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Plant growth regulator and spring nitrogen effects on grass seed crops in western Canada

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Abstract

Plant growth regulators (PGRs) are used in herbage seed crops to mitigate lodging risk and protect seed yield, though their effects on aging perennial grass stands remain understudied. This study evaluated the effect of PGRs on the agronomic characteristics of herbage seed crops of creeping red fescue (*Festuca rubra* L.), meadow brome (*Bromus riparius* Rehmann), and timothy (*Phleum pratense* L.) in their third and fourth years after establishment in western Canada during 2021 and 2022. A split-plot experimental design comprised PGR treatments of trinexapac ethyl (TE), chlormequat chloride (CC), and ethephon (ETH) as main plots with paired subplots of spring nitrogen (N) top-dressed at 40 kg N ha^{-1} and an unfertilized control. TE, CC, and ETH were applied at 0.20, 1.12, and 0.60 kg ha^{-1} active ingredient, respectively, at the Biologische Bundesanstalt, Bundessortenamt and Chemical industry (BBCH) 31-32 crop development stage. TE reduced internode length, tiller height, and lodging severity in meadow brome and timothy, and increased culm diameter in creeping red fescue and timothy. However, no PGRs enhanced seed yield, seed weight, and seed density. Spring top-dressed N increased biomass and seed yield in timothy and meadow brome, and seed density in all grasses, with no interaction between PGRs and supplemental N. Despite modifying plant morphological attributes and reducing lodging risk, PGRs did not consistently improve seed yield under dry to average soil moisture conditions. Future studies should examine weather variables and plant physiological thresholds that contribute to lodging risks to better inform the use of PGRs.

Plain Language Summary

Plant growth regulators are chemical substances that can alter plant growth and enable plants to stand upright against winds and storms, thereby preventing seed yield losses. This study examined the effect of three plant growth regulators—trinexapac

Abbreviations: a.i., active ingredient; BBCH, Biologische Bundesanstalt, Bundessortenamt and Chemical industry; CC, chlormequat chloride; ETH, ethephon; PGR, plant growth regulator; TE, trinexapac ethyl.

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ethyl, chlormequat chloride, and ethephon—on the perennial grass seed crops of creeping red fescue, meadow brome, and timothy in their third and fourth years. Application of trinexapac ethyl to young, actively growing plants at the two-node stage reduced plant height and lodging in meadow brome and timothy, and increased stem girth in creeping red fescue and timothy. However, none of the plant growth regulators consistently improved seed yield under dry to average soil moisture conditions, despite making the plants shorter and sturdier to withstand lodging. Future studies should focus on advanced predictions of crop lodging risks to better realize potential benefits of plant growth regulators.

1 | INTRODUCTION

Seeds of forages and turfgrasses, collectively termed as herbage crops, are key agricultural exports of Canada. Historically, the Peace River region contributed one-third to half of national production (Wong, 2017). Creeping red fescue, timothy, and meadow brome accounted for the bulk of Canada's herbage seed exports, which reached $40 million in 2019–2020 (Wong, 2020b). These cool-season perennials are well suited to the regional climates and have diverse uses. Creeping red fescue is used in lawns, pastures, and land reclamation (Braun et al., 2020). Timothy is primarily grown for hay, pasture, and silage (Berg et al., 1996). Known for rapid regrowth and palatability, meadow brome is suitable for hay and pasture systems (Lardner et al., 2002).

Integrating perennial grasses into annual cropping systems enhances soil health, water conservation, nutrient use efficiency, system productivity, and sustainability (Khanal, 2022). Despite their economic values, the herbage seed production area and volumes are declining in Canada (Wong, 2020a), due primarily to a lack of innovations to address constraints associated with herbage seed crops compared to annual cash crops (Khanal, 2022). Addressing these constraints requires targeted genetic and agronomic strategies.

Changing climate and inherent physiological barriers limit the seed yield potential of temperate grasses (Hampton et al., 2016; Kavka et al., 2024), with yields declining over successive years (Canode & Law, 1975). Lodging, the permanent bending of stems from wind, rain, or overgrowth, is a key factor reducing grass seed yield (Baker et al., 2014). Lodging reduces photosynthesis (Clemence & Hebblethwaite, 1984; Griffith, 2000), impairs pollination (Boelt & Studer, 2010) and seed fill (Berry et al., 2004; Tian et al., 2018), impedes harvest, and reduces seed quality (Macháč, 2013). Seed yield losses can reach 95% (Chynoweth & Moot, 2017). High N and soil moisture exacerbate lodging (Havstad & Aamlid, 2006), underscoring the need for balanced fertility management.

Nitrogen is the most limiting nutrient in Alberta soils (Malhi, 1996). Nitrogen fertilization boosts biomass, seed yield (Chastain et al., 2015), fertile tillers (Koeritz et al., 2015), and floret numbers (Cookson et al., 2001) in perennial grasses. Application of N in fall promotes tiller development for spring flowering, while spring-applied N supports ongoing growth and seed production (Boelt, 1999). However, fall N applications risk losses from ammonia volatilization, denitrification (Malhi, 1996), and reduced plant uptake over winter (Malyshev & Henry, 2012). Excessive N with ample soil moisture increases lodging risk (Bitarafan et al., 2019; Havstad & Aamlid, 2006), making precise timing and rates critical (Borm & van den Berg, 2008; Fairey, 2008).

Where lodging risk of grass seed crops is high, the use of plant growth regulators (PGRs) is an option to limit stem elongation and lodging severity (Chastain et al., 2014; Chynoweth & Moot, 2017; Griffith, 2000; Rolston et al., 2010). By functioning as growth retardants, PGRs mitigate lodging by regulating plant hormone systems to control stem elongation (Rademacher, 2015). This results in shorter, thicker stems that improve structural stability (Rademacher, 2016). The PGRs chlormequat chloride (CC) and trinexapac ethyl (TE) inhibit two distinct steps in the biosynthetic pathway of gibberellic acid that is responsible for stem elongation (Rademacher, 2015, 2016), while ethephon (ETH) inhibits cell division and elongation by releasing ethylene, and restricting auxin biosynthesis (Ma & Smith, 1991). The PGRs TE, CC, and ETH are registered in Canada for various field crops and traded as Moddus (11.3% TE), Manipulator (620 g CC L^{-1}) (https://pr-rp.hc-sc.gc.ca/pi-ip/result-eng.php?1=0&2=501&3=pr&4=n&5=1&6=ASC&7=M&8=E), and Ethrel (240 g ETH L^{-1}) (https://pr-rp.hc-sc.gc.ca/ls-re/lbl_detail-eng.php?p_disp_regn=%2711580%27&p_regnum=11580), respectively.

Past studies indicate TE is effective in reducing lodging and increasing seed production in perennial ryegrass (Rolston et al., 2010), creeping red fescue (Bitarafan et al., 2019; Szczepanek et al., 2021; Zapiola et al., 2014), and tall fescue (Chastain et al., 2015; Rolston & Young, 2009). Combining TE with spring N enhances seed yield across species (Borm & van den Berg, 2008; Chastain et al., 2015; Szczepanek et al.,

2021). The PGRs CC and ETH are more commonly used in cereals (Rajala & Peltonen-Sainio, 2002) and their efficacy varies by species (Farooqi et al., 2005; Fiorani et al., 2002; Hudgins, 2019; Poovaiah & Leopold, 1973). PGRs applied between stem elongation (Biologische Bundesanstalt, Bundessortenamt and Chemical industry [BBCH] 31-32) and early panicle initiation (BBCH 50-51) (see Gustavsson [2011] for growth stage coding) exhibited more pronounced effects (Griffith, 2000).

