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RESEARCH ARTICLE

Seed dormancy and hybridization effect of the invasive species, *Helianthus annuus*

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Abstract

Helianthus annuus is an invasive alien species naturalised in the central region of Argentina where it shares an extended area with the sunflower crop. As this species has also invaded several other sunflower crop growing areas in the world, it severely restricts the use of new technologies, for example herbicide tolerance by genetic modification. The natural seed dormancy of the wild *Helianthus* strains from the centre of origin in North America is well known, but the seed dormancy of the invasive biotypes is still unknown. Dormancy is a fitness trait related to the establishment, dispersion and persistence of invasive weeds. Four experiments were designed to investigate the effect of the pericarp, light, temperature, the after-ripening period and hybridization with the DK3880CL sunflower crop (F1) on the seed dormancy of five invasive *H. annuus* biotypes. The results showed that pericarp scarification increased imbibition of the whole achene by 19%. Light stimulation only increased germination in the wild biotype without any effect on the domesticated sunflower. A period of 12 months after-ripening at 5°C reduced seed dormancy in the wild biotype and its progeny; the optimal temperature for seed germination at this period was found to be 15°C. Mechanical scarification was the best treatment for overcoming seed dormancy with a differential germination, in the biotypes with the highest response, superior to 63%. Hybridization with domesticated sunflower had a minimal or no effect on seed dormancy but the germination rate was improved in three F1 crosses. Wild biotype dormancy appears to be governed by the maternal pericarp and intrinsic hormone regulation. An increased germination rate of some progenies could constitute an advantage during seedling establishment but only in winters without any frost.

Introduction

Seed dormancy – an internal condition that impedes embryo growth even under optimal conditions of water availability, temperature and atmosphere – plays a crucial ecological role in several plant species (Baskin & Baskin, 1998; Benech-Arnold *et al.*, 2000; Bazin *et al.*, 2011). It is an evolutionary trait that adjusts the time of germination to increase the probability of seedling survival in a given habitat (Li & Foley, 1997). During the colonisation of new habitats, strong selection for dormancy could influence the evolutionary trajectories of other adaptive traits (Huang *et al.*, 2010; Chiang *et al.*, 2013).

During seed filling, the abscisic acid concentration prevents germination of the embryo in the sunflower mother plant (Le Page-Degivry & Garello, 1992). In contemporary cultivars this physiological dormancy may only last for a short time after ripening stage, and it is gradually lost within 2 months after harvest maturity. This primary dormancy is an agronomic constraint when off-season nurseries wish to accelerate sunflower breeding. Dormancy could be broken more rapidly if fresh seeds of sunflower crop were treated with ethylene (Fick, 1978). On the other hand, seed dormancy of wild *Helianthus annuus* L. strains is more complex and may persist for more than a year (Seiler, 1998).

Physiological dormancy can be modulated by the concentration of abscisic and gibberellic acids in the embryo or pericarp (Baskin & Baskin, 1998). Also, dormancy may be controlled by the sensitivity of the embryo to these hormones (Finch-Savage & Leubner-Metzger, 2006).

Seed coat permeability might also control dormancy due to its role in gas and water exchange (Corbineau *et al.*, 1989). It thereby protects and increases the persistence of propagules in the soil bank, and it is gradually lost during ageing due to the physical, chemical and biotic attacks to which it is exposed (Cousens *et al.*, 2009; Hu *et al.*, 2009).

Temperature is the main environmental variable involved in development and control of dormancy. Generally, low winter temperatures reduce or increase seed dormancy in spring-summer or autumn-winter species (Milberg & Andersson, 1998). Also, when dormancy is broken, the temperature range for germination widens (Vegis, 1964; Gay *et al.*, 1991). The temperature effect on seed dormancy may be modulated by the maternal environment during seed filling. Species from cooler regions would be likely to show greater dormancy than those adapted to warmer regions because of the expression of *DELAY OF GERMINATION 1* (*DOG1*) gene (Chiang *et al.*, 2011).

Physiological dormancy can be broken chemically by the addition of potassium nitrate, ethylene or gibberellic acid (GA) (Seiler, 1998; Finch-Savage & Leubner-Metzger, 2006). Mechanical scarification (MS), cold stratification (CE) and light stimulation are also effective physical methods that break dormancy (Baskin & Baskin, 1998). International rules for seed analysis recommend CE before testing the germination of sunflower crop seeds (ISTA, 2004).

Treatment with GA has been an effective method for breaking dormancy in wild *H. annuus* and *Helianthus petiolaris* (Seiler, 1998). Other *Helianthus* species may lose their dormancy after MS plus GA (Chandler & Jan, 1985). This combined physical–chemical treatment resulted in germination rates four times higher than in the control in *Helianthus paradoxus* seeds (Van Auken, 2001).

The cosmopolitan invasive *H. annuus* biotypes have limited the release of transgenic sunflower and challenge the durability of new technologies, due to the risk of crop-wild gene flow (Cantamutto & Poverene, 2010). As in several countries, the invasive biotype *H. annuus* ssp. *annuus* coexists with the sunflower *H. annuus* var. *macrocarpus* in Argentina, where there is evidence of hybridization between the two taxa (Ureta *et al.*, 2008; Cantamutto *et al.*, 2010).

Seed dormancy levels have a strong effect on the population dynamics and evolution of invasive plants. Wild–crop hybridization could change the dormancy level by affecting the population dynamics of invasive species (Bagavathiannan & Van Acker, 2008). In the native habitat of the *H. annuus* taxonomic complex, Wild–crop hybridization variably decreased seed dormancy according to the parents' genetic background (Snow *et al.*, 1998; Mercer *et al.*, 2006*a*) but it is unknown how important this effect is in invasive biotypes.

