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Maternal environment and dormancy in sunflower: The effect of temperature during fruit development

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ABSTRACT

A rapid and uniform germination in the field is an important requirement for commercial hybrid sunflower seed. Persistence of dormancy after harvest can negatively affect this aspect of seed quality, and seed lots with some degree of dormancy cannot be commercialized. Seed dormancy intensity and duration can vary greatly among sunflower genotypes, but it is also subject to strong interactions with the maternal environment. In this paper we report results of investigations into the effect of temperature during sunflower fruit development on the level of dormancy after harvest. After conducting controlled (greenhouse) and field experiments (sowing dates and plastic tents), we found that higher temperatures during later stages of achene development significantly increased the level of dormancy at harvest and its persistence during storage. The impact of the maternal (thermal) environment on embryo and coat-imposed dormancy was also investigated. Results showed that although maturation under warmer environments reduced embryo dormancy, this effect was overcompensated for by the enhancement of inhibition imposed by the pericarp and the seed coat. In addition, the results obtained suggest that observed changes in dormancy in response to the maternal environment could be at least partially explained by changes in achene and/or embryo sensitivity to ABA. Results presented here should be useful when choosing a proper environment for the production of hybrid sunflower seed of high quality, avoiding high temperatures during later stages of fruit development.

1. Introduction

At harvest, sunflower fruits (achenes) are usually dormant and germinate poorly (Cseresnyes, 1979; Corbineau et al., 1990). Depending on the genotype and the maternal and storage environments, this dormant state can last for a few weeks or several months (Brunick, 2007). Because hybrid seed destined for sowing cannot be processed and commercialized until germination standards are met, a prolonged dormancy is a problem for the seed industry committed to provide in a timely fashion F1 hybrid seed either for local or counter season markets (Maiti et al., 2006). In addition, industrial costs related to proper seed storage increase.

Mechanisms involved in seed dormancy are determined genetically and this is therefore a heritable trait, but its intensity at harvest and its maintenance afterwards can also be modulated by the maternal environment (Finch-Savage and Leubner-Metzger, 2006). Studies performed in several species indicate that the environmental conditions explored during seed or fruit development and maturation (hereafter, just fruit development) can affect the dormancy level of harvested seeds or fruits (Fenner, 1991). Even though several environmental factors (*i.e.* day length, radiation, water and nutrient availability) have been shown to affect the level of dormancy at harvest, there is strong agreement on the dominant effect of ambient temperature during development on the regulation of seed dormancy at harvest (Benech-Arnold, 2004). The effect of the maternal thermal environment on seed dormancy has been

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studied in many species, and increases in the average temperature during seed development are usually associated with lower levels of dormancy (Fenner, 1991). Examples of this response are winter cereals: low temperatures during grain development result in higher grain dormancy in wheat (Black et al., 1987; Nakamura et al., 2011) and barley (Rodríguez et al., 2001, 2015). However, there are almost no reports regarding the possible effect of the thermal environment explored by sunflower fruits during development on their dormancy level at harvest. Indeed, one previous preliminary study by Fonseca (2000) suggested that the effect of temperature on seed dormancy might be opposite in sunflower to that observed in winter cereals. Even though commercial sunflower seed production takes place in different regions and thermal environments, the precise effect of temperature has not been explored properly under controlled (*e.g.*, greenhouse) or field conditions.

According to the classification proposed by Baskin and Baskin (2004), sunflower achenes display physiological dormancy. The various structures that make up the sunflower achene contribute to dormancy, and their relative contribution varies during fruit development. The embryo is surrounded by a single layer of living endospermatic cells intimately coalesced with the seed coat which is dead at maturity, forming the true sunflower "seed" (Seiler, 1997; Szemruch et al., 2014). This "seed" is further enclosed by the pericarp, forming the fruit. All these structures surrounding the embryo are referred to as "envelopes" in this study. At early stages of fruit development, dormancy is imposed mainly by the envelopes and the embryos germinate readily if they are isolated and incubated in water (Le Page-Degivry and Garello, 1992; Corbineau et al., 1990). As development progresses, the dormancy level of the embryo increases gradually until it reaches a maximum at about 20–22 days after anthesis (DAA). During later developmental stages, embryo dormancy decreases gradually and achene dormancy is mostly due to the envelopes. At harvest maturity, the embryo usually presents low or intermediate levels of dormancy, whereas the achenes are deeply dormant as a result of strong "envelope-imposed" dormancy. Dormancy imposed by the envelopes at harvest maturity usually persists for some time, and may require several weeks or months of dry storage to disappear completely (Corbineau et al., 1990; Bianco et al., 1994). Although several reports have focused on the contribution of the embryo and the seed envelopes to the level of dormancy in sunflower fruits, information is lacking on how these components might be affected by the maternal (thermal) environment. In addition, it is not understood how each of the different structures composing the envelopes (endospermatic layer, seed coat and pericarp) may contribute to dormancy.

Plant hormones have been shown to play a pivotal role in regulating the germination response and the expression of physiological dormancy in many different species (Finch-Savage and Leubner-Metzger, 2006). There is considerable evidence that the plant hormone abscisic acid (ABA) is a positive regulator of both the induction and maintenance of dormancy (Bewley, 1997). It has been shown that, in general, the transition from a high to a low dormancy level is accompanied by a reduction in embryo or seed sensitivity to the inhibitory effect of ABA (Finch-Savage and Leubner-Metzger, 2006). Sensitivity to ABA may vary depending on environmental conditions that prevailed during seed development. Examples of this type of response have been reported for *Sorghum bicolor*, in which certain environmental conditions during grain filling, such as water or nutrient availability, were associated with changes in embryo sensitivity to ABA that corresponded with the resulting dormancy level (Benech-Arnold et al., 1991, 1995). In sunflower, it is still unknown whether the effects of the environmental conditions during fruit development on the level of dormancy are mediated or not by differences in achene sensitivity to ABA.

Therefore, the aims of the present work were: (1) to investigate the effect of the thermal environment to which sunflower plants are exposed during fruit development under both controlled (greenhouse) and field conditions on the level of dormancy of the mature fruits; (2) to evaluate if the observed effect of the thermal environment on achene dormancy is related to changes in embryo and/or coat imposed dormancy; (3) to explore if the effects of the maternal (thermal) environment on fruit dormancy level are related to changes in embryo or achene responsiveness to ABA.

2. Materials and methods

Different experimental systems were used to explore a variety of thermal conditions during fruit development. In Experiment I, plants were cultivated in pots in the field and at full anthesis (R-5.9 according to Schneiter and Miller (1981)) some plants were transferred into a warm greenhouse until harvest time, while control plants remained in the field nearby. In Experiment II, trials were sown in the field on three different dates between early and late spring. In the third experimental system (Experiments III and IV), polyethylene tents (and their corresponding controls) were used to increase the temperature of the air surrounding the plants cultivated in the field; in this case, tents were applied during most of the fruit development phase (Experiment III) or during each of two halves of fruit development (Experiment IV). Experiment I was carried out during 2001–2002, Experiments II and III during 2008–2009 and Experiment IV during 2009–2010.

2.1. Plant materials and sites of the experiments

Plant material used in Experiment I was HA342, an inbred line with high oleic acid content. Plants were cultivated during late spring and summer at the Facultad de Agronomía, Universidad de Buenos Aires, Argentina (34°25′S, 58°25′W). In field experiments (Exp. II, III and IV) two different sunflower inbred lines (female and male) were grown together in the field. The F1 hybrid grain, the main focus of our work, was obtained following the same practices as in the process of commercial hybrid seed production. The experiments were conducted at Fontezuela, Buenos Aires, Argentina (33° 53′S, 60°27′W).

