Reduced stem length increases perennial ryegrass seed yield

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Abstract

The effect of plant growth regulators inducing severe stem shortening on the seed production of perennial ryegrass (*Lolium perenne*) was investigated over two seasons in Canterbury using the tetraploid, late season cultivars 'Halo' (2012-13) and 'Bealey' (2013-14). Stem shortening was achieved through the application of Moddus® (active ingredient 250 g/l trinexapac-ethyl, TE) either alone or in combination with Payback™ (active ingredient 250 g/l paclobutrazol, PB) and 'Cycocel®' (active ingredient 750 g/l chlormequat-chloride, CCC). Trinexapac ethyl applied as a single application at Zadoks growth stage 32 increased seed yield by up to 44% as rates increased from the untreated (1720 kg/ha) to 3.2 l/ha (2470 kg/ha). Where TE was applied in sequences (Zadoks growth stages 30, 32 and 39) seed yield was increased by 59% to 2730 kg/ha. Combinations of TE, PB and CCC increased seed yields by up to 95% (3360 kg/ha) above the untreated control. Seed yield increase was achieved through an increased number of seeds/m^2. Total stem length was reduced from 105 cm to 85 cm by applications of TE alone and further to 65 cm where applications of TE, PB and CCC were applied in combination. On average, each centimetre of stem length reduction increased seed yield by 45 kg/ha. Stem length reduction was associated with delayed onset of lodging and absolute lodging at harvest, where only crops shorter than 71 cm remained standing at harvest. These results suggest that growers should aim to shorten ryegrass stem lengths to approximately 70 cm to reduce lodging and maximise seed yields.

Additional keywords: chlormequat chloride, *Lolium perenne*, paclobutrazole, plant growth regulator, trinexapac-ethyl

Introduction

Plant growth regulators (PGRs) have been the focus of investigations in perennial ryegrass seed crops for many years (Chastain *et al.*, 2014; Hampton *et al.*, 1987; Hebblethwaite, 1980). Prior to 1990 work focused on the use of paclobutrazol (PB) which showed increases in seed yield of between 8 and 136% (Wiltshire *et al.*, 1987). However, the soil residual properties of PB subsequently resulted in possible yield reductions in some following crops and the product was not registered for field crops in New Zealand. Chlormequat chloride (CCC) was used commercially in ryegrass seed crops producing seed yield increases of approximately 10% (Rolston *et al.*, 2004). In the early 2000s trinexapac ethyl (TE) was introduced to the New Zealand market resulting in seed yield responses of up to 70% above untreated controls and rapid grower uptake

(Chynoweth *et al.*, 2010; Rolston *et al.*, 2004; Rolston *et al.*, 2010). Paclobutrazol, CCC and TE are plant growth retardants inhibiting gibberellins (gibberellic acid, GA) biosynthesis. Chlormequat chloride acts in the early steps of GA biosynthesis. Paclobutrazol acts one step later in the pathway while TE acts late in the pathway to inhibit the formation of highly active GA's (Rademacher, 2000). Since stem extension is a response to increased endogenous gibberellins, the use of PGRs can reduce stem length and delay or reduce lodging (Rademacher, 2000).

Following the positive seed yield response of ryegrass seed crops to PB and TE in earlier work, and the positive reported responses of CCC at a lower response level in more recent work, two field experiments were set up to investigate potential additive effects on seed yield to combinations of these compounds which act at three separate locations in the GA pathway.

Materials and Methods

Experiment 1

In 2012-13 a preliminary study was undertaken in a commercial paddock of perennial ryegrass, cultivar 'Halo', a tetraploid late flowering type (head emergence, 'Grasslands Nui' +28 days) near Wakanui (43° 59' 08" S; 171° 47' 17" E). The soil type was a Wakanui silt loam (Cox, 1978) and the paddock was irrigated. The crop was sown on 28 April, 2012 at 7 kg/ha in 30 cm rows. All inputs and crop management except PGRs were undertaken by the grower. In summary the inputs were: spring Nitrogen 135 kg N/ha, three fungicide applications and irrigation at 175 mm water were applied.

Treatments were applied in a randomised complete block design, with four replicates. Plots were 9.6 m long and 3.2 m wide. The final defoliation was achieved through the use of a tractor mounted finishing mower on 26 October. The PGR treatments included TE (applied as Moddus® 250 g/l); CCC as Cycocel® (750 g/l) and PB as Payback™ (250 g/l). Treatments were applied over the Zadoks growth stage 30 - 39 (Zadoks *et al.*, 1974) but did not include an untreated control (Table 1). No fungicide was applied with the PGR treatments.

