Stem shortening plant growth regulators enhance seed yield of annual ryegrass

M.P. Rolston[1,2], R.J. Chynoweth[2], J.A.K.Trethewey[1,3], A.J. Hilditch[1], A.D. Heslop[1] and B.L. McCloy[4]
[1]AgResearch Limited, Springs Rd, Lincoln University 7674, New Zealand
[2]Foundation for Arable Research, 185 Kirk Rd, Templeton, Christchurch 7678, New Zealand
[3]Lincoln AgriTech Limited, Lincoln University, Engineering Drive, Lincoln 7674, New Zealand
[4]New Zealand Arable, 60 Ryans Rd, Christchurch 8042, New Zealand

Abstract

Annual ryegrass cultivar 'Hogan' was sown autumn 2015 and treated with combinations of three gibberellic acid inhibiting plant growth regulators to investigate stem shortening on seed yield. Seed yields of annual ryegrass were increased by up to 55% with three applications of trinexapac-ethyl (TE) each at 200 g TE/ha/application (total 600 g TE/ha). There was a linear seed yield response to increasing TE rates between 0 and 600 g/ha of 135 kg seed/100 g TE. A single application of 200 and 400 g TE at Zadoks Growth Stage 32 increased seed yield by 20 and 32% respectively. Seed yield was not further enhanced by adding either chlormequat or paclobutrazol to the TE application. Seed yield increases were associated with both reduced stem height and reduced lodging. However where cages were used to prevent lodging the data suggests that stem shortening was the more important component contributing to the seed yield response. Seed yields were increased 25.6 kg/ha for every 1 cm decrease in stem length. To optimise seed yield in annual ryegrass growers should apply a split application of TE starting five days after closing.

Additional keywords: chlormequat, paclobutrazol, trinexapac-ethyl, Moddus, *Lolium multiflorum*

Introduction

Trinexapac-ethyl (Moddus® and generic Trinexapac-ethyl (TE) products) are widely used in perennial ryegrass (*Lolium perenne* L.) seed crops to delay lodging and enhance seed yields (Rolston *et al.*, 2010; Chynoweth *et al.*, 2014). In perennial ryegrass seed yield response to TE has been attributed to delayed lodging (Rolston *et al.*, 2010; Chastain *et al.*, 2014) and the associated reduction in light interception in the upper canopy (Rolston *et al.*, 2007). In Italian ryegrass (*Lolium multiflorum* L.) seed yields are increased by 30 to 50% with 200-300 g TE/ha (Trethewey *et al.*, 2016). Recent trials in perennial ryegrass evaluated super-shortening and demonstrated that sequential applications of TE alone or in mixes with chlormequat (Cycocel®) and paclobutrazol (Payback™) caused stem shortening from 90 cm to 60 cm and was as important as delayed lodging in enhancing seed yield (Chynoweth *et al.*, 2014). The research in this paper evaluated the concept of super-shortening on reproductive tiller stem length and lodging on seed yield with multiple applications of plant growth regulators (PGR) on annual ryegrass.

Materials and Methods

The trial was undertaken at the AgResearch Lincoln Research Farm (43º 37' 56" S; 172º 28' 17" E, 13 m above sea level) with the annual tetraploid ryegrass *Lolium multiflorum* cv. 'Hogan'. The trial was sown on 16 April 2015 at 18 kg/ha in 15 cm rows. The soil type was a Wakanui silt loam and the trial was irrigated regularly, applying 25 mm at each application.

Three applications of urea were made: 50 kg N/ha on 17 September, and 70 kg N/ha on the 3 November (closing) and again on 4 December giving a total applied N of 190 kg N/ha. The trial was grazed with sheep and topped after grazing three times between 4 September and 5 October with the final defoliation occurring on the 2 November.

There were nine PGR treatments (Table 1), with four replicates in a randomised block design. Plot size was 3.2 m wide by 10 m long. Three treatments (# 1, 2 and 3) had duplicate plots with cages to support growth without lodging. Zadoks growth stages (GS)

Table 1: PGR treatment rates expressed in g a.i./ha applied to annual ryegrass cv. 'Hogan' at three Zadoks growth stage (GS) and seed yield when grown at Lincoln in the 2015/16 growing season.