Tailored agronomic practices are essential to optimize crop yield under a given environment. In the Peace River region, studies elucidating the efficacy of PGRs combined with top-dressed N in spring on multi-year perennial herbage seed crop stands are limited. A recent study showed that TE and its combination with CC increased creeping red fescue seed yield in the first production year, but not the second (Bhattarai et al., 2024). Drawing on prior studies, we hypothesized that spring application of PGRs, regardless of supplemental N levels, reduces lodging through the development of shorter and thicker stems, thereby improving seed set and enhancing seed yield in aging grass stands. Therefore, this study focused on creeping red fescue, meadow brome, and timothy during their third and fourth years (corresponding to the second and third production years) to (i) assess the effects of PGRs (TE, CC, and ETH) and spring N application grass morphological traits that influence lodging, including internode length, diameter, and tiller height; (ii) evaluate the efficacy of PGRs in controlling lodging; and (iii) quantify key seed yield components and total seed yield in response to PGR and N treatments. The novelty of this study is the simultaneous comparison of the effects of three PGRs (TE, CC, and ETH) combined with spring N fertilization to reduce lodging and improve seed yield in aging stands of perennial herbage crops, creeping red fescue, meadow brome, and timothy. The effect of ETH on temperate grass seed production remains understudied. Most importantly, the study addresses a critical research gap in the Peace River region where such multi-year agronomic evaluations are lacking.

Core Ideas

- Plant growth regulators (PGRs) and nitrogen (N) effects varied by grass seed crops.
- PGRs did not improve seed yield despite reducing plant height and lodging.
- PGRs augmented lodging tolerance under more favourable growing conditions.
- Spring N boosted biomass, seed yield, and seed density in timothy and meadow brome.

2 | MATERIALS AND METHODS

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, Alberta. The study site is located on a gentle east-facing slope with a 2.5% gradient and 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 loamy texture (sand = 31.3%, silt = 46.0%, and clay = 22.7%) with a pH of 5.93 and an organic matter (OM) content of 4.36%. Soil nutrient levels were 4.97 mg kg^{-1} of NH_4^+-N, 14.9 mg kg^{-1} of NO_3^--N, 15.4 mg kg^{-1} of PO_4-P, 10.8 mg kg^{-1} of SO_4 -S, and 139 mg kg^{-1} of K.

Agroclimatic conditions at Beaverlodge typify a short growing season with an average of 117 frost-free days (May 20–September 15), day length ranging between 13.2 and 17 h, warm daytime temperatures (16°C–22°C), cool nighttime temperatures (3°C–9°C), and the occurrence of >60% of an average annual precipitation of 433 mm during the growing season from May to September (Government of Canada, 2013). Grass seed crops mature between late July and mid-August.

Mean temperatures during the growing seasons studied (May to August) were 12.8°C (minimum temperature 6°C and maximum temperature 19.10°C) in 2021 and 11.9°C (minimum temperature 5.8°C and maximum temperature 17.91°C) in 2022. However, the number of days exceeding 25°C during the vegetative to reproductive phases by the third week of July was 12 in 2021 compared to only three in 2022. Though total rainfall during the same period was 167 and 171 mm in 2021 and 2022, respectively, the number of days with >1 mm precipitation was 12 in 2021 compared to 19 days in 2022 (Figure 1).

Weather conditions during 2021 were atypical, with erratic rainfall at the beginning and end of the growing season, and a heatwave (up to 42°C) during the grass reproductive stage in late June. In contrast, 2022 had more favorable growing conditions with better distribution of precipitation and less variation of temperatures (Figure 1). Spring and summer weather conditions (May–July) are major determinants of growth, reproduction, and seed production in cool-season grasses.

2.2 | Experimental design

Forage grass plots of meadow brome, timothy, and creeping red fescue were established in 2019; plots were 50 m long × 12 m wide. Each grass was zero-till seeded with a cross-slot seeder (Cross Slot IP Limited) with 30 cm row spacing. Grass

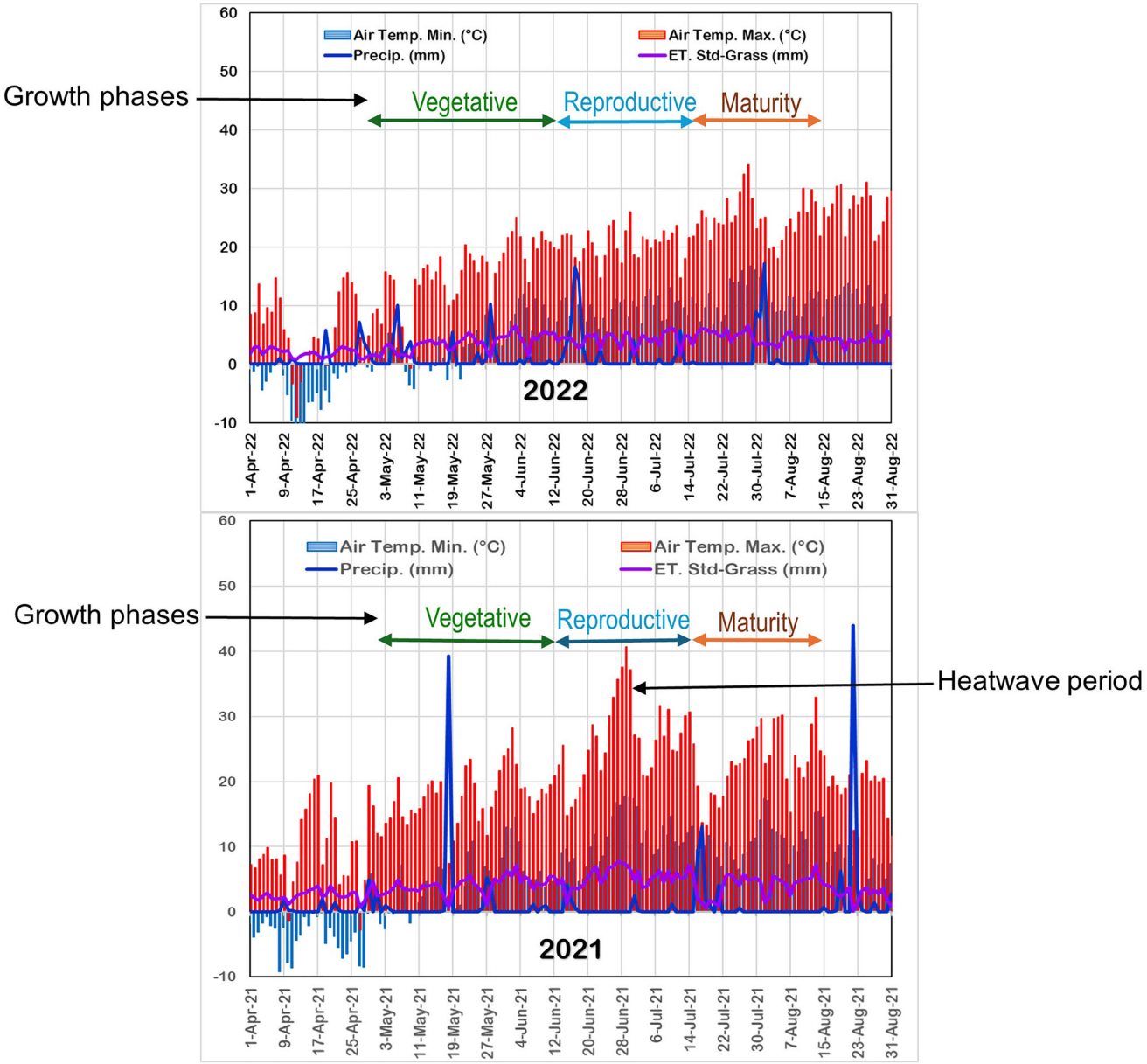


FIGURE 1 Summary of the daily growing season temperatures (minimum and maximum, ˚C), precipitation (mm), and evapotranspiration (ET) (mm) during each of 2021 (A) and 2022 (B) at the AAFC Beaverlodge Research Farm, Alberta, Canada.

