# Vernalisation and photoperiod sensitivity of phenologically diverse Australian wheat cultivars

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## Abstract
Australian wheat growers require a diverse range in cultivar phenology to capture sowing opportunities across a wide range of environments and sowing dates. For growers to maximise yield, it is crucial to know when new cultivars will flower from different sowing times in an environment. However, this information is not currently available for multiple years after a new cultivar is released. The Plant Modelling Framework within APSIM Next Generation allows for phenology parameter inputs derived from controlled environment experiments, meaning parameters can be rapidly obtained to allow accurate prediction of flowering times at point of cultivar release. We grew a phenologically diverse panel of 69 wheat genotypes in four controlled environments (8 or 17-hour photoperiod, ± vernalisation) to derive phenotypes (leaf appearance, final leaf number, time to heading/flowering) required as parameter inputs by the APSIM Next Generation wheat model. Parameters will be derived from the phenotype data and model output will be validated with field data from a network of national field experiments conducted in 2019 and 2020 using the same genotypes.

## Keywords
Flowering time, development, phenotyping, crop modelling, *Triticum aestivum*.

## Introduction
Australian wheat (*Triticum aestivum*) growers require phenologically diverse cultivars to manage variation in timing of sowing opportunities across a broad range of environments. Changing time of sowing of a cultivar in an environment, particularly spring cultivars, will change the time of flowering. There are significant yield penalties incurred when crops flower outside the optimal flowering period (OFP) for a given environment (Flohr et al. 2017). An environment's OFP is defined as the time when combined yield penalties from frost, insufficient radiation, heat and drought are minimised (Flohr et al. 2017). Knowing when a cultivar will flower from different sowing times in different environments is important for growers and advisers to maximise yield. However, this information is not available when new cultivars are released. It requires two to three years of field experiments to refine sowing times, by which time much opportunity has been missed and a cultivar will be nearing the end of its operational life.

Time to flowering is governed by a cultivar's genetic response to the environmental cues of temperature and photoperiod (Pp), largely determined by two *PPD1* and three *VRN1* genes. Increasing temperature and Pp accelerates development, and some cultivars also develop more rapidly after prolonged exposure to cold temperatures, termed vernalisation (Vrn).

Advances in crop modelling software (APSIM Next Generation, hereafter APSIM NG; Holzworth et al. 2014) and the process-based framework to simulate phenology (Brown et al. 2014; Brown et al. 2018) have led to improved model accuracy and useability. Historically, simulation of cultivar phenology in APSIM Classic (Keating et al. 2003) did not perform well outside of environments in which it was parameterised. The Plant Modelling Framework (PMF; Brown et al. 2014) in APSIM NG allows for phenology of wheat genotypes to be parameterised using controlled environment (CE) data that captures genotype-specific responses to the major environmental factors of Pp and Vrn. Key

traits required for the model include the phyllochron (°Cd leaf$^{-1}$), the final leaf number on the main stem and the thermal time taken to reach it, and the thermal time between final leaf emergence on the main stem and heading/flowering.

Here we describe a CE phenotyping methodology to quantify genotype-specific responses to Pp and Vrn which in turn can be used to derive parameters for input in the APSIM NG wheat phenology model, as governed by a genotype's responses to limiting and saturating Vrn and/or Pp conditions. The aim of this study was to phenotype a large and diverse panel of wheat genotypes in four CEs to quantify responses of key phenological processes to Pp and Vrn, and thereby derive parameters for the APSIM NG wheat model.

**Methods**

*Genotype selection*

Sixty-nine wheat genotypes (42 commercial, 17 near-isogenic lines) were assembled to form the Australian Phenology Panel and were grown in the experiment. The genotypes were selected for their diversity of alleles at the *VRN1* and *PPD1* loci with 56 spring (a spring allele in at least one *VRN1* locus) and 13 winter types (winter alleles at all three *VRN1* loci). Seeds were sourced from the Australian Winter Cereals Collection, breeding companies and Dr Ben Trevaskis (CSIRO). A subset of 27 phenologically diverse commercial genotypes are presented here (Table 1). Genotypes were classed into phenology groups according to their time to heading across four field sites (WA, SA, NSW, VIC) and five times of sowing (Apr-Jun) in 2019 (results not shown), as per guidelines developed by Australian Crop Breeders (https://www.australiancropbreeders.com.au/).