Pre-harvest quadrats were cut from each plot to assess crop mass, seed head number and reproductive stem length. Lodging scores were collected weekly from 23 December to 11 January. At harvest the plots were windrowed on 2 February, 2013 at approximately 40% seed moisture content (SMC) and combine harvested on 10 February with a 'Sampo' plot combine at 11% SMC. The field dressed weight was measured in the field and a sub sample taken for seed cleaning to a 1st Generation Seed Certification Standard (AsureQuality, 2008).

Experiment 2

The experiment was undertaken on the tetraploid perennial ryegrass cv. 'Bealey' (head emergence, 'Grasslands Nui' + 25 days) at Wakanui. (43° 58' 49.98"S; 171° 48' 44.62"E) on a Wakanui silt loam soil type (Cox, 1978). The crop was sown on 6 May, 2013 at 18 kg/ha at 15 cm row spacing. All inputs and crop management except for PGRs were undertaken by the grower. Treatments were replicated four times in a randomised complete block design where plots were 10 m x 3.3 m. There was no defoliation. The experiment compared different rates of TE (applied as 'Moddus®', (active ingredient 250 g/l trinexapac-ethyl, TE) ranging from nil to 3.2 l/ha and PB (applied as 'Payback™', active ingredient 250 g/l paclobutrazol, PB) and CCC (applied as 'Cycocel®', active ingredient 750 g/l chlormequat-chloride) combined with TE. Base crop management applied across the whole trial consisted of spring nitrogen fertiliser at 140 kg N/ha, three fungicide applications (Timing 1, 400 ml/ha Proline® (active ingredient 250 m/l prothioconazole) and 400 ml/ha Seguris Flexi® (active ingredient 125 g/l isopyrazam), timing 2, 400 ml/ha Proline® and 500 ml/ha Amistar® (250 g/l azoxystrobin), timing 3, 500 ml/ha Protek™ 500 g/l carbendazim) and 200 mm water applied as irrigation.

Dry matter assessments were made at Zadoks growth stage 32 and 39 and two days prior to windrowing by cutting 3 rows by 50 cm in length and oven drying to a constant weight. Lodging was visually scored each 7-10 days from early head emergence until cutting on a 0 (no lodging) to 100% (fully lodged) linear scale while absolute lodging refers to lodging at harvest. Vegetative tillering was visually assessed at windrowing on a 0 (no vegetative tillers) to 10 (all seed producing tillers have daughter tillers) linear scale. Stem length, the number of heads/m^2, the number of spikelets/head and florets/spikelet were assessed on 10 heads per plot. Thousand seed weight (TSW) was assessed by counting and weighing 200 seeds from each plot from the machine dressed sample.

At harvest, a 1.7 m strip from each plot was cut using a modified plot windrower at approximately 40% seed moisture content on 2 February, 2014. The swath was combined harvested using a 'Sampo' plot harvester on 17 February, 2014 with seed retained for machine dressing. Seed was machine dressed to a 1st Generation Seed Certification standard using a screen separator. Seed yields are reported at 11% seed moisture content.

Statistical analysis was completed with GenStat (version 14, VSN International Ltd, UK) using a general ANOVA model. Average growth rates and days to 50% lodging were determined though regression analysis. Significant differences were separated using least significant difference (LSD) tests ($\alpha = 0.05$).

Results

Experiment 1

Seed yield

Increasing TE rate at Zadoks GS 32 did not increase seed yield compared with a single application of 1.6 l/ha TE. However, splitting 2.4 l/ha of TE between either three or four applications increased seed yield above the single application of 2.4 l/ha by 315 kg/ha (treatments 3 and 5, Table 1). The addition of CCC showed no benefit compared with TE alone. The addition of PB and CCC at early growth stages produced a 1030 kg/ha seed yield increase compared with 2.4 l/ha of TE alone.

The number of seed/m^2 (R^2=0.99) and harvest index (R^2=0.90) were highly correlated to increased seed yield. There was an indication that reduced reproductive stem length was associated (R^2=0.70) with increased seed yield. There was no difference in dry matter yield at harvest or the number of seed heads/m^2 between any of the treatments.

