Quantitative analysis of the thermal requirements for stepwise physical dormancy-break in seeds of the winter annual Geranium carolinianum (Geraniaceae)

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INTRODUCTION

Temperature is the primary factor involved in breaking of physical dormancy (PY) (Taylor, 2005). Depending on the species, PY-break in seeds can take place either in one step or in two steps (Gama-Arachchige et al., 2012). The process of PY-breaking in seeds of certain annual species takes place in two steps controlled by two different temperature (Taylor, 1981, 2005) and/or moisture regimes (Gama-Arachchige et al., 2012). During the first step, PY-seeds become sensitized to dormancy-breaking treatment(s), yet they remain impermeable. During the second step, seeds become permeable upon exposure to the appropriate environmental conditions (Jayasuriya et al., 2008, 2009; Gama-Arachchige et al., 2012).

The concept of thermal time, i.e. the exposure to a temperature above a threshold level for a particular time period, has been successfully applied in determining and comparing the rates of various physiological events in plants and poikilothermal invertebrates (Trudgill et al., 2005). This concept has been employed in describing and quantifying physiological dormancy (PD)-break by after-ripening (Bradford, 2002; Batlla et al., 2009) and single-step PY-break (McDonald, 2000). However, the concept of thermal time has not been used for the explanation of stepwise PY-breaking processes.

Geranium carolinianum is a winter annual weed, native to eastern North America, and is reported to be a naturalized weed in many parts of the world including Australia, China, Great Britain, Japan, Italy and South America. Mature seeds of G. carolinianum exhibit PY and shallow PD (Aedo et al., 1998; Aedo, 2000; Gama-Arachchige et al., 2012).

The water gap region is a morphoanatomically specialized area in the seed or fruit coat in species with PY that opens during imbibition and allows the entry of water into the seed during imbibition. In Geraniaceae, a small opening near the micropyle (hinged valve gap) acts as the water gap (Gama-Arachchige et al., 2010). PY-break in G. carolinianum, a temperature- and time-dependent process, occurs in two temperature-dependent steps. During the first step, seeds become sensitive when stored at temperatures ≥20°C. In the second step, sensitive seeds are made...
permeable when exposed to temperatures ≤ 20 °C (Gama-Arachchige et al., 2012).

On breaking of PY, the water gap region in seeds of *Geranium carolinianum* becomes visible as a brownish orange colour. Application of pressure causes a similar colour change in the palisade cells of the water gap region while making the seeds permeable (Gama-Arachchige et al., 2010).

It has been shown that the pressure that builds up upon heating under the palisade layers of the lens in seeds of *Acacia kempeana* (Hanna, 1984) and under the bulges in seeds of *Ipomoea lacunosa* (Jayasuriya et al., 2008) causes the water gap palisades to pop off, forming the water gap opening(s). However, the colour change in the water gap region of *G. carolinianum* takes place when sensitive seeds are placed at a lower temperature than the sensitivity-inducing temperature. Therefore, a pressure build-up under the water gap palisades in *G. carolinianum* is unlikely.

The objectives of the current study on seeds of *G. carolinianum* were to (1) investigate the role of temperature in driving the two steps of PY-breaking; (2) establish a thermal time (degree-weeks) model to explain sensitivity induction quantitatively; and (3) propose a mechanism to explain PY-breaking, focusing on the water gap region.

**MATERIALS AND METHODS**

**Seed collection and preparation**

Stems of *Geranium carolinianum* bearing mature fruits were collected from plants growing on Spindletop Farm, Lexington, KY, USA, on 1 June 2011. They were covered with a mesh cloth and allowed to dry for 3 d inside a non-heated greenhouse. Seeds released naturally were collected and stored in a refrigerator (approx. 5 °C, dry storage) until used. Experiments were started within 2 weeks of seed collection.

**Step 1: induction of sensitivity**

**Sensitivity induction test.** To calculate the thermal time required for sensitivity induction, seeds were stored dry at constant temperatures of 5, 10, 15, 20, 25, 30, 35 and 40 °C in Petri dishes for 20 weeks. Cool white fluorescent light at 400–700 nm was supplied continuously, at approx. 40 μmol m⁻² s⁻¹.

