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Effect of water availability and seed source on physical dormancy break of *Vicia villosa* ssp. *villosa*

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Abstract

Physical dormancy (PY) plays a crucial role in the control of the reseeding process of *Vicia villosa* Roth, a winter annual species cultivated for pasture and hay, naturalized in several semi-arid temperate agroecosystems. As PY is considered a seed trait modulated by natural selection, populations from different origins are expected to show different responses to environmental regulatory factors. The present study aimed to determine the effect of: (1) water availability on PY-break dynamics of a naturalized population from Argentina (ASC) under both laboratory and field conditions; (2) the seed source on initial PY and dormancy release rate (wet storage at 20°C) of ASC compared to 45 other populations of *V. villosa*, including wild, naturalized, landraces and cultivars. Water availability increased PY loss rate under both storage and field conditions. ASC PY-break dynamics was adequately described by a Gompertz model with a lower thermal-time requirement estimated for dormancy break under fluctuating soil water conditions compared to seeds buried inside impermeable bags. During the field burial experiment, a considerable proportion of seeds ($\sim$70%) became water permeable during the summer season after dispersal, and retained low levels of residual PY for soil seed bank replenishment. Improved populations (i.e. breeding cultivars) showed the lowest percentages of initial PY compared to landraces, naturalized and wild populations. Naturalized populations of Argentina showed similar initial PY compared to landraces, although PY release rate was lower in the former and might be attributed to local environmental selection. Wild types showed the lowest PY release rates.

Keywords: physical dormancy, semi-arid environment, thermal-time, *Vicia villosa* genotypes, winter annual

Introduction

Physical dormancy (PY) is imposed by both physical and chemical impediments to water movement across the seed coat, caused by the presence of phenolic compounds and suberin layers impregnating the palisade cells (Baskin *et al*., 2000). PY is considered one of the main traits responsible for the control of germination of several angiosperms, including the largest, and agronomically valuable, legume family Fabaceae (de Souza *et al*., 2012). Barriers to water movement across the seed are acquired during the end of the seed ripening phase on the mother plant (Hyde, 1954; Rolston, 1978; Kucewicz *et al*., 2010; Gama-Arachchige *et al*., 2011). Under natural conditions, such seeds, usually termed 'hard seeds', remain under PY until the seed coat turns permeable by the action of environmental factors (Hu *et al*., 2009). The specific site of water entry through the seed coat is still a matter of debate. Some legume species have developed specialized structures such as strophioles or lenses. The morphological characteristics of these 'water gaps' can vary between species (Gama-Arachchige *et al*., 2013), but are usually associated with natural openings of the seed, maintained up to the end of the seed development phase (i.e. hilum, micropyle or chalaza) (Smýkal *et al*., 2014).

A number of mechanisms have been proposed for PY break under field conditions, including high and diurnally fluctuating temperatures, relatively short periods of extreme dry heat (e.g. fire exposure) or alternating soil wetting/drying cycles (Hu *et al*., 2009; Santana *et al*., 2010). However, it is not yet well understood how germination is regulated in ecosystems with a temperate climate, where fire occurs very rarely and daily soil temperature fluctuations are rather limited (Van Assche *et al*., 2003). Seeds with PY might overcome their dormant status after mechanical abrasion by soil particles, microbiological decomposition of the seed coat, or seed coat degradation caused by partial or complete consumption by herbivores. However,

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published evidence for these alternative mechanisms of scarification is scarce (Baskin and Baskin, 2014).

Vicia villosa is a cosmopolitan, non-native invader present in several temperate regions of America, where it was introduced intentionally as a forage crop (Gunn, 1979; Aarssen *et al*., 1986). The inclusion of *V. villosa* in agricultural rotations has a crucial economic value in semi-arid ecosystems, where the relatively high cost of nitrogen (N) chemical fertilization affects grain crop yield and quality substantially (Ates *et al*., 2013; Renzi and Cantamutto, 2013). The biological N fixation ability of *V. villosa* as well as its natural reseeding potential are valuable agronomic traits that could contribute to agroecosystem sustainability in semi-arid regions (Renzi and Cantamutto, 2013).

V. villosa has been known to expand from cultivated areas to form spontaneous populations, such as in winter cereals, orchards, pastures and forestry fields (Aarssen *et al*., 1986). It is also found as a ruderal species along roadsides, disturbed areas and fallow fields. Under natural conditions, a healthy stand could generate over 1000 seeds m^{-2}. However, the dispersal potential of *V. villosa* is very limited due its large seed size (length ≈ 4.2 mm, weight ≈ 35 mg $seed^{-1}$) and absence of effective dispersal mechanisms (Jannink *et al*., 1997; Renzi and Cantamutto, 2013).

