Manipulation of cereal crop development by plant hormones

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Abstract
Matching flowering time to the optimal flowering period in the Australian cereal cropping belt is key to maximising yield. Aside from variety selection and sowing time, growers have limited options to alter development in season. A range of plant hormone products were tested to analyse their effects on development in barley and wheat from exogenous sprays. Accelerating barley and wheat development was challenging to achieve with some GA treatments producing minor effects. However, significant delays in the time to flowering were achieved with GA inhibitors. Trinexapac-ethyl applied at 1000 mg/L delayed the time to flowering by up to 200 degree days under controlled conditions for both an early (GS13) and late (GS33) spray. In the field, a trinexapac-ethyl spray delayed flowering enough in early sown wheat to move flowering time into a more optimal period; resulting in significant increases in grain yield compared to the untreated control. More significant delays in flowering and increases in grain yields were found when used in combination with mowing treatments. These results suggest that gibberellin inhibitors could be a practical solution to slow development to better match flowering of early sown crops to the optimal flowering period and increase grain yields in season.

Keywords
plant growth regulators, optimal flowering period, trinexapac-ethyl, gibberellic acid, flowering time

Introduction
Flowering time in cereal crops is an important determinant of final grain yield. For crops to maximise seed size and number (potential yield), cereals must first establish, develop biomass and then flower at a time that coincides with optimal seasonal conditions (Fischer, 1985; Trethowan, 2014; Sadras and Dreccer, 2015). The optimal time to flower is the period when the combined risk of heat, drought and frost are at their lowest, with this period known as the optimal flowering period (OFP) (Flohr et al., 2017). Currently growers match sowing date to variety development speed in order to flower in the OFP. However, late opening rains, seasonal temperature variability, and increasing farm sizes make it difficult to achieve. Growers with mixed farming systems can slow down early sown crops in season by grazing to a certain extent (Virgona et al., 2006). However, this is not an option in monoculture cereal farming systems. There is also no current widely adopted option for speeding up development within a season to compensate for a delay in establishment time.

In cereals, plant growth regulators (PGRs) are used to prevent lodging, but some hormones have been shown to alter crop development. For example: gibberellic acid (GA) was reported to accelerate development (Cottrell et al., 1982) and GA inhibitors delayed development (Grijalva-Contreras et al., 2012), while cytokinins reduced the vernalisation requirement in winter wheat (Barabas and Csepely, 1978). The aim of the study was to investigate developmental effects that PGRs have on Australian cereal varieties to see if they could be used as a management tool to help ensure flowering occurs within the OFP.

Methods
Glasshouse and controlled environment experiments

A range of different PGR products (Table 1) were used to evaluate their initial effects on development in barley. The experiment was organised into a randomised complete block design (RCBD) with four replicates with plants sown into olive pots (17 cm × 7.5 cm × 7.5 cm). Ten different PGR treatments were tested against a control sprayed with water on four barley varieties (RGT Planet, Compass, Schooner and Spartacus CL), with plants sprayed until runoff at GS13 and GS31. Flowering time was recorded as the stage of awn peep.

Table 1: A summary of the different plant hormones and PGR products used with their respective active ingredient or mode of action and spray rate selected from previous studies used in the glasshouse trial.

Active Ingredient	Hormone/Mode of action	Spray rate
250 g/L Paclobutrazol	Gibberellic acid inhibitor	400 mL/100L
400 g/kg Gibberellic acid	Gibberellic acid	80 g/100L
250 g/L Trinexapac-ethyl	Gibberellic acid inhibitor	400 mL/100L
19 g/L Gibberellins A4 + A7 and 19 g/L 6-Benzyladenine	Gibberellic acid and Cytokinin	2 L/100L
20 g/L 6-Benzyladenine	Cytokinin	5 L/100L
0.075 g/L NAA and 0.075 g/L Indole acetic acid	Auxins	5 L/100L
20 g/L NAA	Auxin	500 mL/100L
100 g/kg Prohexadione-calcium	Gibberellic acid inhibitor	70 g/100L
8.84 g/L Indole-3-butyric acid	Auxin	300 mL/100L
99% pure Methyl Jasmonate	Jasmonic acid	0.4 mL/100L

A second experiment was conducted in a controlled environment room under 16 h day lengths at 22°C days and 8°C nights where a concentration response curve was produced with trinexapac-ethyl on Spartacus CL barley. An early (GS13) and late (GS33) spray application were tested at six different concentrations (0, 100, 250, 500, 1000, 5000 mg/L). The experiment was performed in olive pots and was a RCBD with four replicates. Flowering time was recorded as the stage of awn peep and plant height was measured using a ruler.

Field Trial

A field trial was conducted in a cold, frost prone environment at Mintaro, SA (GSR=430mm). The trial was sown on the 27th of April 2020 as a RCBD with three replicates of six different treatments (Table 2) on both barley (cv. Planet) and wheat (cv. Scepter). Sprays were conducted with a handheld sprayer containing 100g/ha of trinexapac-ethyl in a 200L/ha spray volume. A Deutscher HE660 mower was used to replicate a grazing treatment along the top of the furrows at set timepoints outlined in Table 2. Measurements were recorded for flowering date and harvest grain yield.