Lodging

Treatments 1-5 reached 50% lodged between 14 and 21 days following peak anthesis while the TE, CCC and PB treatment did not lodge prior to windrowing.

Table 1: Perennial ryegrass seed yield, cv 'Halo' following treatment with six plant growth regulator treatments when grown at Wakanui, Mid-Canterbury in the 2012-13 growing season.

Trt	Product[1] and application rate (l/ha)				Seed yield (kg/ha)
	Timing 1 (31 October)	Timing 2 (9 November)	Timing 3 GS 32 (19 November)	Timing 4 (30 November)	
1	-	-	1.6 TE	-	2005 a
2	-	-	2.4 TE	-	1950 a
3	0.4 TE	0.4 TE	1.6 TE		2330 b
4	0.4 TE + 1.0 CCC	0.4 + 1.0 CCC	0.4 TE + 1.0 CCC	0.4 TE + 1.0 CCC	2060 a
5	0.4 TE	0.4 TE	1.2 TE	0.4 TE	2305 b
6	0.4 TE + 1.0 CCC + 2.0 PB	0.4 TE +1.0 CCC+ 2.0 PB	1.6	-	2980 c
				Mean	2270
				P value	<0.001
				LSD	190

[1] Note: TE = Moddus (250 g/l Trinexapac ethyl), CCC = Cycocel (750 g/l Chlormequat-chloride) and PB = Payback (250 g/l Paclobutrazol)

Experiment 2

Seed yield

Seed yield was increased ($P<0.001$) by all PGR treatments compared with the control which produced 1720 kg/ha (Table 2). Increasing TE rates at GS32 increased seed yield to approximately 2.4 l/ha. The two TE, CCC + PB combination treatments increased seed yield by 91% to 1580 kg/ha over the untreated control.

Lodging

The level of absolute lodging was affected ($P<0.001$) by treatments that contained PB + CCC (Figure 1). All treatment containing TE were greater than 95% lodged at harvest. The double application of TE, CCC + PB was 56% lodged at harvest compared with 38% for the earlier single application of TE, CCC + PB ($LSD_{0.05} = 15.8$). Many treatments had begun lodging prior to anthesis where the untreated and 1.6 l/ha TE treatments were 50% lodged, or greater, at peak anthesis. Treatments containing TE, CCC + PB approached 50% lodging at windrowing, some 50 days later than the untreated crop (Table 3).

The onset and rate of lodging was affected by the PGR programme where TE delayed the onset of lodging by between 9 and 16 days following which the rate of lodging was constant at a 3.2% increase per day for all TE treatments. The addition of CCC + PB to TE delayed the onset of lodging by 22 days compared with the control and reduced the rate of lodging to 1.2% per day.

Stem length and seeds/m^2

Stem length was reduced ($P<0.001$) by all PGR treatments from 105 cm in the untreated control to 85 cm with TE treatments and further reduced to an average of 67 cm when TE, CCC and PB were combined. The shortest stems produced the highest seed yields with an average increase of 45 kg seed/cm of stem shortening (Figure 2) and the greatest number of harvested seeds/m^2 (Table 4). The number of seeds/m^2 was the main yield component contributing to the final seed yield ($R^2 = 0.99$). Dry matter accumulation was similar for all treatments at approximately 150 kg DM/ha/d. At harvest there was no difference in above ground dry matter yield (Table 3).

Harvest data

Plant growth regulator treatments affected the number of seeds/spikelet ($P<0.001$) and harvest index ($P<0.01$) but did not affect the other harvest components (Table 4). The number of seeds per spikelet increased by 95% from 1.37 in the untreated plots to 2.58 in those treatments containing PB while the harvest index increased by up to 75% as stems were shortened.

Table 2: Perennial ryegrass seed yield, cv 'Bealey' following application of seven plant growth regulator treatments at four possible timings when grown at Wakanui, Mid-Canterbury in the 2013-14 growing season.