Seed dormancy of *V. villosa* is controlled by a combination of physical impediments for water movement across the seed coat (PY) and a physiological component of dormancy (PD) (Renzi *et al*., 2014). Embryos overcome PD and become non-PD after a short afterripening period. Thus, PY could be considered the main component of seed dormancy of *V. villosa*. The combination of a hard seed coat and a dormant embryo is considered as a 'double-safety' mechanism to prevent early germination during summer when soil water content and temperature are not adequate for seedling survival (Van Assche and Vandelook, 2010; Gama-Arachchige *et al*., 2013). Under natural conditions, *V. villosa* pods shatter and disperse the seeds during early summer, fulfilling their thermal-time requirements for PY break by the end of the season. Emergence flushes occur in the following autumn when soil temperature and moisture become favourable for seedling recruitment.

Under laboratory test rules (ISTA, 2011), a prechilling procedure is recommended to improve germination of species with PY dormancy, such as *Lotus, Medicago, Lupinus, Melilotus, Trifolium* and *Vicia*. However, this procedure is not of general applicability. Within each genus, species may have different responses caused by adaptive ecological traits (Grime *et al*., 1981; Van Assche *et al*., 2003; Hu *et al*., 2013). Seed stratification after seed maturity is cited as a successful PY-break treatment for *V. grandiflora* Scop., *V. cracca* L., *V. hirsuta* L. (Gray), *V. bythinica* L. and *V. ervilia* L. Willd. (Elkins *et al*., 1966; Van Assche *et al*., 2003; Samarah *et al*., 2003, 2004; Kucewicz *et al*., 2010; Basaran *et al*., 2012; Uzun *et al*., 2013). Conversely, PY break of *V. villosa* generally occurs by exposure to high temperatures (i.e. afterripening) (Jones, 1928; Renzi *et al*., 2014). Interspecific variability in response might be a result of life form (perennial, winter or summer annual) or adaptation to a local environment (Grime *et al*., 1981; Hu *et al*., 2009).

Temperature and water availability are the main environmental factors regulating PY break (Baskin *et al*., 2000). High moisture availability could be an important environmental cue to promote water permeability of some PY seeds. However, the effect of moisture availability as a dormancy breaking factor has not received much attention (Baskin and Baskin, 2014). In the semi-arid temperate area of Argentina, precipitation regimes are erratic. Thus, inter- and intra-annual variability of soil water content might be an important factor regulating the soil seed bank dynamics of *V. villosa* populations.

The mechanism and environmental factors associated with PY break under natural conditions are poorly understood (Hu *et al*., 2009). It is unknown what the potential relevance of this seed trait is for wild, naturalized or improved populations. As PY could be considered a trait developed under natural selection, populations from different origins should show different responses to environmental regulatory factors. To address this knowledge gap, the present study aimed to determine the effect of: (1) water availability on PY-break dynamics of a naturalized population from Argentina (ASC) under both laboratory and field conditions; (2) the seed source on initial PY and dormancy release rate of ASC compared to 45 other populations of *V. villosa,* including wild, naturalized, landraces and cultivars.

Materials and methods

Hilario Ascasubi population (ASC) seed collection

A naturalized population of *V. villosa* from the EEA Hilario Ascasubi (Buenos Aires, Argentina) was selected as the seed source during three growing seasons (December 2008, 2009 and 2011). ASC was located in a sandy loam soil (pH = 7.5, soil organic matter = 1.2%, available P Bray & Kurtz = 24 mg kg^{-1}) along an internal road margin of a plot cultivated with winter cereals [wheat (*Triticum aestivum*) and rye (*Secale cereale*)]. The predominant climate in this habitat is semi-arid temperate with 489 mm mean annual precipitation and 14.8°C mean annual temperature (EEA H. Ascasubi, 1966–2014; http://inta.gob.ar/documentos/informes-meteorologicos).

Post-harvest PY breaking (only for ASC)

Dry and wet storage

Batches of 50 seeds ($n = 3$) were dry-stored (inside paper bags) at 20 ± 2°C. The proportion of PY seeds was assessed (see the description of the imbibition test in the Data analysis section, below) after 0, 15, 70, 105, 182 and 295 d of storage (DOS) in 2010. For wet conditions, batches of 100 seeds ($n = 3$) were incubated at the same temperature in 9-cm-diameter Petri dishes over two layers of filter paper saturated with distilled water. The time course for PY break under wet storage was registered during 2010 and it was compared with dry storage for the same period. Imbibed seeds (i.e. non-PY seeds) were counted after 15, 70, 105, 182 and 295 DOS.