Table 2: The six different mowing and hormone spray treatments performed to alter phenology in a frost prone landscape at Mintaro, South Australia.

No.	Treatment
1	Control: Untreated
2	Reset GS30: Mowed to ground at GS30
3	Reset GS30 + Trinexapac-ethyl: Mowed to ground at GS30 and then sprayed with trinexapac-ethyl
4	Reset GS32: Mowed to ground at GS32 removing main stem shoot apex
5	Reset GS32 + Trinexapac-ethyl: Mowed to ground removing main stem shoot apex at GS32 and then sprayed with trinexapac-ethyl
6	Trinexapac-ethyl: Sprayed with trinexapac-ethyl at GS13 and GS31

Data Analysis

Differences between treatments were identified with an analysis of variance in the statistical package GenStat (2020) at the 5% significance level. Multiple comparisons were made for the field trial using the Bonferroni test to compare treatments to the control.

Results

Effect of a range of hormonal products on barley time to flowering under controlled conditions

There was a significant variety by PGR interaction (P=0.04). This interaction is shown in Table 3, where a number of GA and auxin products accelerated the time to flowering only in Compass, compared to the control. The other treatment with a significant response was GA3, which accelerated flowering by 2 days in Schooner compared to the control (Table 3). Methyl jasmonate, trinexapac-ethyl and paclobutrazol delayed the time to flowering in almost every variety, except paclobutrazol in Schooner (Table 3). Methyl jasmonate delayed flowering across varieties (on average by 5 days), however, widespread chlorosis/defoliation was noted in days following the spray application with new shoots developing after leaf death.

Table 3: The number of days to awn peep for different barley varieties and the relative difference compared to the control when sprayed at GS13 and GS31 with different PGRs. * indicates that the PGR treatment was significantly different to the control at P>0.05 with four replicates.

PGRs	Compass	Planet	Schooner	Spartacus CL
Control	53.3	52.0	49.5	48.8
Methyl Jasmonate	+5.5*	+6.5*	+4.5*	+7.7*
Trinexapac-ethyl	+4.2*	+3.7*	+3.8*	+5.5*
Paclobutrazol	+3.7*	+1.8*	+1.3	+2.2*
6-Benzyladenine	-0.5	+0.3	0	+0.5
NAA and Indole Acetic Acid	-1.3	+0.3	-0.5	0
NAA	-1.5	0	-0.5	-0.3
GA4 + GA7 and 6-Benzyladenine	-1.8*	0	+0.3	-0.5
GA3	-2*	-0.2	-2.2*	-1.3
Prohexadione-Calcium	-2.3*	-1	-1.2	-0.6
Indole-3-butyric acid	-2.8*	-0.2	+0.8	-0.3
Variety × PGR (LSD 5%)	1.62			
+/- SEM	0.42			

Trinexpac-ethyl concentration response curve and timing response

The effect of concentration on time to flowering was significant (P<0.001) in Spartacus CL under 16 h day length compared to the control (data not shown). There was no timing (P=0.12) or timing by concentration (P=0.11) interaction (data not shown). The GS13 spray produced a similar delay compared to the GS33 spray across most concentrations and as the rate of trinexapac-ethyl increased, the time to flowering also increased compared to the control (Figure 1). The GS13 spray resulted in an increased delay on flowering at the two highest concentrations compared to the GS33 application. The data was log transformed as increased variability across the replicates was observed at higher concentrations. A negative correlation between height and thermal time to flower for trinexapac-ethyl is apparent in Figure 2. The significantly larger increase in flowering time with the GS13 application compared to GS33 at the two highest rates resulted in much shorter plants and the largest difference in the time to flowering. For every 15 degree days delay in flowering time, height was reduced by 1 cm.

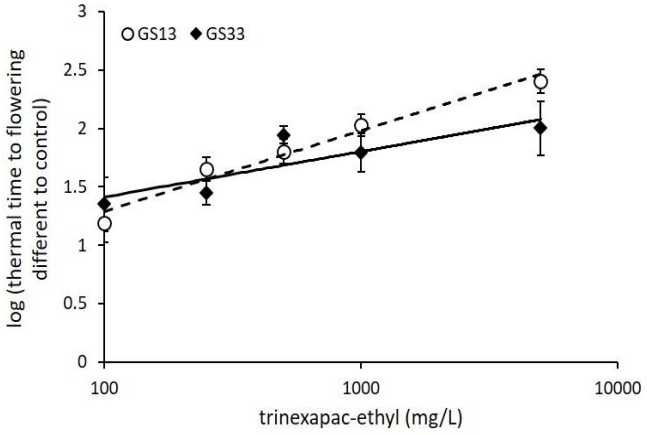

Figure 1: The concentration response curve for trinexapac-ethyl with one spray application at GS13 and GS33 with five different concentrations on a logarithmic scale. Each fitted with a logarithmic trend line and error bars +/- 1 standard error of the mean of four replicates.