Seed dormancy might be responsible for the success of an invasive plant species. Differences in seed dormancy of the alien *H. annuus* strains and its hybrids may be related to differential adaptive ability. In the worst scenario, if crop-wild hybridization increased seed dormancy, the risk of crop traits being transferred to invasive biotypes might be high.

We studied seed dormancy in five invasive *H. annuus* biotypes to address the following questions: (a) What is the role of temperature, water absorption dynamics and light on seed dormancy? (b) What is the effect of ageing on seed dormancy? (c) Would hybridization with domesticated sunflower change seed dormancy of invasive biotypes?

Materials and methods

Plant material

The invasive alien species was represented by five *H. annuus* biotypes collected in central Argentina (Cantamutto *et al.*, 2010): Río Cuarto (RCU; S 33°09′, W 64°20′), Colonia Barón (BAR; S 36°10′, W 63°53′), Adolfo Alsina (AAL; S 37°16′, W 62°59′), Diamante (DIA; S 32°03′, W 60°38′) and Las Malvinas (LMA; S 34°47′, W 68°15′). Following after-ripening for 6 months at room temperature (20–22°C), achenes were placed on wet paper at 5°C for 1 week (ISTA, 2004) and then they were planted in trays on sphagnum peat-based substrate. Seedlings were grown in the greenhouse at 20–25°C for 1 month, then transplanted at the 4–6 leaf stage in a common garden at the Agronomy Department (S 38°41′38″, W 62°14′53″) Universidad Nacional del Sur, Bahia Blanca, Argentina. The sunflower hybrid cultivar DK3880CL (imidazolinone tolerant) was sown directly in the same experimental field. All the achenes were produced during the 2008/2009 growing season.

The achenes of wild biotypes were generated under controlled pollination of the heads of 20–30 plants covered with paper bags at pre-flowering stage. At flowering stage, heads were pollinated with pollen of sibling plants, 3–4 times per head. The F1 progeny (wild × crop) achenes were produced by controlled crosses between the wild biotypes (as female) and the sunflower cultivar DK3880CL (as male) according to Jan & Seiler (2007). Heads of more than 20 plants

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were emasculated in the morning and pollinated with sunflower in the late afternoon. The DK3880CL heads used as pollen source were bagged at the R4 stage and their seed constituted the cultivar offspring.

Fresh achenes, harvested after physiological maturity, were stored in a desiccator with dry silica gel for 2 weeks and then stored in tri-laminar aluminium bags at 5°C during the after-ripening period (cold storage). The wild, F1 and DK3880CL offspring were considered as cross types. Each wild biotype and their corresponding F1 were considered as a half-sibling family.

Effect of pericarp scarification on seed imbibition

The effect of the pericarp on the imbibition dynamics was studied in fresh achenes of the five wild biotypes. MS was carried out using an orbital sander (Hitachi FS 10SA, Tokyo, Japan) with sandpaper (aluminium oxide, 180 grains $inch^{-2}$), without any additional weight, activated for 15 s over a single layer of achenes placed on another sheet of the same sandpaper as the base. After scarification, achenes were placed on filter paper moistened with water in a germination chamber at 20°C (ISTA, 2004) with a 12 h photoperiod. Achenes without scarification (No) were used as a control. The achenes were weighed every 5 h for 30 h of imbibition. The experiment was conducted as a randomised complete design with three replicates and experimental units of 50 achenes. Water imbibition at each time point (ETP) was expressed as the weight increase percentage as follows:

$$\text{Weight increase}_{\text{ETP}}\,(\%) = \left(\frac{\text{final weight}_{\text{ETP}} - \text{initial weight}_{\text{ETP}}}{\text{initial weight}_{\text{ETP}}}\right) \times 100 \quad (1)$$

A new set of achenes were measured at each imbibition time. Data were subjected to ANOVA, after logit transformation, using a randomised complete design with three replicates at each imbibition time. The sources of variation were: biotype (five wild biotypes), and scarification (with or without MS). Data were analysed with Infostat (2008).

Effect of light on seed germination

Plant material was DIA and DK3880 CL offspring achenes. We selected DIA biotype because of the small number of achenes obtained from the other biotypes but a preliminary experiment detected light response in all biotypes (Table S1, Supporting Information). Achenes were placed on moistened paper towels in plastic trays (3.5 cm × 3.5 cm) at 20°C with a 12 h photoperiod. The red light was provided by fluorescent F36W/54 lamps (60 µmol m^{-2} s^{-1}). In the dark treatment, light was excluded by wrapping the trays with aluminium foil (0.014 mm thick). Germinated achenes were counted after 15 days. The test was repeated after 12 months of cold dry storage.

The experiment was conducted as a randomised complete design with three replicates and experimental units of 25 achenes. Data were analysed by ANOVA after logit transformation considering cross type (wild or domesticated), light (with or without light) and storage time (0 or 12 months) as sources of variation. Transformed means of biological interest in significant ($P < 0.05$, F-test) terms from the ANOVA were compared using Student's least significant differences at 0.05 probability. The data were analysed with Infostat (2008).