Due to the lack of visible changes, insensitive seeds cannot be distinguished from sensitive seeds (Gama-Arachchige et al., 2010). Therefore, the ability to imbibe water after exposure to low temperatures (≤ 20 °C) was selected as an indication of sensitivity (Gama-Arachchige et al., 2012). A sample of 100 seeds was retrieved from each storage temperature every week and incubated at 10 °C (under the same light conditions) on moist sand in five replicates of 20 seeds each. The number of imbibed seeds was counted after 2 weeks.

To study the relationship between storage temperature and sensitivity induction, the Arrhenius plot was constructed using sensitivity induction rates 1/Ts0 (where Ts0 = storage time taken for 50% of the seeds to become sensitive) plotted against the reciprocal of storage temperature (1/T).

**Development of the model.** The model was developed based on the assumptions that seed sensitivity induction is irreversible and the base temperature to induce sensitivity is constant for all the sub-populations (Gama-Arachchige et al., 2012).

The induction of sensitivity (step I) was assessed in relation to the accumulation of thermal time. The thermal time units required for induction of sensitivity were calculated using the following function:

\[ \theta_{PY} = (T_s - T_b) \times t_{PY} \]  

where \( \theta_{PY} \) is the thermal time (°Cweeks) to induce sensitivity, \( T_s \) is the temperature at which seeds were stored (°C), \( T_b \) is the base temperature to induce sensitivity (°C) and \( t_{PY} \) is the storage time (weeks).

The base temperature was estimated using the reciprocal of the time required for sensitivity induction (rate of sensitivity induction). The PROBIT procedure in SAS ver. 9.2 was applied to estimate the time required for the induction of sensitivity in sub-populations of 25, 50 and 75% of seeds. The rates of sensitivity induction were plotted against storage temperature and a linear regression model was fitted to estimate the x-intercept for each percentile. The average value of x-intercepts was considered as the base temperature (Steinmaus et al., 2000; Bazin et al., 2011).

To determine the best model that describes the distribution of \( \theta_{PY} \) within a population, Gompertz, Hill, Logistic, Sigmoid and Weibull functions were applied using the global curve fitting option in Sigmaplot ver. 12.0. The best fit was first examined by superimposing the curve on the data points (Motulsky and Ranasnas, 1987). Then, to select the best model, the candidate models were compared with the corrected Akaike Information Criterion (AICc) that considers model complexity and modelling accuracy (Burnham et al., 2011; Symonds and Moussalli, 2011; Eizenberg et al., 2012):

\[ \text{AICc} = 2k + n \left( \ln \left( \frac{\text{RSS}}{n} \right) \right) + \frac{2k(k + 1)}{n - k - 1} \]  

where \( k \) is the number of fitted parameters in the model, \( n \) the number of observations in the model and RSS the residual sum of squares. A lower AICc value indicates better fit of the model to the observed data, with the best approximating model being the one with the lowest AICc value (Symonds and Moussalli, 2011).

The model was evaluated based on root mean square error (RMSE):

\[ \text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} \left( y_{\text{obs}} - y_{\text{pred}} \right)^2} \]  

where \( y_{\text{obs}} \) and \( y_{\text{pred}} \) are the observed and predicted imbibition values, respectively, and \( n \) the number of observations in the model. Smaller RMSE values indicate better fit of the model to the observed data.

**Model validation.** The model validation was performed using the results from sensitivity induction by alternating storage temperatures and non-heated greenhouse experiments.

**Alternating temperatures.** Seeds were stored at alternating temperatures of 15/6, 20/10, 25/15, 30/15, 30/20 and
40/25 °C in Petri dishes. High and low temperatures were supplied on a 12 h/12 h daily basis under light/dark conditions (14/10 h; under the same light conditions as described above). From each storage temperature, a sample of 100 seeds was retrieved every week and incubated at 10 °C on moist sand in five replicates. The number of imbibed seeds was counted after 2 weeks.

Non-heated greenhouse. Twenty seeds each were placed on dry sand in 100 plastic Petri dishes, which were placed on trays filled with potting soil inside a non-heated greenhouse. Air temperature inside the greenhouse was recorded at 30 min intervals using a Thermochron iButton (DS 1921G#F50) and daily average temperatures were calculated. Each week, five Petri dishes were retrieved and the sand was moistened with distilled water. They were incubated at 10 °C under the same light conditions. The number of imbibed seeds was counted after 2 weeks.

