Development of a thermal-time model for combinational dormancy release of hairy vetch (*Vicia villosa* ssp. *villosa*)

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Abstract. Seed dormancy could be a factor related to natural reseeding of hairy vetch (*Vicia villosa* ssp. *villosa* Roth.), a winter annual species cultivated for seed, pasture, hay, green manure and cover crop. The presence of combinational dormancy (physical dormancy + physiological dormancy, PY + PD) in hairy vetch was explored by a model using laboratory and field measures. At the stage of natural dispersal, dry seeds of hairy vetch were stored under laboratory conditions at 5, 10, 20 and 30°C ($\pm$2°C) or buried at 5 cm depth in an experimental field. Germination at 5, 8, 10, 15, 20 and 25°C was assessed at regular intervals up to 295 days after harvest. Following the hypothesis of the existence of a combinational dormancy mechanism, model development was based on the estimation of: (*i*) the fraction of non-PY seed as a function of after-ripening thermal-time accumulation, and (*ii*) seed population thermal parameters associated with a given level of PD. The developed model adequately described the after-ripening thermal-time requirements for PY + PD release of *V. villosa*. Based on model predictions, under a semi-arid thermal regime, >45% of vetch seeds shed during the summer season would be able to germinate during early autumn. Thus, the seed-bank size threshold at the end of the first growing season should be >65 seeds m^{-2} in order to reach a minimum stand of 30 plants m^{-2} necessary for a productive pasture.

Additional keywords: annual pasture, arid-semiarid region, dormancy, modelling, *Vicia* spp.

Received 9 December 2013, accepted 1 May 2014, published online 16 June 2014

Introduction

The genus *Vicia* L., a member of the legume tribe Vicieae of the subfamily Papilionoideae (also known as Faboideae) of Fabaceae, comprises 140–190 species, naturally distributed in the continents of Europe, Asia, North and South America, and Africa (Van de Wouw *et al*. 2001; Bryant and Hughes 2011). Several *Vicia* species, generically named 'vetches', are semi-domesticated crops adopted in several agricultural systems around the world. The total area under vetch cultivation is estimated at 1.3 Mha worldwide (Tate and Enneking 2006).

Hairy vetch (*Vicia villosa*) is an incompletely domesticated species cultivated as a winter annual crop. It is the second most important vetch in the world, because of its high forage production and tolerance to biotic and abiotic stresses (Francis *et al*. 1999). Hairy vetch is a recommended cover crop for conservation agriculture (FAO 2013), mainly because it enhances soil nitrogen (N) content by biological fixation, improving soil structure and reducing soil erosion risk (Clark *et al*. 1995; Brandsæter and Netland 1999; Teasdale *et al*. 2004; Sainju and Singh 2008; Anugroho *et al*. 2009; Vanzolini 2011). *Vicia villosa* is a successful, non-native invader in several temperate regions of America, including Argentina (Gunn 1979; Aarssen *et al*. 1986; Renzi 2009). In the semi-arid temperate

region of Argentina, agroecological systems are subjected to low and erratic precipitation regimes (<500 mm per annum) and are characterised by a severe soil erosion risk (Galantini and Rosell 1997). In this context, hairy vetch is recommended to improve the sustainability of such fragile systems, mainly by enhancing biological fixation of N_2 (Renzi 2009). Another important advantage of *V. villosa* adoption in marginal productive areas is associated with its natural high reseeding potential, a desirable agronomic trait that would reduce production costs (Volesky *et al*. 1995; Renzi and Cantamutto 2009).

The ability to regenerate populations naturally from the soil seed-bank was associated with the presence of primary seed dormancy (Renzi and Cantamutto 2009). According to Jones (1928) and Donnelly *et al*. (1972), at the time of natural dispersal, seeds of *V. villosa* show some degree of physical dormancy (PY) caused by a water-impermeable seed coat. In Fabaceae and other families, water impermeability of the seed is acquired through dehydration during seed ripening in the mother plant (Hyde 1954; Rolston 1978; Kucewicz *et al*. 2010; Gama-Arachchige *et al*. 2011). Seeds with PY do not imbibe water even under environmental conditions favourable for germination (Rolston 1978). For *V. villosa*, preliminary work showed that at the time of seed dispersal the proportion of seeds with PY averaged 74%

of the population, being related to low moisture content of mature seed (Renzi and Cantamutto 2009). PY-release involves disruption or dislodgement of 'water-gap' structures, which act as environmental 'signal detectors' for germination (Gama-Arachchige *et al.* 2010).

Some evidence in *V. sativa*, *V. angustifolia*, *V. amoena*, *V. unijuga*, *V. hirsuta* and *V. cracca* indicates that temperature is the primary factor involved in the PY-release mechanism (Van Assche *et al.* 2003; Hu *et al.* 2013). Depending on the species, PY-break can take place in one or two steps (Gama-Arachchige *et al.* 2012). For the latter case, a given environmental factor allows PY-seed to acquire sensitivity to other/s environmental factor/s, which finally trigger the dormancy-breaking process (Gama-Arachchige *et al.* 2013*a*). Recently, a thermal-time model was developed for the winter annual *Geranium carolinianum* in order to account for acquisition of seed sensitivity during the after-ripening process (Gama-Arachchige *et al.* 2012).