plots were uniformly fertilized with N, P, K, and S at 60, 32, 30, and 14 kg ha^{-1}, respectively, in the fall (mid-September) from 2019 to 2022. To achieve in-plot weed control, herbicide Curtail M (50 g L^{-1} emulsifiable concentrate, containing the active ingredients MCPA [2-Methyl-4-chlorophenoxyacetic acid] and clopyralid) at 2 L ha^{-1} diluted to 100 L of water was applied every fall after the establishment year, and large sporadic weeds were manually removed from time to time during the growing season (Pandey, 2023).

The experiment consisted of eight treatments conducted in a split-plot design, with PGRs applied as the main plot and supplemental spring N as subplot factors (Table 1). Seeded areas of each species were divided into four blocks (replicates) and each block was further subdivided into four plots, measuring 8 m × 2 m in size (after leaving buffer strips around the plots). PGR treatments of TE (0.20 kg ai ha^{-1}), CC (1.12 kg ai ha^{-1}), ETH (0.60 kg ai ha^{-1}), and untreated control were randomly assigned to the plots. The concentrations and application rates of the PGRs were determined based on manufacturers' registration labels for cereals in Canada, which were considered more appropriate than relying on recommendations from unrelated environments. Weblinks to the Canadian registrations of the PGRs are provided in Section 1. Specified active ingredients of each PGR were diluted with water to a spray volume of 100 L ha^{-1} at a pressure of 270 kPa, as previously reported by Bhattarai et al. (2024) for the Peace River region, and applied between 6:00 a.m. and 8:00 a.m. using a backpack boom sprayer. All PGRs were applied

TABLE 1 Summary of experimental treatments, including four plant growth regulators (PGRs) as main plots and two nitrogen (N) treatments as subplots, evaluated on creeping red fescue, meadow brome, and timothy during 2020 and 2021 at the Beaverlodge Research Farm.

Main plot factor: PGRs	Subplot factor: N top-dress in spring[a]	Abbreviations
TE @ 0.20 kg ai ha^{-1}	Without N top-dress	TE + N0
	N top-dress	TE + N40
CC @ 1.12 kg ai ha^{-1}	Without N top-dress	CC + N0
	N top-dress	CC + N40
ETH @ 0.6 kg ai ha^{-1}	Without N top-dress	ETH + N0
	N top-dress	ETH + N40
Control (No PGR application)	Without N top-dress	Control + N0
	N top-dress	Control + N40

Abbreviations: CC, chlormequat chloride; ETH, ethephon; TE, trinexapac ethyl.
[a]Supplemental N was top-dressed at the rate of 40 kg ha^{-1} in spring following the final snowmelt. N0: no spring N top-dressing; N40: N top-dressed at 40 kg ha^{-1}.

at the beginning of stem elongation (BBCH 31-32) stage (see Gustavsson [2011] for detailed growth stage coding in grasses). The PGR main plots were further subdivided into two 4 m × 2 m subplots, one of which was assigned to receive 40 kg N ha^{-1} as spring top-dressed urea fertilizer annually during the first week of May, and the other subplot did not receive spring N (unfertilized control) (Pandey, 2023).

Treatments were assessed separately for each grass due to inherent differences in the growth of each forage. Seed production, lodging, and grass morphology were measured in all subplots during 2021 and 2022.

2.3 | Grass morphological measurements

Tiller height, internode length, and culm diameter were determined from five randomly selected tillers from each subplot at the maturity stage between mid-July and early August in the order of more rapid maturity: creeping red fescue, meadow brome, and timothy. Plant height was obtained by measuring the longest length from the basal crown to the average tip of the inflorescence. At the same time, three uppermost internode lengths were measured, and culm diameters (girths) were taken at the center of each internode using a caliper. The lengths of three uppermost nodes are a major determinant of plant height, while both length and girth influence stem strength and mechanical stability, thereby affecting lodging tendency. The grass sward lodging score was assessed at maturity between late July and mid-August on a five-point scale, where 1 = *not lodged* (fully upright stems) and 5 = *the most severe lodging* (horizontal stems) as described by Corbin et al. (2016).

Grass biomass and seed yield were quantified from a 1 m^{-2} quadrat sample, randomly placed within each subplot when the grasses reached physiological maturity. At harvest, all sample biomass was cut at 2 cm above ground, air-dried, and weighed to determine dry matter yield. Samples were then threshed and sieved to separate seed from the remaining plant material. After cleaning, 1000-seed weights were determined. Finally, seed density (the number of clean seeds recovered from plants harvested from one square meter area, expressed as the number of seeds m^{-2}) from each plot was derived from the total weight of the harvested clean seed yield and the 1000-kernel weight from each subplot (Pandey, 2023).

2.4 | Statistical analysis

Analysis of variance was performed on all response metrics (grass biomass, seed yield, 1000-kernel weight, seed density, plant height, internode lengths, and culm diameter) using the lme4 package (Bates et al., 2015) in RStudio version 1.4.1717. Year of sampling, PGRs, and spring N fertilizer treatments were modeled as fixed effects, while replicate block was modeled as 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 two treatments. Pearson correlation analysis was conducted to examine the relationships among the morphological traits and yield-related variables within each grass species.

3 | RESULTS

3.1 | Effects of PGRs and supplemental nitrogen on grass morphology and lodging

Plant height was affected by the PGRs applied in meadow brome and timothy across both years ($p \leq 0.001$) (Figure 2B,C), with a further interaction of PGR × year observed specifically for meadow brome. Among the PGRs, TE application led to the shortest plant height within each of the three grasses, with this effect most apparent in meadow