Treatment #	GS31 12 Nov	GS32 25 Nov	GS33 4 Dec	TE equiv[1] (g/ha)	Seed yield (kg/ha)
1	0	0	0	0	2280
2	0	200TE	0	200	2740
3	0	400TE	0	400	3020
4	0	600TE	0	600	2770
5	0	800TE	0	800	2770
6	200TE	100TE	100TE	400	2730
7	200TE	200TE	200TE	600	3520
8	200TE+1500CCC	200TE+1500CCC	200TE	800	3250
9	200TE+250P+1500CCC	200TE+1500CCC	200TE	1050	2770
				Average	2870
				$LSD_{0.05}$	410
				P-value	<0.001

TE=Moddus® (trinexapac-ethyl 250 g/l); CCC=Cycocel (chlormequat chloride 750 g/l);
P=Payback (paclobutrazol 250 g/l); [1]750 g CCC = 50 g TE

were used to determine application dates of PGR treatments (Zadoks *et al.*, 1974). A fungicide mixture of epoxiconazole (Opus® 125 g active ingredient (a.i.)/l) at 1.0 l/ha + azoxystrobin (Amistar® 250 g a.i./l) at 0.5 l/ha was applied on 23 December for crown rust control. TE equivalents (g/ha) were calculated by adding the active ingredient (a.i.) weight of TE+P and for CCC 750 g ai (1 l/ha) was equated to 50 g TE, to enable a comparison of rates between different PGR active ingredients and trial treatments.

Cages

At mid-stem elongation, four 'Y type' standards were erected to form a mini-plot of 1.5 x 1 m in each plot of three duplicate treatments: # 1, 2, and 3. Wire mesh was inserted at 50 cm above ground level to support the spikes during elongation and prevent lodging. Balage plastic was wrapped around the standards to prevent seed heads from breaking in the wind. The untreated control (#1) had a second wire mesh inserted at 70 cm to support the long stems. At harvest the caged mini-plot was hand cut and a corresponding quadrat of 1 m^2 was also cut from the plot outside the cage to determine if stem shortening in the absence of lodging was critical.

Assessments

On an adjacent trial herbage mass accumulation of a seed crop of *L. multiflorum* was recorded over a nine week period. Reproductive stem length was measured on 15 December. Lodging percentage was visually assessed (0 = nil lodging; 100 = crop horizontal to the ground) starting at anthesis on 15 December with weekly scoring until 20 January and the days from anthesis to 50% lodging (DL50) were calculated. Pre-harvest mass and head density were assessed from a $0.25m^2$ quadrat cut on 5 January. Spikelets were counted on 15 heads per plot.

Harvest

The plots were windrowed on 21 January with a plot windrower, cutting a 1.65 m wide swath by 10 m length of plot. Rain on the drying crop delayed harvest. Harvest occurred on 2 February 2016 using a 'Sampo' plot combine harvester. Seed moisture content (SMC) was assessed at harvest on a field dressed (FD) seed sample of 10 g per plot, dried at 130°C for 2 hours. The average SMC was 11.7% and there was no significant difference between treatments. From the FD seed sample a weighed subsample of 150 g was cleaned using a Dakota column separator to remove light seed and inert matter. Seed purity of each sample was assessed and seed yield adjusted to 98% purity and to 14% SMC. Thousand seed weight (TSW) was assessed on 100 seeds per sample weighed to 4-decimal places. Seed harvest loss was assessed by vacuuming a 0.5 x 0.3 m quadrat. The samples were air dried in paper bags before being sieved and blown on a Dakota column separator. The ratio of pure seed and soil/inert were assessed on a 2 g subsample to determine the loss of pure seed.

Data analysis used Genstat version 17 (VSN International Ltd, UK) for ANOVA statistical analysis.

Results

Seed yield and harvest loss

The trial average machine dressed yield was 2,870 kg/ha, despite having rain after windrowing and taking 12 days from cutting to combine harvest. Seed yield was increased ($P<0.05$) from 2,280 kg/ha in the untreated control to 3,250 and 3,520 kg/ha for split applications of 600 g TE/ha or 600 g TE/ha plus 1500 g/ha CCC respectively (a 43 and 55% increase over the untreated control) (Table 1). On average PGR treatments increased (P<0.05) seed yields by 690 kg/ha, a 30% increase over the untreated control. Adding chlormequat and/or paclobutrazol to the TE treatment (#8 and 9) did not enhance seed yields, compared with TE alone.

Seed yields from single application of TE gave a linear plateau response with a breakpoint of 277 g TE/ha (Figure 1). For all TE treatments between 0 to 600 g/ha the seed yield response can be described as a linear response with a 135 kg seed/100 g TE. At current prices this represents a benefit/cost ratio of 5:1.