Trt	Product[1] and application date				Seed yield (kg/ha)
	Timing 1 (30 October 2013)	Timing 2 (4 November 2013)	Timing 3 GS32 (13 November)	Timing 4 (27 November)	
1	-	-	-	-	1720 a
2	-	-	1.6 TE	-	2100 b
3	-	-	2.4 TE	-	2335 bc
4	-	-	3.2 TE	-	2470 cd
5	-	0.8 TE	1.6 TE	0.8 TE	2730 d
6	-	0.4 TE + 2.0 CCC + 1.0 PB	1.6 TE + 2.0 CCC + 1.0 PB	1.2 TE	3360 e
7	1.0 TE + 2.0 CCC + 2.0 PB	-	2.4 TE	-	3250 e
				Mean	2567
				P-value	<0.001
				$LSD_{0.05}$	283.2

[1] Note: TE = Moddus (250 g/l Trinexapac ethyl), CCC = Cycocel (750 g/l Chlormequat-chloride) and PB = Payback (250 g/l Paclobutrazol).

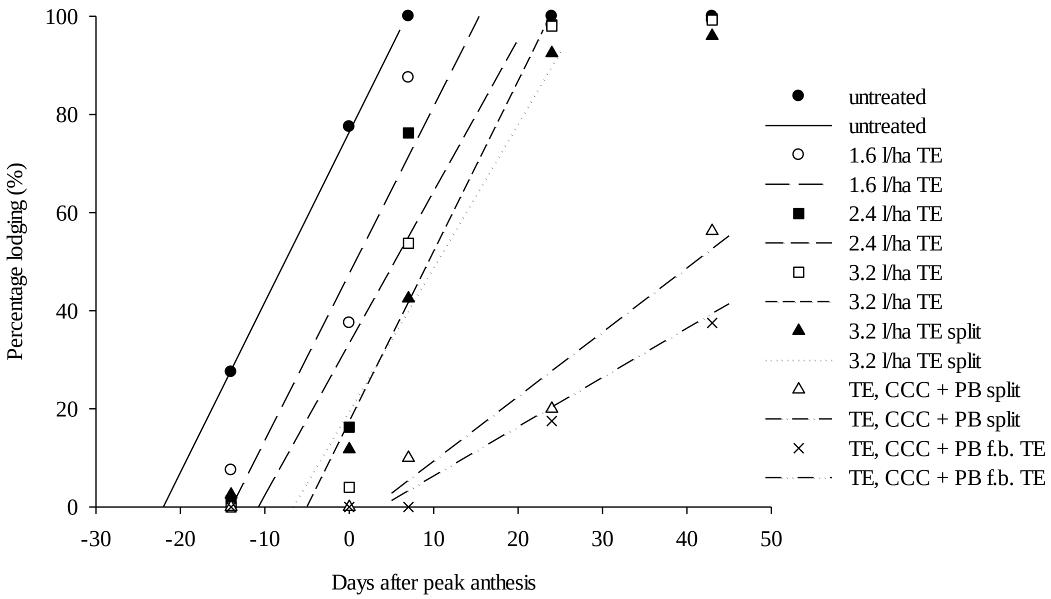

Figure 1: Lodging pattern of perennial ryegrass cv ‘Bealey’ following seven plant growth regulator treatments when grown at Wakanui, Mid-Canterbury in the 2013-14 growing season. Peak anthesis was 16 December 2013. Note: TE only treatments were applied at growth stage (GS) 32; TE was split applied at GS 31, 32 and 39; TE, CCC + PB split were applied at GS31 and 32 respectively; and TE only at GS 39: TE, CCC + PB followed by (f.b.) TE was applied at GS30 f.b. TE only at GS 32. TE = Trinexapac ethyl, CCC = Chlormequat-chloride and PB = Paclobutrazol. Note 2: 0% = fully upright, 100% = horizontal.

Table 3: Parameters of growth for perennial ryegrass, cv ‘Bealey’ following treatment with seven plant growth regulators when grown at Wakanui, Mid-Canterbury in the 2013/14 season.

Treatment[1]	Stem length (cm)	Dry matter[2] (t/ha)	Harvest index (%)	Days to 50% lodging[3]
Untreated	105	15.9	10.9	-8.3
1.6 l/ha TE	85.8	15.4	14.0	0.9
2.4 l/ha TE	85.5	18.0	13.0	5.2
3.2 l/ha TE	85.7	15.3	16.6	9.4
3.2 l/ha TE split	82.0	16.9	16.4	10.4
TE, CCC + PB split	64.4	18.8	18.1	45[4]
TE, CCC + PB f.b. TE	71.0	17.5	19.1	45[4]
P-value	<0.001	0.268	0.006	<0.001
$LSD_{0.05}$	12.1	NS	4.2	14.5

[1] TE only treatments applied at growth stage (GS) 32, TE split applied GS 31, 32 and 39, TE, CCC + PB split applied GS31, 32 and TE only at 39, TE, CCC + PB followed by (f.b.) TE applied GS30 f.b. TE only at 32. TE = Trinexapac ethyl, CCC = Chlormequat-chloride and PB = Paclobutrazol. [2] Note: includes seed weight. [3] Note: relative to peak anthesis. [4] Note: crops less than 50% lodged at windrowing.