The timing and extent of germination might also be regulated by physiological dormancy (PD) at the embryo level (Van Assche and Vandelook 2010). As stated by Baskin and Baskin (2004), these systems could co-exist in the so-called combinational dormancy (PY + PD). In such a case, seed germination does not start immediately after softening or scarification because of the PD component (Hu *et al.* 2013). The combination of a hard seed coat and a dormant embryo was interpreted as a double safety mechanism to prevent early germination after out-of-season rains (Van Assche and Vandelook 2010).

Alleviation of PD is generally associated with a widening of the thermal permissive range for germination (Batlla and Benech-Arnold 2010). For winter annual species in temperate environments, PD is a selective trait that prevents early germination in the summer season (Schütz *et al.* 2002).

Some population-threshold-based models have been developed for describing seed dormancy changes in weed species. For example, for the summer annual *Polygonum aviculare*, Batlla and Benech-Arnold (2004) developed a model to describe primary seed dormancy release based on the accumulation of stratification thermal-time. Chantre *et al.* (2009), working with the winter annual *Lithospermum arvense*, developed an after-ripening thermal-time model for PD release. For the latter case, seed dormancy level in the population was quantified by describing changes in the germination mean maximum temperature and the thermal-time requirements during the after-ripening process.

As previously mentioned, in marginal agroecosystems, such as those of the semi-arid temperate region of Argentina, *V. villosa* reseeding capacity is a desirable trait that would allow production of a valuable forage source at low production costs. Empirical evidence provided by Renzi and Cantamutto (2009) indicates that not all the seeds incorporated to the soil in early summer after seed dispersal (i.e. pod shattering) were capable of germinating and producing new seedlings. In fact, after a summer rain, a fraction of the seed-bank remained hydrated (imbibed) without germinating.

Based on these facts, we hypothesise that *V. villosa* ssp. *villosa* Roth. seed shows combinational dormancy (*sensu* Baskin and Baskin 1998) at the time of natural dispersal in the

field. There is scarce knowledge about seed dormancy-release requirements of *V. villosa*; thus, we aim to study the effect of temperature on the dormancy release process thorough the development of a thermal-time model.

Materials and methods

Plant material

Seeds were collected from a *V. villosa* ssp. *villosa* population at the Agricultural Experimental Station (EEA) of Hilario Ascasubi (39°22′S, 62°39′W), in Buenos Aires province, Argentina.

Seeds were removed by hand from mature pods before natural dispersal (29 December 2009). After harvest, seeds were cleaned by sieving, air-dried under laboratory conditions and stored in paper bags at 7–8% (dry weight basis) of water content. Seed biomass estimated from samples of 50 seeds each was 28 ± 1.7 mg ($n = 8$).

After-ripening treatments

Clean seeds were dry-stored in chambers at 5, 10, 20 and 30°C (± 2°C). Seed germinability was assessed at 0, 15, 70, 105, 182 and 295 days of after-ripening.

Field experiment

Batches of 50 clean seeds were placed inside impermeable nylon bags (10 by 10 cm) then buried in the experimental field of the EEA Hilario Ascasubi at 1 week after manual harvest (6 January 2010). Seed bags were buried at 5 cm depth following a completely randomised experimental design (three replicates) and exhumed at intervals of 40, 60, 140 and 230 days after burial. Field temperature was recorded at 5 cm depth at 2-h intervals using digital temperature data-loggers (Thermochron Ibuttons, Model DS1921G-F50; Maxim Integrated Products Inc., San Jose, CA, USA).

Germination test

Germination tests for seeds after-ripened in the laboratory and exhumed from the field were conducted on a thermo-gradient bar designed according to Chatterton and Kadish (1969). Batches of 50 seeds were incubated at constant temperature regimes of 5, 8, 10, 15, 20 and 25°C. Three replicates (i.e. batches) were used at each incubation temperature. The number of germinated seeds was counted daily over 15 days. A seed was considered germinated when the radical protruded from the seed coat at least 1 mm.

Seed viability test was performed on ungerminated seeds by slicing them longitudinally and further incubating in a 0.1% (wt/vol.) tetrazolium chloride (2,3,5-triphenyltetrazolium chloride) solution for 24 h at 30°C in the dark (International Seed Testing Association 2011). Seeds with pink- or red-stained embryos were considered viable. Germination percentages were calculated for the viable fraction of the seed population.

After 15 days, the total numbers of dead, germinated and ungerminated (imbibed and hard-unimbibed) seeds were counted. When a seed imbibed, there was a visible change in its size : volume ratio; thus, imbibed seeds were easily distinguished from unimbibed ones. Hard-unimbibed seeds represent the fraction of the population with PY.