Split PGR application

Both treatments #4 and #7 had 600 g TE/ha, with treatment #4 applied once and #7 as a triple split application (3 x 200 g TE). The split application increased (P<0.05) seed yield by 760 kg/ha from 2,770 to 3,520 kg/ha, an extra 27% more seed from the split application compared with the same rate applied as a single application. In contrast when the lower rate 400 g TE/ha was split (#6) the seed yield was not different from applying the amount once at GS32 (#3) (Table 1).

Harvest loss of saleable seed averaged 770 kg/ha representing 20.9% of the seed at cutting time (Table 2). Seed loss was high on the highest seed yield treatment (#7) with 1,060 kg/ha representing 23% loss. The average potential seed yield (seed harvested plus seed lost at harvest) was 3,640 kg/ha, with the potential yield of treatment #7 being 4,580 kg/ha (Table 2). It rained during crop drying with 27 mm of rain during the 26 and 27 January on the windrowed crop.

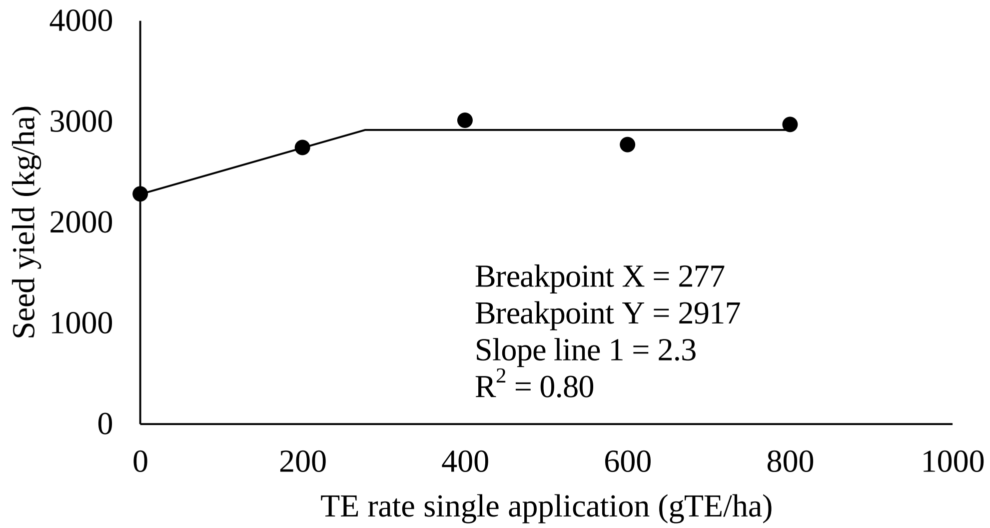

Figure 1: Seed yield response of annual ryegrass cv. 'Hogan' to single application treatments of trinexapac-ethyl applied at growth stage 32 at Lincoln in the 2015/16 growing season.

Table 2: Harvest loss, total seed yield (harvested seed+loss) and seed loss as a percent of the total for annual ryegrass cv. 'Hogan' when treated with nine plant growth regulator treatments in the 2015/16 season at Lincoln, Canterbury.

Trt #	GS31 12 Nov	GS32 25 Nov	GS33 4 Dec	Loss	Total (kg/ha)	%loss
1	0	0	0	850	3130	27.2
2	0	200TE	0	900	3640	24.7
3	0	400TE	0	740	3760	19.7
4	0	600TE	0	620	3390	18.3
5	0	800TE	0	630	3400	18.5
6	200TE	100TE	100TE	640	3370	19.0
7	200TE	200TE	200TE	1060	4580	23.1
8	200TE+1500CCC	200TE+1500CCC	200TE	550	3800	14.5
9	200TE+250P+1500CCC	200TE+1500CCC	200TE	770	3540	21.7
			Average	770	3640	20.9
			$LSD_{0.05}$	255	440	8.1
			P-value	0.005	<0.001	0.28

TE=Moddus® (trinexapac-ethyl 250 g/l); CCC=Cycocel (chlormequat chloride 750 g/l); P=Payback (paclobutrazol 250 g/l)

Crop height, lodging and regrowth

Increasing rates of PGR reduced ($P<0.05$) stem lengths and delayed ($P<0.05$) lodging (Table 3). For every 1 cm reduction in the length of the reproductive seed head stem, seed yield was increased by 25.6 kg/ha for stems in the range of 52 to 90 cm (Figure 2). The reduction in stem length with increasing PGR was 4.1 cm per 100 g TE. Increasing the PGR rate also increased days to 50% lodging from 11 days from anthesis to 38 days in the highest yielding seed treatment (Table 3). Not surprisingly there was a strong relationship between reduced stem length and delayed lodging. The development of lodging over time for increasing TE rates is shown in Figure 4.