Field burial inside permeable and impermeable bags

During 2009/10, 2010 and 2012/15 periods, at the onset of the summer season after natural seed dispersal (i.e. January 2009, 2010 and 2012), batches of 100 seeds were placed inside permeable nylon mesh bags (10 × 10 cm) in order to create natural soil conditions (diffusion of air and water, micro-organisms, etc.) and were buried in the field at depth 5 cm in a bare-soil site (without vegetation or litter). The burial site was near the natural habitat of ASC (<100 m). Seeds were exhumed after 0, 30, 78, 122, 157, 184, 333 and 421 days of burial (DOB) in 2009/10; after 0, 40, 60, 140 and 230 DOB during 2010; and after 84, 174, 272, 357, 453, 459, 543, 628, 719 and 747 DOB during 2012/15. During 2010, a set of seeds was buried inside impermeable nylon bags (102 microns) and further exhumed at the same time intervals. A completely randomized factorial design was used during 2009/10 ($n = 6$) and 2012/15 ($n = 3$) periods. Field temperature was recorded at seed burial depth every 2 h using digital temperature data loggers (Thermochron Ibuttons, Model DS1921G-F50; Maxim Integrated Products, Inc., San Jose, California, USA). Data obtained from permeable bags were used to develop a thermal-time model.

Thermal-time requirements for PY break under field conditions

In order to quantify the effect of the afterripening time and temperature on *V. villosa* physical dormancy break process under natural soil conditions (i.e. permeable bags), an afterripening thermal-time index (θ_{ATPY}) was implemented. The accumulation of afterripening thermal-time was calculated according to Renzi *et al.* (2014):

$$\theta_{ATPY} = \sum_{i=1,n} \theta_n$$

where

$$\theta_n = \begin{cases} T_i - T_b & \text{if} \quad T_b < T_i \\ 0 & \text{otherwise} \end{cases}$$

θ_{ATPY} is the afterripening thermal-time at day n, T_i is the estimated mean daily soil temperature during the afterripening time period, and T_b is the base temperature, estimated as 9.8°C (Renzi *et al.*, 2014) below which afterripening does not occur.

The fraction of non-PY seeds (i.e. population fraction without PY) was plotted as a function of burial time and temperature. A thermal-time model for PY break was developed by fitting a non-linear function to relate the proportion of non-PY seeds as a function of thermal-time accumulation (θ_{ATPY}). A non-linear fitting routine was performed using the Levenberg–Marquardt algorithm with GraphPad Prism Software version 6.0 (GraphPad, San Diego, California, USA).

Seed collection sources

In order to determine the representativeness of the ASC seed collection, it was contrasted with other seed sources, including 45 spontaneous and cultivated accessions from Argentina and Europe. Germplasm from Argentina consisted of 17 naturalized populations collected during December 2013 from a representative plant sample ($n > 30$). A sample of Argentinian landraces ($n = 11$) maintained by farmers was also included (Fig. 1). Wild and cultivated germplasm of *V. villosa* from Europe was represented by 18 populations. Matured seeds of three wild populations of *V. villosa* were collected by J.P.R. in August 2013 from plants growing in natural habitats of Serbia, in Radenka II (21°46′E, 44°34′N), Petrovo Selo (22°24′E, 44°38′N) and Senokos (22°54′E, 43°08′N). The country of origin and accession number of each cultivar, provided by the Research Institute of Crop Production (CRI) of the Czech Republic, are shown in Table 1 (for more information see http://genbank.vurv.cz/genetic/resources/).

Geographic altitude and climatic conditions of collected populations from Argentina and Europe are detailed in Table 2. The mean temperature of the hottest and coolest months and the average annual rainfall during growing seasons (nearest locality) were obtained from de Fina (1992) and www.worldclimate.com. The growing season for *V. villosa* was defined as from March to November for Argentinian populations and from November to July for wild populations from Serbia.

Seed production for comparative PY experiments

Seeds from each population source (Tables 1 and 2) were cultivated at the Experimental Agricultural