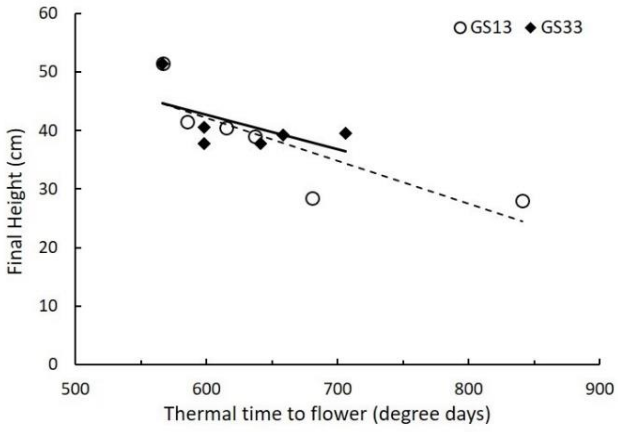

Figure 2: The difference that a change in flowering time has on the final height of Spartacus CL from a spray application of trinexapac-ethyl at GS13 and GS33. Each point represents a concentration of trinexapac-ethyl with its respective thermal time to flower and final height.

Wheat and barley response to PGRs and grazing in the field

Trinexapac-ethyl delayed flowering of both early sown Scepter and Planet by approximately 3-4 days in the field at Mintaro. In combination with the mowing treatment which also created more asynchronous flowering from more secondary tiller growth, the delay was extended out to 6-7 days. The flowering delay created in the early sown Scepter, enabled it to flower at a more optimal time. This resulted in trinexapac-ethyl and mowing treatments significantly increasing grain yield compared to the control (Figure 3b). The trial site experienced a couple of frosts (-0.2°C and -3.2°C) during the flowering period which may have influenced the yields in this experiment. The delay in flowering time in Planet was similar to Scepter, however there was no significant yield improvement across the treatments (Figure 3a). This suggests that delaying

flowering of early sown barley may not be as valuable as it is in wheat, due to its potentially wider optimal flowering window.

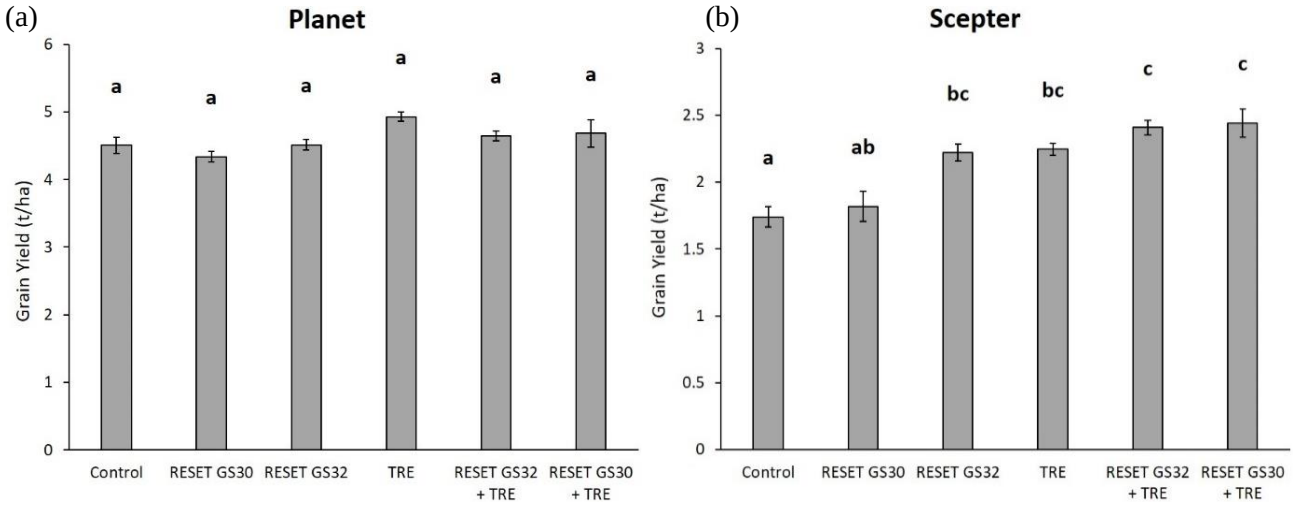

Figure 3: The grain yields of (a) Planet barley and (b) Scepter wheat for the different management treatments (TRE = trinexapac-ethyl) used at the field trial at Mintaro, South Australia. Different letters represent significant differences (P<0.05) and error bars +/- 1 standard error of three replicates.

Conclusion

The use of plant hormones to alter development and flowering time provides a potential option for aligning flowering time to the OFP. Trinexapac-ethyl significantly delayed flowering of barley under controlled conditions and delayed flowering of early sown wheat enough to move flowering time into a more optimal period. Yields were increased compared to untreated controls in wheat, but further work is required to demonstrate its reproducibility across sites and seasons. Future investigation of the synchronous nature of flowering in cereals and how hormones or mechanical manipulation alter development, may also provide further practical management options for growers. However, the findings of this study suggest that speeding up development from late sowing does seem to be more challenging and unlikely compared to slowing down crop development. This highlights that crop type, variety selection and time of sowing still have the largest effect on influencing relative flowering time by management.

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