Modelling temperature, photoperiod and vernalization responses of \textit{Brunonia australis} (Goodeniaceae) and \textit{Calandrinia} sp. (Portulacaceae) to predict flowering time

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**Background and Aims** Crop models for herbaceous ornamental species typically include functions for temperature and photoperiod responses, but very few incorporate vernalization, which is a requirement of many traditional crops. This study investigated the development of floriculture crop models, which describe temperature responses, plus photoperiod or vernalization requirements, using Australian native ephemerals \textit{Brunonia australis} and \textit{Calandrinia} sp.

**Methods** A novel approach involved the use of a field crop modelling tool, DEVEL2. This optimization program estimates the parameters of selected functions within the development rate models using an iterative process that minimizes sum of squares residual between estimated and observed days for the phenological event. Parameter profiling and jack-knifing are included in DEVEL2 to remove bias from parameter estimates and introduce rigour into the parameter selection process.

**Key Results** Development rate of \textit{B. australis} from planting to first visible floral bud (VFB) was predicted using a multiplicative approach with a curvilinear function to describe temperature responses and a broken linear function to explain photoperiod responses. A similar model was used to describe the development rate of \textit{Calandrinia} sp., except the photoperiod function was replaced with an exponential vernalization function, which explained a facultative cold requirement and included a coefficient for determining the vernalization ceiling temperature. Temperature was the main environmental factor influencing development rate for VFB to anthesis of both species and was predicted using a linear model.

**Conclusions** The phenology models for \textit{B. australis} and \textit{Calandrinia} sp. described development rate from planting to VFB and from VFB to anthesis in response to temperature and photoperiod or vernalization and may assist modelling efforts of other herbaceous ornamental plants. In addition to crop management, the vernalization function could be used to identify plant communities most at risk from predicted increases in temperature due to global warming.

**Key words:** \textit{Brunonia australis}, \textit{Calandrinia} sp., modelling, flowering, phenology, temperature, photoperiod, vernalization.

INTRODUCTION

\textit{Brunonia australis} and \textit{Calandrinia} sp. (Mt Clere: not yet fully classified, AQ no. 743890) are Australian native herbs with commercial potential as flowering potted or bedding plants. Both species are best grown as annuals and flower naturally during spring and early summer. However, many ornamental plants are grown outside their natural flowering period to align flowering with peak market demand, which requires the capacity to predict flowering date under changing or different environments. The precision of flowering time estimates can be improved by using dynamic crop scheduling tools, which have not been developed for \textit{B. australis} or \textit{Calandrinia} sp.

Scheduling crop production using quantitative flowering time models can have considerable advantages as they can be tailored for individual requirements, unlike traditional scheduling methods that are typically based on calendar date and have no particular reference to the environment (Adams et al., 1997). These models can be used for long-term strategic planning when completed well before the crop is grown or for short-term predictions when completed during production to reassess or change the flowering date (Larsen, 1990). While most modelling efforts have concentrated on field crops, some development rate models have been generated for ornamental species, including \textit{Salvia splendens} ‘Vista Red’ and \textit{Tagetes patula} ‘Bonanza Yellow’ (Moccaldi and Runkle, 2007), cineraria (Yeh et al., 1999), \textit{Dahlia pinnata} ‘Royal
Dahlietta Yellow’ (Bryndum and Heins, 1993), Viola × wittrockiana ‘Universal Violet’ (Adams et al., 1997) and chrysanthemum (Larsen and Persson, 1999).

Most development rate models for ornamental species predict flowering time in relation to temperature, photoperiod and/or daily light integral as observed for the above models. However, there are few flowering time models (e.g. Larsen, 1988) for ornamental plants that include a vernalization function. Vernalization is important for early and complete flowering of many traditional herbaceous crops, such as Aquilegia × hybrida ‘Remembrance’, A. flabellata ‘Cameo’, Salvia × superba ‘Blaukönigin’ and cineraria (Yeh et al., 1997; Garner and Armitage, 1998; Niu et al., 2002; Waaseth et al., 2006). Plant responses to vernalization have been incorporated into some models for field crops (e.g. Clarkson and Russell, 1979; Ellis et al., 1989; Habekotté, 1997; Liu, 2007; McMaster et al., 2008) and arabidopsis (e.g. Chew et al., 2012), which reportedly improved accuracy. These models described vernalization responses using functions that were linear (Ellis et al., 1989), exponential (Clarkson and Russell, 1979; Larsen, 1988), dent-like (Habekotté, 1997; McMaster et al., 2008), sigmoidal (Liu, 2007) or a combination of beta and broken linear (Chew et al., 2012). The use of non-linear functions typically allows the relationships between temperature and the level of vernalization saturation and the duration required for complete vernalization to be defined (Liu, 2007; McMaster et al., 2008).