Effect of temperature on seed germination

Response to temperature was evaluated in the five wild biotypes and their half-sibling (F1). Data were collected on fresh achenes immediately after harvest, and at 6 and 12 months after cold dry storage. The thermo-gradient was generated by an aluminium plate (28 cm wide × 100 cm long), with an automatic cooling and heating system at the extremes, covered with a moistened paper (Hernández & Paoloni, 1998). The temperature in each gradient section was monitored with an infrared thermometer.

The achenes were randomly placed in groups of 25 achenes aligned in 5, 10, 15, 20, 25 and 30°C mean temperature segments (average ± 0.5°C) without any contact between them. Light on a 12 h photoperiod was provided by fluorescent lamps (60 µmol m^{-2} s^{-1}). Germinated achenes were counted daily for 15 days.

The results were expressed as means (± SE) of each cross type (wild or F1, $n = 5$), as data were not replicated per cross.

Promoting germination treatments

Dormancy breaking treatments were evaluated in the five families of wild biotypes and their half-sibling F1. Germination was evaluated after 0, 6, and 12 months of cold storage. The dormancy breaking treatments were: (a) CE: achenes were placed in trays on wet filter paper, covered with transparent polyethylene bags and stored for 1 week at 5°C (ISTA, 2004); (b) GA: achenes were placed in trays on filter paper moistened with 1 mM GA_3 (Seiler, 1998); (c) MS: achenes were scarified with an orbital sander operated as previously described; (d) Achenes without any treatment were used as control (No). Germination was evaluated every 2 days during 15 days on filter paper moistened with distilled water

or GA solution, at 20°C with a 12 h photoperiod. Light was provided by fluorescent lamps (60 µmol m^{-2} s^{-1}). To estimate the total seed viability, a tetrazolium test was performed on the non-germinated achenes at the end of each experiment (ISTA, 2004). Percent dormancy was calculated as the difference between the percent of viable achenes and the percent of germinated achenes.

The experiment was conducted as a randomised complete design with four replicates and experimental units of 25 achenes. Data were subjected to ANOVA, after logit transformation. Sources of variation were family, cross type, storage time and dormancy breaking treatment. Transformed means of biological interest in significant ($P < 0.05$, F-test) terms from the ANOVA were compared using Student's least significant differences at 0.05 probability.

With the aim of comparing the effect of hybridization and scarification on the germination rate, the time to reach 50% of maximum seed germination (TG50) was calculated for each family. Data (control, MS and hybridization) of each family obtained after 12 months of storage at 5°C were used to calculate TG50. Data were fitted to a logistic model with three parameters (Chauhan *et al.*, 2006) according to the following equation:

$$G\,(\%) = \frac{G_{\max}}{\left[1 + (x/\mathrm{TG50})^{G\mathrm{rate}}\right]} \qquad (2)$$

where G is the total germination (%) at time x, $G_{\max}$ is the maximum germination value (%), TG50 is the time to reach 50% of maximum germination, and G_{rate} indicates the slope around TG50. The logistic dose–response curves were fitted using the *drc* package of the R version 2.9.0 statistical software (R Core Development Team, 2009). The function *SI*, used to compare the relative differences of the TG50 values between the curves, compared the TG50 values between treatments (control, MS and hybridization). Data were analysed with Infostat (2008) and R version 2.9.0 (R Core Development Team, 2009).

After storage for 1 year at 5°C, the tetrazolium test on the non-germinated achenes showed 99% viable achenes. Hence, non-germinated achenes were considered to be alive.

Estimation of crop introgression

A conservative estimation of crop introgression was assessed by considering the gene flow rate (Ureta *et al.*, 2008), fitness parameters without competition for water or space (Presotto *et al.*, 2012), and the dormancy parameters measured in this experiment. Hybridization rate was estimated using a crop specific isozyme marker in an experiment where a stand of cultivated sunflower was surrounded by plots of wild plants at increasing distances (Ureta *et al.*, 2008). Further increases in the frequency of the wild biotype in the soil seed bank and migration were not considered. The composition of the population was estimated in three successive seasons under two extreme scenarios. Scenario 1: all seedlings emerging from non-dormant achenes (F1 and wild biotype) that germinated during the postharvest-spring period survive and form the cohort. Scenario 2: the seedlings that emerged postharvest are killed by winter frost and the cohort is only formed from the seedlings that emerged from non-dormant achene after chilling. Each of the five biotypes was considered as a replication.

Introgression event (Generation 0, G0): The F1 achene production per mother plant (wild biotype) was estimated considering the estimated crop gene flow rate when the crop was 3 m far from wild plants (0.18, Ureta *et al.*, 2008).

First cohort: The frequency of introgressed plants (F1) in the first cohort was estimated as follows:

$$P\,(\mathrm{F1}) = \frac{G\,(\mathrm{F1})}{G\,(\mathrm{Wild}) + G\,(\mathrm{F1})} \qquad (3)$$

P (F1) is the proportion of F1 seedlings over the total. Germination (G) was the percentage of achenes germinated after 6 months at 5°C (Scenario 1) or the achene germinated after 6 months of cold storage minus the fresh germination (Scenario 2).

The achene contribution to the second generation (G2) was calculated as the mating probability corrected by the relative fitness of both cross types. F1 fitness was calculated as the achene production per plant relative to the wild biotype (fitness = 1).

Second cohort: the proportion of introgressed plants in the second cohort was estimated by the proportion of F2 and BC1 in the G2 generated from the G1 plants plus the F1 achenes that had not germinated in the previous spring (carry-over).