2.2. Growth conditions in Experiment I

Thirty sunflower plants were cultivated individually in 50 l plastic pots. Plants were fertilized and watered manually, and fungicides and insecticides were applied whenever necessary. Soil mixture included natural top soil and sand (2:1) for optimal drainage. Plants (21 in total) that reached the R-5.1 stage (according to the scale proposed by Schneiter and Miller (1981)) on the same day were selected for the experiment. At R-5.9 stage, six plants were transferred to a warm greenhouse and 15 plants were kept in the field (together with remaining plants which were kept as border plants). The greenhouse was approximately 60 m^3 in volume and temperature was set at 32 °C. Temperature above this limit was prevented by forcing external air through wet straw cooling pads. Meteorological data (temperature, radiation and relative humidity) inside and outside the greenhouse was recorded hourly in a weather station (Campbell Scientific Inc., USA). At harvest (60 days after R-5.1; plants had already passed the R-9 stage) heads were threshed manually and achenes from the outer two-thirds of the head were collected separately and used in germination tests (as described in Section 2.4). After harvest achenes were stored dry in paper bags and kept in a chamber at 15 °C. For germination tests, achenes from different plants were pooled to obtain experimental units. For the "field" treatment, plants were assigned randomly to three groups of five plants each, and these three pools were treated as experimental units. For the "greenhouse" treatment, achenes

from three pairs of plants were pooled into three experimental units.

2.3. Growth conditions and treatments in field trials

Three different experiments were conducted in the field (Experiments II, III and IV). In these three experiments the parental lines were planted at a crop population density of 7.14 plants/m^2, and row spacing was 0.7 m, inserting a row of male parent every two rows of female parent. The flowering date was recorded when 50% of the heads had reached the R-5.1 stage (Schneiter and Miller, 1981).

In Experiment II, parental lines were sown on three different dates (S_1, S_2 and S_3): September 22nd (early spring, S_1), October 22nd (mid spring, S_2) and December 2nd (late spring, S_3). Experimental design was completely randomized, and four plots per sowing date were randomly located within the experimental field. In this experimental system it is clear that not only temperature but other environmental factors differ among sowing dates. At harvest maturity, F1 achenes from different female plants within each plot were pooled and used as biological replicates.

In Experiments III and IV manipulation of temperature was achieved with the use of transparent tents heated by incident solar radiation during the daytime. These tents were used to increase air temperature of experimental sunflower plots during fruit development in Experiment III (between R-5.10 and few days after R-9 according to developmental scale by Schneiter and Miller (1981)), or during different intervals of fruit development in Experiment IV. In both Experiments III and IV, treatments with tents began after R-5.10 (13 days after anthesis, DAA) to avoid abortion of newly fertilized fruits by heat stress. Treatments were ended (plastic covers removed) 46 DAA, around one week after R-9 stage. In Experiment IV, treatments during intervals of fruit development were named IFD1 (tents from 13 to 26 DAA, or R-5.10 to *ca.* R-8), IFD2 (from 27 to 46 DAA; *ca.* R-8 to after R-9) and IFD1-2 (from 13 to 46 DAA; R-5.10 to one week after R-9). In both Experiments III and IV, the experimental design was completely randomized with four replications (tents) per treatment. At harvest time, fruits from all plants within each plot were harvested, pooled together and stored for germination trials.

Tents consisted of a cubic, rigid structure built with iron profiles, 2 m tall and 2 m wide, encased with transparent plastic (polyethylene 100 μm thick). When placed over the crop, each tent defined a "high temperature" (HT) plot (Fig. S1). Four "high temperature" (HT) and four control tents were placed at random positions in the field. Control tents (Fig. S1) consisted of the same iron structure with a plastic rooftop, but sides open except for a 30 cm plastic lateral strip near the top. The area covered by each tent was 4 m2 (8 m3 volume) and included about 25–28 plants, and was considered an experimental unit. The tents could be opened at the bottom and top to improve ventilation. Each side of the HT tents had an opening 30 cm high near the ground and side slits near the top to reduce accumulation of excessive moisture and allow gas exchange. In addition, the roofs were opened daily for one hour (from 8 AM to 9 AM) and partially opened whenever the internal temperature reached 42 °C to avoid higher temperatures. During the period of fruit development that did not correspond to the "high temperature" treatments, plots remained covered only by the plastic roofs with a polyethylene 30 cm-wide strip around the upper portion of the sides. All field experiments (II, III and IV) were irrigated and were kept free of insects, weeds and diseases.

To establish the timing of physiological maturity, 20 fruits from each treatment and replicate plot were sampled from the intermediate portion of the floral disk, dried at 80 °C for 72 h, and weighed. This procedure was repeated every five days starting 15 DAA (*ca.* R-6) and until at least three points with similar dry weight values were recorded. Harvest time was determined when grain moisture content was below 11%. Heads were cut and threshed manually. Each head was divided into three concentrical regions and grains from the middle third from capitulum of similar first anthesis date (less than two days difference) were dried at 40 °C until 6% grain moisture was reached. After drying, the samples from the different treatments were stored at 25 °C and subsamples were taken to perform germination tests (see Section 2.4). Germination tests began at harvest and were repeated every 15 days for Experiments II and III (achenes were incubated at 11 °C and 25 °C), or 12 days after harvest and repeated every 20 days thereafter for Experiment IV (achenes were incubated at 25 °C).

Throughout the growing season, temperature, radiation and daily rainfall were recorded with a weather station (I-Metos, Pessl Instruments, Austria) located 200 m from the experimental plots in Experiment II. In Experiments III and IV, temperature and humidity inside the tents were registered on an hourly basis using dataloggers (Hobo U10, HOBO, USA) placed inside white PVC pipes located at capitulum height. In turn, radiation levels (above the plants) inside and outside the tents were measured by using a radiometer (Cavadevices, Argentina). During Experiment IV, meteorological variables were only recorded in two of the four plots (tents) belonging to each treatment.

Mean diurnal vapor pressure deficit (VPD) was estimated for treatments in Experiments III and IV using hourly records of temperature and relative humidity between 8 AM and 8 PM, and mean daily VPD was estimated for treatments in Experiment I following the method described by Abbate et al. (2004). Meteorological data obtained for replicate tents/plots in Experiments III and IV were used to calculate mean and standard deviation values for each treatment.

2.4. Germination tests

Germination trials from Experiments II, III and IV were done as follows: for each replicate plot (four replicates) 25 fruits (intact achenes), seeds (without pericarp) and/or naked embryos (without seed coat and endospermatic layer) were placed in 9 cm diameter plastic Petri dishes on two discs of filter paper moistened with 5 ml of distilled water and incubated at 25 or 11 °C for a period of 20 days. Petri dishes were sealed with plastic film to reduce evaporation during incubation, and water was added whenever necessary. In treatments where the pericarp was removed, this was done manually and avoiding damage to the embryo. In treatments where the seed coat plus endospermatic layer were also removed, seeds (without the pericarp) were placed for two hours in Petri dishes on a disk of filter paper containing 3 ml of distilled water, in order to hydrate these tissues to facilitate their separation from the embryo. Fruits were considered as germinated when radicle was visible outside the envelopes, while embryos and seeds were considered as germinated when radicle had elongated at least 3 mm.

Germination trials in Experiment I followed a different incubation protocol: three replicates of 25 achenes from each biological sample were sown on a layer of sterile cotton placed in rectangular plastic trays (1 × 8 × 16 cm) soaked with distilled water (excess water was added first and then allowed to briefly drain by gravity). Trays were wrapped with plastic film with small perforations and kept in incubation chambers (at constant 15 °C) in the darkness. Germinated fruits were scored daily using the same criteria as described above. Triplicate germination values obtained for each biological replicate were averaged, and then used to calculate the mean and SE (n = 3). Also, another incubation experiment was done with intact achenes and isolated embryos (after manually removing the pericarp and the seed coat). In this case, achenes and embryos were first incubated at 10 °C for 11 days, and then transferred to another chamber set with an alternating temperature regime (12 h

20 °C, 12 h 30 °C). This thermal regime is known to stimulate germination of dormant seeds of *Cynara cardunculus*, another member of the *Asteraceae* familiy (Huarte and Benech-Arnold, 2010). Here, it had the purpose of demonstrating that non-germinated achenes after incubation at 10 °C were not dead, but dormant.