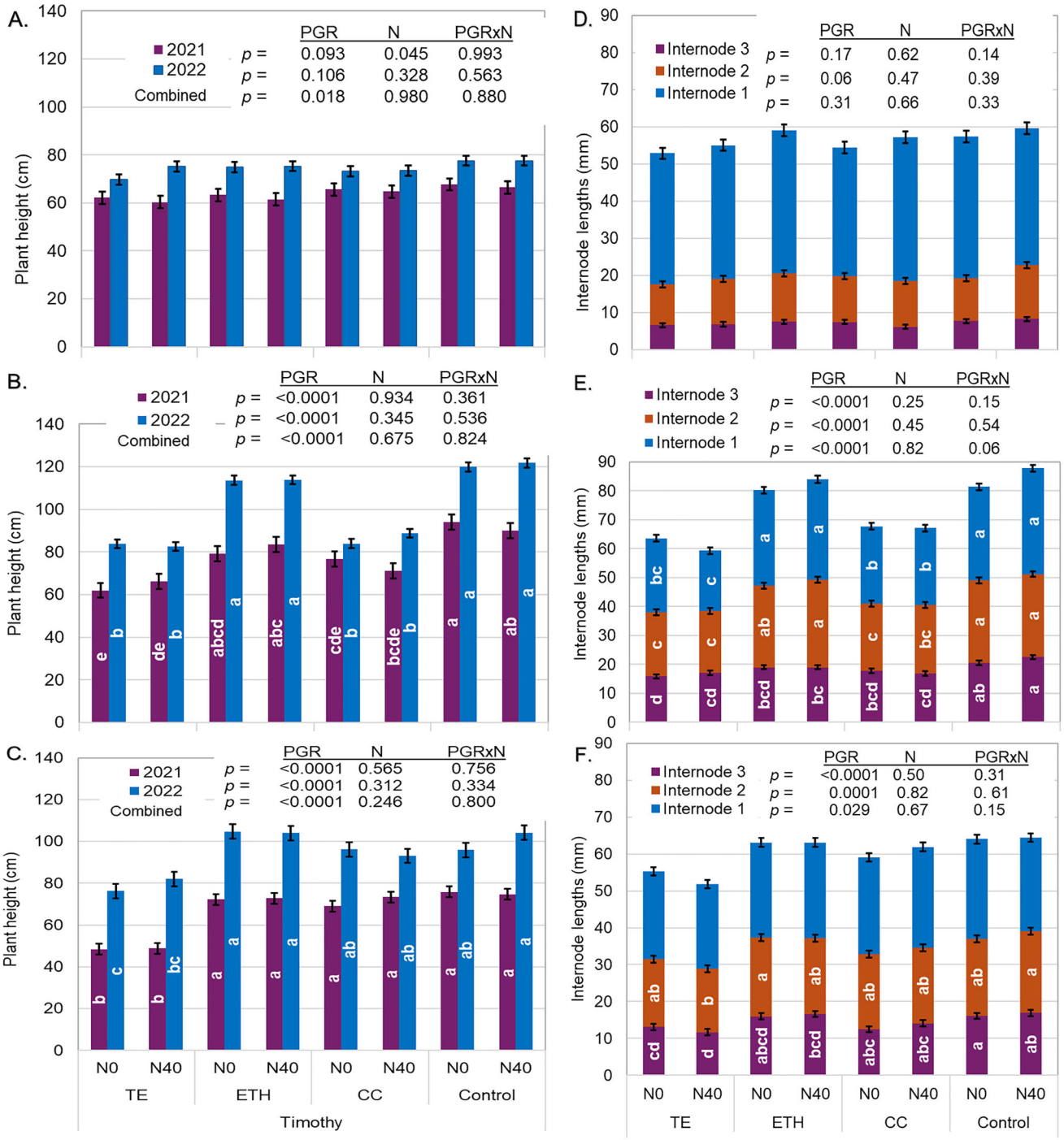


FIGURE 2 Effect of plant growth regulators (PGRs) and supplemental spring nitrogen (N) on plant height (cm) and the length of three uppermost internodes (numbered in ascending order from top) in creeping red fescue (A and D), meadow brome (B and E), and timothy (C and F) on the same perennial seed crop stands in 2021 and 2022. Bars in the graphs represent yearly means of plant heights across four replicates; internode lengths are pooled means from 2 years ($n = 8$). The error bars indicate standard error of the mean. Means with different letters denote significant differences at $p \leq 0.05$. CC, chlormequat chloride; ETH, ethephon; TE, trinexapac ethyl; N0, no spring nitrogen top-dressing; N40, nitrogen top-dressed at 40 kg ha^{-1}.

brome (−31% compared to the control) (Figure 2B), followed by timothy (−27% compared to the control) (Figure 2C), and then creeping red fescue (−7% compared to the control) (Figure 2A). ETH and CC did not reduce the height of timothy or fescue relative to the control ($p \geq 0.05$) but did reduce the height of meadow brome, with CC being more effective than ETH. Plant heights were markedly greater ($p < 0.001$) in all three grasses during 2022, while PGRs reduced meadow brome height to a greater extent in 2022 than in 2021. Spring N addition did not affect plant height in any of the grasses, except for creeping red fescue in 2021 (Pandey, 2023).

Changes in plant heights were linked to the degree of elongation of the upper three internodes. Application of PGRs affected the upper three internode lengths, specifically in meadow brome (Figure 2E) and timothy (Figure 2C) ($p < 0.001$), but not in creeping red fescue (Figure 2A). Internode lengths were also longer during 2022 for all grasses compared to the previous year (only means over years are shown), though PGR effects remained similar between years. Overall, internodes were shortest under TE application, and this effect was apparent for all three uppermost internodes in both meadow brome and timothy. In contrast, ETH and CC had a negligible effect on the internode length of timothy, but CC reduced internode lengths in meadow brome, particularly those of the third internode. Spring N fertilization did not affect internode lengths in any of the grasses, both alone and in combination with the PGRs.

Culm diameter, measured as the width of the three uppermost internodes, was significantly affected by PGR application in timothy and creeping red fescue ($p < 0.05$), but not meadow brome (Table 2). TE produced the thickest-diameter culms in both timothy and creeping red fescue. Spring-applied N did not affect the culm diameter of grass crops, although culm diameters were generally higher in 2022 compared to 2021 ($p < 0.001$). There was also no interaction between PGRs and spring N in relation to culm diameter in any year (Pandey, 2023).

Consistent with the effects observed on plant heights, internode length, and internode diameters, lodging in meadow brome and timothy was significantly influenced by PGR application ($p < 0.001$) and the PGR × year interaction (Table 3). In contrast, PGRs had no significant effect on lodging in creeping red fescue. Lodging scores decreased in meadow brome after TE application, and this response was particularly apparent in 2022. Severity of lodging in timothy declined under both TE and CC applications, and was lowest under TE treatment, and these responses were again more apparent during 2022 when lodging of grasses was overall more common ($p \leq 0.001$). Application of supplemental N in spring did not induce changes in lodging for any of the grass crops. In general, the control and ETH treated plots for meadow brome and timothy exhibited the most severe lodging during the 2022 production year, though lodging occurred near maturity, which is less critical for yield effects (Pandey, 2023).

3.2 | Effect of PGRs and supplemental nitrogen on biomass and seed attributes of grasses

Total aboveground grass biomass was significantly affected by the PGRs when applied to meadow brome ($p \leq 0.001$), but not timothy and creeping red fescue ($p \geq 0.2$) (Table S1). More specifically, TE application reduced brome biomass by 30%, while CC and ETH reduced brome biomass by 19% and 11%, respectively. Spring N fertilization increased the biomass yield of meadow brome and timothy ($p \leq 0.001$), but not creeping red fescue ($p \geq 0.4$). Supplemental spring N application did not alter grass responses to PGRs. Biomass production was higher during 2022 for meadow brome and timothy ($p \leq 0.001$). Spring N application led to particularly large increases in biomass of both meadow brome and timothy during 2022 but had no effect on biomass yields in 2021 (Pandey, 2023).