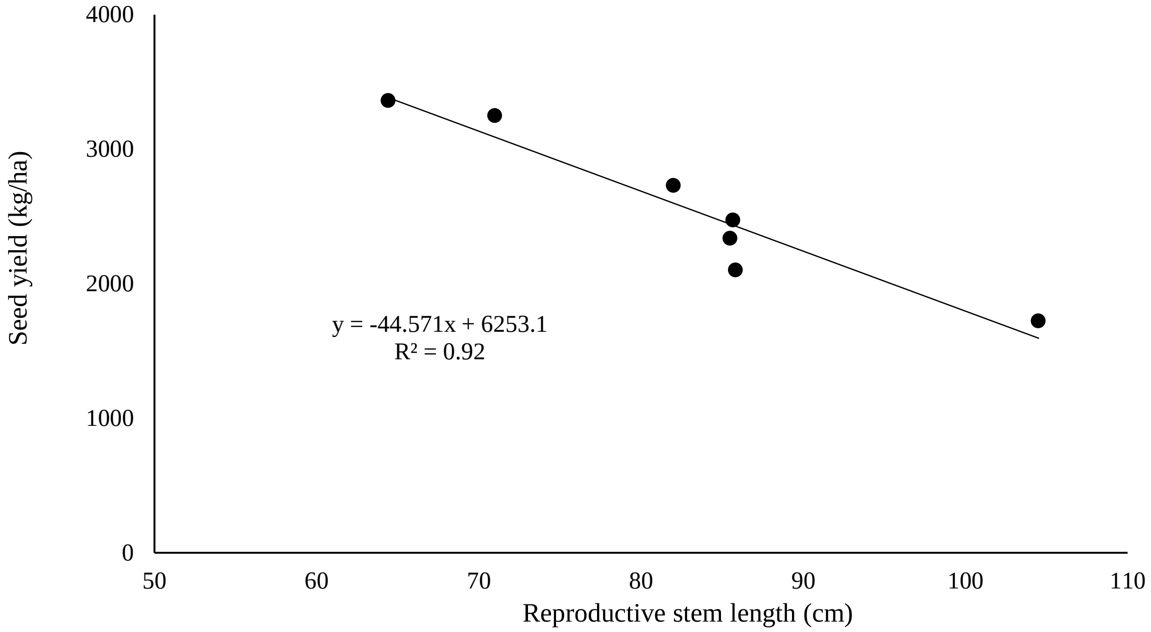

Figure 2: Influence of stem length on seed yield of perennial ryegrass cv 'Bealey' following treatment with seven plant growth regulators when grown at Wakanui, Mid-Canterbury in the 2013-14 season.

Table 4: Components of seed yield in Perennial ryegrass, cv 'Bealey' following treatment with seven plant growth regulators when grown at Wakanui in the 2013-14 season.

Treatment[1]	Heads/m^2	Spikelets/ head	TSW (g)	Harvested seeds/spikelet	Seeds /m^2 (1000)
Untreated	1670	20.5	3.936	1.37	44.4
1.6 l/ha TE	1580	21.8	4.15	1.07	50.6
2.4 l/ha TE	1950	22.0	4.042	1.55	58.1
3.2 l/ha TE	1630	22.2	4.244	1.69	58.3
3.2 l/ha TE split	1820	22.5	4.144	1.64	66.1
TE, CCC + PB split	2000	21.5	3.939	2.25	93.9
TE, CCC + PB f.b. TE	1955	21.4	3.785	2.58	85.9
P-value	0.471	0.411	0.166	<0.001	<0.001
$LSD_{0.05}$	NS	NS	NS	0.58	13.67

1 TE only treatments applied at growth stage (GS) 32, TE split applied GS 31, 32 and 39, TE, CCC + PB split applied GS31, 32 and TE only at 39, TE, CCC + PB followed by (f.b.) TE applied GS30 f.b. TE only at 32. TE = Trinexapac ethyl, CCC = Chlormequat-chloride and PB = Paclobutrazol