2.5. Achene and embryo response to exogenous abscisic acid (ABA)

Responsiveness of achenes to exogenous ABA was tested on samples obtained in Experiment IV. Forty days after harvest, four replications of 20 fruits from the IFD1-2 and control treatments were incubated at 25 °C in Petri dishes with filter paper and 5 ml of different media: distilled water, Fluridone 100 μM, Fluridone 100 μM + ABA 10 μM and Fluridone 100 μM + ABA 50 μM. Fluridone (Pestanal ®, analytical standard, SIGMA-ALDRICH) is an inhibitor of ABA synthesis and was used to assess the existence of ABA synthesis during incubation, and also to prevent changes in ABA levels (due to possible synthesis) while testing germination under fixed concentrations of ABA. Preparation of 1 mM stock of ABA was as follows: Abscisic acid (from SIGMA-Aldrich, (±)-ABA) was dissolved first in an aliquot of ethanol before being added to water, and ethanol evaporated afterwards. Germinated fruits or seeds were counted and removed daily, during 20 days.

Effect of ABA on germination of isolated embryos was tested in samples from Experiment I as follows: Samples of 25 embryos were incubated in rectangular plastic trays over a cotton layer soaked with distilled water or two different ABA solutions (5 and 50 μM) at 10 °C, with three replicates per incubation media. Germinated embryos were counted daily.

2.6. Statistical analysis

Germination and meteorological data was subject to analysis of variance and Tukey's or Dunnett's post-test (see figure legends) for mean separation with a significance level of 5%. Statistical analysis was performed using InfoStat software (InfoStat 2010 version. InfoStat Group, FCA, National University of Córdoba, Argentina) assisted by R (R version 2.11.1 Copyright 2010; The R Foundation for Statistical Computing) and GraphPad Prism 6 (GraphPad Software, San Diego California USA, www.graphpad.com).

Grain dry weight data as a function of time (days) after anthesis was used to establish the time of physiological maturity by adjusting bi-linear regressions with a breakpoint model (Ploschuk and Hall, 1995). Bi-linear regressions were fitted with GraphPad Prism 6 and the date of physiological maturity was taken to be the breakpoint value of the function. The breakpoint value was unknown and fitted by the software. The R2 values for the fitted functions were in every case greater than 0.8.

3. Results

3.1. Achene development in a warm greenhouse enhanced coat-imposed dormancy

During fruit development in Experiment I, mean air temperature inside the greenhouse was about 5.8 °C above that measured in the field nearby (Fig. 1a; Table 1). Other environmental variables that differed between both treatments were mean daily radiation and mean daily vapor pressure deficit (VPD; Table 1), which were respectively reduced and increased inside the greenhouse as compared to the field.

Final germination percentage measured 25 days after harvest (DAH) was lower (*i.e.* dormancy level was higher) for "greenhouse" achenes (2.7% ± 1.3) as compared to "field" achenes (30.4% ± 11.9; Fig. 1b). Dormancy release proceeded for "field" achenes and germination percentage at 15 °C increased from *ca* 30% to 75% after four months of storage but not for "greenhouse" achenes in which final germination at 15 °C remained below 10% after the same period. Incubation under a fluctuating temperature regime stimulated ger-

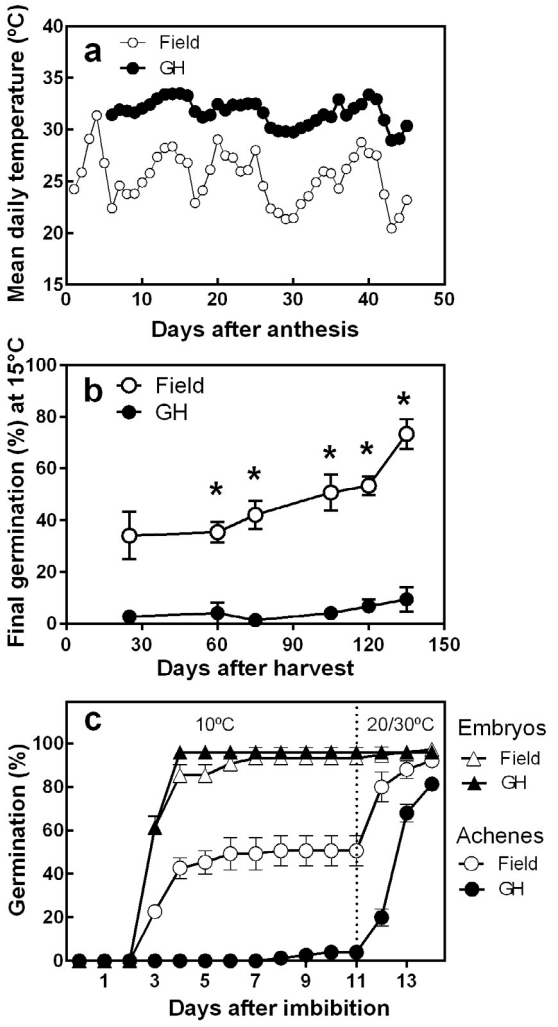

Fig. 1. Experiment I. (a) Mean daily temperature of the air in the field and inside the greenhouse (GH) during the 45–day period following first anthesis (R-5.1). (b) Final germination percent of achenes incubated at 15 °C and tested at different times after harvest. Asterisks indicate significant difference (multiple *t*-tests) between GH and Field values ($p < 0.01$). (c) Cumulative germination percentage of 3-month after-ripened achenes and isolated embryos during incubation at 10 °C constant (until day 11), followed by a 3-day period at an alternating regime (12 h at 20 °C – 12 h at 30 °C). Capped vertical lines in b and c indicate ±1 SE of the mean (n = 3) and are not shown when smaller than the symbol.

Table 1
Average values for environmental variables measured during the 45-day period after first anthesis (R-5.1) in Experiment I.

Environmental variables	Field	Greenhouse
Mean daily temperature (°C)	25.4 (2.5)	31.2 (1.9)
Mean diurnal temperature (10AM to 6PM) (°C)	27.9 (3.0)	33.5 (1.2)
Average minimum temperature (°C)	20.9 (2.8)	27.6 (2.7)
Average maximum temperature (°C)	30.3 (3.0)	35.7 (3.0)
Absolute minimum temperature (°C)	14.0	24.1
Absolute maximum temperature (°C)	38.4	38.6
Daily radiation (MJ m^{-2})	21.2 (6.6)	14.3 (5.6)
Average daily VPD (KPa)	1.43 (0.49)	1.93 (0.30)
PAR (mol m^{-2} d^{-1})	8.5 (2.6)	5.3 (2.3)

Standard deviation of daily values are shown in parentheses.

mination of 3-month afterripened achenes from both maternal environments, and germination increased from 51% (±7.1, SEM) to 92% (±2.3) for "field" achenes, and from 4% (±2.3) to 81% (±1.3) for "greenhouse" achenes (Fig. 1c). Removal of the fruit envelopes (pericarp, seed coat and endospermatic layer) also promoted germination of embryos incubated at 10 °C to high and similar levels for both maternal environment treatments (Fig. 1c).