Increasing PGR rate delayed days to 50% lodging, linear from 0 to 600 g TE by 5.6 days per 100 g TE (Figure 3). Treatments with shorter stems also had delayed lodging (Figure 4). For every days delay in the crop reaching 50% lodging a 24.8 kg/ha seed yield increase occurred.

Crop supported v no support

The seed yield data of the crop supported in the cages was lower than the lodged, unsupported crop outside the cage (Table 4). An interpretation of this result is that stem shortening is more important than lodging as an impact on seed yield.

Table 3: Influence of nine growth regulator treatments on reproductive stem length, days to 50% lodging, secondary heads and vegetative regrowth scores of annual ryegrass cv. 'Hogan' grown at Lincoln in the 2015/16 season.

Trt #	GS31 12 Nov	GS32 25 Nov	GS33 4 Dec	Length (cm)	Days to 50% lodging	Regrowth (score 0-5*)
1	0	0	0	91	11	4.5
2	0	200TE	0	69	34	0
3	0	400TE	0	62	36	0
4	0	600TE	0	60	39	0
5	0	800TE	0	57	39	0
6	200TE	100TE	100TE	67	30	0
7	200TE	200TE	200TE	60	38	0
8	200TE+1500CCC	200TE+1500CCC	200TE	52	38	0
9	200TE+250P+1500CCC	200TE+1500CCC	200TE	44	40	0
		$LSD_{0.05}$		5	6	1.3
		P-value		<0.001	<0.001	<0.001

TE=Moddus® (trinexapac-ethyl 250 g/l); CCC=Cycocel (chlormequat chloride 750 g/l); P=Payback (paclobutrazol 250 g/l); *Scores 0=nil, 5=maximum

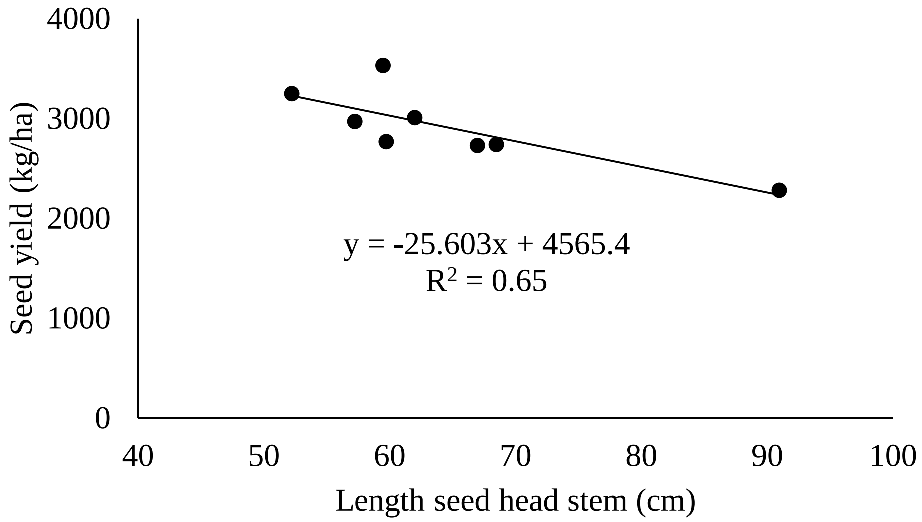

Figure 2: Relationship between seed yield and reproductive stem length for annual ryegrass cv. 'Hogan' when treated with nine plant growth regulator treatments in the 2015/16 season at Lincoln, Canterbury.