Previous studies for *Brunonia australis* and *Calandrinia* sp. indicated that a prediction model for flowering time must incorporate temperature and photoperiod or vernalization responses. Cave and Johnston (2010) reported that long days (LDs), which included 11 h of ambient light plus a 5-h low light-intensity night break, increased inflorescence numbers of *B. australis* by 59 % and reduced the time to first visible floral bud (VFB) by 34 % compared with short days (SDs; 11 h), indicating a facultative LD response. Plants were found to perceive the LD stimulus for early flowering 18–22 d after seed germination (Cave et al., 2011b). In contrast, *Calandrinia* sp. showed a small (13 %) reduction in time to VFB when grown under LDs, but exhibited a strong vernalization requirement (Cave and Johnston, 2010). For example, 6 weeks of vernalization at 4·8 °C reduced flowering time by 37 % and increased flower number by 73 % compared with non-vernalized plants (Cave and Johnston, 2010). In a separate study, plants of *Calandrinia* sp. remained vegetative for 90 d (duration of the experiment) when grown at 35/20 °C (day/night), whereas floral initiation commenced 47 d after seed germination at 25/10 °C (Cave et al., 2010). Further studies showed that plants were vernalization-sensitive 4 d after seed germination when chilled at 9·3 °C for 21 d (Cave et al., 2011b).

The objectives of the present study were to quantify temperature and photoperiod or vernalization responses of *B. australis* and *Calandrinia* sp. and model development rate from planting to VFB and VFB to anthesis for the purpose of scheduling year-round flowering. The effects of temperature and photoperiod or vernalization on plant quality characteristics, including flower and branch number, were defined.

**MATERIALS AND METHODS**

**Plant material**

Seeds of *Brunonia australis* were collected from a wild population in southern Queensland (27°54′-90.5′S 149°51′-139°E) in October 2003. *Calandrinia* sp. seeds were collected from plants grown at The University of Queensland, Gatton Campus (UQG) in 2005. Seed of both species was stored in a specialized seed store at UQG. Seeds were germinated at 21 °C, as per requirements identified in an earlier study (Cave et al., 2011a). When ≥60 % of seeds had germinated (3–4 d after sowing), they were transferred to trays containing standard UQG nursery propagation media that consisted of perlite, vermiculite and peat (6 : 3 : 1), plus 2 g L⁻¹ of controlled-release fertilizer 13N-6P-16K-2Mg-10S (Basacote Mini 3M; COMPO GmbH & Co. KG, Münster, Germany) and 1 g L⁻¹ of dolomite 14Ca–8Mg (Dolomite; Flinders Trading Pty Ltd, Strathpine, Australia). Freshly transplanted seedlings were placed in a greenhouse under ambient conditions. Four days later, when the seedlings had emerged and the cotyledons were fully expanded, they were transferred to 125-mm (1-L) pots that contained standard UQG nursery potting medium and consisted of 100 % pine bark, plus controlled release fertilizers: 2 g L⁻¹ of 15N–4P–7·5K–1·8Mg (Osmocote Plus 8–9M), 1 g L⁻¹ of 16N–5P–9·2K–1·8Mg (Osmocote Plus 3-4M), 2 g L⁻¹ of 16N–4·4P–8·3K (Nurtricote 7M; Yates, Paddstown, Australia), 1·3 g L⁻¹ of 18N–2·2P–11K–1·2Mg (Osmoform 4M), 1·3 g L⁻¹ of 28Fe–17S (Coated Iron), 1·3 g L⁻¹ of dolomite 14Ca–8Mg (Dolomite; Flinders Trading Pty Ltd) and 1·2 g L⁻¹ of granular wetting agent (SaturAid; Debco, Tyabb, Australia). All nutrients were supplied by Scotts, Baulkham Hills, Australia, unless stated otherwise. Seedlings were established in the greenhouse for a further 3 d before being placed on a raised outdoor bench. Plants were irrigated daily as required, depending on plant size and environmental conditions. At 3-month intervals, 3·5 g of slow release fertilizer (16N–8P–12K–2Mg–5S, Basacote Plus 3M; COMPO GmbH & Co. KG) was applied to each *Calandrinia* sp. plant. *Brunonia australis* flowered within 3 months of planting and did not require supplementary nutrients.