The thermal time units required for induction of sensitivity for the alternating temperatures 30/20, 35/20 and 40/25 °C, and non-heated greenhouse experiments were calculated using eqn (4) and for alternating temperatures where the low temperature period is ≤15 °C using eqn (5):

\[ \theta_{\text{PY}} = (T_{\text{avg}} - T_h) \times t_{\text{PY}} \]  
\[ \theta_{\text{PY}} = (T_h - T_h) \times t_{\text{PY}} / 2 \]

where \( \theta_{\text{PY}} \) is the thermal time (°Cweeks) to induce sensitivity, \( T_{\text{avg}} \) the average temperature on seeds were stored/average daily temperature in the greenhouse (°C), \( T_h \) the high temperature period (°C), \( T_b \) the base temperature to induce sensitivity (°C) and \( t_{\text{PY}} \) the storage time (weeks).

Cumulative percentages of sensitive seeds from alternating temperature and greenhouse experiments were plotted against thermal time, and the developed thermal time model (three-parameter Gompertz) was superimposed to compare the actual thermal induction and predicted thermal time by the model. Goodness of fit of the developed model was estimated for each alternating storage temperature and non-heated greenhouse data according to RMSE values.

Step II: breaking of PY

Effect of temperature on PY-break. To determine the effect of temperature on PY-break in step II, seeds were stored at 30 °C in Petri dishes for 4 months to induce sensitivity. An approx. 2 mm layer of moulding clay was spread inside the Petri dish and 20 sensitive seeds without the colour change in the water gap region were embedded so that the water gap was pointing upwards. The open Petri dish was immersed in a temperature-controlled water bath and observed for colour change during early imbibition, the number of seeds with a water gap blister was recorded.

Morphological changes during early imbibition. To observe the morphological changes during early imbibition, 40 untreated seeds were soaked in water for 2 h and the outer permeable layers were removed with a toothpick (Gama-Archchige et al., 2010). They were made permeable by drying at 40 °C for 2 months followed by exposure to 10 °C for 24 h. To observe the morphological changes during early imbibition, seeds were allowed to imbibe water under ambient conditions for 0–20 min. Three seeds each were removed from the water at 2 min intervals for 20 min of imbibition and blotted dry. Three sensitive (impermeable) seeds (outer permeable layers

Determination of separation force. To determine the force required for separation of the water gap palisade cell layer from the sub-palisade layer during the PY-breaking step, 50 seeds made sensitive by storing them at 25 °C for 5 months and 50 insensitive seeds (untreated) were cut transversely into two halves. The halves with the micropyle were glued at the cut surface onto wooden blocks (Supplementary Data Fig. S1). The separation force was measured with a Chatillon® DFM10 penetrometer. A probe with a blunt tip 0·2 mm in diameter was fixed to the penetrometer and the micropyle was touched with the tip of the probe. As the stage of the penetrometer was moved upwards, observations were made under a microscope until a colour change was seen at the micropyle–watergap region, at which time the maximum force reading was recorded.

The effect of external cooling. To determine the internal temperature of seeds upon external cooling, 60 seeds were made sensitive by storing them for 5 months at room temperature (approx. 23 °C). Using a 0·45 mm drill bit, a hole was drilled in each seed up to the sub-palisade layer of the water gap end, starting at the widest point of the seed at the end opposite the water gap. The probe of a type K microthermocouple (0-432 mm width) was inserted into the drill hole of a seed. The water gap end of the seed was placed on the water surface in a temperature-controlled water bath and the internal temperature of the seed was recorded at 1 s intervals for 1 min using LASCAR EL-USB-TC data loggers. The minimum temperature recorded during the 1 min period was used for calculation. The procedure was repeated for 15 seeds each, for 0, 5, 10, 15 and 20 °C. For 0 °C, ice was used instead of water.

Role of moisture in opening of the water gap. To evaluate the role of moisture level in opening of the water gap, permeable (heat-treated) seeds were incubated at different relative humidity (RH) levels. Eight hundred seeds were made permeable by storing them at 30 °C for 5 months followed by exposure to 10 °C for 24 h. Five replicates (20 seeds each) were placed on a wire mesh platform suspended in accelerated ageing plastic boxes filled with 100 mL of saturated salt solutions as follows, to maintain different RH levels: H2O, 100 %; KCl, 83·5 %; NaCl, 75 %; MgCl, 32 %; and LiCl, 11·5 % at 30 °C; NaNO₂, 65 % at 25 °C; Mg(NO₃)₂, 50·5 % at 35 °C; and CaCl, 40 % at 5 °C (Weston et al., 1992; Fang and Moore, 1998; Baalbaki et al., 2009). After 24 h of incubation, the number of seeds with a water gap blister was recorded.