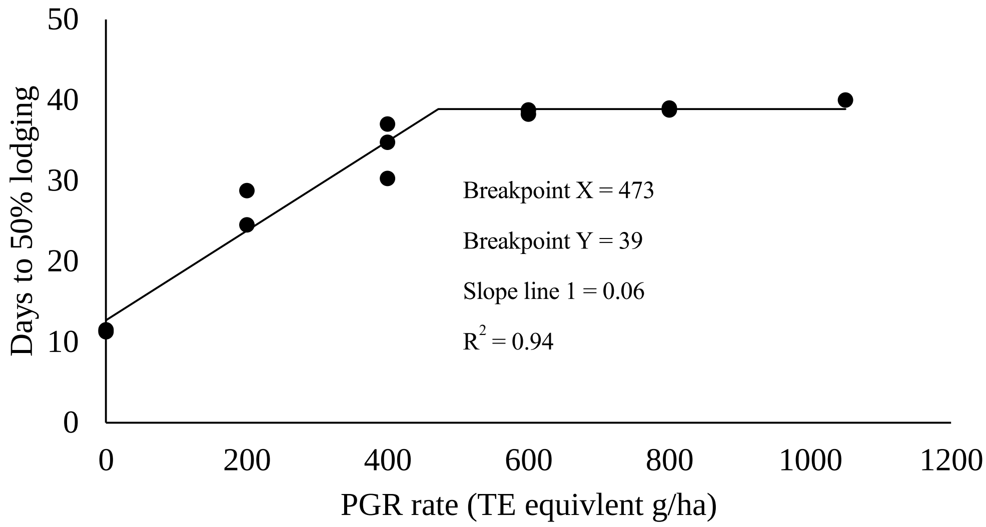

Figure 3: Relationship between PGR rate expressed as g TE equivalent and days to 50% lodging for annual ryegrass cv. 'Hogan' grown in the 2015/16 season at Lincoln, Canterbury.

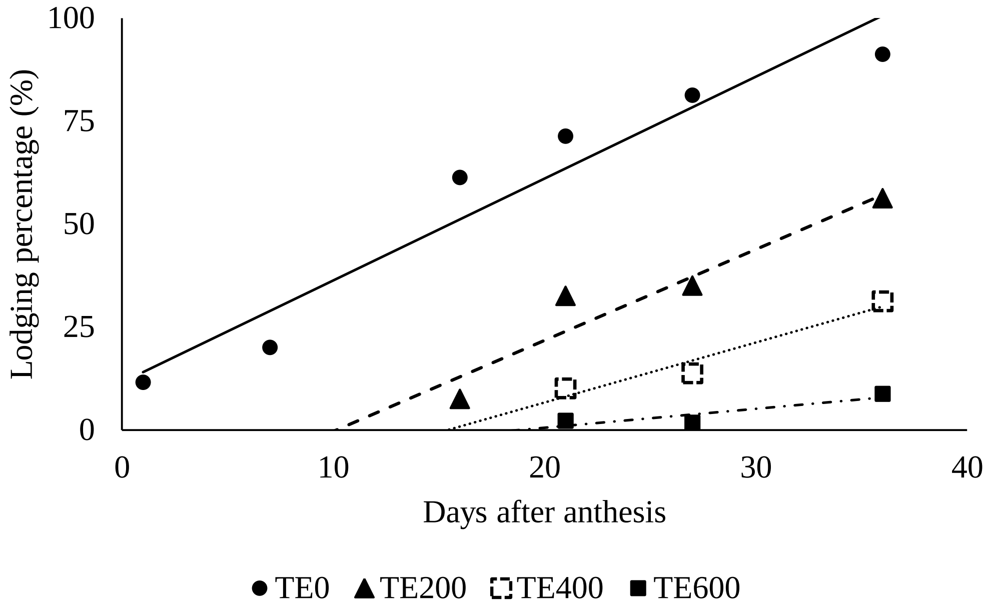

Figure 4: Weekly lodging score for annual ryegrass (cv. 'Hogan') treated with four rates of TE from 0 to 600 g/ha with slopes of TE0=2.47; TE200=2.21; TE400=1.46; TE600=0.26 ($LSD_{0.05}$ = 0.93; P-value <0.001).

Table 4: Hand harvested seed yield (kg/ha) of annual ryegrass cv. 'Hogan' when treated with three plant growth regulator treatments and supported by mesh cages compared with unsupported (lodged) seed heads outside the cage in the 2015/16 season at Lincoln, Canterbury.

TE (g/ha)	Cage	Outside	Mean
0	3120	3510	3310
200	3770	4270	4020
400	3380	4180	3780
Mean	3420	3990	
$LSD_{0.05}$	357		438
P-value	0.004		0.012

Table 5: Number of seeds per area ('000/m^2) and per spikelet for annual ryegrass cv.'Hogan' when treated with nine growth regulation treatments at Lincoln in the 2015/16 growing season.