Dormancy release index

In order to quantify the effect of temperature and after-ripening time on the dormancy-release process in *V. villosa*, an after-ripening thermal-time index (θ_{AT}) was implemented. The accumulation of after-ripening thermal-time units (degree-days) was defined according to the following equation:

$$\theta_{AT} = \sum_{i=1,n} \theta_n \quad \text{if} \quad T_1 < T_n \tag{1a}$$

$$\theta_{AT} = 0 \quad \text{otherwise} \tag{1b}$$

where θ_{AT} is the thermal-time requirement for after-ripening, T_n is the estimated mean daily soil temperature during the after-ripening period, and T_1 is the lowest or base temperature (at or below which after-ripening does not occur).

Physical dormancy release

The proportion of non-PY seeds (imbibed + germinated) for the different after-ripening treatments was evaluated at the end of the germination test to account for the effect of after-ripening thermal-time accumulation on the PY-release process. The fraction of non-PY seeds was plotted as a function of after-ripening time and temperature. An after-ripening thermal-time model for PY-release was developed by fitting a nonlinear function to relate the proportion of non-PY seeds as a function of after-ripening thermal-time accumulation (θ_{ATPY}). The after-ripening base temperature value for PY-release (T_{IPY}) was obtained by an iterative method until maximum fit was obtained between observed and predicted data.

Estimation of population thermal parameters related to physiological dormancy and germination

Population thermal parameters were estimated by implementing the mathematical approach proposed by Washitani (1987). Such a model allows the estimation in the seed population of two types of thermal parameters: (*i*) PD-related parameters, i.e. the lower (T_1) and upper (T_h) temperature limits for germination, which define the amplitude of the permissive germination range; and (*ii*) germination-related parameters, i.e. the cardinal temperatures (T_b, T_o, T_m) and the thermal-time (θ) requirement for germination.

The implemented model is based on the following assumptions (Washitani 1987):

(1) A given seed can germinate within the temperature range delimited by both T_1 and T_h. Such thresholds are assumed to vary in the seed population following a normal distribution. Thus, the fraction of the seed population (F_1) with lower limit temperatures below a given temperature (T) can be calculated as (Eqn 2):

$$F_1(T) = \int_{-\infty}^{(T-\mu_{Tl})/\sigma_{Tl}} (1/\sqrt{2\pi}) \exp\left(-x^2/2\right)dx \tag{2}$$

where μ_{Tl} and σ_{Tl} are the mean and the standard deviation of T_1, and x stands for the expression $[(T-\mu_{Tl})/\sigma_{Tl}]$.

The fraction of the population with a higher limit temperature (F_h) than T is defined as follows (Eqn 3):

$$F_h(T) = \int_{(T-\mu_{Th})/\sigma_{Th}}^{\infty} (1/\sqrt{2\pi}) \exp\left(-x^2/2\right)dx \tag{3}$$

where μ_{Th} and σ_{Th} are the mean and standard deviation of T_h, respectively.

By assuming the distributions T_1 and T_h as mutually independent, the seed population germinable fraction for a given PD level at a given temperature (G_{PD} (T)) was calculated as (Eqn 4):

$$G_{PD}(T) = F_l(T)F_h(T) \tag{4}$$

(2) The temperature dependency of the rate of germination (r) of individual seed can be approximated by a bilinear equation with four parameters (Eqn 5): the base temperature (T_b), optimal (T_o) and maximal or ceiling (T_m) temperatures, and thermal-time requirement for germination (θ).

$$r = (T - T_b)/\theta \ T_b < T < T_o \tag{5a}$$

$$r = [(T_o - T_b)(T_m - T)/(T_m - T_o)]/\theta \ T_o < T < T_m \tag{5b}$$

(3) Cardinal parameters (T_b, T_o and T_m) are assumed to be constant for the different fractions of the population, whereas θ is assumed to be distributed according to Eqn 6:

$$F(\theta_g) = 1 - [3D^{-3}(\theta - \theta_{50} + D_\theta)^3 + 1]^{-1/2} \tag{6}$$

where θ_{50} is the median of the distribution (i.e. the thermal-time requirement from seed imbibition until 50% germination) and D_θ corresponds to the differential thermal-time between 0 and 50% germination (i.e. thermal-time accumulation from the onset of germination until 50% percentile).

(4) Distributions of T_1, T_h and θ within the population are assumed independent of each other.

From the assumptions depicted above, cumulative germination percentages (G) after time (*t*) at constant temperatures (*T*) can be calculated by combining Eqns 2, 3 and 6, as described by Washitani (1987):

$$G(T, t) = F_1(T)F_h(T)F_t((T - T_b)t)$$
$$\text{if} \quad T_b < T < T_o \quad \text{and} \quad (T - T_b)t > \theta_{50} - D_\theta \tag{7a}$$

$$G(T, t) = F_1(T)F_h(T)F_t([(T_m - T)(T_o - T_b)/(T_m - T_o)]t)$$
$$\text{if} \quad T_o < T < T_m \quad \text{and} \quad (T - T_b)t > \theta_{50} - D_\theta \tag{7b}$$

$$G(T, t) = 0 \quad \text{if} \quad T < T_b \quad \text{or} \quad T > T_m$$
$$\text{or} \quad (T - T_b)t < \theta_{50} - D_\theta \tag{7c}$$

Dormancy release and germination model development

Model development is summarised in the following points:

A. Estimate the fraction of non-PY seeds as a function of after-ripening thermal-time accumulation for physical dormancy release (θ_{ATPY}).