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removed) were used as a control. All the seeds were mounted on scanning electron microscopy specimen stubs using double-sided carbon tapes. Then, the samples were sputter-coated with gold–palladium (15 nm), scanned with an S-3200 Hitachi scanning electron microscope at an acceleration voltage of 5.0 kV and micrographs were taken.

RESULTS

Step I: induction of sensitivity

Sensitivity induction test. Seeds stored at temperatures \( \leq 15^\circ C \) did not become sensitive even after 20 weeks of storage (Fig. 1A; results for storage under 5 and 10\(^\circ C\) not shown). The minimum storage temperature at which seeds became sensitive was 20\(^\circ C\). With increasing storage temperature and time, the fraction of sensitive seeds increased. The time required for 50 % of the seeds to acquire sensitivity decreased exponentially with increasing temperature (\( R^2 = 0.98; \) Fig. 2A).

The Arrhenius plot for sensitivity induction (step I) showed a negative relationship between the rate of sensitivity induction and the reciprocal of storage temperature. Temperature coefficient values (Q\(_{10}\)) were between 3.5 and 2.0 (\( R^2 = 0.97; \) Fig. 2B).

Development of the model. Linear extrapolation of the sensitivity induction rate data of three sub-populations resulted in an average x-intercept of 17.22\(^\circ C\) (Fig. 3). Based on the RMSE values, all the candidate functions strongly fitted with the sensitivity induction data at constant temperatures (Table 1). However, four-parameter Weibull and three-parameter Gompertz functions were the best two models based on AICc values, with 265.002 and 265.388, respectively (Table 1). Therefore, the model with fewer parameters (Gompertz) was selected as the best model due to the ease of interpretation.
of explanation of data:

\[ S(\%) = 100 \times e^{-\left(\frac{x-x_0}{b}\right)} \]  

(6)

where \( S \) is the cumulative percentage of sensitive seeds, \( b \) the rate of increase, \( x \) the thermal time (°C weeks) and \( x_0 \) the lag phase until the induction of sensitivity.

The values of parameters of the best fitted model are \( b = 19.8380 \pm 1.2937 \) and \( x_0 = 54.6846 \pm 0.9600 \) (\( n = 65 \), RMSE = 7.43; Fig. 4A). Therefore, the three-parameter Gompertz model for the sensitivity induction in PY-seeds of \( G. \) carolinianum can be expressed as:

\[ S(\%) = 100 \times e^{-\left(\frac{\theta_{PY} - 54.6846}{19.8380}\right)} \]  

(7)

During the lag phase, sensitivity was not detected in seeds until a thermal time of 24.39 °C weeks was supplied (Fig. 4A). Thereafter, 25, 50 and 75 % of the seeds became sensitive at 48.02, 61.96 and 79.40 °C weeks, respectively.

**Model validation.** Seeds stored at 15/6 and 20/10 °C alternating temperatures did not become sensitive even after 20 weeks of storage (Fig. 1B; results for storage under 15/6 and 20/10 °C not shown). At 25/15 °C, approx. 60 % of seeds were sensitive by 20 weeks while, at all the other storage temperatures, 100 % of seeds were sensitive by 20 weeks.

The developed three-parameter Gompertz model fitted well for the observed values of the sensitivity induction at alternating temperatures, with RMSE values ranging from 4.28 to 16.33 (Fig. 4B; Table 2). Moreover, the fitted model showed good agreement with the non-heated greenhouse data, RMSE = 11.91, and with the 40/25 °C (average summer soil temperature; Gama-Arachchige et al., 2012) data, RMSE = 12.40 (Fig. 4B; Table 2). However, the fitted model slightly overestimated the

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**Table 1. Summary of the model selection statistics for models fitted to sensitivity induction at constant temperature storage**