**Experimental design**

The study was conducted at UQG plant nursery (27°34′S 152°20′E). Seeds were sown in weeks 3 (P13), 8 (P2 and P14), 12 (P3), 15 (P4), 19 (P5), 23 (P6), 27 (P7), 32 (P8), 37 (P9), 41 (P10), 45 (P11) and 51 (P12) from February 2009 to February 2010. The value following ‘P’ usually corresponds to the calendar month in which plants were sown; e.g. P2 was sown in February. Final data collection was in September 2010. There were 12 and 13 planting dates for *B. australis* and *Calandrinia* sp., respectively, with three replicates each containing four plants. Plants were placed on a bench that was 3 × 1·5 m and partitioned lengthwise to give three blocks. Replicates for each sowing date were randomized within a block. The long axis of the bench ran approximately north–south. Netting was used to prevent bird damage and reduced the transmission of natural sunlight by 14 %. The daily light integral for planting to VFB was 23–54 mol m⁻² d⁻¹.
Data collection and analysis

Data on date and number of days to first VFB, leaf number of the rosette at VFB, and the date and number of days from VFB to anthesis were collected. At anthesis, floral bud and branch number were recorded, except for branch number of *B. australis* during the early stages of the experiment (P3 and P4). Plant diameter of *Calandrinia* sp. was measured where the plant was widest, including the tip of the floral bud. Temperature of the medium (3 cm below the surface) and air were measured at 1-min intervals and the average recorded every 30 min using sensors (TA10) connected to a data logger (EasiData Mark4; Environdata Environmental Monitoring and Management, Warwick, Qld). Total solar radiation was measured using a sensor (SR10) connected to the same data logger. Daily photoperiod included civil twilight (solar elevation of –6.0°) and was computed by Geoscience Australia (http://www.ga.gov.au/bin/astro/sunrisenset) using Sunrisenset version 2.2. Data were analysed using the General Linear Model in SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Means calculated for each replicate were used to model development rate responses to temperature and photoperiod or vernalization. Plant death resulted in some replicates having fewer than four plants. To reduce the influence of these replicates on results for leaf appearance rates, and for leaf, floral bud and branch number, means for each planting date were weighted according to the number of plants within the replicate and the least-square adjusted means (LSMEAN) calculated. Correlations between every pairwise combination of the variables: days to VFB, VFB to anthesis, and leaf, floral bud and branch number, were determined using Pearson correlation coefficients generated using the CORR procedure in SAS.

Modelling development rate from planting to VFB

A multiplicative model was used to describe development rate (*R*) of *B. australis* as a function of temperature and photoperiod:

\[
R = R_{\text{opt}} \times f(T) \times f(P)
\]

where *R*\(_{\text{opt}}\) is the rate of development under optimum temperature and photoperiod, *f*(T) is a function that best represents developmental responses to temperature and *f*(P) is a function that best represents developmental responses to photoperiod. A vernalization function was added to the model to describe development rate from planting to VFB for *Calandrinia* sp. Photoperiod was omitted from the model, since previous studies clearly showed that vernalization was the most important floral stimulus (Cave et al., 2011b). The resultant model was:

\[
R = R_{\text{opt}} \times f(T) \times f(V)
\]

where *f*(V) is a function that best represents developmental responses to vernalization. Functions were selected based on biological suitability and by inspecting the rate of development in response to temperature and photoperiod or vernalization. The temperature function was curvilinear:

\[
f(T) = \left\{ \begin{array}{ll}
\alpha \times (T - T_b) \times (T_m - T)^\beta & \text{where,} \\
\alpha = 1/[(T_o - T_b) \times (T_m - T_o)^\beta] \\
\beta = (T_m - T_o)/(T_o - T_b)
\end{array} \right.
\]

and where *T* is temperature, *T*\(_b\) the base temperature, *T*\(_o\) the optimum temperature and *T*\(_m\) the maximum temperature (Holzworth and Hammer, 1996). This function has been used to model plant development in response to temperature for a number of ornamental species, including *Portulaca grandiflora* ‘Margarita Apricot’, *Cosmos sulphureus* ‘Cosmic Orange’, *Antirrhinum majus* ‘Montego Orange Bicolour’ (Blanchard and Runkle, 2011) and Dahlia pinnata ‘Royal Dahliaetta Yellow’ (Brundum and Heins, 1993). The photoperiod function was triple broken linear:

\[
f(P) = \left\{ \begin{array}{ll}
\text{for} (P_{\text{sens}} \geq 1), 1 - P_{\text{crit1}} - P_{\text{crit2}} & \text{if} \ P < P_{\text{crit1}} \\
1 - P_{\text{crit2}} & \text{if} \ P_{\text{crit1}} < P < P_{\text{crit2}} \\
1 & \text{if} \ P > P_{\text{crit2}}
\end{array} \right.
\]

\[
\text{for} (P_{\text{sens}} < 0), 1 + (P - P_{\text{crit1}})P_{\text{sens}} & \text{if} \ P < P_{\text{crit1}} \\
1 + (P_{\text{crit2}} - P)P_{\text{sens}} & \text{if} \ P_{\text{crit1}} < P < P_{\text{crit2}} \\
1 + (P_{\text{crit2}} - P_{\text{crit1}})P_{\text{sens}} & \text{if} \ P > P_{\text{crit2}}
\]

where *P* is photoperiod, *P*\(_{\text{crit1}}\) the lower critical photoperiod, *P*\(_{\text{crit2}}\) the upper critical photoperiod and *P*\(_{\text{sens}}\) the photoperiod sensitivity coefficient (Holzworth and Hammer, 1996). The vernalization function was modified exponential:

\[
f(V) = a + (1 - a)[1 - \exp(-V_{\text{sens}} \times \Sigma H_{\text{vern}})]
\]

where *a* is the extent of the vernalization effect 0 < *a* < 1, \(\Sigma H_{\text{vern}}\) the accumulated sum of vernalizing hours below a critical temperature and \(V_{\text{sens}}\) is the vernalization sensitivity coefficient. The use of accumulated hourly data is more definitive than summing the number of days at vernalizing temperatures (Sharma and D’Antuono, 2011). To accommodate autonomous flowering, previously reported for *Calandrinia* sp. (Cave et al., 2011b), the function allowed plant development to progress (via the coefficient ‘a’), albeit slowly, when vernalizing temperatures were absent.

Modelling development rate from VFB to anthesis

A weak photoperiodic response for rate of development from VFB to anthesis was observed for *B. australis* and *Calandrinia* sp. Therefore, development rate was modelled as a function of temperature using:

\[
R = R_{\text{opt}} \times f(T)
\]

The temperature function was linear:

\[
f(T) = T_{\text{sens}}(T - T_b)
\]
where $T_{\text{sens}}$ is the temperature sensitivity coefficient (Holzworth and Hammer, 1996).

Parameter estimation

Parameters were estimated for eqns (3)–(5) and (7) using DEVEL2, an optimization program, which employs an iterative process that minimizes sum of squares residual (SSR) between estimated and observed timing (days) for the phenological event (Holzworth and Hammer, 1996). The procedure uses observed dates for VFB and anthesis, daily minimum and maximum temperature, photoperiod and the sum of hours below a critical temperature (vernalization function). DEVEL2 contains nine standard functions, including curvilinear, triple broken linear, exponential and linear for both temperature and photoperiod that the user can select based on the best statistical fit for environmental responses. The time to VFB and anthesis can be predicted by calculating $R$, based on the responses derived from DEVEL2 using daily climate data, and then repeating the process for each calendar day until accumulated $R$ is equal to one (Holzworth and Hammer, 1991). Development rate is defined as the proportional increment in the chosen phenological period (d$^{-1}$). Parameter profiling and jack-knifing are included in DEVEL2 to remove bias from parameter estimates and introduce rigour into the parameter selection process (Holzworth and Hammer, 1996).

RESULTS

Maximum air temperatures during the day ranged between 16 and 43°C and night temperatures were between 0 and 25°C during the 18-month experimental period (Fig. 1). The shortest reported photoperiod was 11.25 h and the longest was 14.75 h (civil twilight was included).

Development rate of Brunonia australis from planting to VFB and VFB to anthesis

Planting dates P11 to P13 were excluded from the model $R = R_{\text{opt}} \times f(T) \times f(P)$ for planting to VFB due to atypical development rates that were more rapid and variable (Fig. 2A, C) and prevented model convergence. A possible explanation could be heat stress, since these plants accumulated about 50–70 h at $\geq 35^\circ$C, whereas most other planting dates received $<15$ h at $\geq 35^\circ$C. With these allowances, a curvilinear function eqn (3) was used to describe temperature responses, while photoperiod responses were best represented by a triple broken linear function eqn (4).