*Experimental design*

One experiment was conducted in four CEs in the AgriBio building at La Trobe University in 2018-19, with environment (E; 8 or 17-hour photoperiod, ± vernalisation) and genotype (G) as factors. Each environment was a randomised complete block design with three replicates. The four environments were long (17-hour) day, not vernalised (LN); long day, vernalised (LV); short (8-hour) day, not vernalised (SN); and short day, vernalised (SV). Seeds were pre-germinated on filter paper wet with reverse osmosis water in petri dishes for two days (24 hours at 5°C and 24 hours at 22°C) to break dormancy. Seeds for the vernalised treatments were sown in seedling trays and grown in a Humiditherm growth cabinet (Thermoline) at 5°C for eight weeks with either S or L day length. After eight weeks, seedlings were transplanted into 90 mm olive pots and moved to the CE room with S or L day length and 22°C constant temperature. Seeds for the non-vernalised treatments were sown into 90 mm olive pots and placed in the CE room with S or L day length and 22°C constant temperature. Growth medium for all trays and pots was a standard potting mix containing slow-release fertiliser.

Plants were monitored twice a week to record decimal leaf number as Haun stage (HS; Haun 1973), and daily during critical times to record emergence date; final leaf number on the main stem and the date it fully extended from the preceding leaf; dates the first spike fully emerged and 50% of culms with spikes fully emerged; and dates the first spike flowered (extrusion of anthers or yellow/white anthers in spikelets) and 50% of culms flowered. Temperatures in each environment were recorded on Tinytag Plus 2 loggers (Gemini Data Loggers) in radiation shields at pot height at 30 min intervals. Lights in the cabinet and CE emitted 180 and 300 µmol m$^{-2}$ s$^{-1}$ of photosynthetically active radiation (PAR) at pot height, respectively.

*Statistical analyses*

Linear Mixed Models techniques used for Multi-Environment Trials were adopted. The latter allow modelling the variance-covariance structure of G × E, assume different genetic variance for each E and simultaneously account for the effects of G, E and G × E and, where present, spatial variation. Thermal time (TT) was calculated for each environment assuming a base temperature of 0°C. Final leaf number (FLN), TT from emergence to flag leaf (TTFL) and TT from emergence to flowering (TTF) were analysed using ASReml-R (VSN International). Phyllochrons were calculated as the slope

of the regression of TT vs HS (HS 3 to 7) for each genotype in each environment in GENSTAT 19 (VSN International).

## Results and discussion

Vrn and Pp responses of a subset of 27 genotypes are presented in Table 1. LV is considered as the least limiting environment and values from it describe a genotype's most rapid development. Vrn response is determined as the difference in values between the LN and LV environments, and Pp response is determined as the difference between the SV and LV environments (Brown et al. 2018).

**Table 1. Vernalisation (Vrn; LN-LV) and photoperiod (Pp; SV-LV) responses of 27 diverse wheat genotypes for phyllochron, final leaf number (FLN), thermal time to flag leaf (TTFL), and thermal time to flowering (TTF).**

Habit indicates spring (S) or winter (W) habit type. Phenology indicates mean time to heading from national field experiments in 2019 (very quick, VQ; quick, Q; quick-mid, Q-M; mid, M; mid-slow, M-S; slow, S; slow-very slow; S-VS; very slow, VS). Standard errors (SE) in parentheses are the larger error of the two environments compared. Average standard error of differences (SED) are for environment × genotype.