<table>
<thead>
<tr>
<th>Candidate models</th>
<th>Equation</th>
<th>RSS</th>
<th>RMSE</th>
<th>k</th>
<th>n</th>
<th>AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Gompertz, 3 parameter</td>
<td>( y = a \times \exp\left{-\exp\left(-\left(x-x_0\right)/b\right)\right} )</td>
<td>3473.6894</td>
<td>7.43</td>
<td>3</td>
<td>65</td>
<td>265.002</td>
</tr>
<tr>
<td>2 Sigmoid, 3 parameter</td>
<td>( y = a/[1 + \exp\left(-\left(x-x_0\right)/b\right)] )</td>
<td>3789.4080</td>
<td>7.76</td>
<td>3</td>
<td>65</td>
<td>270.656</td>
</tr>
<tr>
<td>3 Logistic, 3 parameter</td>
<td>( y = a/[1 + \text{abs}(x/x_0) \times b] )</td>
<td>3587.7317</td>
<td>7.55</td>
<td>3</td>
<td>65</td>
<td>267.101</td>
</tr>
<tr>
<td>4 Logistic, 4 parameter</td>
<td>( y = y_0 + a/[1 + \text{abs}(x/x_0) \times b] )</td>
<td>3567.8018</td>
<td>7.59</td>
<td>4</td>
<td>65</td>
<td>269.012</td>
</tr>
<tr>
<td>5 Weibull, 4 parameter</td>
<td>( y = a \times {1 - \exp\left[-\left(\text{abs}(x-x_0 + b \times \ln(2)(1/c)/b)\right)\right}} )</td>
<td>3374.2916</td>
<td>7.38</td>
<td>4</td>
<td>65</td>
<td>265.388</td>
</tr>
</tbody>
</table>

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**Fig. 4.** Cumulative sensitivity induction (%) of seeds of \( G. \) carolinianum as a function of sensitivity induction thermal time (°Cweeks). (A) Symbols represent the observed percentage of sensitive seeds at different constant storage temperatures. The line corresponds to the three-parameter Gompertz model (eqn 7). (B) Validation of the developed thermal time model for sensitivity induction (line) for alternating temperatures and non-heated greenhouse conditions.
sensitivity induction under non-heated greenhouse conditions and 40/25 °C. Also the model slightly underestimated the sensitivity induction under 30/15, 30/20 and 35/20 °C.

**Step II: breaking of PY**

Effect of temperature on PY-break. $T_{50}$ colour (min), the time taken for the colour change (= PY-break) in 50 % of the seed population, decreased exponentially from approx. 62 min at 25 °C to approx. 15 s at 5 °C (Fig. 2A). At temperatures ≥30 °C, none of the seeds indicated a colour change even after 24 h.

The Arrhenius plot for PY-break (step II) showed a positive relationship between the rate of PY-break and the reciprocal of storage temperature with temperature coefficient (Q10) values between 0.02 and 0.1 ($R^2 = 0.98$; Fig. 2B).

**Determination of separation force.** The force required for the separation of the water gap palisade cell layer from the sub-palisade cell layer was significantly reduced from insensitive seeds (2.59 ± 0.07 N) to sensitive seeds (1.60 ± 0.05 N) ($P < 0.05$).

The effect of external cooling. The temperature difference between the water bath and seed interior decreased linearly with increasing water bath temperature (Fig. 5A).

**Role of moisture in opening of the water gap.** The relationship between the storage relative humidity and the fraction of seeds with a water gap blister followed a sigmoidal pattern ($RMSE = 4.32$; Fig. 5B). The water gap blister formed in seeds stored under an RH of >40 %. With increasing RH, the fraction of seeds with a water gap blister increased rapidly, and reached 100 % at 80 % RH.

**Morphological changes during early imbibition.** No difference was observed in the morphology of water gap palisade cells of sensitive (impermeable) and permeable (heat-treated) seeds prior to imbibition (Fig. 6A, B). After 2 min of imbibition, a slight rise began to appear in the water gap palisade layer of permeable seeds and it had risen further by 4 min of imbibition (Fig. 6C). Upon further imbibition, the water gap palisades continued to rise, until a crack was formed in the periphery of the raised area by 8 min (Fig. 6D). On continued imbibition, the raised area detached at the crack while still hinges to the palisades at the micropyle (hinged valve) after 12 min (Fig. 6E). By 20 min, the hinged valve was completely dislodged, revealing the water gap (Fig. 6F).