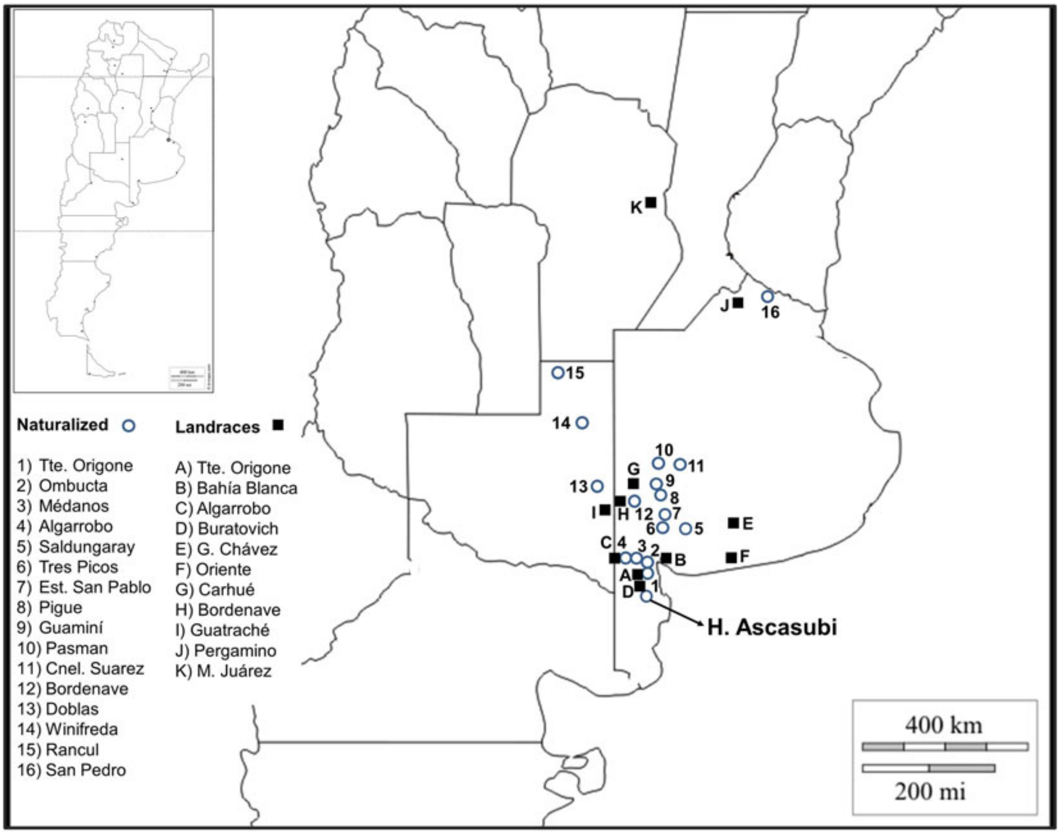

Figure 1. Seed collection sites of naturalized (circles) and landrace (squares) *Vicia villosa* populations from Argentina.

Station (EEA) of Hilario Ascasubi (62°37′W, 39°23′S), located in the south of the semi-arid temperate region of Argentina, during the 2014 growing season. The soil of the experimental site was an entic haplustoll, sandy loam, slightly alkaline (pH 7.6), high in phosphorus (P) content (19.4 ppm P Bray & Kurtz) and low organic matter content (1.84%). A common garden was established in an experimental plot (2 m^2) which was sown on 10 April 2014, and consisted of ≈40 plants per accession ($n = 3$). Seeds from mature pods (brown colour) were harvested manually on 18 December 2014.

Table 1. Passport information of the investigated European cultivar accessions

Country of origin	Accession		Year of inclusion into collection*
	Number	Name	
Hungary	05L1700047	Simabuekkoeny	1967
Yugoslavia	05L1700036	Sarajevo	1966
Poland	05L1700015	Sielecka	1961
Australia	05L1700046	617 81	1966
Bulgaria	05L1700059	266 99	1969
Czechoslovakia	05L1700075	Modra	1987
	05L1700071	Viola	1981
German Democratic Republic	05L1700066	Oregon	1974
	05L1700032	Welta	1964
Germany	05L1700062	Polyp	1973
Poland	05L1700067	Rea	1976
	05L1700068	Rod MPI	1976
Union of Soviet Socialist Republics	05L1700070	Pridesnjanskaja	1973
	05L1700061	Stavcanka	1970
	05L1700054	Stenskaja 24	1969

*EVIGEZ: Plant Genetic Resources Documentation of the Czech Republic.

Table 2. Macro-environmental conditions of the naturalized, landrace and wild populations of *Vicia villosa*

Locality	Status	Altitude (m asl)	Mean temperature (°C)		Rainfall (mm)	
			Hottest month	Coldest month	Year	Growing season
H. Ascasubi (ASC)	Naturalized	16	22.6	7.2	443	308
Saldungaray		213	20.7	6.4	643	463
Bordenave		217	22.6	6.5	603	424
Doblas		150	24.1	7.2	542	366
Winifreda		163	24.5	7.7	535	351
Rancul		246	24.9	8.1	664	395
San Pedro		36	23.4	9.8	960	689
Tte. Origone		14	22.5	7.2	466	335
Ombucta		19	22.5	7.2	476	347
Tres Picos		243	21.0	6.1	587	424
Est. San Pablo		357	21.0	6.1	615	442
Pigue		274	21.5	6.1	680	471
Guamini		116	23.8	7.3	674	475
Pasman		165	22.6	6.8	699	485
Cnel. Suárez		225	21.5	6.3	697	482
Médanos		42	22.9	7.2	474	348
Algarrobo		47	23.3	7.1	454	326
Bahia Blanca	Landraces	22	23.4	7.6	585	404
Pergamino		71	23.3	9.2	944	640
Guatrache		136	23.5	6.7	580	379
M. Juárez		144	23.7	9.0	841	534
Bordenave		217	22.6	6.5	603	424
Carhue		120	23.8	7.2	632	419
Oriente		33	20.2	7.8	700	532
Buratovich		12	22.5	7.2	453	319
Tte. Origone		14	22.5	7.2	466	335
G. Chavez		205	20.7	7.0	717	505
Algarrobo		47	23.3	7.1	454	326
Radenka II.	Wild	490	21.0	0.0	621	496
Petrovo Selo		484	21.5	−0.5	731	584
Senokos		781	19.0	−0.5	863	662

Data analysis

After harvesting from the common garden, seeds were cleaned, and seed biomass and moisture content were estimated. Seed biomass was determined for a sample of 100 individuals (Table 3). Moisture content was determined by weighing a batch of 100 seeds, which were then oven-dried at 105°C for 24 h before being weighed again.

