhouse study was conducted to investigate the effects of PGRs on vegetative growth, root-shoot allometry, chlorophyll pigmentation, and photosynthetic efficiency. The use of PGRs such as TE and CC limited the early growth (root and shoot biomass) of timothy grass and creeping red fescue but had no long-term effects on plant morpho-physiological attributes as they recovered under (optimal) greenhouse conditions. Rajala and Peltonen-Sainio (2001) reported similar results where PGR reduced root and shoot biomass at an early stage, but plants recovered and remained unchanged in the long term. Since the final biomass of these grasses did not change, root growth such as root biomass, root length, and total root surface area also remained stable within each grass species. Previous studies have shown that PGRs did not change root growth in creeping red fescue (Fagerness and Yelverton 2001) as well as root length and surface area in Kentucky bluegrass (Beasley et al. 2005). Likewise, growth parameters such as relative growth rate, net assimilation rate, leaf weight ratio, and specific leaf area remained constant throughout the growth period.

While the PGRs were expected to inhibit the photosynthetic activities with an immediate effect, there was no difference in maximum quantum efficiency ( $F_v/F_m$ ) between PGRs and non-treated controls at 4 hours through 12 days after application, suggesting no negative effects occurred on the health of plants after PGR application. This result was consistent with Steinke and Stier (2003) and Beasley and Braham (2007) who suggested PGRs had no effects on

quantum efficiency in turf grasses. The chlorophyll and carotenoid content measured at 70 days after PGR application, showed that chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid, were all higher in timothy grass crops, and total chlorophyll was elevated in meadow brome but not creeping red fescue. Increased chlorophyll a, chlorophyll b and total chlorophyll content increased the greenish colouration and photosynthetic activities of plants (Heckman et al. 2001). However, a stable ratio of photosynthesis pigment, chlorophyll *a* to chlorophyll *b* in this study indicated that these PGRs did not influence the photosynthetic capacity of these grass crops as suggested by Li et al. (2018) where the ratio Chl (*a:b*) directly correlated with the photosynthetic capacity of plants. Similarly, the unaltered Carotenoid to Chlorophyll (Car:Chl) ratio in these perennial forage grasses after PGR application suggested that PGR has no role in the photoinhibition photoprotection of the photosystems II. Higher carotenoids (i.e., lower Car:Chl ratio) are needed for photoprotection of the photosystems II (Ma et al. 2015) and stress condition (Strzałka et al. 2003). Higher carotenoids act as antioxidants to make plants more resistant to environmental stress (Young 1991).

However, PCA in this study revealed that TE and CC treatments had higher chlorophyll and carotenoid contents compared to the non-treated CHECK and ETH treatments. Several previous studies (McCann and Huang 2007; Xu and Huang 2010) showed that TE increased the chlorophyll content and photochemical efficiency of creeping bentgrass. Further understanding is warranted on how PGRs affect the photosynthetic efficiency and photoprotection of PS II, including the preventive mechanisms and their relationship to various environmental scenarios of varying temperature, light, water availability and intensity.

### **4.3 Future research needs**

The present study showed that the PGR TE was more efficient and efficacious in reducing plant height and lodging severity of both timothy and meadow brome grass, and this was evident under greenhouse and field conditions. While the PGRs CC and ETH also exhibited impacts on grass morphology, they were less consistent in reducing lodging within these forage grasses. None of the PGRs had a modulating effect on root and shoot growth, as well as growth parameters, but TE and CC both inhibited the early growth of timothy and creeping red fescue without affecting their photosynthetic quantum yield under greenhouse conditions. Despite the reduction in lodging, PGRs did not contribute to the seed composition and seed yield of any of the three perennial forage grasses tested here. These grasses lodged commonly when rainfall was abundant compared to dry years. Thus, in drought year or non-lodging conditions, the use of PGRs may not be necessary and could be discouraged. By considering the benefits and drawbacks of using PGRs and considering environmental factors, farmers can work towards more sustainable and profitable farming practices, benefiting both themselves and the wider community. Consequently, additional long-term studies are needed to assess the effect of PGRs on grass growth and yield, including forage seed harvestability under field conditions. This information can be used to inform the development of more efficient PGR use strategies that enhance forage yield and maximize seed harvestability of grass seed crops.