B. Determine seed population thermal parameters that maximise the fit between experimentally obtained germination time-course curves and predicted data for

seeds after-ripened in the laboratory at 5, 10, 20 and 30°C for 0, 15, 70, 105, 182 and 295 days. Following the hypothesis of the existence of a combinational dormancy (PY+PD) mechanism, cumulative germination curves for non-PY seed fractions (g) of the population could be obtained by modifying Eqn 7 as:

$$G_{(g)non\text{-}PY} = [(\%non\text{-}PY_{seeds})/100]G(T, t) \quad (8a)$$

where

$$\%non\text{-}PY_{seeds} = f(\theta_{ATPY}) \quad (8b)$$

C. Characterise changes in the seed population thermal parameters over time as a function of storage temperature, and derive thermal-time equations relating rate of change of these parameters to the after-ripening thermal-time. Germination curves obtained by implementing Eqn 8a would allow the estimation of PD-related population parameters (i.e. each non-PY seed fraction of the population would be associated with a specific set of thermal parameters according to a given PD status). Thus, after thermal requirements for PY loss of a given population fraction is achieved according to Eqn 8b, such a fraction should have accumulated a given amount of after-ripening thermal-time for PD release (θ_{ATPD}) in order to allow germination. Thus, cumulative germination of the seed population at a given after-ripening time, according to its combinational dormancy status, could be represented by Eqn 9:

$$G_{(\theta\,AT)} = f(\theta_{ATPY})f(\theta_{ATPD})G(T, t) \quad (9)$$

where $f(\theta_{ATPD})$ is the PD-release function associated with specific dormancy-related population thermal parameters. The basal temperature for PD-release (T_{lPD}) was calculated by maximising the fit of the derived thermal-time equations.

Experimental cumulative germination curves obtained from the different after-ripening treatments were simulated according to Eqn 9. Optimal population thermal parameters for germination were obtained by a non-linear least-squares curve-fitting method using the Levenberg–Marquardt optimisation algorithm (Premium Solver Platform 7.0; Frontline Systems Inc., Incline Village, NV, USA).

Model evaluation

Model evaluation was performed on independent data obtained from the soil seed-bank experiment. By using thermal-time equations derived above (see *Dormancy release and germination model development*, C), dormancy-related population parameters were estimated for seed exhumed from the field at different time intervals. Germination time-course curves were simulated using the above-mentioned parameters and further compared with experimentally obtained independent data. Predicted values of the above-mentioned parameters were used to simulate germination time-course curves and compare with experimentally obtained independent data.

Results

Physical dormancy release model

As observed in Fig. 1, the PY-release process for seed after-ripened under laboratory conditions was adequately described by a second-order polynomial model. The T_{lPY} was estimated to be 9.8°C. Thus, θ_{AT} accumulation for PY-release was possible at storage temperatures ≥ 10°C. After harvest, the proportion of non-PY seed was estimated to be 30%; thereafter as θ_{ATPY} was accumulated, the total amount of non-PY seeds reached 90% of the population after 295 days of dry storage at 30°C.

Estimation of seed population thermal parameters and PD-release model

A noticeable increment in the upper limit temperature for germination ($T_{h(50)}$) was observed as after-ripening time and temperature increased (Table 1). The increment in $T_{h(50)}$ was described as a function of θ_{ATPD} accumulation as observed in Fig. 2. Despite the fact that σ_{Th} values showed considerable variation among treatments, no statistically significant trends
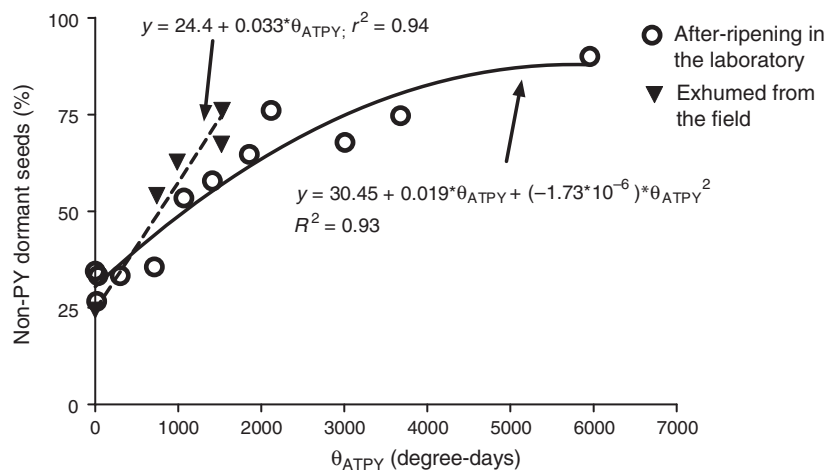

Fig. 1. Percentage of non-physically dormant seeds of *Vicia villosa* as a function of after-ripening thermal-time accumulation for PY release (θ_{ATPY}) at constant temperatures in the laboratory (—) and for field exhumed seeds (- - -).