PY seeds (i.e. 'hard' or impermeable) were determined by an imbibition test performed at 20 ± 2°C for 3 d (Baskin and Baskin, 2014). Intact and ungerminated seeds of each replicate were placed on moist filter paper in Petri dishes and watered daily with tap water. Imbibed seeds showed a visible change in their size/volume ratio, and were easily distinguished from unimbibed ones. The seed coat colour was always lighter for imbibed seeds than for unimbibed ones (Jones, 1928).

Seed viability was assessed for ungerminated seeds by slicing them longitudinally and immersing them in a 0.5% tetrazolium chloride (2,3,5-triphenyltetrazolium chloride) solution for 24 h at 30°C in the dark (ISTA, 2011). Seeds with pink or red stained embryos were considered viable. The total number of viable seeds consisted of germinated + stained.

For all populations, cumulative percentages of PY break (i.e. non-PY seeds = germinated + imbibed seeds) were analysed by linear regression. Initial PY (%) (19 December 2014) and PY-break dynamics as a function of storage time (38 d) and temperature (20°C) under wet conditions were analysed by analysis of variance (ANOVA) (LSD test) and linear regression analysis using GraphPad Prism Software version 6.0 (GraphPad). Differences were considered significant at P value < 0.05 when compared to ASC. Accessions were grouped by status and were compared by

Table 3. Seed weight and initial PY (mean and standard deviation), PY-release rate (wet-20°C for 38 d) and viability of *Vicia villosa* accessions compared to ASC (H. Ascasubi population), and mean for each status

Accessions of *V. villosa*		Seed weight (mg)	Physical dormancy					Seed viability (%)
			Initial			Release		
Locality/Acc. number	Status		(%)	SD	P value^2	slope (a)^1	P value^2	
H. Ascasubi (ASC)	Naturalized	**27.6**	**80.0**	**6.9**	–	−0.68	–	99
Saldungaray		29.8	85.3	6.1	ns	−0.93	ns	97
Bordenave		24.7	78.7	6.1	ns	−1.75	**	95
Doblas		30.4	84.0	6.9	ns	−1.21	ns	100
Winifreda		26.4	86.7	8.3	ns	−0.99	ns	97
Rancul		31.5	83.3	0.0	ns	−0.78	ns	100
San Pedro		22.8	70.7	8.3	ns	−1.13	**	99
Tte. Origone		32.3	88.0	0.0	ns	−0.54	ns	100
Ombucta		27.1	77.3	2.3	ns	−0.75	ns	100
Tres Picos		26.3	78.7	6.1	ns	−1.13	ns	100
Est. San Pablo		31.7	87.0	8.2	ns	−0.50	ns	100
Pigue		26.3	85.3	4.6	ns	−1.08	*	99
Guaminí		30.2	92.0	10.6	ns	−1.30	ns	100
Pasman		39.5	89.4	5.3	ns	−1.39	*	100
Cnel. Suarez		35.9	90.7	2.3	ns	−1.17	ns	100
Médanos		32.4	82.7	8.3	ns	−0.80	ns	95
Algarrobo		37.4	94.4	5.6	ns	−1.13	ns	100
Mean		30.1a	84.4b			−1.02b		99a
Bahía Blanca	Landraces	42.0	84.0	10.6	ns	−1.46	*	99
Pergamino		29.9	87.7	11.2	ns	−0.85	ns	100
Guatraché		27.1	72.0	13.9	ns	−1.48	*	92
M. Juárez		41.4	68.8	6.3	ns	−1.28	**	98
Bordenave		31.2	80.5	13.6	ns	−1.90	**	100
Carhué		29.4	73.3	2.3	ns	−1.19	**	97
Oriente		35.0	90.9	4.6	ns	−1.55	*	100
Buratovich		32.4	89.3	8.3	ns	−1.64	**	100
Tte. Origone		35.5	89.3	2.3	ns	−1.25	**	100
G. Chavez		27.8	86.7	2.3	ns	−0.62	ns	97
Algarrobo		30.0	85.3	2.3	ns	−1.41	**	99
Mean		32.9a	82.5b			−1.33a		98a
Radenka II.	Wild	10.3	97.0	2.7	*	−0.37	*	100
Petrovo Selo		16.1	100.0	0.0	**	-0.12^{ns}	–	100
Senokos		14.2	95.3	4.6	*	−0.48	ns	83*
Mean		13.5b	97.4a			−0.32c		94a
05L1700047	Cultivar	30.8	85.3	10.1	ns	-0.38^{ns}	–	100
05L1700036		32.4	57.3	10.1	*	−0.90	ns	92
05L1700015		30.1	58.7	12.2	ns	−1.13	ns	99
05L1700046		36.4	34.7	12.9	**	−0.79	ns	100
05L1700059		38.7	72.0	6.9	ns	−0.99	ns	100
05L1700075		37.2	21.3	10.1	**	-0.31^{ns}	–	95
05L1700071		33.3	61.3	2.3	**	−1.11	ns	100
05L1700066		30.1	32.0	4.0	**	−0.64	ns	96
05L1700032		31.1	46.7	4.6	**	−0.88	ns	92
05L1700062		21.8	84.0	16.0	ns	−1.37	ns	96
05L1700067		32.5	39.3	4.0	**	−0.78	ns	100
05L1700068		35.8	64.0	10.6	ns	−1.04	*	99
05L1700070		33.9	62.7	6.1	*	−1.04	ns	92
05L1700061		31.4	54.1	20.3	ns	−1.10	ns	99
05L1700054		39.2	41.3	2.3	**	−0.81	ns	99
Mean		33.0a	54.3c			−0.88b		97a