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## Appendices

### Appendix A

**Table A1:** The partial variable cost for gross margin analysis of plant growth regulators and nitrogen from 2021 to 2022

<b>Plant growth regulators</b>	PGR cost (CAD \$ ha <sup>-1</sup> )	PGR Spraying (CAD \$ ha <sup>-1</sup> )	Urea Cost (CAN \$ ha <sup>-1</sup> )		Urea spraying cost (CAN \$ ha <sup>-1</sup> )		Total variable cost (CAN \$ ha <sup>-1</sup> )	
			No spring N	Spring N (40 kg ha <sup>-1</sup> )	No spring N	Spring N (40 kg ha <sup>-1</sup> )	No spring N	Spring N (40 kg ha <sup>-1</sup> )
<b>TE</b>	63	40	0	85.3	0	40	103	228.3
<b>ETH</b>	65	40	0	85.3	0	40	105	230.3
<b>CC</b>	59	40	0	85.3	0	40	99	224.3
<b>Check</b>	0	0	0	85.3	0	40	0	125.3

Note. The partial variable costs included the cost of inputs such as plant growth regulators, spraying cost and urea cost and their spraying cost with and without spring nitrogen. Variable input costs are calculated based on purchase receipts from local suppliers. Variable input application costs were adapted from custom rates surveys of the Peace region, conducted by Alberta Agriculture and Forestry (2016).