Table 1.　Estimated population thermal parameters for *V. villosa* seeds incubated in the laboratory at constant temperatures of 5, 8, 10, 15, 20 and 25°C at 0, 15, 70, 105, 182 or 295 days of after-ripening

$T_{l(50)}$ and $T_{h(50)}$, Mean lower and upper limit values (°C) for germination, respectively; σ_{Tl} and σ_{Th} are corresponding standard deviations; θ_{50}, mean thermal-time requirement (degree-days) for germination; D_θ, deviation parameter; CV, coefficient of variation. –, Estimation of population thermal parameters was not possible due to very low germination values associated with absence of after-ripening (storage temperature $< T_{lPY}$, basal temperature for PY-release). † Missing data of after-ripening treatment

| After-ripening time (days)	Storage temp. (°C)	$T_{l(50)}$	σ_{Tl}	$T_{h(50)}$	σ_{Th}	θ_{50}	D_θ	RMSE
0 (recently matured seed)		2.2	0.5	11.9	2.2	30.0	30.0	24.1
15	5	–	–	–	–	–	–	–
	10	2.2	0.5	11.9	1.7	30.0	30.0	31.4
	20	2.2	0.5	13.1	2.0	30.0	30.0	36.7
	30	2.2	0.5	11.9	2.5	30.0	30.0	50.3
70	5	–	–	–	–	–	–	–
	10	†	†	†	†	†	†	†
	20	2.2	0.5	14.8	5.6	25.0	17.5	65.7
	30	2.2	0.5	16.0	5.1	28.0	16.0	43.5
105	5	–	–	–	–	–	–	–
	10	2.2	0.5	11.9	2.8	30.0	23.0	27.1
	20	2.2	0.5	15.6	2.8	25.0	19.4	39.5
	30	2.2	0.5	16.1	3.1	28.0	19.8	48.8
182	5	–	–	–	–	–	–	–
	10	2.2	0.5	16.9	1.6	29.7	25.0	30.8
	20	2.2	0.5	19.4	0.5	25.0	10.0	57.5
	30	2.2	0.5	18.6	0.8	28.4	8.1	44.7
295	5	–	–	–	–	–	–	–
	10	2.2	0.5	17.0	3.9	29.1	11.5	30.8
	20	2.2	0.5	21.2	0.1	25.0	13.0	45.5
	30	2.2	0.5	23.1	0.9	26.7	6.3	45.2
CV		0.23	2.88	22.14	67.33	7.52	43.77	
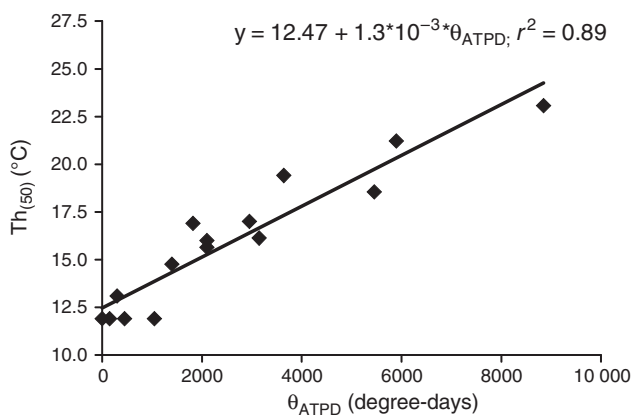
Fig. 2. Upper limit temperature for germination as a function of after-ripening thermal-time for physiological dormancy release (θ_{ATPD}) accumulation for seeds after-ripened at constant temperatures under laboratory conditions. A basal temperature for PD release (T_{lPD}) value of 0°C was used for θ_{ATPD} calculation.

were obtained when regressed as a function of after-ripening time ($P = 0.19$) or storage temperature ($P = 0.99$). As observed in Table 1, $T_{l(50)}$ and σ_{Tl} values remained almost constant during the after-ripening process. Mean thermal-time values for germination (θ_{50}) showed a slight variation among treatments,

although not related to either to time of storage ($P = 0.11$) or after-ripening temperature ($P = 0.40$). Conversely, the deviation parameter (D_θ) was significantly influenced by after-ripening time ($P < 0.01$; $r^2 = 0.66$). Changes in D_θ values were adequately described by a negative exponential function of θ_{ATPD} accumulation (Fig. 3). The T_{lPD} was estimated to be 0°C.