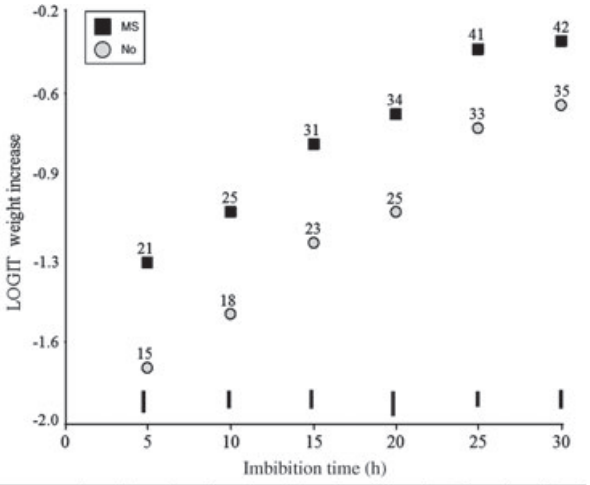
Third cohort: to estimate the achene contribution to the composition, the BC1 seed dormancy was considered to be the same as in G1. F2 was excluded because achene production was less than 0.1%.

The contribution of achene of introgressed plants to the third cohort was calculated as the mating probability, taking into account the relative fitness of parents, wild biotype, F1 (carry-over) and BC1 to wild biotype. The G2 carry-over was added.

Results

Effect of pericarp scarification on seed imbibition dynamics

Interaction between biotype and scarification on each imbibition time was not significant ($P \geq 0.13$; $F \leq 2.1$).



ANOVA source	5		10		15		20		25		30	
	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value
Biotype (B)	30.3	<0.001	35.6	<0.001	39.9	<0.001	19.7	<0.001	39.4	<0.001	22.6	<0.001
Scarification (S)	175.1	<0.001	305.0	<0.001	232.1	<0.001	127.8	<0.001	224.0	<0.001	93.9	<0.001
B x S	0.5	0.71	2.1	0.13	1.7	0.18	0.6	0.68	1.5	0.23	1.2	0.34

Figure 1 Imbibition dynamics (expressed as Logit weight increase) of invasive *Helianthus annuus* achenes soaked in distilled water at 20°C, with (MS, mechanical scarification) or without (no) mechanical scarification. Bars above *x*-axis indicates least significant difference ($P = 0.05$). Numbers above points indicates back-transformed (%) values. Statistics (F) with their respective P-value are shown below figure.

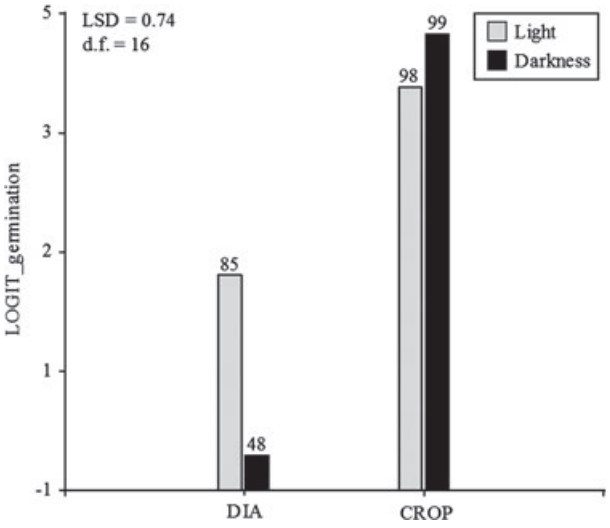


Figure 2 Effect of light on germination of an invasive alien species biotype (DIA, Diamante) and DK3880CL offspring (CROP). Bars show average germination of fresh seeds and 12-month stored seeds. LSD: least significant difference ($P = 0.05$) of logit transformed data. Numbers above bars indicates back-transformed (%) values.

Scarification significantly increased water absorption with maximum effects at 20–25 h of imbibition (Fig. 1). At the end of this period, scarified achenes absorbed 19% more water than non-scarified achenes (41.7 vs 35.1).

Effect of light and temperature on seed dormancy

Differences between cross type were highly significant ($P < 0.001$; $F = 191.0$) and differences between light treatment was significant ($P < 0.01$; $F = 12.1$), while storage time was not significant ($P = 0.25$; $F = 1.4$). Interaction between cross type and light was highly significant ($P < 0.001$; $F = 18.0$). Light significantly decreased dormancy of DIA biotype while there was no response in the domesticated sunflower (Fig. 2). In darkness only 48% wild seeds germinated, whereas under light stimulation germination exceeded 80%.

During the after-ripening period, germination was generally lower for the wild biotypes in the temperatures tested, particularly at 20 and 25°C (Fig. 3a and Fig. 3b). The after-ripening period not only decreased dormancy but it also widened the germination temperature range. The highest germination was observed at 15°C, and exceeded 75% at 12-months storage. At 10°C the germination rate was slow, but it reached similar values as at 20°C. At 5 and 30°C, germination was minimal and at 25°C germination was fast but only a few achenes germinated. At 15°C the germination dynamics were not different between wild and F1 generation (Fig. 3c and Fig. 3d). At 12-months of after-ripening period, the accumulated germination exceeded 50% 2 days before that in fresh seeds.

Promoting germination treatments

Differences between family, cross type, storage time and dormancy breaking treatment were highly significant. All interactions, except quadruple and storage time*treatment*cross type, were significant (Table S2). Because of the interactions data were treated separately.