Grass seeds yield attributes such as seed density (number of seeds per unit area, seeds m^{-2}) (Table 4) and 1000-seed weight (g) (Table 5) were not affected by the PGR applications. Spring N addition increased the seed density ($p \leq 0.02$) in all grasses but did not affect seed weight in any of the three grasses ($p \geq 0.06$). Grass seed density and seed weight were higher in 2022 in both meadow brome and creeping red fescue ($p < 0.001$) but not timothy ($p \geq 0.06$). There was no interaction between the PGRs tested and N application on the seed density of any grasses, even though seed densities were higher under spring-applied N fertilization during 2022 for both meadow brome and timothy (Pandey, 2023).

The total seed yield of all grasses remained unchanged with respect to PGR application ($p \geq 0.1$) (Figure 3). However, spring-applied supplemental N increased seed yield for timothy and meadow brome ($p < 0.001$), but not creeping red fescue ($p \geq 0.09$). Spring N application did not enhance seed yields in any of the grass species in 2021. During 2022, however, meadow bromegrass showed a significant increase in seed yield following supplemental spring N application ($p < 0.001$). Notably, PGRs and N treatment did not interact to affect seed yield in any of the grass species studied. (Figure 3; Pandey, 2023).

3.3 | Relationship between grass plant height, lodging, and seed yield

Across 2 years of data collected under contrasting weather conditions in 2021 and 2022, the third- and fourth-year stands

TABLE 2 Effect of plant growth regulators (PGRs) and supplemental spring nitrogen (N) on culm diameter (mm) of creeping red fescue, meadow brome, and timothy on the same perennial stands in 2021 and 2022. Values are means of four replicates from 2 years ($n = 8$) of seed production.

	Creeping red fescue			Meadow brome			Timothy		
Treatment	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**
Plant growth regulators (PGRs)									
TE	1.33	1.08	1.21a	1.56	2.19	1.88	1.38a	2.04a	1.71a
ETH	1.13	1.06	1.09b	1.59	2.22	1.91	1.13ab	1.98ab	1.56b
CC	1.15	1.04	1.09b	1.84	2.19	2.02	1.15ab	1.84ab	1.50b
Control	1.10	1.08	1.09b	1.49	2.04	1.77	1.1b	1.92c	1.51b
SEM (±)	0.062	0.023	0.033	0.13	0.067	0.074	0.066	0.0451	0.04
Supplemental nitrogen (N)									
N0	1.18	1.06	1.13	1.61	2.11	1.92	1.19	1.93	1.58
N40	1.18	1.07	1.12	1.64	2.11	1.86	1.19	1.97	1.56
SEM (±)	0.044	0.016	0.023	0.0918	0.0494	0.05	0.0467	0.0319	0.028
Year									
2021	–	–	1.06b	–	–	1.62b	–	–	1.19b
2022	–	–	1.18a	–	–	2.16a	–	–	1.95a
SEM (±)	–	–	0.023	–	–	0.05	–	–	0.028
ANOVA (*p*-value significance tests)									
PGRs	0.056	0.552	0.041	0.277	0.192	0.12	0.021	0.029	0.0013
N	0.904	0.678	0.79	0.825	0.124	0.37	0.959	0.334	0.55
Year			0.001			<0.0001			<0.0001
PGRs × N	0.346	0.676	0.49	0.188	0.996	0.28	0.435	0.543	0.69
PGRs × year	–	–	0.09	–	–	0.49	–	–	0.25
N × year	–	–	0.97	–	–	0.62	–	–	0.61
PGRs × N × year	–	–	0.27	–	–	0.23	–	–	0.30

Note: Within a grass species and treatment grouping, treatment means followed by different letters indicate significant differences using a Tukey test ($p \leq 0.05$). N0: no spring N top-dressing; N40: N top-dressed at 40 kg ha^{-1}.
Abbreviations: ANOVA, analysis of variance; CC, chlormequat chloride; ETH, ethephon; SEM: standard error of the mean; TE, trinexapac ethyl.

TABLE 3 Effect of plant growth regulators (PGRs) and supplemental spring nitrogen (N) on lodging score[a] of creeping red fescue, meadow brome, and timothy on the same perennial stands in 2021 and 2022. Values are means of four replicates from 2 years ($n = 8$) of seed production.

	Creeping red fescue			Meadow brome			Timothy		
Treatment	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**
Plant growth regulators (PGRs)									
TE	1.0	1.38	1.19	1.0	1.0c	1.50b	1.25	1.0c	1.19
ETH	1.12	1.62	1.38	1.12	2.88b	2.31a	1.0	3.0a	1.38
CC	1.0	1.38	1.25	1.12	1.62c	1.94ab	1.0	2.12b	1.25
Control	1.38	1.62	1.50	1.62	3.88a	2.31a	1.25	3.0a	1.50
SEM (±)	0.114	0.197	0.121	0.14	0.165	0.11	0.114	0.126	0.121
Supplemental nitrogen (N)									
N0	1.12	1.44	1.28	1.12	2.25	1.91	1.06	2.25	1.72
N40	1.12	1.56	1.38	1.31	2.44	2.12	1.19	2.31	1.69
SEM (±)	0.0807	0.147	0.08	0.111	0.117	0.078	0.081	0.097	0.076
Year									
2021	–	–	1.12	–	–	1.66a	–	–	1.12b
2022	–	–	1.30	–	–	2.38b	–	–	2.28a
SEM (±)	–	–	0.08	–	–	0.078	–	–	0.076
ANOVA (*p*-value significance tests)									
PGRs	0.0928	0.617	0.11	0.0067	<0.0001	<0.0001	0.2155	<0.0001	<0.0001
N	1.00	0.507	0.24	0.131	0.268	0.052	0.284	0.587	0.69
Year			0.053			<0.0001			<0.0001
PGRs × N	0.506	0.716	0.87	0.844	0.933	0.54	0.331	0.822	0.40
PGRs × year	–	–	0.87	–	–	0.0016	–	–	<0.0001
N × year	–	–	0.24	–	–	0.16	–	–	0.24
PGRs × N × year	–	–	0.33	–	–	0.10	–	–	0.63

Note: Within a grass species and treatment grouping, treatment means followed by different letters indicate significant differences using a Tukey test ($p \leq 0.05$). N0: no spring N top-dressing; N40: N top-dressed at 40 kg ha^{-1}.
Abbreviations: ANOVA, analysis of variance; CC, chlormequat chloride; ETH, ethephon; SEM: standard error of the mean; TE, trinexapac ethyl.

[a]Lodging was scored on a five-point scale, where 1 indicated not lodging (fully upright stems) and 5 represented the most severe lodging (horizontal stems).

TABLE 4 Effect of plant growth regulators (PGRs) and supplemental spring nitrogen (N) on seed density (number of seeds/square meter area) of creeping red fescue, meadow brome, and timothy on the same perennial stands in 2021 and 2022. Values are means of four replicates from 2 years ($n = 8$) of seed production.