Model evaluation

Germination curves obtained from seeds exhumed from the field at different time intervals were used to evaluate the model performance based on both PY-release and expected changes in $T_{h(50)}$ and D_θ values. Specifically, the polynomial model presented in Fig. 1 was used for estimating non-PY seed fractions as a function of θ_{ATPY} accumulation in the field from seed burial date until exhumation date. The mean daily temperature at 5 cm depth was used for θ_{AT} calculation. In addition, changes in population thermal parameters (i.e. $T_{h(50)}$ and D_θ) were estimated by implementing the equations presented in Figs 2 and 3, respectively. After-ripening thermal-time accumulation for PD-release (θ_{ATPD}) was calculated as the daily sum of the difference between mean temperature and the T_{lPD}, which was previously estimated to be 0°C.

As observed in Fig. 4, model predictions largely underestimated observed data (Fig. 4*a, d, f, j, l, m, o*), except for some combinations of exhumation dates and incubation
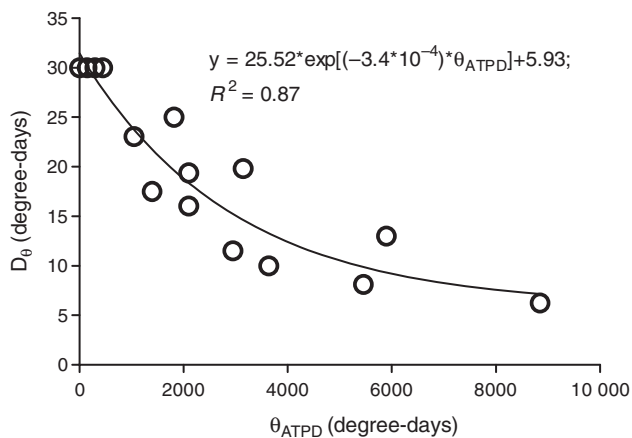
Fig. 3. Differential thermal-time between 0 and 50% germination (D_θ) as a function of after-ripening thermal-time for physiological dormancy release (θ_{ATPD}) accumulation for seeds after-ripened at constant temperatures under laboratory conditions. A basal temperature for PD-release (T_{IPD}) value of 0°C was used for θ_{ATPD} calculation.

temperatures were obtained RMSE values were rather acceptable (Fig. 4*i, k, n, r–t*).

Based on these results, the percentage of non-PY seeds at each exhumation date was plotted as a function of after-ripening thermal-time accumulation. A linear model was fitted to the available dataset to describe the PY-release process under field conditions (Fig. 1). As shown in Fig. 1, by comparing both PY-release functions, a higher rate of PY-release was observed under field conditions (0.033% non-PY seeds per θ_{ATPY} unit) than for seed after-ripening under constant temperatures in the laboratory (0.019% non-PY seeds per θ_{ATPY} unit). Thus, in order to evaluate the general model performance based on a more representative estimation of PY-release under field environmental conditions, the polynomial model was replaced by the linear model presented in Fig. 1.

Results of simulated germination curves assuming a linear relation between the percentage of non-PY seeds and θ_{ATPY} are presented in Fig. 4. As observed by comparing RMSE values between seed after-ripened in the field and under constant temperature regimes, significant (Fig. 4*c, g, l, o, p*) to moderate (Fig. 4*b, d, f, i, j, m, n*) reductions in the predictive error were obtained. Despite the improvement in the general model outcome obtained by the above-mentioned adjustment of the PY-release function, simulated germination curves considerably underestimate observed data in some cases (Fig. 4*a, d, m*).

Discussion

We confirmed our working hypothesis that *V. villosa* ssp. *villosa* presents a double mechanism for regulating dormancy release. In the present work, an after-ripening thermal-time model for combinational dormancy release (PY+PD) was developed. The model consisted of a sub-model for describing PY-release as a function of an after-ripening thermal-time index (θ_{ATPY}) combined with the description of changes in the PD status of *V. villosa* associated with specific thermal parameters ($T_{h(50)}$ and D_θ).

Our results indicate that PY-release in *V. villosa* ssp. *villosa* did not take place at temperatures <10°C ($T_{IPY} = 9.8°C$). However, PY-release was promoted at increasing storage temperatures as after-ripening time progressed (Fig. 1). These results agree with previous reports showing the positive effect of after-ripening temperature on PY-release in winter annual vetches (Van Assche *et al*. 2003; Van Assche and Vandelook 2010; Hu *et al*. 2013). The mechanisms to sense environmental signals by water-gap structures (i.e. the water-gap complex) differ between species (Gama-Arachchige *et al*. 2013*b*). PY-release in several legume species is associated with loss or changes of lipids in the seed coat due to weakening of hydrophobic bonds, which increases the thermal degradation of lipids due to exposure to high summer temperatures (Gama-Arachchige *et al*. 2013*a*).