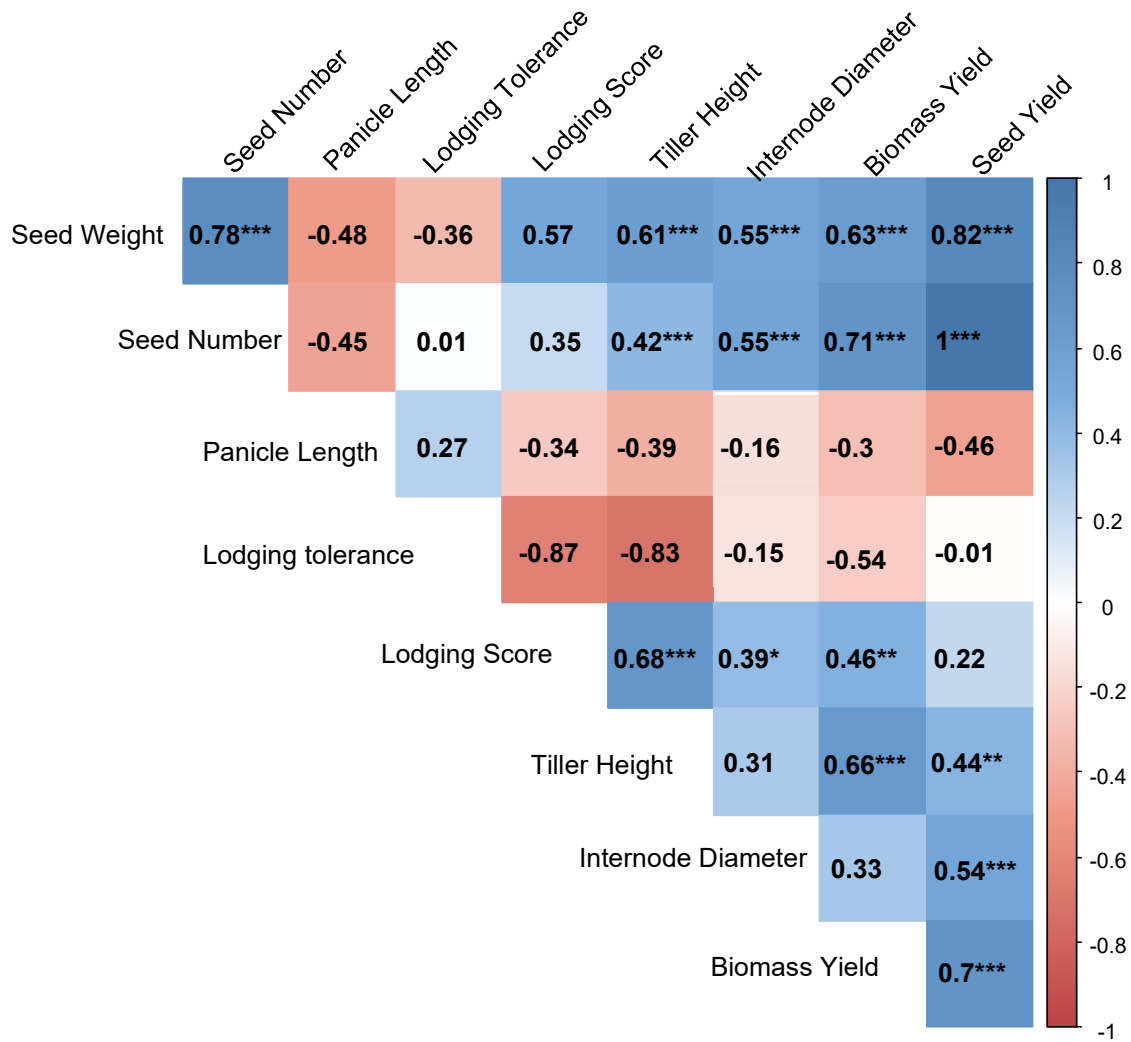


Figure A1: Correlation coefficients (r) between plant traits and seed yield of meadow brome over two crop years. \*P ≤ 0.05. \*\*P ≤ 0.01. \*\*\*P ≤ 0.001