The loss of seed dormancy during the after-ripening period was dependent on the wild biotype (Table 1). AAL, LMA and RCU showed the greatest increase in germination after 12 months of dry storage. DIA showed a minimal effect due to its high initial germination and BAR accession was the least sensitive in the after-ripening period, with more than 50% dormant achenes, even after 12-months dry storage (Table 1). On the whole, there were differences in dormancy between F1 and wild biotypes. F1 showed similar dormancy values in fresh seed and 6-month stored achenes while in the wild biotype, germination increased after 6-months cold storage.

With regard to the treatments, MS showed the largest increase on germination (Table 2). The highest increase in germination was observed in AAL and LMA biotypes, which had an initial germination less than 20% and it was increased to over 75%. RCU and BAR showed

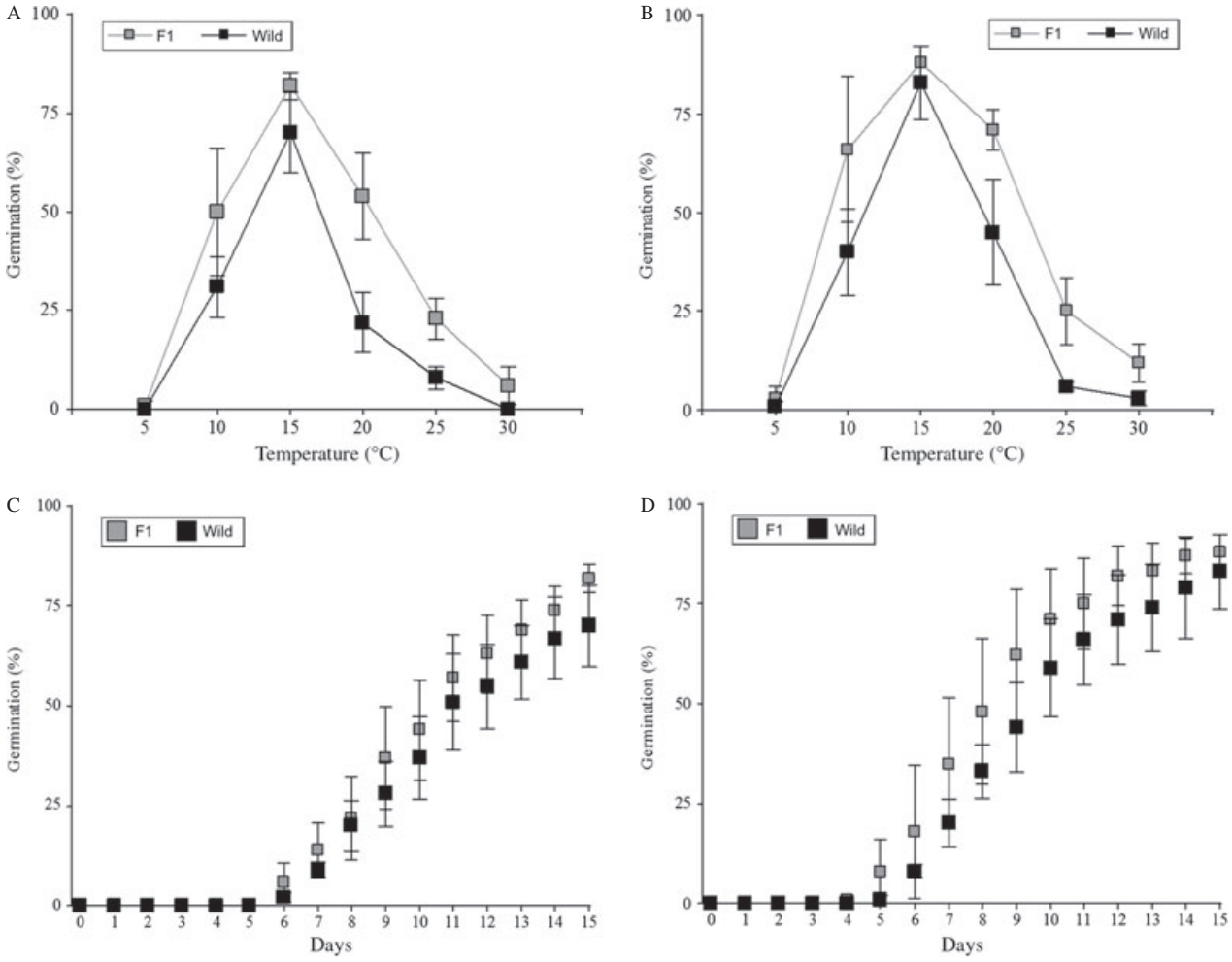


Figure 3 Germination (mean ± SE, $n = 5$, averaging across each cross type, wild or F1) under six temperatures and neutral photoperiod and accumulated germination at 15°C of invasive *Helianthus annuus* biotypes (wild) and their hybrids with sunflower crop (F1) in fresh seed (a and c) and after 12-month of cold storage (b and d).

intermediate responses, having an initial germination less than 20% and it was increased to over 40%. DIA showed no response due to its low dormancy. MS showed a similar pattern with the F1 biotypes. The germination with GA was improved in BAR (wild and F1) and LMA wild biotype but by a lesser proportion compared to MS. The CE treatment improved germination only in BAR and LMA wild biotypes but with lower effect than MS. After 12-months of cold storage, in general germination in untreated achenes exceeded 75% and the differences between dormancy breaking treatments were statistically significant in BAR (wild and F1) and LMA F1, only with MS (Table S3).

Germination dynamics of all biotypes fit the logistic model with three parameters (Table 3). The TG50 varied between biotypes and treatments. TG50 in scarified achenes was less than half of the intact achenes. AAL and DIA achenes showed similar behaviour. TG50 in wild biotypes was ca. 6 days whereas hybridization with sunflower reduced this parameter by 1 day (Table 3).