	Creeping red fescue			Meadow brome			Timothy		
Treatment	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**
Plant growth regulators (PGRs)									
TE	31,927	47,501	31,066	4705	24,034	15,048	145,116b	178,632a	160,217
ETH	26,713	35,419	39,714	4705	19,585	12,145	145,212b	154,943ab	150,078
CC	26,740	43,871	35,305	5731	20,502	13,369	170,672a	165,477ab	161,075
Control	25,126	44,534	34,830	6236	19,487	12,609	163,336ab	161,515 c	162,426
SEM (±)	3040	4110	2403	1110	1860	1218	6750	5750	5033
Supplemental nitrogen (N)									
N0	22,666a	42,030	32,348b	5586	16,755b	11,170b	149,280	150,188b	148,040b
N40	32,588b	43,633	38,110a	5781	25,050a	15,415a	162,888	180,095a	172,358a
SEM (±)	2280	3400	1732	910	1370	1001	4770	4070	3757
Year									
2021	–	–	27,627b	–	–	5683b	–	–	156,084
2022	–	–	42,832a	–	–	20,902a	–	–	164,314
SEM (±)	–	–	1732	–	–	1001	–	–	3757
ANOVA (*p*-value significance tests)									
PGRs	0.376	0.0852	0.094	0.637	0.266	0.18	0.026	0.049	0.073
N	0.0022	0.6286	0.018	0.831	<0.0001	<0.0001	0.055	<0.0001	<0.0001
Year			<0.0001			<0.0001			0.092
PGRs × N	0.462	0.066	0.082	0.935	0.807	0.91			0.053
PGRs × year	–	–	0.42	–	–	0.43	–	–	0.055
N × year	–	–	0.084	–	–	0.00016	–	–	0.029
PGRs × N × year	–	–	0.43	–	–	0.73	–	–	0.96

Note: Within a grass species and treatment grouping, treatment means followed by different letters indicate significant differences using a Tukey test ($p \leq 0.05$). N0: no spring N top-dressing; N40: N top-dressed at 40 kg ha^{-1}.
Abbreviations: ANOVA, analysis of variance; CC, chlormequat chloride; ETH, ethephon; SEM: standard error of the mean; TE, trinexapac ethyl.

TABLE 5 Effect of plant growth regulators (PGRs) and supplemental spring nitrogen (N) on 1000-seed weight (g) of creeping red fescue, meadow brome, and timothy on the same perennial stands in 2021 and 2022. Values are means of four replicates from 2 years ($n = 8$) of seed production.

	Creeping red fescue			Meadow brome			Timothy		
Treatment	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**
Plant growth regulators (PGRs)									
TE	0.899	1.27	1.07	4.01	4.85	4.43	0.426a	0.411	0.42
ETH	0.878	1.29	1.08	3.96	4.96	4.46	0.419ab	0.408	0.41
CC	0.878	1.26	1.09	3.91	4.93	4.42	0.408b	0.393	0.40
Control	0.925	1.3	1.11	4.05	4.99	4.52	0.409b	0.408	0.41
SEM (±)	0.0261	0.0248	0.018	0.0704	0.106	0.076	0.00382	0.0108	0.0053
Supplemental nitrogen (N)									
N0	0.869	1.27	1.07	3.97	4.92	4.45	0.414	0.403	0.41
N40	0.921	1.29	1.11	3.99	4.94	4.47	0.417	0.408	0.41
SEM (±)	0.0191	0.0175	0.013	0.0504	0.0971	0.068	0.00291	0.0088	0.0038
Year									
2021	–	–	0.895b	–	–	3.98b	–	–	0.41
2022	–	–	1.281a	–	–	4.93a	–	–	0.42
SEM (±)	–	–	0.013	–	–	0.068	–	–	0.0038
ANOVA (*p*-value significance tests)									
PGRs	0.522	0.706	0.41	0.530	0.447	0.47	0.0045	0.505	0.13
N	0.05385	0.5151	0.060	0.777	0.698	0.64	0.485	0.589	0.82
Year			<0.0001			<0.0001			0.058
PGRs × N	0.133	0.0774	0.054	0.467	0.674	0.81	0.463	0.666	0.95
PGRs × year	–	–	0.87	–	–	0.54	–	–	0.72
N × year	–	–	0.33	–	–	0.97	–	–	0.49
PGRs × N × year	–	–	0.19	–	–	0.36	–	–	0.48

Note: Within a grass species and treatment grouping, treatment means followed by different letters indicate significant differences using a Tukey test ($p \leq 0.05$). N0: no spring N top-dressing; N40: N top-dressed at 40 kg ha^{-1}.
Abbreviations: ANOVA, analysis of variance; CC, chlormequat chloride; ETH, ethephon; SEM: standard error of the mean; TE, trinexapac ethyl.

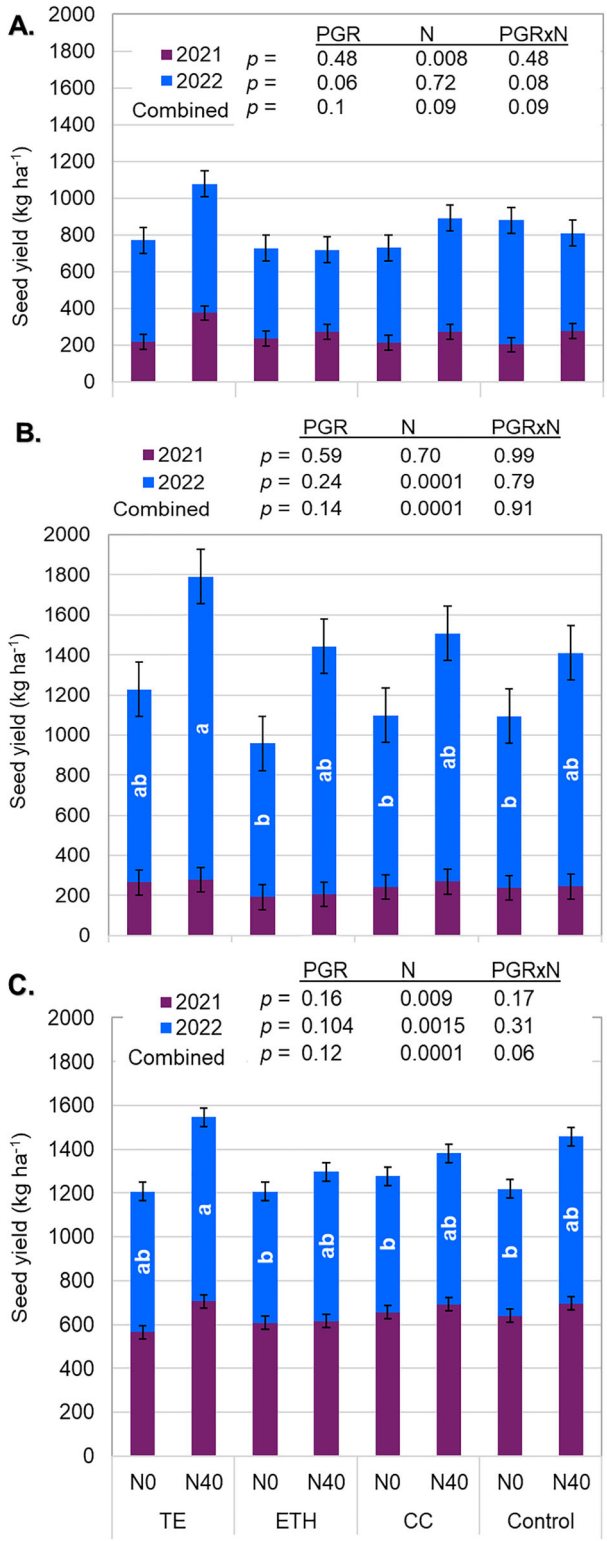


FIGURE 3 Effect of plant growth regulators (PGRs) and supplemental spring nitrogen (N) on seed yield of creeping red fescue (A), meadow brome (B), and timothy (C) on the same perennial seed crop stands in 2021 and 2022. Bars in the graphs represent yearly means across four replicates. The error bars indicate standard error of the mean. Means with different letters denote significant differences at $p \leq 0.05$. CC, chlormequat chloride; ETH, ethephon; TE, trinexapac ethyl; N0, no spring nitrogen top-dressing; N40, nitrogen top-dressed at 40 kg ha^{-1}.