The rate of PY-release of *V. villosa* ssp. *villosa* was clearly influenced by the after-ripening environment. The higher rate of PY-release observed under field conditions suggests that thermal fluctuations of the soil environment should have promoted seed-coat breakage. These observations were further confirmed with data obtained from a previous experiment with *V. villosa* ssp. *villosa* accessions buried in the field under natural environmental conditions. A significantly better fit (RMSE = 0.09) was obtained between observed data and the estimated amount of non-PY seeds when the linear PY-release model was implemented than with the after-ripening laboratory-based model (RMSE = 0.18). Similarly, overestimations of PY rupture models (step 1, induction of sensitivity) were also observed under alternating temperatures (30/15°, 30/20° and 35/20°C) compared with seed stored at constant temperatures in *Geranium carolinianum* (Gama-Arachchige *et al*. 2013*a*).

In many hard-seeded Fabaceae, once PY is overcome (i.e. seed coat becomes permeable), seeds are capable of germinating over a wide range of temperatures under both light and dark conditions (Baskin and Baskin 1998; Hu *et al*. 2013). For *V. villosa*, a PD component regulating the amplitude of environmental conditions permissive for germination could be devised. A noticeable increment in the upper limit temperature ($T_{h(50)}$) for germination was observed as after-ripening thermal-time was accumulated (Table 1; Fig. 2), thus indicating an increment in the permissive thermal range for germination. In addition, an exponential reduction in the deviation of the thermal-time requirement for germination was also registered as θ_{ATPD} increased (Table 1; Fig. 3), indicating a more synchronous germination as after-ripening thermal-time for PD-release progressed. A widening in the thermal range permissive for germination and a reduction in thermal-time requirements were also observed during after-ripening in the winter annual species *Lithospermum arvense* L. (Chantre *et al*. 2009). Similar results were obtained in other winter annual species that require an after-ripening period for physiological dormancy loss (Foley 1994; Bauer *et al*. 1998; Bair *et al*. 2006).

From a natural reseeding perspective, the alleviation of dormancy (PY + PD) of *V. villosa* ssp. *villosa* seeds, which are naturally dispersed in late spring and early summer, occurs by exposure to high summer temperatures during a relatively short after-ripening period (<3 months). Based on our model predictions, a 5% increment in the mean soil temperature during field after-ripening would increase the germination of <5% of
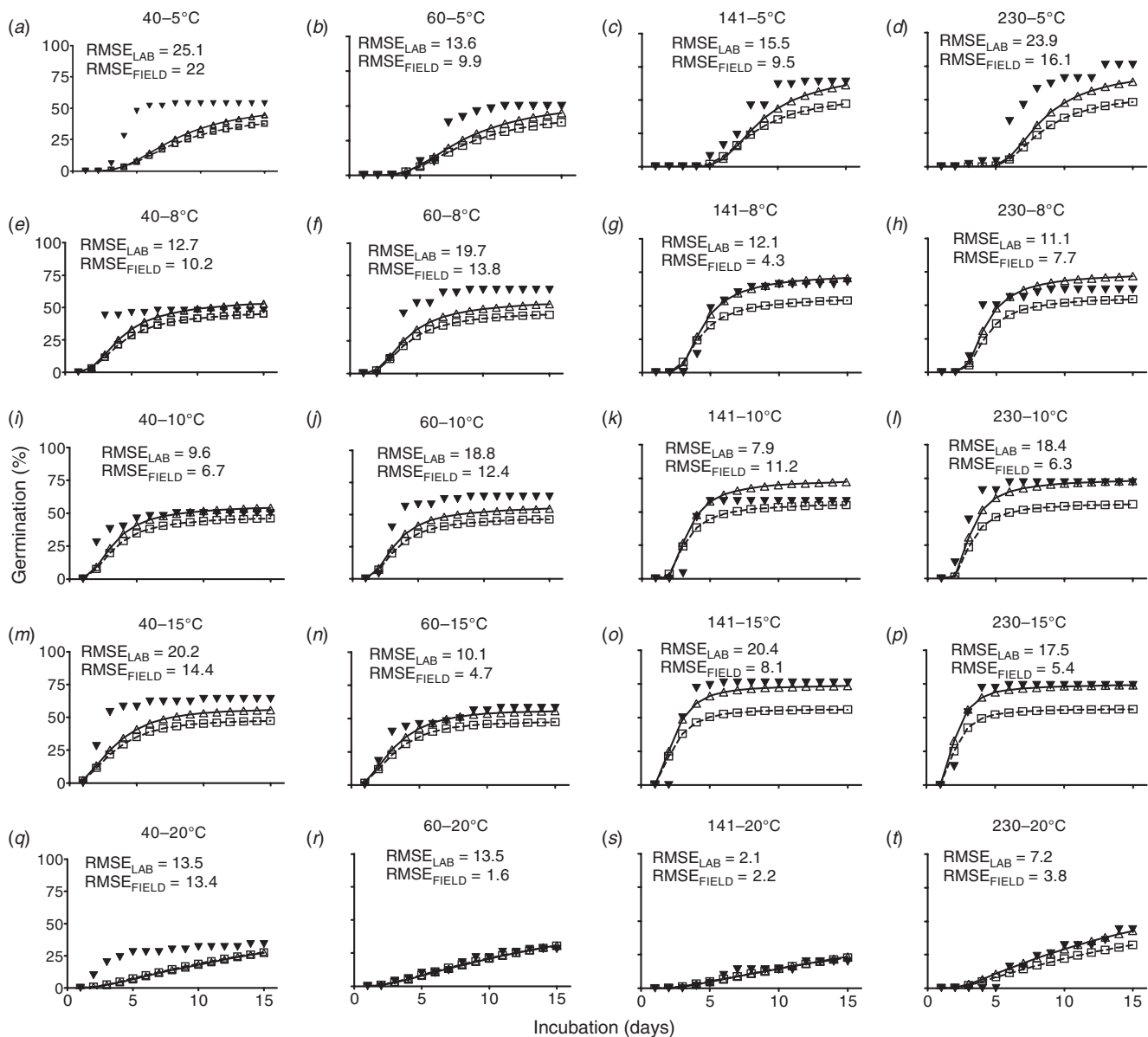
Fig. 4. Model evaluation performed on *V. villosa* seeds exhumed from a field experiment at different time intervals. Seeds were buried in nylon bags for 40, 60, 141 and 230 days and further incubated at 5, 8, 10, 15, 20 and 25°C for 15 days. Observed germination values (▼) as well as models predictions based on physical dormancy (PY) release estimates for seed after-ripened in the laboratory (□) and field exhumed seed (△). Germination time-course curves with cumulative values <5% are not shown.