| Genotype | Habit | Phenology | Phyllochron (°Cd leaf$^{-1}$) (SE) | | FLN (SED = 0.5) | | TTFL (°Cd) (SED = 78) | | TTF (°Cd) (SED = 130) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Vrn | Pp | Vrn | Pp | Vrn | Pp | Vrn | Pp |
| Axe | S | VQ | 4.1 (9.9) | 24.6 (9.9) | 0.0 | 1.0 | -18 | 262 | -41 | 244 |
| Emu Rock | S | VQ-Q | 3.5 (9.5) | 37.8 (7.8) | 0.0 | 1.3 | -48 | 340 | -53 | 372 |
| Grenade CL Plus | S | Q | 1.3 (7.4) | 45.5 (7.4) | 1.3 | 2.7 | 106 | 717 | 49 | 871 |
| H45 | S | Q | 9.1 (7.0) | 38.7 (7.0) | 0.7 | 1.7 | 30 | 393 | 15 | 523 |
| Young | S | Q | -15.4 (6.4) | 25.2 (5.7) | 1.0 | 0.7 | 11 | 253 | -23 | 354 |
| Mace | S | Q-M | 37.3 (6.8) | 31.7 (6.8) | 4.3 | 1.3 | 488 | 295 | 323 | 283 |
| Scepter | S | M | 23.2 (6.5) | 27.3 (6.5) | 5.0 | 1.7 | 511 | 357 | 360 | 346 |
| Suntop | S | M | -1.3 (6.3) | 27.9 (5.8) | 1.0 | 1.3 | 66 | 375 | 56 | 445 |
| Trojan | S | M | 32.1 (6.2) | 53.0 (5.9) | 4.0 | 3.3 | 383 | 883 | 280 | 1145 |
| Gregory | S | M-S | 23.7 (6.0) | 36.4 (5.6) | 3.7 | 1.7 | 349 | 351 | 305 | 411 |
| Lancer | S | M-S | -3.6 (7.1) | 19.6 (7.1) | 0.7 | 3.7 | 26 | 630 | -19 | 650 |
| Yitpi | S | M-S | 28.7 (7.4) | 51.0 (7.4) | 3.3 | 5.0 | 372 | 1187 | 327 | 1810 |
| Bolac | S | S | 23.9 (5.8) | 30.8 (5.7) | 2.3 | 4.3 | 326 | 903 | 329 | 1066 |
| Braewood | S | S | 6.6 (5.9) | 20.9 (5.5) | 1.3 | 3.6 | 78 | 782 | 55 | 1840 |
| Mitch | S | S | 17.4 (6.1) | 42.5 (5.6) | 4.0 | 2.0 | 424 | 488 | 377 | 586 |
| Beaufort | S | S-VS | 12.0 (6.0) | 21.1 (5.6) | 6.3 | 1.0 | 787 | 316 | 736 | 391 |
| Ellison | S | S-VS | 21.0 (7.8) | 52.6 (7.8) | 4.7 | 4.0 | 598 | 1114 | 475 | 1353 |
| Sunbri | S | S-VS | 7.3 (6.0) | 21.2 (5.9) | 1.0 | 4.0 | 111 | 901 | 114 | 1715 |
| Eaglehawk | S | VS | 45.8 (7.5) | 63.3 (7.5) | 4.3 | 3.0 | 564 | 896 | 460 | 1214 |
| Sunlamb | S | VS | 18.5 (6.4) | 29.6 (5.4) | 6.7 | 3.7 | 790 | 778 | 726 | 2437 |
| Longsword | W | Q | 45.4 (6.0) | 53.4 (6.0) | 8.0 | 1.7 | 1158 | 398 | 1136 | 434 |
| Whistler | W | Q | 30.4 (5.7) | 26.5 (5.5) | 6.2 | 2.0 | 974 | 457 | 999 | 684 |
| Kittyhawk | W | M | 22.7 (5.6) | 36.1 (5.5) | 9.0 | 3.0 | 1415 | 727 | 1503 | 1350 |
| Rosella | W | M | 25.8 (6.7) | 35.5 (6.7) | 8.8 | 2.7 | 1623 | 871 | 1686 | 931 |
| Wedgetail | W | M | 32.6 (5.5) | 35.7 (5.6) | 6.7 | 2.0 | 1385 | 476 | 1312 | 542 |
| Manning | W | S | 11.6 (6.1) | 21.7 (5.9) | 5.0 | 2.3 | 1158 | 680 | 1335 | 1380 |
| Revenue | W | S | 19.5 (6.0) | 26.6 (6.4) | 7.5 | 1.5 | 1343 | 481 | 1344 | 652 |

Clear separation of winter and spring habit is seen in Vrn response of TTF. The most responsive spring type, Beaufort, flowered 742°Cd quicker, whereas all winter types flowered ≥1000°Cd quicker when vernalised. Increased phyllochron and FLN from short Pp led to increased TTF for all

genotypes including the quickest commercial type, Axe. Axe is one of the quickest commercially available genotypes in Australia when grown in the field, indicating its insensitivity to both Vrn and Pp under field conditions.

A potential constraint with the methodology in its current form is the 8-hour Pp (S). Some replicates of Pp-sensitive genotypes failed to produce a spike on the main stem, because either the main stem died during leaf production or following the appearance of the final leaf. This can have implications on the mean values that are used to parameterise the model. This was demonstrated by large values for some genotypes for the TT from flag leaf to flowering (results not shown), where TTFL is measured on the main stem but TTF is measured on another culm because the main stem died before heading/flowering. Although day length in the Australian wheat belt is never lower than ~9.5 hours, it is important to ensure physiological response to extreme limiting Pp is captured. Growing plants under higher PAR could potentially improve growth of Pp-sensitive genotypes. In addition, factoring the photothermal quotient into the thermal time calculations could improve model accuracy.

These results also showed Vrn and/or Pp responses under controlled conditions do not necessarily correlate with phenology in the field. For example, mid spring type Trojan has a small Vrn response and a very large Pp response, similar to spring types in the slow to very slow range. However, Vrn response clearly separated spring and winter types.

**Conclusion**

Growers and advisers require accurate cultivar sowing guides to ensure crops are managed to flower during the OFP, especially when a new cultivar is released. CE phenotyping captures cultivar-specific responses to Vrn and Pp that can be used to derive wheat phenology parameters for the PMF within APSIM NG. We outlined methods describing conditions of four CEs to capture phenology data needed to parameterise the APSIM NG wheat model. Vrn and Pp had varying effects across genotypes, with Pp generally affecting spring types more than Vrn. Conversely, winter types were more affected by Vrn. Results from the experiment will be used to derive genotype-specific parameters in APSIM NG, which will be validated with phenology data gathered in national field experiments in 2019 and 2020.

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