(Continues)

of creeping red fescue, meadow brome, and timothy exhibited differential correlation patterns among plant morphological traits, lodging, and seed yield attributes (Table S2). Plant height was strongly correlated with lodging in meadow brome ($r = 0.86$, $p < 0.001$), and moderately correlated in timothy ($r = 0.59$, $p < 0.001$) and weakly correlated in creeping red fescue ($r = 0.35$, $p = 0.005$). Similarly, plant height showed a significant positive correlation with seed yield in creeping red fescue ($r = 0.57$, $p < 0.001$) and meadow brome ($r = 0.44$, $p < 0.001$), but no correlation was observed in timothy ($r = 0.09$, $p = 0.41$). Lodging was weakly correlated with seed yield in meadow brome ($r = 0.38$, $p = 0.002$), while no significant correlations were found in creeping red fescue ($r = 0.21$, $p = 0.10$) and timothy ($r = -0.07$, $p = 0.59$). Except for meadow brome ($r = 0.55$, $p = 0.002$), culm diameter did not show significant correlations with seed yield. Seed density (number of seeds per unit area) exhibited strong to perfect correlations with seed yields in creeping red fescue ($r = 0.94$, $p < 0.001$), meadow brome grass ($r = 1.0$, $p < 0.001$), and timothy ($r = 0.95$ $p < 0.001$).

4 | DISCUSSION

4.1 | Relationship among morphological traits, lodging, and seed yield components

Previous studies have identified a close relationship between grass seed yield and various aspects of plant growth habit (Sartie et al., 2018), including plant height, growth rate, leaf width, time to heading (Iwanska et al., 2019), and plant biomass (Pasquale, 1998). These findings suggest that both plant morphology, phenology, and components of biomass can significantly influence seed yield in grasses. Vigorous growth leads to a tall stem, which bears larger heads with a higher number of seeds per head and consequently results in higher yields. However, lodging, as discussed by (Baker et al., 2014), can disrupt these relationships by reducing light interception and photosynthesis in shaded plants, thereby limiting the availability of photo-assimilates necessary for pollination and floret fertilization, ultimately reducing seed yield (Bitarafan et al., 2019; Boelt, 1999; Chastain et al., 2014; Clemence & Hebblethwaite, 1984; Griffith, 2000). Moreover, lodging also makes harvesting difficult and causes further loss in seed recovery due to shattering (Macháč, 2013).

The underlying hypothesis of this study is that spring application of PGRs, regardless of supplemental N levels, reduces

lodging through the development of shorter and thicker stems, thereby improving seed set and enhancing seed yield in aging grass stands. Therefore, this study investigated if PGRs and supplemental N can impact lodging and thereby yield responses through the alteration of key morphological variables as antecedent factors for lodging in the aging stands of creeping red fescue, meadow brome, and timothy. These variables, selected based on previous PGR-related studies (Chastain et al., 2014, 2015; Szczepanek et al., 2021; Young et al., 2007; Zapiola et al., 2014), include internode lengths contributing to overall plant height, culm diameter as a proxy for stem strength and lodging tolerance, total plant biomass, and seed yield components such as seed density (number of seeds per unit area) and 1000-seed weight.

In this study across two contrasting seasons, the third- and fourth-year stands of creeping red fescue, meadow brome, and timothy revealed distinct relationships among morphological traits, lodging, and seed yield. The subsequent subsections discuss the seed yield responses and underlying mechanisms of effects of PGRs, supplemental N, and their interactions.

4.2 | Influence of PGRs and N fertilization on seed yield across species

None of the PGRs affected seed weight, seed density, or seed yield in any of the grasses tested. Additionally, supplemental spring N increased seed yields, especially in 2022 when a typical seasonal precipitation pattern, similar to the long-term average, prevailed. However, no interactions were observed between N treatment and PGRs. This contrasts earlier studies showing that TE was effective in improving seed yield in creeping red fescue (Bitarafan et al., 2019; Zapiola et al., 2014), perennial ryegrass (Rolston et al., 2010), and tall fescue (Chastain et al., 2015; Rolston et al., 2004) grown under higher fertility and precipitation regimes.

Use of TE is known to improve seed yield of grasses where lodging risk was high due to luxurious growth conditions (Chastain et al., 2015; Trethewey et al., 2016). However, in our study with 40 kg ha^{-1} of supplemental N, PGRs did not influence seed yield or related traits, as lodging risk was very low with restricted growth during the drought year of 2021 and remained moderate after seed development in 2022. Frahm et al. (2018) also reported that TE had no effect on improving seed yield of intermediate wheatgrass unless lodging severity was high. Similarly, no advantage of CC was reported in creeping red fescue (Bhattarai et al., 2024) and meadow brome (Yoder et al., 2019) seed crops. In contrast, ETH inhibited head formation in zoysiagrass (*Zoysia japonica* Steud.), but its effect on temperate grass seed production remains understudied. Wenhua et al. (2008) reported that both CC and ETH decreased seed yield of alfalfa in the first production year and had no impact on yield in the following year.

We did not detect PGR-induced changes to any specific yield attributive traits in our study. This finding was supported by earlier studies showing no effect of TE on the number of florets in perennial ryegrass (Rolston et al., 2010), together with the seed weight in tall fescue (Chastain et al., 2015) and perennial ryegrass (Borm & van den Berg, 2008). However, Chastain et al. (2015) reported that the application of TE improved seed density, contributing to higher seed yield of perennial ryegrass and tall fescue.

While high N fertilization is known to exacerbate lodging in grasses (Jungers et al., 2017; Koeritz et al., 2015), the addition of supplemental spring N to forage grasses did not increase lodging despite increasing grass biomass and seed yield in meadow brome and timothy. Spring N fertilization enhances biomass, promoting solar energy capture and photosynthate allocation to seeds (Chastain et al., 2014). Young et al. (1997) reported differential responses of perennial ryegrass cultivars to N rates. However, in this study, no benefit of split application was observed. A heatwave coupled with a long dry spell during the growing season of 2021 created stressful conditions that likely masked the treatment effects of both PGRs and supplemental N.

4.3 | Morphological drivers of biomass and lodging

Among all PGRs, TE consistently reduced plant height across all three grasses. Shorter plant heights of meadow brome and timothy were linked to reduced lengths of the three uppermost internodes. In contrast, CC and ETH showed less consistent effects on internode lengths and plant heights. These results align with TE-induced reductions in height and internode lengths in creeping red fescue (Szczepanek et al., 2021), tall fescue (Chastain et al., 2014, 2015), perennial ryegrass (Chastain et al., 2014), and intermediate wheatgrass (Frahm et al., 2018). Although internode length was not reduced in creeping red fescue, the modest height reduction suggests that the effects of TE are subtle and distributed throughout plant's vertical profile.