non-PY seeds, thus producing a small change in the potential density of the plant stand. During summer, *V. villosa* seeds are progressively released from dormancy and emerged seedlings would increase at the beginning of the field germination 'time window' in early autumn. This is mainly due to the widening in the germination-permissive thermal range as previously mentioned. Hence, the seedling stand obtained by reseeding should be mainly determined by soil seed-bank size rather than by the effect of temperature variability in the field among the summer seasons.

Based on our results, we infer that for the semi-arid temperate region under study, early germination of *V. villosa* ssp. *villosa*

during summer would be prevented after 'out-of-season' rains by the combinational dormancy mechanism: an impermeable seed coat (i.e. PY) and PD. Combinational dormancy seems to have evolved in Mediterranean climates, where cool and wet winters are safe times for seedlings to survive (Van Assche and Vandelook 2010). Combinational dormancy may also act by reducing environmental risks associated with habitat uncertainty, by forming soil seed-banks that persist over several years (Baskin and Baskin 1998). During the field burial experiment in this study, a considerable proportion of seeds exposed to the after-ripening treatment during the summer of 2010 became water-permeable; therefore, most of

the seed-bank would be able to germinate during the next autumn if soil moisture was not a restrictive factor for germination. The average soil temperature in summer at this location was ~25°C, and the proportion of non-PY seeds by mid-autumn averaged 75% of the population, with an estimated upper limit temperature for germination of 16.5 ± 2°C and an average season temperature of 13.4°C. Therefore, under such conditions, it would be expected that *V. villosa* seeds would remain viable for no more than a couple of years. However, according to Warwich (2011) and Crockett *et al.* (2012), seed-bank persistence of *V. villosa* seems to be >2 years, with a fast release of dormancy within the first 6 months. According to our results, we might infer a type III persistent seed-bank for *V. villosa* ssp. *villosa* (*sensu* Baskin and Baskin 1998). In the type III seed-bank, many seeds germinate soon after dispersal, but a small reserve of viable seed remains ungerminated. However, this behaviour might only be confirmed on seeds exhumed from the field during a more extended burial period (after 230 days of burial); thus, this remains a hypothesis to be tested in future work.

In the present work, an after-ripening thermal-time model for combinational dormancy release of *V. villosa* seed was developed. The model was capable of predicting the fraction of the seed population that will germinate according to its dormancy status. The model performance for predicting *V. villosa* germination patterns was acceptable, especially when the rate of PY-release was explicitly considered for field-exhumed seeds.

According to Siddique and Loss (1996) and Renzi (2009), a minimum stand of 30 plants m^{-2} would be necessary to obtain a productive vetch pasture. Based on our model predictions, at the onset of the field emergence period (i.e. early autumn), >45% of vetch seeds shed during the previous summer would be able to germinate under a semi-arid thermal regime. Thus, the seed-bank size threshold at the end of the first growing season should be >65 seeds m^{-2} (~2 g seeds m^{-2}). The developed model suggests that for semi-arid systems, farmers could rely upon natural reseeding, with the proviso that the soil seed-bank has been replenished above the recommended threshold.

Further research is needed to improve the present model within the context of a vetch–cereal system. Predicting the influence of seed burial depth, soil water content fluctuation and surface residue cover on *V. villosa* seedling emergence would be necessary to develop decision-management tools. Enhancing the capacity of *V. villosa* to self-regenerate their populations in fragile ecosystems becomes an important issue for conservation agriculture (Taylor *et al.* 1991).

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