**DISCUSSION**

Physical dormancy-break in *G. carolinianum* is a moisture-independent, two-step process controlled by temperature (Gama-Arachchige et al., 2012). In the present study, a mathematical model was developed for the induction of sensitivity during step I in PY-breaking in *G. carolinianum* seeds. The time required to attain sensitivity in seeds held under constant temperatures was described well by the developed three-parameter Gompertz model ($RMSE = 7.43$). The developed model was robust enough to predict successfully the acquisition of sensitivity for alternating temperatures and semi-natural non-heated greenhouse conditions ($RMSE = 4.28–16.33$). Thus, the developed model described the thermal requirements for sensitivity induction in each fraction of the seed population (Fig. 4A, B).

The parameter values of the model indicate that induction of sensitivity takes place at temperatures above the base temperature of 17.22 °C, and the higher the temperature above this value, the higher would be the rate of sensitivity induction.
The base temperature for thermal models for dormancy break in PD seeds is assumed to be constant for all seed fractions of a population (Bradford, 2002). Similarly, in this study, the value for the base temperature obtained by extrapolating the rates of sensitivity showed a constant value for all seed fractions (Fig. 3).

Mott et al. (1981) and McDonald (2000) reported that the base temperature for PY break in several tropical and subtropical legume species growing in northern Australia ranged between 40 and 55 °C. The high summer soil temperatures of those sites usually exceed this base temperature, hence a considerable number of seeds becomes permeable during summer and they germinate in the autumn (McDonald, 2000). Similarly, >90% of the G. carolinianum seeds buried at a depth of 2 cm in an open area on the campus of the University of Kentucky became sensitive during the summer of 2011 and germinated in autumn 2011 (Gama-Arachchige et al., 2012). The average summer soil temperature at this location was approx. 28 °C, and therefore all the seeds can become sensitive within approx. 12 weeks during summer. Thus, the formation of a long-term seed seed bank in Lexington is highly unlikely.

FIG. 6. Scanning electron micrographs of the micropylar–water gap region of G. carolinianum seeds without the outer permeable cell layers: (A) sensitive seed (impermeable); (B) seed with colour change in the water gap (permeable); (C) permeable seed soaked in water for 2 min with slightly raised water gap palisades forming a blister; (D) permeable seed soaked in water for 8 min with raised water gap palisades; (E) permeable seed soaked in water for 12 min with raised water gap palisades (hinged valve) still attached at the micropylar end; (F) permeable seed soaked in water for 20 min with water gap opening after the dislodgement of the hinged valve. Abbreviations: Cr, cracks on the palisade layer; Mi, micropyle; Pa, palisade cells; PaL, elongated palisade cells of the water gap; Wpa, water gap palisades; *, sub-palisade cells of the water gap opening after the dislodgement of the hinged valve; **, sub-palisade cells with a smooth outer periclinal cell wall; ***, sub-palisade cells with a corrugated outer periclinal cell wall.

A negative correlation between the reciprocal of storage temperature and the rate of sensitivity induction during step I can be observed in Arrhenius plots (Fig. 2B). In this study, Q10 values for step I ranged between 2.0 and 3.5. Q10 values for chemical processes are generally in the range of 2–3 (Atwell et al., 1999). Therefore, the involvement of a chemical process(es) during the sensitivity induction stage can be inferred in seeds of G. carolinianum. A similar observation (Q10 values 3.4–5.1) has been obtained for PY-break in Medicago arabica (Van Assche and Vandeloek, 2010).

Significant reduction in the force required to separate water gap palisade cells from sub-palisade cells indicates weakening of the bond between these two cell layers during the sensitivity induction step. Zeng et al. (2005) demonstrated that when seeds are exposed to field conditions, PY-break in several legume species is related to loss of lipids in the seed coat. Further, they suggested that the polymeric structure of lipids changes on exposure to high summer temperatures, due to weakening of hydrophobic bonds which increase the thermal degradation of lipids. In G. carolinianum seeds, a similar process can be expected to take place. During step I, weakening of the polymeric lipids in the seed coat loosens the bonding between palisade and sub-palisade layers. This phenomenon may be more prominent in the water gap region, where the connection between the two layers is weak. The thermal requirements to complete the weakening process (sensitivity induction) can be estimated from the model developed in this study. According to the results, weakening of the seed coat takes place at temperatures >17 °C, and approx. 135 °C weeks are required for all the seeds to become sensitive.