Reduced plant height and internode length were expected to lower biomass. However, only meadow brome showed reduced biomass, particularly with TE. Biomass reductions corresponded with height reductions for each PGR (TE > CC > ETH > Control). Earlier studies have found that TE did not affect biomass in tall fescue (Chastain et al., 2014), intermediate wheatgrass (Frahm et al., 2018), and perennial ryegrass (Borm & van den Berg, 2008; Chynoweth et al., 2014). In this study, treatment with TE increased culm diameter in creeping red fescue and timothy, potentially offsetting biomass loss from reduced height, a result also reported for intermediate wheatgrass (Frahm et al., 2018). Meadow brome showed no change in culm diameter in

response to any PGR, which likely contributed to its biomass decline.

Lodging severity was reduced by the PGRs, especially in meadow brome and timothy, with TE generally most effective, followed by CC. ETH did not reduce lodging in any grass. Reduced lodging from TE and CC likely reflects the morphological changes by those PGRs. Height reduction and lodging control in meadow brome and timothy support findings that tiller height is key to lodging resistance (Shah et al., 2017). TE reduced stem length by 28% and controlled lodging in perennial ryegrass (Chastain et al., 2014). In this study, thicker culms from TE may have further reduced lodging in timothy and creeping red fescue. Prior studies show TE and CC shorten and thicken internodes, enhancing lodging resistance in creeping red fescue (Szczepanek et al., 2021), common bent (*Agrostis capillaris* L. syn. *A. tenuis* Sibth.) (Aamlid et al., 2007), intermediate wheatgrass (Frahm et al., 2018), and wheat (Y. Zhang, Su, et al., 2017). However, CC showed inconsistent lodging reduction, and ETH was ineffective in reducing height, internode length, and lodging in the third- and fourth-year grasses, consistent with findings in common bent (Aamlid et al., 2007) and creeping red fescue (Szczepanek et al., 2021). A combination of CC and TE was more effective in reducing lodging and improving seed yield in meadow brome (Yoder et al., 2019), creeping red fescue (Bhattarai et al., 2024), chewing fescue (Szczepanek et al., 2021), and orchard grass (Rolston et al., 2014; R. Zhang et al., 2024). This synergistic effect may result from the inhibition of two distinct steps in the gibberellin biosynthetic pathway (Hedden et al., 2001; Rademacher, 2015), warranting further studies on varying TE and CC application rates across diverse conditions and species.

4.4 | Interannual variation and environmental modulation

The application of TE and CC markedly altered plant morphology, particularly during the average precipitation year of 2022. Lodging reductions were more evident in 2022 under normal (similar to long-term monthly average) rainfall, while drought in 2021 clearly limited PGR benefits, highlighting their greater utility under favorable growing conditions. These results align with the previous findings that growth regulators can differentially modulate crop morphology and yield in response to varying environmental conditions (W. Zhang, Yu, et al., 2017). Under those conditions, added spring N did not increase internode length, plant height, and lodging risk in third- and fourth-year perennial grass stands. A study on Mombasa grass (*Megathyrsus maximus*) elucidated that drought alone as well as the combination of drought and heat stress reduces plant N uptake and N use efficiency (Olivera Viciedo et al., 2021).

Seed yield responses varied markedly between the 2 years of study, driven by contrasting weather conditions. In 2021, an extended dry spell and intense heatwave during the reproductive phase led to pollen desiccation, accelerated senescence, and reduced seed yields, particularly in meadow brome and creeping red fescue. While focused studies investigating yield-reducing effects of stresses on these grasses are lacking, co-occurrence of drought and heat stress has been shown to reduce leaf photochemical efficiency and root dry weight (Jiang & Huang, 2000), thereby creating source-limited conditions that constrain the resource supply to growth and reproductive sinks (White et al., 2016). Timothy, however, maintained consistent seed yield across both years, likely due to staggered reproductive tiller development and greater tolerance to heat stress.

In 2022, cooler temperatures and more evenly distributed precipitation supported better plant growth and higher seed yields, despite more pronounced lodging, especially in ETH and untreated plots. Under the more favorable growing conditions of 2022, supplemental spring N was more effective in increasing seed density and seed yield in meadow brome and timothy. Growing body of literature shows that PGR success varies with environmental conditions, especially under high N and favorable soil moisture, which make the crops susceptible to lodging (Podolska, 2011; Szczepanek et al., 2021). These findings underscore the importance of environmental context in modulating efficacy of agronomic interventions such as PGRs and supplemental N applications.

4.5 | Implications and future directions

Although no direct effects of PGRs on seed yield were detected, the reduction in lodging severity, particularly with TE, was associated with improved seed yield in meadow brome during 2022. This suggests that under favorable growing conditions, PGRs can enhance seed yield indirectly by improving plant architecture and reducing mechanical stress, which corroborates previous findings reviewed by Podolska (2011) and (Wu et al., 2022). Since crop lodging interferes harvesting, and decreases seed recovery, and seed quality (Rehman et al., 2024; Vera et al., 2011), reduced lodging may confer additional benefits beyond yield, such as improved harvestability. Although this aspect was not examined in the present study, it warrants further investigation.

The lack of interaction between PGRs and N fertilization across species and years highlights the complexity of plant responses of combined agronomic treatments. Genetic variation, environmental stressors, and physiological plasticity likely contribute to these outcomes (Nunes-Nesi et al., 2016). Future research should explore the synergistic effects of PGR combinations (e.g., TE + CC), varying N rates, and broader

climatic conditions to optimize seed yield and crop resilience in perennial forage grasses.

5 | CONCLUSION

The application of the PGRs TE, CC, and ETH exhibited differential modulating effects on the seed crop stands of creeping red fescue, meadow brome, and timothy in their third and fourth growing seasons. TE had a more prominent and consistent effect on shortening internode length and overall plant height while reducing lodging severity. Although CC and ETH also altered grass morphology, their effects were less consistent and did not reduce lodging to the same extent. The effects of PGRs were more pronounced in meadow brome and timothy than in creeping red fescue. Despite their effectiveness in reducing lodging, PGRs did not increase grass seed yield attributes such as 1000-kernel weights and seed density, resulting in no significant changes in overall seed yield. Growth-retarding PGRs provided little to no benefit during a drier and hotter growing season but improved lodging tolerance under more favorable moisture conditions. While supplemental spring N application did not induce lodging, it increased biomass and seed yield in timothy and meadow brome, as well as seed density across all grass crops. The study findings indicate that the effectiveness of PGRs can vary depending on weather conditions, soil fertility, grass species, and stand age. Therefore, PGR application should be considered as a strategic, rather than routine, agronomic practice in rain-fed cropping systems. Future studies should make use of seasonal weather forecasts to predict soil moisture and evapotranspiration patterns and identify plant physiological thresholds that increase lodging risks—factors that may warrant the use of PGRs to safeguard seed yield. Investigating the efficacy of different PGR combinations on seed yield, quality, growth synchrony, and crop harvestability also presents promising avenues for future studies.

AUTHOR CONTRIBUTIONS

Bishnu Pandey: Data curation; formal analysis; investigation; methodology; software; visualization; writing—original draft. **Edward W. Bork**: Supervision; validation; visualization; writing—review and editing. **Malinda S. Thilakarathna**: Validation; visualization; writing—review and editing. **Nityananda Khanal**: Conceptualization; formal analysis; funding acquisition; methodology; project administration; resources; supervision; validation; visualization; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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