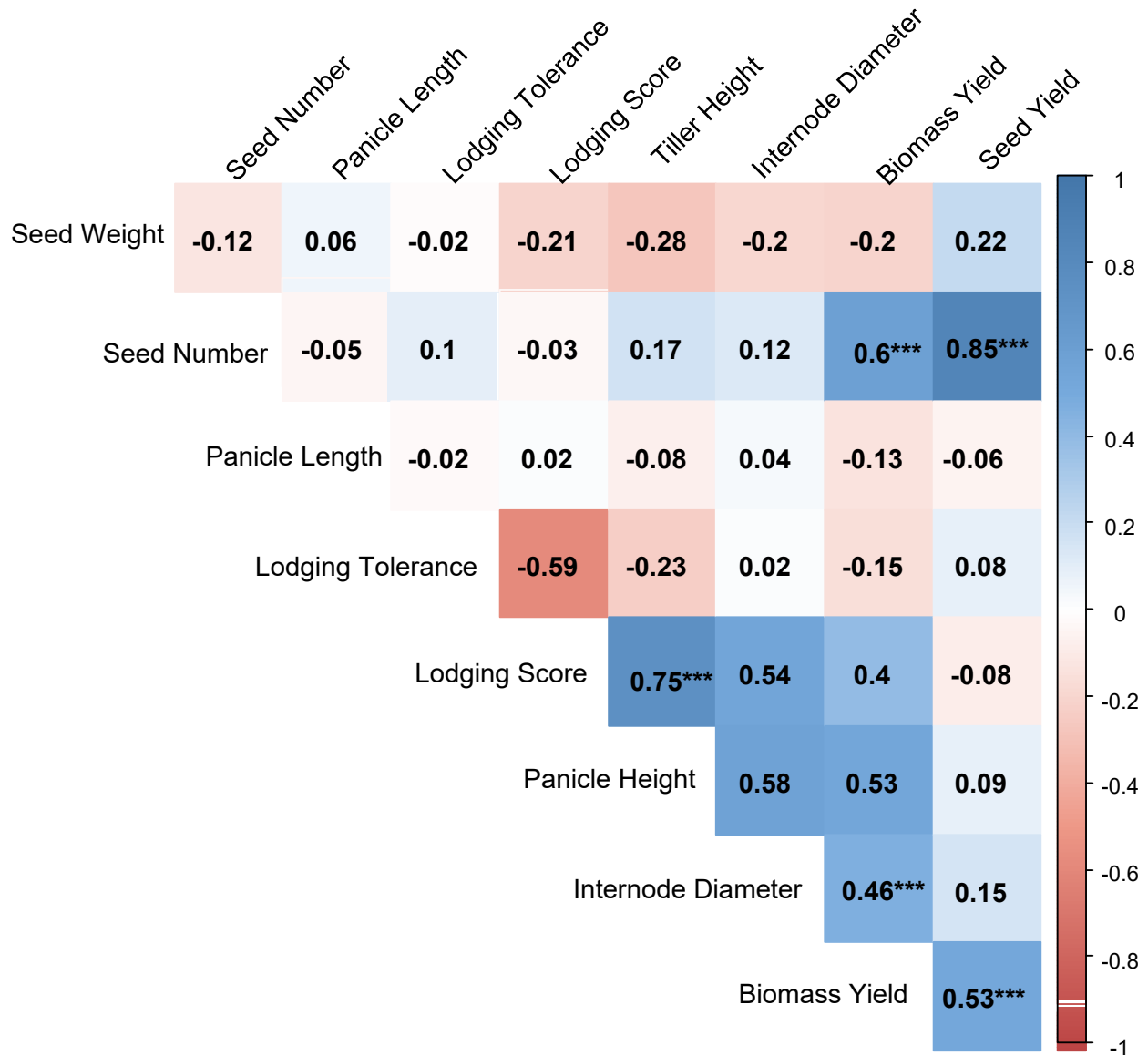


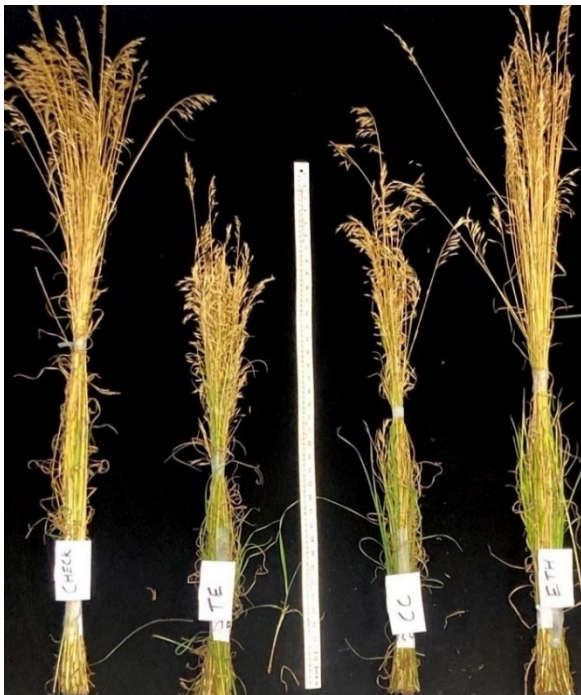
Figure A2: Correlation coefficients (r) between plant traits and seed yield of timothy over two crop years. \*P ≤ 0.05. \*\*P ≤ 0.01. \*\*\*P ≤ 0.001.



Figure A3: Correlation coefficients ( $r$ ) between plant traits and seed yield of creeping red fescue over two crop years. \* $P \leq 0.05$ . \*\* $P \leq 0.01$ . \*\*\* $P \leq 0.001$ .



A



B

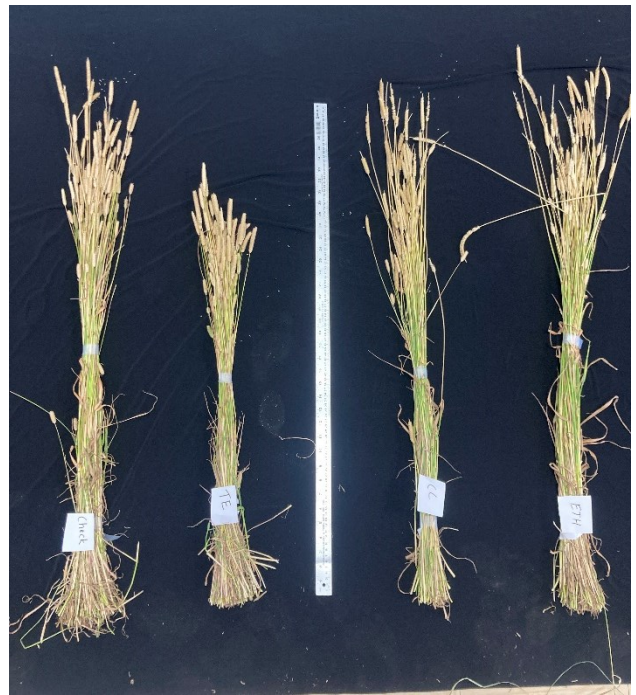


Figure A4: The plant height comparison in order of (Check, TE, CC and ETH) of meadow bromegrass (A) and timothy grass (B) from field study.