The initial optimization run using DEVEL2 showed the fitted model was over parameterized as the optimization did not converge. An iterative process, which reduced the number of parameters until the parameter profile plots were approximately quadratic (Jones and Carberry, 1994; Soltani...
et al., 2006; Ravikumar et al., 2009), was performed. The process resulted in two out of three parameters of \( f(T) \) and \( f(P) \) being fixed at estimated (biologically plausible) values. Values were estimated by inspecting the rate of development in response to temperature (Fig. 2A). Incorrect values selected by the operator should become obvious during model validation. Cross-validated goodness-of-fit (\( R^2 \) and SSR) and optimized estimates of parameter values for the rate of development model are provided in Table 1. Differences between fitted and observed days for each replicate were biologically small, except for one clear outlier in P5 (Fig. 3A).

Atypical development rates were observed for P10 to P12 in response to temperature and photoperiod (Fig 2B, D) and were excluded from the VFB-to-anthesis model to assist model convergence. The increased development rates of P10 to P12 were possibly caused by heat stress, given that plants accumulated about 85–138 h at \( \geq 35^\circ\text{C} \) from VFB to anthesis, whereas most other planting dates received <24 h at \( \geq 35^\circ\text{C} \). Additionally, one replicate of P3 was excluded due to death of three out of four plants. With these allowances, the rate of

![Graphs showing development rates for different planting dates](image)

**Fig. 2.** Development rates for different planting dates (P3 to P14) of *Brunonia australis*, (A, C) from planting to first visible floral bud (VFB), and (B, D) from VFB to anthesis, in response to average air temperature and photoperiod.

**Table 1.** Optimized parameter estimates and goodness-of-fit (\( R^2 \) and SSR) for the rate of development models from planting to visible floral bud (VFB) and from VFB to anthesis for *Brunonia australis*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Development stage</th>
<th>Planting to VFB</th>
<th>VFB to anthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_{zp} )</td>
<td>0.026 (0.0009)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( T_b )</td>
<td>5.0*</td>
<td>–</td>
<td>10.2 (0.43)</td>
</tr>
<tr>
<td>( T_o )</td>
<td>25.2 (1.59)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( T_m )</td>
<td>30.0*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( T_{sen} )</td>
<td>–</td>
<td>–</td>
<td>0.002 (0.0002)</td>
</tr>
<tr>
<td>( P_{sen} )</td>
<td>0.242 (0.159)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( P_{crit1} )</td>
<td>11.0*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( P_{crit2} )</td>
<td>12.0*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>0.81</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>SSR</td>
<td>0.43</td>
<td>0.48</td>
<td></td>
</tr>
</tbody>
</table>

The number in parentheses following each fitted parameter value is the estimated s.e.

\* Fixed values for which standard errors were not calculated.
development model \( R = R_{\text{opt}} \times f(T) \), which consisted of a linear function (eqn 7) to describe temperature responses, was a good statistical fit (Table 1). Cross-validated parameter estimates for the model are provided in Table 1. Overall, the numbers of days from VFB to anthesis were well fitted, except for one replicate of P14 (Fig. 3B).

**Influence of temperature and photoperiod on plant morphology and flowering of Brunonia australis**

Leaf number at VFB ranged from 8 to 22 \((P < 0.05)\) and increased with increasing average temperature and photoperiod, except for P11 to P13, which were probably affected by heat stress (Table 2). Similarly, leaf appearance rate \((d^{-1})\) from planting to VFB generally increased as temperature and photoperiod increased. The rate of leaf appearance for P3 and P4 was at least 20% greater than that observed for other planting dates. Floral bud number at anthesis was strongly correlated to the number of days from VFB to anthesis \((0.89)\) and branch number \((0.71; \text{Fig. 2B and Table 2})\). Plantings P3 and P4 produced at least 72% more floral buds than other planting dates, which coincided with the lowest average temperature for VFB to anthesis.