Data from the non-heated greenhouse fitted well (RMSE = 11.91) with the developed thermal time model. Therefore, this model is capable of predicting sensitivity induction under semi-natural conditions. However, further field experiments are required to test the application of this thermal time model to predict sensitivity induction under natural conditions.

Step II

A positive correlation was observed for the reciprocal of incubation temperatures and the rate of step II (PY-break) with Q10 values between 0.02 and 0.1. As Q10 values <1.5 indicate purely physical processes (Clearwater et al., 2000), it can be assumed that a physical process is responsible for step II. Based on the results from the present study and Gama-Arachchige et al. (2012), it was observed that the base temperature for PY-break in step II varied with the storage temperature in step I. Therefore, in the present study, no thermal time models were developed for this step. However, further studies should be carried out to evaluate the possibility of developing a thermal time model for this step.

Sensitive G. carolinianum seeds can be made permeable when exposed to temperatures lower than the sensitivity induction temperature (Gama-Arachchige et al., 2012). In the present study, the internal seed temperatures were always higher by several degrees than the external temperature of the seed coat (= temperature of the water bath). This temperature difference can create a tensile stress across the seed coat. Mott (1979) observed that seeds of several species of
**Stylosanthes** became permeable only at the lens when in contact with a high temperature (140–150 °C) metal plate for a short period (15–60 s). He also found that when seeds were made to contact the 145 °C metal plate, the internal temperature of the seeds reached only approx. 100 °C after 60 s. Since the seeds were agitated during the high heat treatment, only a very small portion of them were momentarily heated, while a larger portion remained cooler. This differential temperature might have imposed a considerable mechanical stress on the seed coat, causing the metastable (weak) palisade cells at the lens to fracture.

Based on the previous observations by Taylor (1996a, b) and Jayasuriya et al. (2008), the completion of step II in the PY-break is much faster than step I in *Medicago polymorpha* and *Ipomoea lacunosa*, respectively. A similar pattern was observed in the PY-break of *G. carolinianum*. However, the completion of step II in *G. carolinianum* takes place at a rate much faster than that in other species studied. At 5 °C, only 15 s were required for 50 % of the seed population to complete step II. Therefore, it can be assumed that step I enables the seeds to maintain impermeability and thus survive in adverse conditions while progressing towards sensitivity.
sensitive and permeable) (Fig. 6A, B). Water can enter walls of the palisade cell layer of mature seeds (insensitive, micrometres in depth) can be found in the upper periclinal region has previously been reported in

Opening of the water gap is controlled by the availability of moisture during imbibition (Fig. 5B). A web of cracks (a few micrometres in depth) can be found in the upper periclinal walls of the palisade cell layer of mature seeds (insensitive, sensitive and permeable) (Fig. 6A, B). Water can enter through these cracks, causing the upper part of the palisades to swell. However, the lower part of the palisades cannot expand since the lower periclinal walls of the palisades are tightly bound to sub-palisades throughout the seed coat in sensitive and insensitive seeds. This stops further imbibition. After PY is broken, the water gap palisade cells can continue to swell since they are not connected to the water gap sub-palisade cells (Fig. 8C). This causes the deepening of the cracks in palisades and makes the cells permeable. Subsequently, as imbibition proceeds, the palisades of the whole water gap region swell and bend outward, forming a blister (hinged valve) (Fig. 8D). Then, due to the tension, the water gap palisades separate from sub-palisades along the water gap margin. Eventually, with further swelling, the hinged valve dislodges from the seed coat, revealing the water gap (Fig. 8E, F).

In conclusion, induction of sensitivity by temperature during the first step of PY-break in G. carolinianum can be best explained by the thermal time model using a three-parameter Gompertz model. The developed thermal time model is also able to predict sensitivity induction in G. carolinianum under semi-natural conditions. Differential thermal contraction of the palisade layer in the water gap region may be the reason for the colour change and PY-break. Thus the water gap region acts as a thermal sensor that detects the onset of autumn.

**SUPPLEMENTARY DATA**

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Figure S1: experimental set-up for measurement of the separation force of palisade cells from sub-palisade cells in the water gap in seeds of G. carolinianum. Video. S1: time-lapse video of the colour change pattern in the water gap region of a sensitive seed of G. carolinianum immersed in a temperature-controlled water bath at 10 °C. The photographs were taken at 1 s intervals for 10 min and the video length was compressed to 15 s. The white box demarcates the micropylar water gap region of the seed.

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**LITERATURE CITED**