3.2. Effect of different sowing dates on achene dormancy level after harvest

Thermal variables during the fruit development in Experiment II were very similar for both early (S_1) and mid-spring (S_2) sowing dates, but differed from those observed for the late-spring sowing (S_3; Table 2). Average mean temperature values during fruit development for S_1 and S_3 (21.5 and 19.7 °C, respectively) differed in 1.8 °C. A greater contrast (2.8 °C) between S_1 and S_3 was observed for the average mean temperature between 10 AM and 6 PM (*i.e.* diurnal temperature), which was 26.6 °C for S_1 and 23.8 °C for S_3. Other environmental variables during fruit development are shown in Table 2 and also reflect a great similarity between S_1 and S_2, as compared to S_3. S_3 had a lower daily radiation level and a shorter photoperiod compared to both S_1 and S_2. Consistent with this, final grain dry weight (GDW) attained in S_3 (39.3 ± 1.65 mg) was significantly lower as compared to GDW in S_1 (66.2 ± 2.53 mg) and S_2 (65 ± 2.68 mg; Fig. 2a).

Final germination percentages of achenes were generally lower at 11 °C as compared to 25 °C indicating that the expression of dormancy was enhanced at low incubation temperatures (Fig. 2b and c). Achenes from both S_1 and S_2 behaved similarly and expressed a higher level of dormancy at both incubation temperatures as compared to achenes from S_3 (Fig. 2b and c). At harvest time, fruits from S_1 and S_2 showed almost no germination at 25 °C, while fruits from S_3 showed germination values close to 50% at harvest, and reached full germination (100%) after 30 days of dry storage. In contrast, dormancy release was delayed in achenes from S_1 and S_2 and full germination at 25 °C was achieved 60–75 DAH. Early dormancy release for achenes from S_3 was also evident when incubation was performed at 11 °C; full germination was observed 75 DAH, a time at which fruits from S_1 and S_2 showed almost no germination at all (Fig. 2c).

Removal of the fruit envelopes (pericarp, seed coat and endospermatic layer) followed by incubation at 11 °C (Fig. 2d) allowed germination of *ca.* 50% of the embryos from S_1, and remaining embryo-related dormancy disappeared by 45 DAH. On the other hand, germination of S_1 achenes at 11 °C remained completely

Table 2
Experiment II: Environmental variables recorded during fruit development phase (between R-5.1 and R-9) for the early spring (S_1), mid spring (S_2) and late spring (S_3) sowing dates.

Environmental variables	S_1	S_2	S_3
Average mean diurnal temperature (10AM to 6PM) (°C)	26.6 (3.7)	26.3 (3.7)	23.8 (3.6)
Average mean daily temperature (°C)	21.5 (5.5)	21.1 (5.6)	19.7 (4.6)
Average minimum temperature (°C)	20.5 (5.5)	20.1 (5.6)	18.8 (4.5)
Average maximum temperature (°C)	22.6 (5.6)	22.3 (5.6)	20.6 (4.6)
Absolute minimum temperature (°C)	7.5	6.9	9.5
Absolute maximum temperature (°C)	35.3	35.0	33.9
Average radiation (MJ m^{-2})	32.8	32.9	26.7
Photoperiod 15 DAA (h)	14.2	14.1	13.1

For average values, SD (shown in parentheses) was calculated using daily values measured during the fruit filling period. Environmental data was recorded in a weather station 200 m from the experimental plots. DAA means days after anthesis.

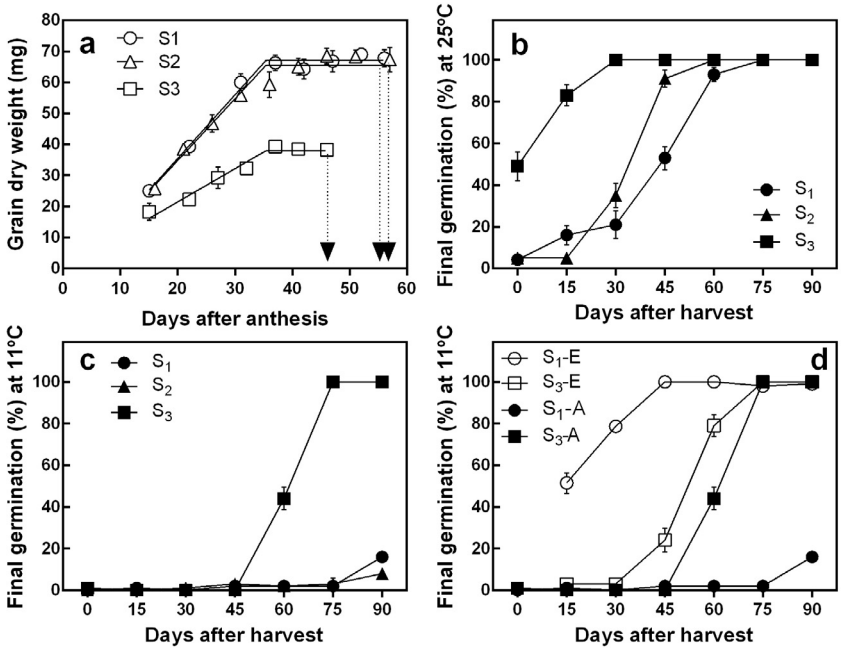

Fig. 2. Experiment II. (a) Dynamics of achene dry weight as a function of time after first anthesis (R-5.1) for sowing dates S_1, S_2 and S_3 (early, mid and late spring, respectively); harvest time is indicated with an arrow. (b) and (c) Final germination percentage as a function of time after harvest for fruits from S_1 to S_3 sowing dates. Achenes were incubated at 25 °C (b) or at 11 °C (c). (d) Final germination percentage (at 11 °C) as a function of time after harvest for achenes (A) and embryos (E) from the S_1 and S_3 sowings. Mean values of four plots are shown, and capped vertical lines in (a) to (d) indicate ±1 SE of the mean (n = 4) and are not shown when smaller than the symbol.

Table 3
Experiment III. Characterization of the thermal environment during fruit development (between 13 and 46 days after anthesis) for control and high temperature (HT) plots.

Environmental variables	HT	Control	
Mean daily temperature (°C)	27.2 ± 2.8	25.2 ± 2.7	*
Mean diurnal temperature (10 AM to 6 PM) (°C)	37.7 ± 4.8	32.1 ± 4.1	*
Average minimum temperature (°C)	15.2 ± 3.1	16 ± 3.0	*
Average maximum temperature (°C)	43.7 ± 4.2	36.5 ± 3.9	*
Absolute minimum temperature (°C)	8.3 ± 0.12	9.1 ± 0.14	*
Absolute maximum temperature (°C)	48.8 ± 0.65	42.8 ± 0.39	*
Average VPD (KPa)	3.04 ± 0.10	2.19 ± 0.09	*

Meteorological data was collected with sensors located in each experimental plot. Values shown are the average of four tents ± 1 SE (n = 4). Asterisks indicate significant difference ($p<0.05$) between treatments (HT and Control) for each variable (1-way ANOVA).

inhibited until 75 DAH, indicating that, in this case, dormancy was imposed by the fruit envelopes. In contrast, final germination percentage of both intact achenes and isolated embryos belonging to S_3 (cooler maternal environment) evolved similarly after harvest. In this case, embryo-related dormancy was stronger and had a major role in the germination response of the achene, with a very small contribution of the fruit envelopes.

3.3. Effect of plastic tents in the field on temperature during achene development and on dormancy level after harvest

Transparent tents used in Experiment III effectively increased the average temperature in the high temperature plots (HT) by about 2 °C as compared to the control plots (Table 3). This difference was even higher when comparing the average for mean diurnal temperature (between 10 AM and 6 PM; Table 3 and Fig. 3a). Estimated average diurnal VPD was higher inside the HT plots as compared to the control plots. Solar radiation in the HT plots was reduced by *ca.* 18% as compared to the control (which only had a roof of film). Grain filling dynamics were also affected in the HT plots (Fig. 3b). Final dry weight of fruits from the HT treatment (45.7 ± 1.5 mg) was significantly lower than that of the control (61.2 ± 2.9 mg).