The germination rate of LMA was high, and it was also increased by MS, but it was not affected by hybridization with sunflower. BAR and RCU showed a low germination rate and MS reduced TG50 by two or more days. Crop hybridization only decreased the TG50 in the BAR biotype (Table 3).

Estimation of crop introgression

Considering a hypothetical situation where the sunflower crop was 3 m away from the wild biotypes, hybridization would originate 18% Wild–crop hybrid plants (Ureta *et al.*, 2008). If F1 and wild achenes fall to the

Table 1 Effect of storage time (months) on untreated achene germination (%) of five invasive *Helianthus annuus* biotypes (wild) and their half sibling with a sunflower cultivar (F1) at 20°C. Statistics (*F*) with their respective *P*-value are shown. d.f. = 9

ST	AAL	BAR	DIA	LMA	RCU
Wild					
0	14.7[a] (−1.85[b])	12.0 (−2.09)	77.0 (1.20)	14.7 (−1.96)	18.7 (−1.56)
6	43.0 (−0.34)	36.0 (−0.64)	96.0 (3.28)	60.3 (0.39)	45.0 (−0.28)
12	83.0 (1.53)	44.0 (−0.30)	97.0 (3.36)	78.7 (1.30)	76.0 (1.33)
$LSD_{0.05}$	0.68	0.78	1.42	1.01	1.57
ANOVA	$F = 64.5, P < 0.001$	$F = 14.3, P < 0.01$	$F = 8.4, P < 0.01$	$F = 28.5, P < 0.001$	$F = 8.7, P < 0.01$
F1					
0	40.0 (−0.48)	29.7 (−1.10)	73.0 (1.02)	22.7 (−1.42)	38.7 (−0.52)
6	52.0 (0.12)	26.0 (−0.86)	74.7 (1.06)	74.0 (1.07)	50.0 (−0.06)
12	88.7 (2.06)	53.8 (0.12)	89.0 (2.22)	93.0 (2.53)	77.3 (1.17)
$LSD_{0.05}$	1.56	0.71		1.17	1.00
ANOVA	$F = 7.8, P = 0.01$	$F = 9.5, P < 0.01$	$F = 3.3, P = 0.09$	$F = 28.8, P < 0.001$	$F = 8.2, P = 0.01$

AAL, Adolfo Alsina; BAR, Colonia Barón; DIA, Diamante; LMA, Las Malvinas; RCU, Río Cuarto, ST, storage time; LSD, least significant difference ($P = 0.05$).
[a]Untransformed means.
[b]Logit transformed means.

Table 2 Germination (%) of fresh achenes of five invasive *Helianthus annuus* biotypes (wild) and their progenies with a sunflower cultivar (F1) with different treatments (T). Statistics (*F*) with their respective p-value are shown. d.f. = 12

T	AAL	BAR	DIA	LMA	RCU
Wild					
MS	92.3[a] (2.78[b])	43.5 (−0.32)	90.0 (2.52)	77.7 (1.30)	65.0 (0.63)
GA	20.0 (−1.45)	26.7 (−1.08)	88.0 (1.93)	41.3 (−0.39)	34.7 (−0.71)
CE	33.3 (−0.74)	24.0 (−1.22)	78.7 (1.25)	32.0 (−0.80)	21.3 (−1.39)
No	14.7 (−1.85)	12.0 (−2.09)	77.0 (1.20)	14.7 (−1.96)	18.7 (−1.56)
$LSD_{0.05}$	1.39	0.64		0.97	0.96
ANOVA	$F = 26.8, P < 0.001$	$F = 14.5, P < 0.001$	$F = 2.1, P = 0.17$	$F = 21.8, P < 0.001$	$F = 12.3, P < 0.01$
F1					
MS	98.0 (4.02)	76.5 (1.15)	93.0 (3.10)	97.0 (3.62)	88.0 (2.37)
GA	48.0 (−0.12)	46.7 (−0.18)	76.0 (1.12)	36.0 (−0.62)	65.3 (0.61)
CE	38.7 (−0.54)	32.0 (−0.80)	82.7 (1.60)	30.1 (−0.93)	41.3 (−0.40)
No	40.0 (−0.48)	29.7 (−0.86)	73.0 (1.02)	22.7 (−1.42)	38.7 (−0.52)
$LSD_{0.05}$	1.42	0.48	1.36	1.52	1.68
ANOVA	$F = 28.2, P < 0.001$	$F = 43.3, P < 0.001$	$F = 5.5, P = 0.02$	$F = 27.5, P < 0.001$	$F = 7.2, P < 0.01$

AAL, Adolfo Alsina; BAR, Colonia Barón; CE, treatments were cold stratification; DIA, Diamante; GA, gibberellic acid 1 mM; LMA, Las Malvinas; LSD, least significant difference ($P = 0.05$); MS, mechanical scarification; No, control; RCU, Río Cuarto, ST, storage time; T, different treatments.
[a]Untransformed means.
[b]Logit transformed means.

soil, temperature would play a crucial role in seed dormancy and germination dynamics. Taking into account two contrasting winter temperatures, chilling temperatures would be detrimental for F1 in G1 and the proportion of seed would be reduced. In addition, a previous experiment had shown that F1 seed production represented on average 3% of wild achene production (Presotto *et al.*, 2012). Then, F1 would mate each other or to wild biotypes, but in the worst scenario, F1 contribution to the second cohort would be less than 9%. In this second cohort, introgressed seedlings would mainly originate from F1 achenes that were dormant in the previous spring (carry-over) so low fitness will play a major role over seed bank formation. In addition, backcrosses to the wild biotype would be in a low proportion and they also would have low fitness, which in the worst case should be 30% of wild plants (Presotto *et al.*, 2012). Therefore, in the third cohort the introgressed seed bank would decrease to less than 4.5%, regardless of freezing temperatures.