Discussion

Seed yield was increased in the respective experiments by 49% and 60% above the lowest PGR input of TE 1.6 l/ha (Table 1 and Table 2). In Experiment 2, the best PGR treatment increased seed yield by up to 95% above the untreated control (Table 2). The number of seed heads/m^2 and the number of spikelets per head were similar for all treatments resulting in a similar number of spikelets/m^2 in each crop. Therefore, differences in harvested seed yield arose either from changes in individual seed weight or the number of seeds/spikelet increasing the number of seeds/m^2. As there was no treatment effect on TSW (Table 4) the increase in the number of seeds produced per spikelet was responsible for the increase in seed yield. In Experiment 1, 1.3 seeds/spikelet were produced at the lowest PGR rate increasing to 2.2 in the highest yielding treatment (Treatment 6), while in Experiment 2, 1.4 seeds/spikelet were produced without PGR input compared greater than 2.3 seeds/spikelet for treatments containing PB, CCC + TE. An increase in the number of seeds/spikelet reaching a saleable weight after application of PGRs was also shown by Hampton and Hebblethwaite (1985), Rolston, *et al.* (2010) and Chastain, *et al.* (2014).

The application of TE at various rates reduced stem length by approximately 20 cm while the combinations of TE, CCC + PB showed a 40 cm reduction compared with the control. The TE reduction in stem length is consistent with data presented by Borm and van den Berg (2008) and Silberstein *et al.* (2003). No comparison was found in the literature for the combined TE, CCC + PB treatments but reductions in stem length of approximately 40% are large. However Hampton and Hebblethwaite, (1985) demonstrated stem length reductions of approximately 50% from PB alone where untreated stems were approximatley half the length of those in Experiment 2, an indication of diffenent genetic material and environmental conditions during stem extension. Stem length was well correlated to seed yield (Figure 2). Stem length was associated with the onset of lodging and the lodging at harvest, similar to data presented for PB by Hampton and Hebblethwaite (1985) and TE by Chastain *et al.* (2014). Untreated plots were 80% lodged at anthesis potentially influencing pollination (Wright and Hebblethwaite, 1979), however all other treatments were at or less than 50% lodged at anthesis (Figure 1). Each days delay to 50% lodging added 25 kg seed/ha (data not presented), similar to the 24 kg/ha for each days delay presented by Rolston, *et al.* (2010) when combining data from eight experiments in Canterbury New Zealand. Lodging has been reported to reduce seed yield through reduced assimilate supply to developing seeds (Clemence and Hebblethwaite, 1984) presumably from reduced ability to capture and utilise incoming solar radiation.

The shorter stems, which lodged later (Figure 1), produced higher harvest index values (Table 3). Dry matter production and potential sink size were the same for all treatments suggesting the sink in treatments with shorter stems was stronger or more assimilate was available for seed fill though reduced stem competition or reduced shading of seed heads (Trethewey and Rolston, 2009). Hampton and Hebblethwaite (1985) presented seed yield increases from PB and reported that the source size was the same in treated and untreated crops but treated crops mobilised less reserve into vegetative tillers. In the current experiments, vegetative tillers assessed at harvest showed plots which lodged early produced more vegetative tillers compared with those which remained standing. Vegetative tillers will compete for resources, sunlight, nitrogen and water, thus reducing the source for seed filling. Vegetative tillering occurs when the red to far red ratio at the base of the crop canopy is favourable for tiller release (Casal *et al.*, 1985), as occurs under a lodged environment. Therefore reducing stem length and reducing the lodging is critical to achieving high seed yields.

Paclobutrazol is currently not registered for use on field crops in New Zealand as there are issues with soil residues and crop plant back intervals. Future research needs to evaluate alternative products with similar activity to PB or by refined management of TE and CCC with multiple split applications from Zadoks GS31 to reduce stem length and lodging.

Conclusions

The TE, CCC and PB combination improved seed yields above products currently used by seed producers (TE) through further reductions of stem length. Shortening of stems reduced lodging and the proliferation of daughter tillers without reducing DM production while concurrently increasing seed yield, resulting in increased harvest index. The use of TE, CCC and PB provides the potential to further reduce stem length as a method for improving seed yield in ryegrass crops.

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