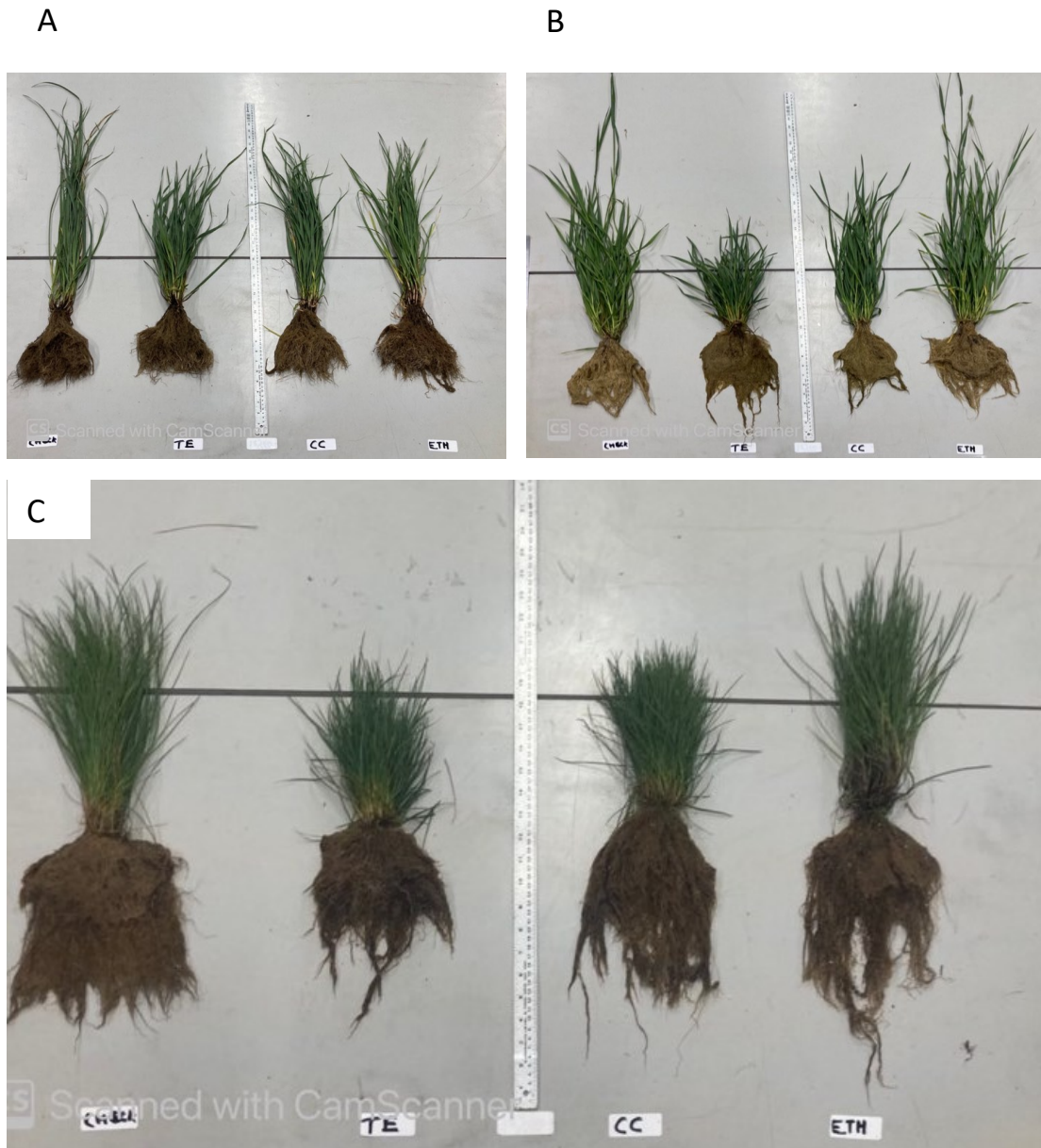


Figure A5: The root and shoot biomass comparison in order of (check, TE, CC and ETH) of meadow bromegrass (A) and timothy grass (B) and creeping red fescue (C) after 40 days after treatment in greenhouse conditions.