[1] PY (%) = a*days + b (wet storage at 20°C; 38 d).

[2] P value among accessions when compared to ASC (Hilario Ascasubi population).

*,** Significant at the 0.05 and 0.01 probability level, respectively.

For mean values, different letters (a, b and c) indicate significant differences ($P < 0.05$) among status.

LSMEANS for seed biomass, initial PY and PY release traits (INFOSTAT Software; Di Rienzo *et al.*, 2013).

Results

Post-harvest PY breaking (ASC)

No interaction was observed among storage/burial condition × time ($P > 0.05$) on PY-break dynamics during the evaluated period. A faster rate of PY loss was observed under wet storage compared to dry storage (Fig. 2A). PY break was also faster for seeds buried in the field inside permeable bags exposed to thermal and soil water content fluctuations (Fig. 2B).

PY-break dynamics under field burial conditions were adequately described by a Gompertz function (Fig. 3). θ_{ATPY} values to achieve 50% of non-PY seeds was 685°Cd for field burial inside permeable bags.

Seed production for comparative PY experiments

Rainfall during the 2014 growing season in EEA Hilario Ascasubi (62°37′W, 39°23′S) was within the range of conditions for Argentinian *V. villosa* populations and also the Radenka II wild population (Fig. 4). Although the coldest monthly temperature and rainfall from the original sites in Petrovo Selo and Senokos for wild populations were lower and higher, respectively, than those recorded in EEA Hilario Ascasubi, such environmental conditions did not affect pod setting or seed maturation.

Initial PY and dormancy release rate

At harvest, the seed moisture content of all *V. villosa* accessions was less than 14%. Seed viability was over 92% in all cases. Only the wild population Senokos showed lower viability than ASC ($F_{1,4} = 18.01$; $P < 0.01$). The initial PY of ASC showed no statistical differences from all naturalized populations from Argentina (Table 3). Conversely, wild populations showed higher PY values than ASC, while 61% of the foreign cultivar showed lower PY figures compared to ASC (Table 3). PY-break dynamics of all populations showed a linear decrease rate under wet/20°C storage, except for the cultivars 05L1700047 and 05L1700075, and the wild population of Petrovo Selo. The ASC PY-release rate showed no differences compared to 75 and 80% of the naturalized and improved cultivars, respectively. However, ASC showed a lower dormancy release rate compared to 81% of landraces (Table 3).

The initial PY was highest for wild populations, showing intermediate values in naturalized and landraces from Argentina, and the lowest percentages in breeding cultivars ($F_{3,134} = 56.5$; $P < 0.001$). PY-release rates under wet storage at 20°C were landraces > naturalized = cultivar > wild genotype ($F_{3,134} = 17.8$; $P < 0.001$) (Table 3).

Discussion

Our work demonstrates that humidity fluctuations during seed ageing interact with temperature to promote PY break. A similar response had been observed in other legumes (Santana *et al.*, 2010). The interactions between PY-seed mechanisms and soil conditions determine whether, and what fraction of, the seed bank will germinate at a given time. PY-break dynamics under burial conditions in the field was adequately described by a Gompertz function (Fig. 3). This equation arises from self-regulated models where PY-release rate decreases exponentially with thermal-time after reaching the tipping point. This curve was