**Development rate of Calandrinia sp. from planting to VFB and VFB to anthesis**

Flowering time of *Calandrinia* sp. was strongly influenced by vernalization, whereby summer, early autumn and late spring plantings failed to produce floral buds until the onset of lower temperatures \((\leq 15^\circ C; \text{see paragraph below})\), after which flowering occurred simultaneously. For example, P2 and P3 reached the VFB stage around the same time in early June 2009 (Fig. 1). Likewise, VFB stages for P11 to P14 were recorded mid- to late June 2010. Figure 4A shows the negative linear relationship between temperature and development rate for these plants that reflected the duration from planting to vernalization, which increased sequentially with

### Table 2. The influence of average air temperature (T) and photoperiod (P) on leaf number at first visible floral bud (VFB), leaf appearance rate \((\text{RLA}; \text{d}^{-1})\), and floral bud and branch number at anthesis of the first floret for Brunonia australis

<table>
<thead>
<tr>
<th>Planting</th>
<th>Average T (°C)</th>
<th>Average P (h)</th>
<th>Leaf no. at VFB</th>
<th>RLA (d⁻¹)</th>
<th>Floral bud no.</th>
<th>Branch no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VFB Anthesis</td>
<td>VFB Anthesis</td>
<td>VFB Anthesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>22.0</td>
<td>15.9</td>
<td>12.4</td>
<td>11.4</td>
<td>21.5⁺</td>
<td>0.56⁺</td>
</tr>
<tr>
<td>P4</td>
<td>18.9</td>
<td>15.5</td>
<td>11.9</td>
<td>11.5</td>
<td>18.5⁺</td>
<td>0.43⁻</td>
</tr>
<tr>
<td>P5</td>
<td>15.5</td>
<td>18.4</td>
<td>11.4</td>
<td>12.0</td>
<td>13.5⁻</td>
<td>0.18⁸</td>
</tr>
<tr>
<td>P6</td>
<td>14.9</td>
<td>20.3</td>
<td>11.4</td>
<td>12.4</td>
<td>8.8⁸</td>
<td>0.14⁸</td>
</tr>
<tr>
<td>P7</td>
<td>17.0</td>
<td>21.2</td>
<td>11.7</td>
<td>12.8</td>
<td>8.0⁺</td>
<td>0.14⁺</td>
</tr>
<tr>
<td>P8</td>
<td>20.4</td>
<td>22.8</td>
<td>12.4</td>
<td>13.5</td>
<td>10.7⁺</td>
<td>0.22⁺</td>
</tr>
<tr>
<td>P9</td>
<td>22.2</td>
<td>24.9</td>
<td>13.2</td>
<td>14.1</td>
<td>11.9⁺</td>
<td>0.31⁺</td>
</tr>
<tr>
<td>P10</td>
<td>24.2</td>
<td>27.5</td>
<td>13.9</td>
<td>14.5</td>
<td>12.5⁺</td>
<td>0.34⁺</td>
</tr>
<tr>
<td>P11</td>
<td>26.7</td>
<td>27.4</td>
<td>14.3</td>
<td>14.7</td>
<td>8.5⁻</td>
<td>0.37⁺</td>
</tr>
<tr>
<td>P12</td>
<td>27.1</td>
<td>26.7</td>
<td>14.7</td>
<td>14.2</td>
<td>9.9⁺</td>
<td>0.30⁺</td>
</tr>
<tr>
<td>P13</td>
<td>26.7</td>
<td>25.4</td>
<td>14.0</td>
<td>13.0</td>
<td>12.1⁺</td>
<td>0.39⁺</td>
</tr>
<tr>
<td>P14</td>
<td>25.1</td>
<td>20.0</td>
<td>13.0</td>
<td>11.9</td>
<td>22.2⁺</td>
<td>0.51⁺</td>
</tr>
</tbody>
</table>

Average temperature and photoperiod values for VFB included the period from planting to VFB and values for anthesis were from VFB to anthesis of the first floret.

Values followed by different letters within a column are significantly different \((P < 0.05)\) according to probabilities for all pairwise comparisons.

* Branch number was not recorded during the early stages of the experiment.
planting date until the onset of cold. Data for P10 was omitted from the final analysis due to likely partial vernalization as indicated by a rapid and atypical development rate from VFB to anthesis and a significant reduction in flower bud production (Fig. 4B and Table 3).