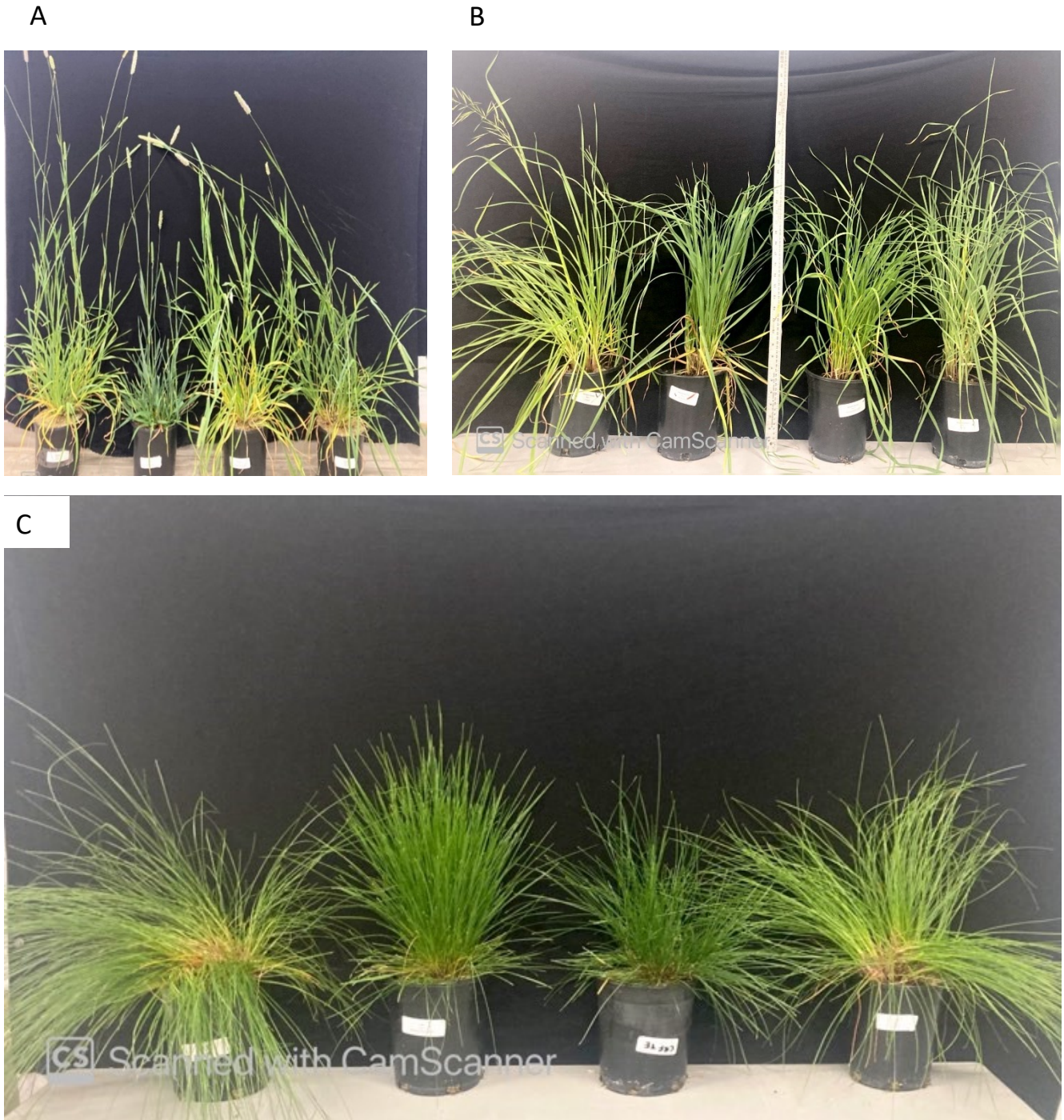


Figure A6: The plant lodging comparison in order of (Check, TE, CC and ETH) of meadow bromegrass (A) and timothy grass (B) and creeping red fescue (C) after 70 days after treatment in greenhouse condition.