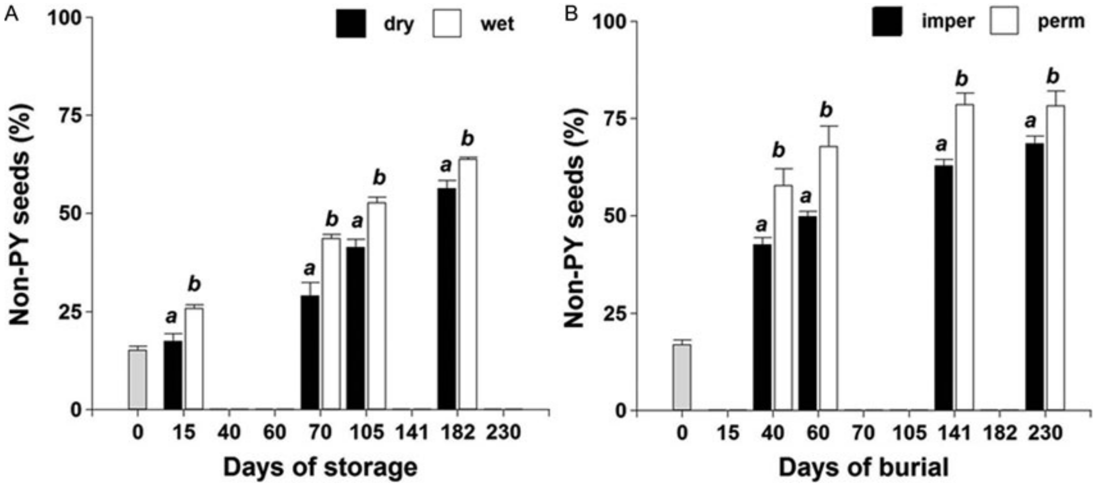

Figure 2. PY-break dynamics of the ASC *Vicia villosa* population under dry and wet storage at 20°C in the laboratory (A); or buried in the field inside permeable (perm) or impermeable (imper) bags (B).

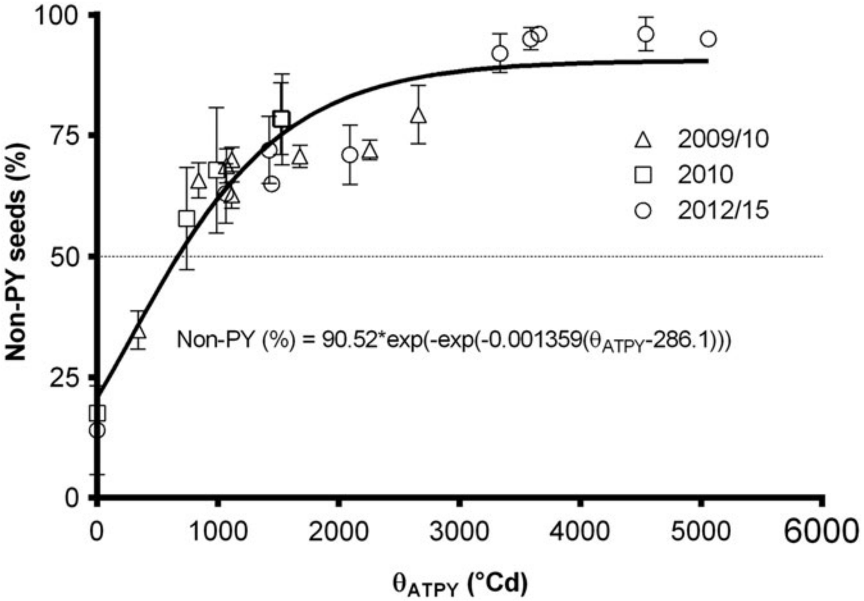

Figure 3. PY-break dynamics of *Vicia villosa* seeds as a function of thermal-time accumulation (θ_{ATPY}) under conditions of field burial inside permeable bags in 2009/10, 2010 and 2012/2015.

applied successfully to describe field emergence of different legumes in Australia (Probert, 2000; van Klinken and Flack, 2005).

Water availability influenced thermal-time requirements for dormancy break in the ASC population. Seed exposure to a continuous wet storage condition, as well as a fluctuating soil moisture environment in the field, promoted PY break (Fig. 2). As reported by Jones (1928), high relative humidity promotes seedcoat breakage of *V. villosa*. The effect of humidity on PY break was also highlighted by Jayasuriya *et al.* (2008) and van Klinken *et al.* (2008). Weather and soil physical characteristics largely determine the microclimate to which seeds are exposed (Smýkal *et al.*, 2014). This has important implications under natural conditions. According to the hypothesis of van Klinken and Goulier (2013), species with PY would have habitat-specific dormancy-release mechanisms. *V. villosa* genotypes with larger seeds might require substantially more time to imbibe prior to germination (J.L. Ekpo, pers. comm.). This adaptive disadvantage would be a trade-off for their ability to emerge from deeper soil layers. In sandy-loam soils it was observed that *V. villosa* could emerge effectively from a depth of 15 cm (Renzi and Cantamutto, 2013). The promoting effect of seed burial under semi-arid conditions could be related to higher moisture levels and lower temperature fluctuations (Owens *et al.*, 1995). This could