Trt #	GS31 12 Nov	GS32 25 Nov	GS33 4 Dec	Seeds '000 (/m^2)	Seeds (/spikelet)
1	0	0	0	53.5	1.6
2	0	200TE*	0	62.6	2.1
3	0	400TE	0	67.9	2.2
4	0	600TE	0	65.1	2.1
5	0	800TE	0	71.4	2.2
6	200TE	100TE	100TE	66.4	2.4
7	200TE	200TE	200TE	86.8	2.6
8	200TE+1500CCC	200TE+1500CCC	200TE	79.5	2.6
9	200TE+250P+1500CCC	200TE+1500CCC	200TE	61.6	2.0
		$LSD_{0.05}$		9.5	0.4
		P-value		<0.001	0.004

TE=Moddus® (trinexapac-ethyl 250 g/l); CCC=Cycocel (chlormequat chloride 750 g/l); P=Payback (paclobutrazol 250 g/l)

Harvest mass and components of yield

There was no effect of the PGR treatments on harvest mass (average 14,530 kg DM/ha), seed head density (average 1,270 heads/m^2), spikelets per head (average 25.8/head) and TSW (average 4.17 g). The herbage mass growth rate from closing to assessment (94 days) averaged 155 kg DM/ha/day.

PGR treatment increased ($P<0.05$) the number of seeds per m^2 and per spikelet (Table 5). Seeds per spikelet increased from

1.6 seeds for untreated control to between 2.0 and 2.6 seeds for the PGR treatments and was highly correlated (R^2=0.78) to seed yield (SY=1009*seeds/spikelet+683).

Discussion

Seed yield was increased by up to 55% above the untreated control (Table 1) when multiple PGR applications were made. This is similar to results reported by (Trethewey *et al.*, 2016) who showed 51-65% seed yield increases from single applications of TE up to 800 g/ha. The increase in seed yield was attributed to an increase in the number of seeds/m^2 and per spikelet (Table 5) while all other components of yield remained constant. Seed head density and spikelets per head are set before PGR's are applied and are not normally affected by PGR treatments. In this study the optimum application rate for a single application was 277 g a.i./ha which provided a 28% increase above the control (Figure 1). These responses are large compared with the 10% increases reported from the northern hemisphere (Mellbye *et al.*, 2007; Rijckaert, 2010). However none of these previous studies compared split applications with a standard single application. Stem shortening was an important component of the seed yield increase, with an extra 25.6 kg seed/cm of stem shortening (Figure 2) while plants held upright had lower seed yields than their lodged counterparts (Table 4). Chynoweth *et al*. (2014) reported up to 45 kg seed/cm stem shortened in the late flowering perennial ryegrass trial. The application of TE delayed the onset of lodging (Figure 4) and slowed the progression. Delayed lodging increased seed yield by 24.8 kg/ha for every days delay in reaching 50% lodging and this is similar to values reported in perennial ryegrass (Rolston *et al*., 2010). Delayed or reduced lodging reduced (P<0.05) the amount of secondary regrowth (Table 3) in all treatments where PGR was applied. Secondary regrowth is associated with increased radiation reaching buds in the base of the canopy, and is therefore wasted radiation.

Annual ryegrass seed crops closed at the beginning of November have very high DM accumulation rates. In this trial the average mass accumulation from closing to harvest was 155 kg DM/ha/day. An adjacent trial with weekly cuts recorded 250 kg/ha/day from closing to GS32 (traditional PGR timing). Early/mid flowering diploid perennial ryegrass e.g. cv. Grasslands 'Nui', and cultivars with similar head emergence dates are closed four weeks earlier than annual ryegrass. These cultivars are growing in cooler conditions with lower radiation receipts and at lower daily growth rates during the same developmental stages. It is hypothesised that the difference in DM accumulation leads to split-applications of TE, starting five days after closing, being more effective than single applications at GS32. Chynoweth *et al.* (2014) evaluated TE in a late flowering tetraploid perennial ryegrass (cv. 'Bealey') and also showed enhanced seed yield with split TE applications. However unlike the late flowering perennial ryegrass trial (Chynoweth *et al*. 2014) this trial found no advantage in adding chlormequat and paclobutrazol to the TE treatment, suggesting differences between annual and perennial ryegrass.

The use of TE equivalents and the equating of 750 g chlormequat to 50 g TE to allow a comparison of chlormequat and paclobutrazol treatments is supported by the lodging data presented in Figure 3. This is the first published data using a TE equivalents approach of data interpretation.

Conclusions

This is the first work to show that split applications of TE increase seed yield above single applications in annual ryegrass. Three applications, each of 200 g/ha, increased seed yield by 27%, compared with untreated plots and gave a seed yield that was 750 kg/ha more than applying the same rate as a single application. Stem shortening was a major driver of seed yield response.

Acknowledgements

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