Discussion

In contrast to cultivated sunflower, the germination of invasive *H. annuus* biotypes increased with light stimuli. This response was also observed in other wild species such as *Helianthus bolanderi*, *Helianthus exilis* (Olivieri &

Table 3 Effect of scarification (MS) and cross type (CT) on germination rate expressed as the time required to reach 50% of maximum seed germination (days, means ± SE) of five invasive *Helianthus annuus* biotypes (four replicates per biotype by treatment combination). The p-logmodel is the *P*-value of the ANOVA lack-of-fit test which explores if the model provides good data description. Different letters indicate significant differences ($P < 0.05$, *F*-test) between TG50 values within biotypes

Treatment	AAL	BAR	DIA	LMA	RCU
No	5.8 ± 0.2 c	8.8 ± 0.5 c	5.7 ± 0.1 c	4.0 ± 0.1 b	6.9 ± 0.2 b
MS	2.3 ± 0.6 a	6.0 ± 0.3 a	2.6 ± 0.5 a	3.5 ± 0.2 a	4.7 ± 0.2 a
CT	4.9 ± 0.1 b	7.5 ± 0.3 b	4.9 ± 0.1 b	4.1 ± 0.1 b	6.6 ± 0.3 b
P-logmodel	0.99	0.87	0.99	0.99	0.59

AAL, Adolfo Alsina; BAR, Colonia Barón; CT, cross type; DIA, Diamante; MS, effect of scarification; LMA, Las Malvinas; No, control; RCU, Río Cuarto, ST, storage time.

Jain, 1978) and *H. paradoxus* (Van Auken, 2001). It is considered that red light activates type II phytochromes, which function as red light receptors (Quail, 1998). Active phytochromes trigger PHYTOCHROME-INTERACTING FACTOR3-LIKE5 protein (PIL5) degradation, leading to a decrease in abscisic acid and an increase in GA levels and consequently it induces germination (Oh *et al.*, 2007). This germination control system could be an advantage for soil seed bank persistence when soil is under agricultural disturbance so that buried seed would mainly germinate after cultivation and exposure to light (Benech-Arnold *et al.*, 2000). In contrast, no-till practices would reduce seed persistence in the soil given the higher germination probability (by continuous exposure to light), predation (by birds, rodents, and several invertebrates) and more variable climatic conditions (Alexander & Schrag, 2003).

The highest germination rate of invasive *H. annuus* and F1 was at 15°C, whereas sunflower optimal germination temperature is 20°C (ISTA, 2004). The highest germination rate in the wild biotype and F1 was achieved at 15°C; hybridization slightly increased germination between 10°C and 25°C. Previous studies have shown that non-dormant sunflower seeds showed high germination levels at a wide range of temperatures between 5°C and 40°C (Gay *et al.*, 1991; Hernández & Paoloni, 1998).

In temperate regions, spring-summer germinating species require exposure to low winter temperature to break dormancy (Baskin & Baskin, 1998). This probably explains why cold storage for 1 year increased *H. annuus* seed germination. This response has also been detected in *H. paradoxus* achenes stored for more than a year at 4°C (Van Auken, 2001). Low temperatures might promote positive and negative gene regulation involved in gibberellin synthesis and germination induction (Yamauchi *et al.*, 2004).

Moreover, temperature during seed ripening influences natural selection of the *DOG1* gene linked to primary dormancy in *Arabidopsis thaliana*. The *DOG1* gene expression was affected by temperature during seed maturation (Chiang *et al.*, 2011). This could explain the absence of dormancy in DIA, which is the invasive population in the highest temperatures during seed ripening and the warmest winter (ICyA, 2013).

Despite the fact that BAR, LMA and RCU are populations located geographically distant from each other, the mean temperatures during achene maturation are on average 2°C lower than the temperature of the DIA habitat (De Fina, 1992; ICYA, 2013). Furthermore, AAL biotype achenes mature in a cooler environment which is on average 4°C below DIA. These temperature differences during ripening might be the cause of the low dormancy of DIA. In the natural habitat, their seedlings can survive the winter and dormancy would not be a positively selected trait.

Achene ageing led to a loss in dormancy. In fresh achenes, the most effective treatment for breaking dormancy was MS. Although addition of GA increased germination, the response was less than for MS and it was variable among biotypes. MS not only broke seed dormancy but it also increased the seed germination rate (Table 3). Possibly MS decreased the inhibitors present in the maternal pericarp (Baskin & Baskin, 1998) and increased the rate of water absorption (Fig. 1) which allowed a faster triggering of the processes involved in germination.