Fruits from HT and control plots germinated poorly at harvest (*i.e.* dormancy was strong at this time), either when incubated at 25 °C or 11 °C (Fig. 4a, b). Dormancy release proceeded during dry storage for achenes from control plots, but was significantly delayed in fruits from the HT plots. Within 30 DAH fruits from the control plots reached 62% (±14.7) germination when incubated at 25 °C, while germination of fruits from HT plots was only 23% (±8.2) (Fig. 4a). At lower incubation temperature (11 °C) germination of fruits from the control treatment reached 20% (±9.2) after a three month storage period, while those exposed to the "high temperature" environment did not germinate at all (Fig. 4b).

Removal of the fruit envelopes (pericarp, seed coat and endospermatic layer) increased germination at 11 °C (Fig. 4b). Nevertheless, germination response of the embryos was contrary to the observed response of intact achenes for both HT and control plots. Although the HT treatment delayed achene dormancy release as compared to the control, the isolated embryos from HT plots were less dormant when compared to control embryos. These results are in agreement with those reported above for early (S_1) and late-spring sowings (S_3) (Fig. 2d).

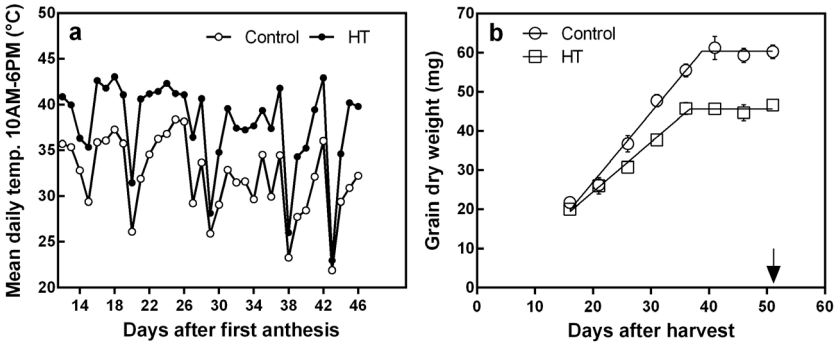

Fig. 3. Experiment III. (a) Mean diurnal temperature (between 10 AM and 6 PM) for the high temperature plots (HT, covered with plastic tents) and control plots during the field treatments (between 13 and 46 days after anthesis, R-5.1). Each value is the mean of four replicate plots (see Section 2.3). (b) Achene dry weight as a function of time after anthesis for both HT and control plots. Harvest time is indicated with an arrow in (b). Mean values of four plots are shown, and capped vertical lines in (b) indicate ±1 SE of the mean (n = 4) and are not shown when smaller than the symbol.

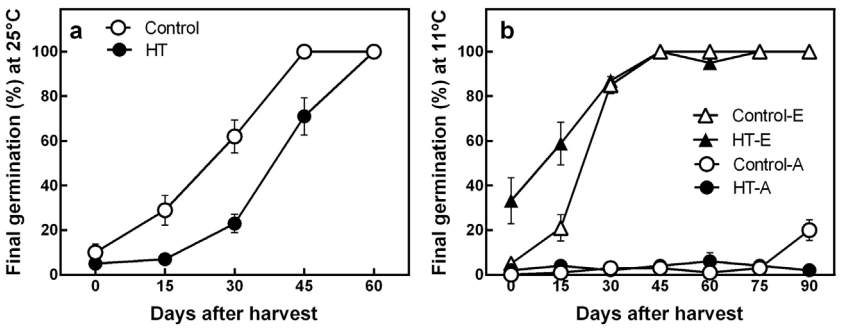

Fig. 4. Experiment III. (a) Final germination percentage at 25 °C for achenes from control and high temperature (HT) plots tested at different times after harvest. (b) Final germination percentage at 11 °C for achenes (A) and embryos (E) obtained from the HT and control plots, and tested at different times after harvest. Capped vertical lines in a) and b) indicate ±1 SE of mean (n = 4) and are not shown when smaller than the symbol.

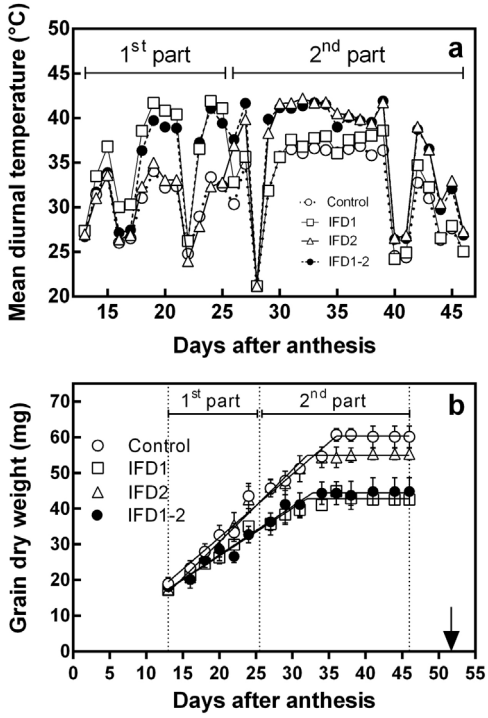

Fig. 5. Experiment IV. (a) Mean diurnal temperature (between 10 AM and 6 PM) in the experimental plots covered with plastic tents, applied between 13 and 46 (IFD1-2), 13 and 25 (IFD1) and between 26 and 46 days after anthesis (IFD2), and for the control plots. (b) Evolution of achene dry weight as a function of time after anthesis (R-5.1) for each field treatment (IFD1, IFD2, IFD1-2, and control). Harvest time was the same for all treatments, and is indicated with an arrow. Horizontal lines indicate 1st and 2nd part of fruit development as defined for the application of treatments. Capped vertical lines indicate mean value of four repetitions ±1 SE of mean and are not shown when smaller than the symbol.

3.4. *Effect of increasing temperature during different stages of achene development on dormancy level after harvest*

After finding that increasing temperature during the whole fruit development phase had an impact on achene dormancy, we proceeded to explore the effect of high temperature on dormancy when applied during different sub-phases of fruit development. With this aim, tents were applied during different time intervals (Experiment IV; see Section 2.3). If compared to the observed grain filling dynamics, IFD1 began when fruits had reached 30–40% of final GDW and ended with 75–79% of final GDW, while IFD2 began with 75–79% of final GDW and extended shortly into the dehydration phase (several days after the maximum GDW had been reached) (Fig. 5b). Inside the tents the average diurnal temperature (between 10 AM and 6 PM) was significantly increased, as well as mean daily, average daily maximum and average absolute maximum temperatures (Table 4 and Fig. 5a). Average mean diurnal VPD (also calculated for both sub-phases of fruit development) was increased inside the tents, although differences between treatments and control were not significant (Table 4). The use of tents had an impact on final GDW (Fig. 5b): The highest GDW (60.7 ± 1.8 mg) was observed for the control plots, followed by IFD2 (54.4 ± 2.9 mg). In the IFD1-2 and IFD1 treatments, final GDW was further reduced (44.4 ± 2.7 mg and 42.4 ± 4.4 mg, respectively). The IFD1-2 treatment reduced germination percentage (increased dormancy) at 25 °C as compared to the control (Fig. 6a) when tested at different times after harvest but, curiously, dormancy was even stronger for the IFD2 treatment. On the other hand, the IFD1 treatment had only a slight effect on final germination percentage at 25 °C when compared to the control treatment tested at 12, 32 and 52 DAH.