When the above allowances and $\Sigma H_{\text{vern}}$ values of $\leq 15\, ^\circ \text{C}$ were employed, initial optimization runs with the model $R = R_{\text{opt}} \times f(T) \times f(V)$ resulted in all parameters ($T_{b\,r}, T_0$, and $T_m$) of the curvilinear temperature function eqn (3) needing to be fixed before stable parameter estimates were obtained. Parameters for the modified exponential vernalization function eqn (5) were not fixed. Value selection for $f(T)$ parameters were based on inspection of development rate in response to temperature (Fig. 4A). When all three parameters of $f(T)$ were fixed, the model provided robust parameter estimates for the development rate model (Table 4). Generally, the difference between fitted and observed days was $< 8 \, \text{d}$ for planting to VFB (Fig. 5A). The same iterative optimization procedure was performed using $\Sigma H_{\text{vern}}$ values of $\leq 14\, , \leq 16\, , \leq 17$ and $\leq 18\, ^\circ \text{C}$, but resultant parameter estimates were less statistically robust compared with the use of $\Sigma H_{\text{vern}}$ at $\leq 15\, ^\circ \text{C}$ or the model failed to converge (data not shown). When the predictability of the model at suboptimal vernalization duration was assessed by including P10, the model predicted flowering dates that were far later than observed, suggesting that flowering time of partially vernalized plants may be poorly predicted.

Planting date P10 was omitted from the VFB-to-anthesis model for reasons explained above. Additionally, P2 and P3 and P11 to P14, which coincided with at least a 4-week delay between planting and the onset of vernalizing temperatures, were excluded because development rates from VFB to anthesis were clearly influenced by vernalization during the planting to VFB phase (Fig. 4B) and prevented model convergence. With the above allowances, development rate from VFB to anthesis was modelled using $R = R_{\text{opt}} \times f(T)$, which included a linear function eqn (7) to explain development rate in response to temperature. Robust parameter estimates were obtained in the first run of DEVEL2 and are provided in Table 4. Fitted and observed days were biologically similar as shown in Fig. 5B.

**Influence of temperature and vernalization on plant morphology and flowering of Calandrinia sp.**

When planting coincided with vernalizing conditions (P5 to P10), leaf number at VFB decreased with increasing vernalization hours until plants had received 644 h at $\leq 15\, ^\circ \text{C}$, after which there was no significant reduction in leaf number following longer durations of vernalization (Table 3). Leaf appearance rate for plants exposed to cold immediately after planting increased rapidly as temperature increased from about 16 to 26 $^\circ \text{C}$ and then stabilized at about 0.5 $\, \text{d}^{-1}$. Photoperiod had little influence on leaf number or appearance rate (data not shown).

Average flower bud number at anthesis was strongly correlated (0.75) with the duration from planting to VFB, which was influenced by vernalization (Fig. 4A and Table 3). When plants were exposed to cold immediately after planting, flower number ranged from 52 to 72 ($P > 0.05$), except for P10 which produced only 27 floral buds. These results may be due to partial vernalization of P10. In contrast, P2 and P11 to P13, for which the onset of cold was delayed by at least 7 weeks, produced about 146 to 171 ($P > 0.05$) floral buds. These plants were about 3-fold greater in diameter (1.2 m; data not shown) than plants that were vernalized shortly (P4) or immediately after planting and which produced fewer flowers. Branch number was variable, but there was no clear relationship between temperature and vernalization. Photoperiod had little influence on flower bud or branch number (data not shown).

**DISCUSSION**

**Influence of temperature and photoperiod or vernalization on development rate**

Temperature affected development rate of both species from planting to anthesis, but different temperature functions were needed to describe development rate responses for different
as expected, the duration to floral bud initiation for B. australis under a 12-h photoperiod when supplementary lighting is required. Additionally, delaying the LD treatment until after the end of juvenility would offer further cost benefits, since a juvenile phase length of 18–22 d was reported for B. australis (Cockshull, 1985). Development rate from planting to VFB for B. australis increased with increasing photoperiod until an upper critical threshold was reached (12 h), after which no further increase in development rate was observed, suggesting a facultative LD requirement. The LD plant, Lobelia × speciosa ‘Compliment Scarlet’, showed similar responses to photoperiod in that the duration from forcing to VFB decreased with increasing photoperiod until a plateau was reached at 14:2 h (Runkle et al., 1999b). Photoperiod influenced the duration from planting to VFB of B. australis, whereas a weak photoperiodic response was observed for VFB to anthesis as observed for Antirrhinum majus (Cockshull, 1985). Development rate from planting to VFB for B. australis increased with increasing photoperiod until an upper critical threshold was reached (12 h), after which no further increase in development rate was observed, suggesting a facultative LD requirement. The LD plant, Lobelia × speciosa ‘Compliment Scarlet’, showed similar responses to photoperiod in that the duration from forcing to VFB decreased with increasing photoperiod until a plateau was reached at 14:2 h (Runkle et al., 1999b). Photoperiod influenced the duration from planting to VFB of B. australis, whereas a weak photoperiodic response was observed for VFB to anthesis as observed for Antirrhinum majus (Cockshull, 1985). 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interaction between temperature and photoperiod was reflected from planting to first visible floral bud (VFB), and (B) from VFB to anthesis for *Calandrinia* sp. The 1:1 line is shown.