On the basis of these results, different levels of dormancy were found in Argentine *H. annuus* populations. DIA had the lowest dormancy; BAR had the greatest dormancy and AAL, LMA and RCU showed intermediate levels of dormancy. Differences are essentially attributed to the environment where they were selected (Luzuriaga *et al.*, 2006). Moreover, the dormancy of invasive *H. annuus* biotypes would be of a physiological nature. Dormancy might be governed by the abscisic acid : GA ratio in the embryo (Finch-Savage & Leubner-Metzger, 2006, Finkelstein *et al.*, 2008), that possibly interplays with signal molecules such as ROS (reactive oxygen species, namely superoxide anions and hydrogen peroxides) which may or may not accumulate, and which plays a major role in embryonic dormancy. In the dormant state, the abscisic signalling pathway is active and prevents

germination. A high amount of abscisic acid may maintain a low level of ROS during imbibition of dormant seeds. During after-ripening there is an accumulation of ROS. This accumulation might reduce the abscisic level and/or block abscisic signalling, stimulate gibberellin signalling and alter protein function through oxidative modifications. Subsequent imbibition of after-ripened seeds would therefore be associated with the completion of germination (Oracz *et al.*, 2007; El-Maarouf & Bailly, 2008). However, data also showed that the maternal pericarp also imposes restrictions on germination. Some studies suggest that the composition of the fruit pericarp or coat (i.e. phenolic compounds) would make them less permeable to oxygen uptake and/or inhibit the exit of, for example abscisic acid (Corbineau *et al.*, 1989; Debeaujon *et al.*, 2000; Cousens *et al.*, 2009).

Among the biotypes evaluated, DIA and RCU are established in environments where the sunflower crop is not frequent; whereas LMA is established within a valley of fruit trees and vineyards where sunflower seed production fields have occasionally been found (Poverene *et al.*, 2008). On the other hand, AAL and BAR biotypes are established within the sunflower crop area together with another related wild species, *H. petiolaris*. In both environments it is common to find hybrid zones where wild *H. annuus*, *H. petiolaris* and sunflower crop coexist (Poverene *et al.*, 2008). Hybridization between *H. annuus* and *H. petiolaris* has been documented (Rieseberg *et al.*, 1995; Gutiérrez *et al.*, 2010) and it is known that *H. petiolaris* has greater seed dormancy than *H. annuus* (Seiler, 1998). Thus, introgression with *H. petiolaris* could explain the deep seed dormancy of the BAR accession.

Although hybridization with domesticated sunflower did not reduce dormancy to a degree that was statistically significant, the data did show a slight reduction in seed dormancy, especially in the AAL, BAR and RCU accessions. These results are consistent with Snow *et al.* (1998) and Mercer *et al.* (2006*a*) who found that germination increased in Wild–crop hybrids as compared to the original wild populations. Despite the increased germination due to hybridization, these authors conclude that the effect of the wild and domesticated genetic backgrounds might be variable.

The effect of the genetic background was not only shown by the differential behaviour to dry storage at 5°C but also in the germination rate. The crop hybridization of AAL, BAR and DIA increased the germination rate, but the F1s of RCU and LMA did not show the same response (Table 3).

Seeds from Wild–crop hybrids were mainly dispersed in disturbed areas (i.e. agro-ecosystems) where both parents were present. In such environments, the lack of dormancy may reduce the ability of these hybrids to persist. Therefore, the behaviour of Wild–crop seed that resembles that of their wild parents most closely will have the best chance to produce hybrid plants and backcross plants in the wild population (Landbo & Jørgensen, 1997).

When the wild/hybrid germination ratio is less than 1 it is possible that a high number of Wild–crop hybrids will grow in a wild population due to the higher proportion of germinated seeds. In contrast, when the ratio is greater than 1, wild plants will prevail. Therefore, wild populations with relatively low germination in comparison to crop–wild hybrids are more likely to incorporate crop genes (Mercer *et al.*, 2006*a*). For example, if all the wild and F1 seeds germinate in spring, AAL, LMA and RCU populations would have the greatest risk of incorporating crop traits. However, in the fall (after shattering) if environmental conditions allowed germination the seedlings would be killed by winter frost, so seedlings would come from seeds that broke dormancy just after the winter. Under this scenario LMA seems to be the only wild biotype with a greater risk of incorporating crop traits (Table 1).

Despite that F1 fitness was lower than that of wild plants, there was a rapid fertility recovery in the following generations (Presotto *et al.*, 2012). Also it has been shown that, under competition, fecundity in Wild–crop hybrids is less affected than in wild plants (Mercer *et al.*, 2006*b*). In contrast, the similar patterns of germination between wild and Wild–crop hybrids found in our results suggest that in the absence of gene flow, crop traits into the wild biotypes would tend to disappear. This would be so because of the low fitness of plants that bear crop genes.

In conclusion, the results of this study establish that there is variability in the level of dormancy in the Argentine biotypes that would be related to the original habitat. It is also known that light, after-ripening time and MS promote germination and that hybridization with sunflower crop had a minimal or no effect on seed dormancy. For future investigation, the effect of the maternal achene pericarp or coat on seed dormancy in wild *H. annuus* should be studied in more depth.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Preliminary experiment to detect light response in fresh achenes of five wild biotypes of *Helianthus annuus*. The experiment was conducted as a randomised complete design with three replicates and experimental units of 25 achenes. Data were analysed by ANOVA, within each biotype, after logit transformation considering light (with or without light) as source of variation

Table S2. Four-way analysis of variance for effect of biotype, cross type, storage time and treatment on seed germination in *Helianthus annuus* L.

Table S3. Germination (%) of 12-months stored achenes of five invasive *H. annuus* biotypes (wild) and their progenies with a sunflower cultivar (F1) with different treatments (T). Treatments were cold stratification (CE); gibberellic acid 1 mM (GA); mechanical scarification (MS) and control (No). LSD: least significant difference ($P = 0.05$), d.f. = 12. Statistics (F) with their respective P-value are shown