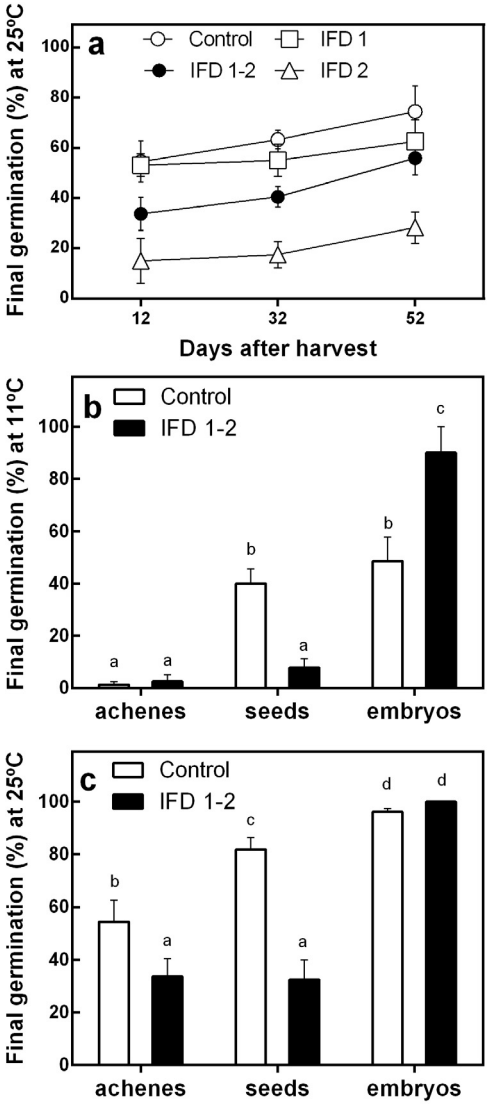

Fig. 6. Experiment IV. (a) Final germination percentages at 25 °C for fruits from plants exposed to high temperature during the first part (IFD1; from 13 to 25 days after anthesis) or second part (IFD2; from 26 to 46 days after anthesis) of fruit development, during both parts (IFD1-2; from 13 to 46 days after anthesis) and from control plants. Final germination percentages at 11 °C (b) and 25 °C (c) of fruits, seeds and embryos from IFD1-2 and control treatments. Capped vertical lines indicate ±1 SE (a) or +1 SE (b and c) of mean (n = 4 in a, b and c) and are not shown when smaller than the symbol. Different letters in (b) and (c) indicate significant differences between treatments (Tukey's test, α = 0.05).

The relative contribution of the seed envelopes to dormancy was assessed at incubation temperatures of 11 and 25 °C soon after harvest in achenes from the control and IFD1-2 treatments. Germination of intact achenes at 11 °C was very low for both treatments (Fig. 6b). Removal of the pericarp did not affect germination of seeds from the IFD1-2 treatment as compared to the intact achenes, but increased germination by almost 40% in the control treatment. Instead, removal of all envelopes surrounding the embryo (*i.e.*, pericarp, seed coat and endospermatic layer) promoted germination of the IFD1-2 embryos to above 90%, while only a slight increase was observed for control embryos (Fig. 6b). At an incubation temperature of 25 °C, dormancy expression was reduced (Fig. 6c) as compared to 11 °C (Fig. 6b). When incubated at 25 °C, fruits from IFD1-2 treatment germinated less (*i.e.* showed a higher dormancy level) than control fruits (Fig. 6c). In agreement with results obtained at 11 °C, the removal of the pericarp had no sig-

Table 4
Experiment IV. Environmental variables recorded during fruit development.

Treatment	Thermal variables during grain filling			
	(1st part)	(2nd part)	(1st part)	(2nd part)
	Mean diurnal temperature (10 AM to 6 PM) (°C)		Mean daily temperature (°C)	
IFD1	35.6 ± 5.5 (b)	32.9 ± 5.7 (a)	26.1 ± 3.2 (b)	26.4 ± 2.5 (a)
IFD2	30.9 ± 3.8 (a)	36.5 ± 6.4 (b)	24.4 ± 2.9 (a)	28.0 ± 2.8 (b)
IFD1-2	34.4 ± 5.5 (b)	36.3 ± 6.6 (b)	25.7 ± 3.1 (b)	27.6 ± 2.7 (b)
Control	30.3 ± 3.1 (a)	32.1 ± 5.2 (a)	24.1 ± 2.7 (a)	26.0 ± 2.2 (a)
	Daily minimum temperature (°C)		Daily maximum temperature (°C)	
IFD1	16.2 ± 3.7 (a)	18.7 ± 1.9 (a)	39.4 ± 5.1 (b)	36.1 ± 5.8 (a)
IFD2	16.1 ± 3.7 (a)	18.6 ± 1.8 (a)	35.6 ± 4.4 (a)	40.6 ± 6.2 (b)
IFD1-2	16.2 ± 3.7 (a)	18.6 ± 1.8 (a)	37.4 ± 5.6 (a)	40.1 ± 6.4 (b)
Control	16.2 ± 3.8 (a)	18.7 ± 1.9 (a)	33.6 ± 3.6 (a)	34.7 ± 5.2 (a)
	Absolute minimum temperature (°C)		Absolute maximum temperature (°C)	
IFD1	10.3 ± 3.7 (a)	15.2 ± 1.9 (a)	46.0 ± 5.1 (b)	42.2 ± 5.8 (a)
IFD2	10.4 ± 3.7 (a)	15.5 ± 1.8 (a)	42.7 ± 4.4 (a)	47.0 ± 6.2 (b)
IFD1-2	10.1 ± 3.7 (a)	15.6 ± 1.8 (a)	44.0 ± 5.6 (a)	46.1 ± 6.4 (b)
Control	10.3 ± 3.8 (a)	15.2 ± 1.9 (a)	38.7 ± 3.6 (a)	40.4 ± 5.2 (a)
	Diurnal VPD (KPa)			
IFD1	2.11 ± 0.17 (a)	2.60 ± 0.18 (a)		
IFD2	1.88 ± 0.10 (a)	3.09 ± 0.45 (a)		
IFD1-2	1.82 ± 0.23 (a)	3.10 ± 0.40 (a)		
Control	1.65 ± 0.18 (a)	2.30 ± 0.26 (a)		

Data was collected with sensors located inside two of four tents belonging to each treatment (see treatments in Section 2.3). Average values (±SD; n = 2) for each treatment were calculated separately for the 1st part (from 13 to 25 days after anthesis) and for the 2nd part (26 to 46 days after anthesis) of fruit development. Different letters indicate significant difference between each treatment and the control within each column (Dunnett's multiple comparison test, α = 0.05).

nificant effect on germination of IFD1-2 seeds incubated at 25 °C, while the germination percentage was increased significantly in the control seeds. Finally, isolated embryos from both IFD1-2 and control treatments reached almost 100% germination, evidencing no expression of embryo dormancy under these conditions. In summary, results obtained at both incubation temperatures (11 and 25 °C) showed a major effect of the seed coat plus endospermatic layer in the expression of dormancy for achenes from the IFD1-2 treatment, while the major restraint to germination in control achenes was the pericarp.

3.5. *Effect of temperature during fruit development on achene and embryo responsiveness to ABA*

The effect of exogenously applied ABA was tested using achenes that developed under contrasting thermal environments in Experiment IV (Fig. 7a). Fruits from IFD1-2 treatment showed lower germination percentages at 25 °C than those from the control treatment 40 days after harvest. Incubation in the presence of Fluridone strongly promoted germination for both treatments, indicating that ABA *de novo* synthesis is involved in the expression of dormancy in this genotype. The promoting effect of Fluridone was mostly reverted by the addition of ABA 10 μM and to a similar level for both IFD1-2 and control treatments, while the addition of ABA 50 μM further inhibited germination but to a greater extent in the IFD1-2 treatment. Therefore, achenes from the IFD1-2 treatment were significantly more responsive to 50 uM ABA than those from the control treatment.

Embryo sensitivity to ABA was also evaluated in 5-month after-ripened achenes obtained in Experiment I (Fig. 7b). Embryo germination percentage data showed that the maternal environment had a significant effect (2-way ANOVA, $p < 0.0005$), while both the effect of ABA concentration and the interaction were not significant (p-values 0.24 and 0.16 respectively). Despite these results, germination of "greenhouse" (but not "control") embryos showed a tendency to decrease at higher ABA doses, suggesting a higher sensitivity to ABA of the former. In addition, incubation of after-ripened achenes (105 DAH) from both maternal environments in presence of an ABA biosynthesis inhibitor (100 ppm Fluridone) did not affect the germination response (data not shown).