**Figure 5.** Fitted versus observed days for the rate of development models, (A) from planting to first visible floral bud (VFB), and (B) from VFB to anthesis for *Calandrinia* sp. The 1:1 line is shown.

Influence of temperature or vernalization on flower number and plant morphology

Flowering and plant morphology was mostly influenced by temperature and vernalization for *B. australis* and *Calandrinia* sp., respectively. Increased branching and flowering of *B. australis* is highly desirable in floriculture species to achieve good pot fill, was observed when the duration from VFB to anthesis increased, which was temperature dependant. However, there were exceptions, which suggest that other factors, apart from temperature, may be involved in determining floral bud number. Overall, floral bud production increased 8-fold following an 11°C decrease in temperature, but coincided with a 4.5-fold increase in the duration for VFB to anthesis. Similar increases in flower number, due to a reduction in temperature, have been observed for some wild-type and cultivated *Petunia* spp. (Mattson and Erwin, 2003; Warner, 2010), and for *Campanula carpatica* ‘Blue Clips’ (Whitman et al., 1997).

Flowering and growth habit of *Calandrinia* sp. reflected the timing of vernalization. When vernalization was delayed by at least 7 weeks, plants produced on average 2.5 times more flowers than those vernalized immediately after planting, which suggested a positive relationship between plant age at time of vernalization and flower number. Yuan et al. (1998a) reported a similar increase in floral bud number when older plants of *R. fulgida* ‘Goldsturm’ and *G. × grandiflora* ‘Goblin’ were vernalized for 10 or 15 weeks, respectively. However, *Calandrinia* sp. plants, which produced more flowers due to delayed vernalization, had a sprawling habit and were visually unattractive and commercially unacceptable. These results indicated that vernalization should be applied within 7 weeks after planting.

**Model applications and limitations**

The development rate models in the present study can be used to generate production schedules and predict flowering time within the experimental data range for temperature and photoperiod. However, it is worth noting that the accuracy of predictions may become less reliable outside the range of data upon which the models were based (Marcelis et al., 1998). In addition to temperature and photoperiod or vernalization, daily light integral could influence plant development of *B. australis* and *Calandrinia* sp. outside the experimental range of 23–54 mol m⁻² d⁻¹. Irradiance has been widely
reported to influence development of many herbaceous ornamental plants (Niu et al., 2001, 2002; Pramuk and Runkle, 2005; Warner and Erwin, 2005; Moccaldi and Runkle, 2007). Therefore, further studies on the critical range for daily light integral of both species may enhance the predictability of the models, particularly at higher latitudes where the daily light integral can be low during winter.

The vernalization function may provide a foundation for future modelling efforts of other ornamental species grown under natural vernalizing conditions. The present study is the first work, to the authors’ knowledge, to model natural vernalization of herbaceous ornamental plants. In addition to crop management, the vernalization function may be useful for conservation efforts when used for screening plant populations to investigate the vernalization ceiling temperature and duration of cold required to promote flowering. This information could be used to assist in the translocation of species due to habitat loss or to identify species and plant communities most at risk from predicted increases in temperature as a result of climate change. For example, the data obtained in the present study for Calandrinia sp. indicated that plants in their natural habitat, in the Gascoyne region of Western Australia, received vernalizing temperatures from late autumn to early spring (Bureau of Meteorology, 2012). However, climate change predictions for Australia suggest that this area is likely to experience some of the highest increases in average temperature (Waterson et al., 2007), which could result in partial vernalization of Calandrinia sp. Unfortunately, the species’ ability to adapt to future warming and reduced vernalization may be limited because there are only two known naturally occurring populations (F. Obbens, Western Australian Herbarium, WA, pers. comm.).

In conclusion, the phenological models for B. australis and Calandrinia sp. described development rate in response to temperature, plus photoperiod or vernalization and may assist future modelling efforts of herbaceous crops grown under controlled and ambient environments. In addition, the vernalization function could provide a foundation for screening plant populations vulnerable to global warming.

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