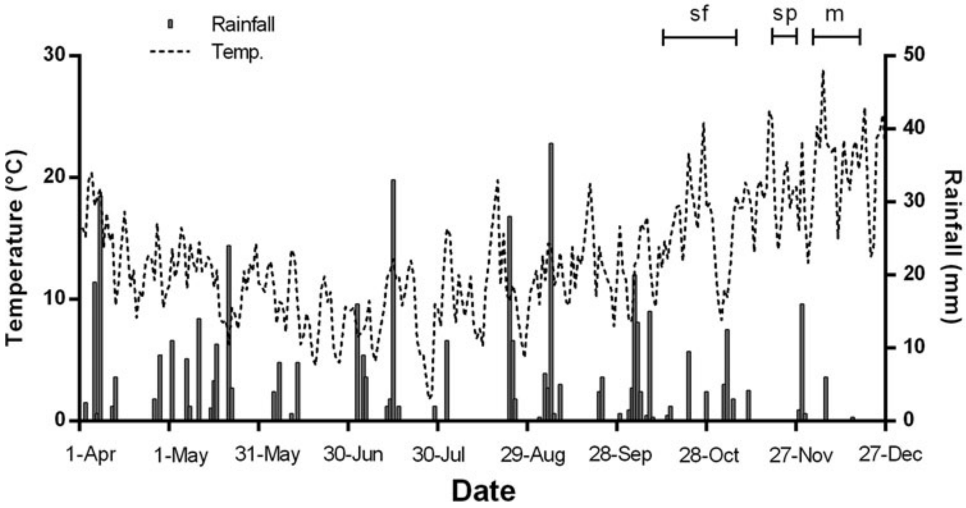

Figure 4. Mean daily temperatures and rainfall at EEA Hilario Ascasubi during the 2014 growing season. Phenological development of *Vicia villosa*: sf, start of flowering; sp, start of podding; m, seed maturity.

explain why PY release occurs at a faster rate under wet field conditions, being less affected by soil temperature fluctuations. In addition, *V. villosa* has no specific light requirement for germination, hence being able to germinate from deep burial (J.L. Ekpo, pers. comm.). Thus, from an ecological perspective, it is expected that naturalized populations of *V. villosa* could establish in anthropogenic soil-disturbed areas.

Initial PY values of ASC did not differ significantly from those of other natural populations from Argentina. Thus, ASC might be useful as a model to study PY of *V. villosa* from Argentina. However, sensitivity of dormancy-release mechanisms can also differ among species (van Klinken and Goulier, 2013). Genetic differences in seeds of *Trifolium subterraneum* and *Medicago polymorpha* influenced percentages and rates of PY break (Baskin and Baskin, 2014). Similarly, Hu *et al*. (2009) observed contrasting PY-release patterns between two populations of *Sophora alopecuroides*. The rates of PY release for Argentinian genotypes under laboratory wet conditions showed significant differences compared to ASC in 25 and 82% of naturalized and landrace populations, respectively (Table 3). Moreover, 65% of the foreign accessions of *V. villosa* differed in their initial values of PY compared to ASC, but only three genotypes (20%) differed in the rate of dormancy release (Table 3). It is probable that accession differences could be explained by genetic adaptations of *V. villosa* to the local environment (Baskin and Baskin, 2014), considering habitat-specific dormancy-release mechanisms (van Klinken and Goulier, 2013), or by selection for improved genotypes (Fuller and Allaby, 2009; Kluyver *et al*., 2013). Such selection traits are linked to successful early growth of planted seeds and include dormancy loss and seed size increase (Smýkal *et al*., 2014). More-improved genotypes, such as the cultivars, showed lower initial PY figures (54.3 ± 17%) compared to landraces (82.5 ± 9.8%), naturalized (84.4 ± 7.9%) and wild accessions (97.4 ± 2.6%) (Table 3). Such a high level of PY after seed dispersal is typical of wild progenitors of cultivated legumes (Fuller and Allaby, 2009). In addition, the rate of PY release for wild types was the lowest (0.32 ± 0.04) of all studied groups. Interestingly, regression slopes were significantly different, emphasizing genotype PY differences at an intra-specific level. While naturalized populations of Argentina showed a similar initial PY compared to landraces, the rate of PY release was lower, and might be attributed to local environmental selection. This study showed that wild genotypes of *V. villosa* have a small seed weight and the response to wet–20°C conditions was less effective in breaking dormancy compared to naturalized, landrace or cultivar genotypes. Our results showed that wild genotypes with low seed weight might require additional environmental conditions to promote PY-loss rate. This could be related to low temperatures, which have been suggested as an effective method for breaking PY in previous studies (Dunn, 1939; cited by Baskin and Baskin, 2014).

In conclusion, results obtained in this study could be used for the development of models to predict physical dormancy changes of *V. villosa* populations, and could be applied to increase our understanding of the naturalization process and the self-regeneration capacity of such populations in fragile agroecosystems of Argentina.

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Conflicts of interest

None.

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