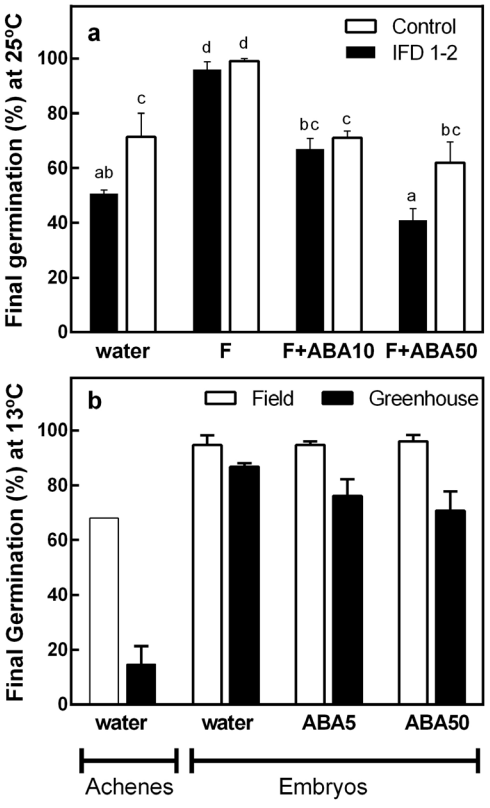

Fig. 7. (a) Effect of maternal environment on achene sensitivity to ABA in Experiment IV. Final germination percentages of achenes from IFD1-2 treatment and control plots (see treatments in Section 2.3). Forty days after harvest, achenes were incubated at 25 °C in water, Fluridone 100 μM (F), and Fluridone 100 μM + ABA 10 μM (F+ABA10) or 50 μM (F+ABA50). Different letters above the bars in (a) indicate significant differences between treatments (Tukey's test, α = 0.05). After harvest, fruits were kept at 5 °C in dry conditions. (b) Final germination percentage at 13 °C for intact achenes incubated in water, and for isolated embryos incubated in water and ABA solutions of 5 (ABA5) and 50 μM (ABA50). Fruits were obtained from Experiment I and had been stored dry at 15 °C for 105 days after harvest. Capped vertical lines are +1 SE of mean (n = 4 in a and n = 3 in b).

4. Discussion

4.1. Higher temperatures during fruit development enhanced dormancy of sunflower achenes

It has been known for long that the environmental conditions prevailing during fruit development and maturation on the mother plant (*i.e.* grain filling) can affect the level of seed dormancy at harvest or dispersal time (Fenner, 1991). Among the different factors acting on the mother plant, temperature has been shown to play a major role in modulating the level of dormancy in several species. In this work we investigated a possible effect of the thermal environment during sunflower fruit development on achene dormancy at harvest and its persistence during storage.

After using a variety of experimental systems to generate different thermal environments, and two different genotypes (an inbred line and a F1 hybrid), a general pattern was clear and in agreement with the hypothesis that higher temperatures during fruit development result in higher levels of dormancy at harvest and its persistence during dry storage (Figs. 1, 2, 4 and 6).

Results presented here are, to the best of our knowledge, the first to show that temperature during fruit development can affect achene dormancy level at harvest under field conditions. These results are consistent with preliminary results obtained by Fonseca (2000) who reported significant changes in dormancy level for sunflower achenes from plants grown in greenhouses set at constant, contrasting temperatures (12.9 *vs* 35 °C, and 24.4 *vs* 31.6 °C). Differences in mean temperature values between "cool" and "warm" treatments in the experiments reported by Fonseca, and in our greenhouse experiment (Experiment I; Table 1), are larger than the observed for treatments applied in the field (Experiments II, III and IV; Tables 2–4, respectively). Nevertheless, results presented here show that even small differences in mean diurnal temperature (*e.g.* ~2 °C in Experiment II and III; Tables 2 and 3, respectively) under field conditions can produce a strong impact in the level of dormancy at harvest and in the dormancy release pattern afterwards.

The possibility that regulation of dormancy level by the maternal (thermal) environment is exerted within a particular phase of fruit development was explored in Experiment IV. Results suggest that higher temperatures towards the final stages of the fruit development period are associated to increased dormancy levels at harvest (Fig. 6). A similar response pattern was reported in barley, where high temperatures by the end of grain filling (*i.e.* 300–350 °Cd from the beginning of grain filling, base temperature 5.5 °C) were those that exhibited the highest correlation with the dormancy level observed at harvest in this species (Rodríguez et al., 2001).

Unexpectedly, applying high temperature strictly during the final sub-phase of fruit development (IFD2) had a stronger effect on dormancy as compared to the whole fruit development treatment (IFD1-2; Fig. 6a). A possible explanation to this observation is that temperature during early development might have an effect on some component of dormancy as well. For example, lower temperature during early development might enhance embryo dormancy, while higher temperatures during later development enhance coat-imposed dormancy. Differential effects of temperature (according to the timing and the "direction") might exist on embryo and coat-imposed dormancy. In addition, observed differences could also be associated with the fact that the occurrence of abrupt raises in temperature during fruit development may have a differential effect on dormancy than prolonged exposures to "high temperatures" (*i.e.* all fruit development) in which acclimation responses could be operating. Further research is required in order to test these hypotheses.

The magnitude of the effects observed in the different field experiments (Experiments II, III and IV), showed that different sowing dates (Experiment II) had the strongest impact on the level of dormancy (Fig. 2) as compared to more direct manipulations of the thermal environment through the use of plastic tents (Figs. 4 and 6). This stronger response suggests that, in addition to temperature, other environmental factor/s that differed among sowing dates affected achene dormancy. The different sowing dates in Experiment II produced not only variations in the thermal environment but also had an impact in other factors like photoperiod and radiation (Table 2). Maternal effects of radiation and/or photoperiod on seed dormancy have been reported for several species (Fenner, 1991). For example, a short photoperiod during grain filling in quinoa has been associated with lower levels of dormancy at harvest (Ceccato et al., 2011). The same response was reported for *Portulaca oleracea* (Gutterman, 1974) and *Lactuca sativa* (Gutterman, 1973), among others reported by Fenner (1991). Further experiments should be conducted in order to test the possible effects of these factors (and their interaction with temperature) on the dormancy level of sunflower fruits.

To increase temperature during grain development under field conditions we used transparent plastic shelters. A usual concern regarding the use of this methodology is the creation of a confined atmosphere. Depending on how limited gas exchange rate is, plant transpiration and photosynthesis can increase vapor pressure and reduce CO_2 levels during daytime (Rattalino Edreira et al., 2014). In our experiments, transient increases in vapor pressure inside the tents were observed during daytime indicating some degree of confinement; nevertheless, ventilation was enough to avoid saturation with water vapor, even at times in which highest transpiration rates were expected to occur (see Fig. S2). It could be argued that reduced CO_2 levels inside the tents that limit carbon fixation can also affect dormancy or any other studied variable using this experimental setup. Additionally, increasing temperatures above 20 °C have been shown to further reduce CO_2 uptake in sunflower leaves as a consequence of negative, direct effects of temperature on true photosynthesis and increased photorespiration (Hew et al., 1969). Both factors ("confinement" and temperature) may affect dormancy through their impact on photoassimilate supply, or operate on dormancy through different, independent pathways. Evidence presented here supports this last possibility, as no relation between limitation of carbon supply during grain development (reflected in reduced final GDW values) and fruit dormancy became apparent when comparing the results presented throughout this work. Finally, a similar effect of higher temperature on increased dormancy was observed in Experiments I and II (with no expected changes in CO_2 levels), suggesting that temperature is the most relevant environmental factor influencing dormancy at least within the range of experimental conditions explored in this work. On the other hand, recent experiments conducted with sunflower support that limitation of carbon supply during fruit development by shading of the leaf area reduces dormancy of the harvested fruits (Pizzorno, Batlla and López Pereira, unpublished results). Therefore, possible effects of reduced C supply (resulting from transient depletion of CO_2 inside the tents) on dormancy could be expected to be opposite to those observed for high temperature.

4.2. The maternal thermal environment affected the relative contribution of the fruit structures to dormancy

Removal of the fruit envelopes (pericarp, or pericarp plus seed coat and endospermatic layer) usually increased germination percentage (Figs. 1 c, 2 d, 4 b, 6 b and c, and 7 b). Nevertheless, the relative contribution of the envelopes and the embryo to the dormancy level of the intact achene showed a strong interaction with the thermal environment, as observed in Experiments II, III and IV. As a general pattern, warmer maternal environments produced

stronger coat imposed-dormancy, and weaker embryo-related dormancy (Figs. 2 d, 4 b and 6 b).

Early (S_1) and late-spring (S_3) sowings (Experiment II) produced important changes in embryo germination capacity, and which were opposite to the observed for intact achenes (Fig. 2d). Similarly, in Experiments III and IV, embryo and coat imposed dormancy were affected in opposite ways by the "high temperature" treatment (Figs. 4 b, and 6 b, c): although achenes and seeds from the "high temperature" plots were more dormant than those from the control, the isolated embryos were less dormant when compared to their control counterparts. This indicates that the "high temperature" treatment decreased embryo dormancy, but increased dormancy imposed by the envelopes to an extent sufficient to produce a higher level of achene dormancy. As a result of fruit development under warmer maternal environments, embryo dormancy decrease after harvest was accelerated, while persistence of dormancy imposed by the envelopes was extended (Figs. 2 d and 4 b). Similar results have been reported by Ceccato et al. (2011) in Quinoa: in this species, warmer maternal environments during fruit development have also been associated with a higher level of dormancy imposed by the envelopes and a lower level of embryo dormancy. As far as it was discussed previously, the most common response for different species is that increased temperatures during fruit development are associated with reduced levels of dormancy (Fenner, 1991), as in winter cereals (Rodríguez et al., 2015). According to our results, this response in sunflower was observed for the embryos, but not for the entire fruit, for which the response was exactly the opposite, and similar as reported for Quinoa (Ceccato et al., 2011).

Results from Experiment IV also suggest that the relative contribution of both the pericarp and the seed coats to achene dormancy can change as a result of the maternal thermal environment (Fig. 6b and c). For instance, the seed coat (plus the endospermatic layer) can play a critical role in the imposition of higher levels of dormancy by a warmer maternal environment, since removal of the pericarp alone had no effect on germination, while the isolated embryos reached full germination values in the IFD1-2 treatment (indicating no embryo dormancy; Fig. 6b and c). In parallel, almost no effect of the seed coat was observed in seeds from control plots, but dormancy level of these achenes was determined almost equally by both the pericarp and the embryo (Fig. 6b).

Regarding the mechanisms involved in the observed responses to the maternal thermal environment, these might include specific changes in the structure of the pericarp and/or the seed coat layers (including the endospermatic layer). These structures have been shown to develop differently in response to changes in the maternal environment. For example, Lindström et al. (2007) and Franchini et al. (2010) observed changes in the pericarp in response to post-anthesis shading and water stress conditions in the field, respectively (although these works did not address effects on dormancy level). Changes in the structure of the endospermatic cell layer in relation to sunflower fruit dormancy were investigated by Szemruch et al. (2014). These authors observed that desiccation treatments intended to advance harvest time also enhanced dormancy, and this was related with changes in the thickness of the outer side of the wall of endospermatic-layer cells. Future experiments should be done to assess the possibility that similar changes take place under thermal environments that promote dormancy imposed by the envelopes.

4.3. Warmer maternal environments increased achene sensitivity to ABA

The role of ABA in the expression of achene dormancy was also investigated in this work. The possibility of a differential response to exogenous ABA (50 uM) during incubation was investigated with fruits obtained in Experiment IV (40 days after harvest) belonging to IFD1-2 and control treatments. The inhibitory effect of ABA was more pronounced in fruits from the IFD1-2 treatment as compared to the control, suggesting that a warmer maternal environment may affect achene dormancy through a higher embryo or seed sensitivity to ABA (Fig. 7a). The promotion of germination by incubation in presence of fluridone (ABA synthesis inhibitor) supports the occurrence of *de novo* ABA synthesis in the F1 hybrid used in this work (Fig. 7a). This is in agreement with work by Le Page-Degivry and Garello (1992) who reported that *de novo* synthesis of ABA in the embryonic axis takes place in imbibed dormant sunflower embryos. Nevertheless, direct measurements of ABA content are still lacking and would be necessary to confirm if differential ABA accumulation (resulting from synthesis/inactivation rates) exists or not in achenes with different depth of dormancy as observed in our work.

When the effect of ABA was tested in isolated embryos from 3-month afterripened achenes (Experiment I), "Greenhouse" embryos showed a slight decrease in germination with increasing ABA doses, while "Field" embryos showed no reduction in germination when incubated under the same conditions. Although this effect was not significant, it suggests that differences in embryo sensitivity to ABA between maternal thermal environments could be also operating in this genotype. Nevertheless, responsiveness to ABA of the isolated embryo may not reflect that of the embryo within the intact achene (more detailed studies with another sunflower genotype suggest that the fruit coats are necessary for embryo responsiveness to ABA, especially at low incubation temperatures; Rodríguez and Batlla, unpublished results). Unfortunately, the effect of ABA was not tested for intact achenes from Experiment I, as was done in latter experiments.

These results with ABA, although preliminary, suggest that the effect of the maternal environment on the resulting dormancy level could be at least partially explained by changes in sensitivity to ABA. Changes in embryo sensitivity to ABA during dormancy release or in response to environmental dormancy-modulating factors (such as drought) have been previously reported for other species (Benech-Arnold et al., 1991, 1995).

Previous works by Le Page-Degivry et al. (1990) suggested that dormancy imposition in sunflower embryos is determined by a peak of ABA content during the first stages of fruit development. According to this evidence, maternal environmental effects on the level of dormancy at harvest were expected to be found during the early stages of fruit development. However, results obtained in the present study suggest that it is during later stages of fruit development when the thermal environment influences the outcoming dormancy level (Fig. 6a). Results shown on Fig. 7 support that changes in dormancy level in response to temperature are mediated by changes in achene sensitivity to ABA, which might be defined during later stages of fruit development. The latter could be a possible argument for explaining why "high temperature" treatments showed a greater effect when applied during later stages of fruit development, as observed in the present work and also in Rodríguez et al. (2001) for barley.

5. Conclusions

In this work we describe different experiments under controlled and field conditions using two different genotypes, and performed in different years, and which provide evidence supporting the hypothesis that temperature during later stages of sunflower fruit development can affect dormancy level. We also showed that the contribution of the pericarp, seed coat and embryo to achene dormancy may vary in response to the environment explored by the mother plant during fruit development. And finally, we also pro-

vide evidence suggesting that observed changes in dormancy level in response to the maternal environment could be at least partially explained by changes in achene and/or embryo sensitivity to ABA.

Altogether, this information should prove useful to establish management strategies during sunflower seed production in order to reduce the level of dormancy of the harvested seed lots. For example, high levels of dormancy at harvest may be diminished, at least in part, through the selection of planting dates and/or sites showing lower probability of occurrence of high temperatures during the late stages of fruit development. In addition, these results are also relevant to understand some of the physiological mechanisms associated with environmental control of primary dormancy level in this species, such as the sensitivity to ABA and the effect of the different fruit components (pericarp, seed coats and embryo) in the regulation of dormancy.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eja.2016.10.007.

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