The effect of maternal environment on sunflower (*Helianthus annuus*) achenes dormancy level at harvest: the thermal environment

Paula Bodrone[1], Diego Batlla[2], Verónica Rodríguez[2], Sebastian Arisnabarreta[1], Monsanto Argentina, Fontezuela Research Station, Ruta 8 Km 214 B2700, Pergamino, Argentina, paula.bodrone@monsanto.com, sebastian.arisnabarreta.dupuy@monsanto.com [2]Faculty of Agronomy-IFEVA, University of Buenos Aires-CONICET, Av San Martin C1417DSE, Ciudad de Buenos Aires, Argentina, batlla@agro.uba.ar, mvr@agro.uba.ar.

ABSTRACT

- Thermal environment during seed development and maturation (thereafter just development) can affect the dormancy level of harvested seeds in several species. Field experiments were conducted with sunflower to investigate how the thermal environment during achene development affected their dormancy level at harvest, and evaluated if the observed response was related to changes in embryo and/or coat imposed dormancy.
- Three experiments were conducted during 2008 and 2009 in order to impose different thermal conditions during achene development, and to evaluate their effect on their dormancy level at harvest: i- different sowing dates ii- installation of polyethylene plastic tents covering whole plants that increased temperature artificially during the entire period of achene development, and iii- polyethylene plastic tents installed during each of three intervals within the period of achene development. Mature achenes were harvested for all experiments and dormancy level was assessed periodically for whole achenes and/or isolated embryos.
- Significantly higher levels of dormancy were observed in achenes from the earliest sowing dates (1st and 2nd), in which higher temperatures occurred during fruit development, as compared to achenes from the latest sowing date (3rd) in which lower temperatures occurred. Similarly, fruits from plots in which temperature was increased artificially with the use of plastic tents showed a higher dormancy level than seeds from control plots, although differences in dormancy level between treatments were somehow smaller than those observed between sowing dates. Experiments in which temperature was artificially increased at different intervals within grain development showed that dormancy was enhanced with respect to control plots only when temperature was increased during the last half of grain filling period, but not during the first half. The higher levels of dormancy in response to higher temperatures during development were mainly explained by an increase in coat imposed dormancy.
- Our results with sunflower indicate that higher temperatures during achene development can produce higher levels of dormancy at harvest due to an increase in coat imposed dormancy. On the other hand, results also suggest that this effect of temperature takes place at later stages of achene development. Finally, the fact that treatments in which temperature was increased artificially with plastic tents explained only partially the differences obtained between sowing dates suggests that other factors of the maternal environment (for example radiation and photoperiod) might also affect dormancy level of sunflower achenes.

Keywords: Achenes development, dormancy, maternal environment, seed coat, temperature.

INTRODUCTION

At harvest time sunflower seeds are dormant and germinate poorly (Corbineau et al., 1990). Depending on the genotype and the environment, this dormant state can last for weeks or even months. This issue represents a significant inconvenient to the seed industry because it prevents the utilization of the recently harvested seed lots to provide commercial hybrid seed either for local or counter season markets and increases industrial costs. Studies performed in several species indicated that the environmental conditions explored during seed development and maturation (thereafter just development) might affect the dormancy level of harvested seeds (Fenner, 1991). One of the most significant influences on seed germinability is the temperature they experience during their development (Batlla 2004). Regarding sunflower, results from greenhouse experiments indicated that higher temperatures during fruit development were related to higher levels of dormancy at harvest (Fonseca, 2000; Rodríguez et al., 2003). However, this effect had not been explored under field conditions yet. Therefore, the aims of the present work were to investigate under field conditions the effect of the thermal environment to which sunflower plants are exposed during fruit development on the level of dormancy of the mature achenes, and to evaluate if the observed effect is due to changes in embryo and/or coat imposed dormancy (pericarp and seed coat).

MATERIALS AND METHODS

Plant material

Plant material consisted of two different inbred lines (female and male) that were crossed to obtain the hybrid achenes following the same practices as in the process of commercial hybrid seed production. Experiments were also conducted without water limitations, free of insects, weeds and diseases. The experimental design was completely randomized with four replicates per-treatment.

Growth conditions and thermal environment treatments:

In order to evaluate the effect of temperature during fruit development on their dormancy level at harvest, three different experiments to impose contrasting thermal environments during achene development were conducted: i) different sowing dates (*i.e.* Sept 22nd, Oct 22nd and Dec 2nd of 2008), ii) installation of polyethylene plastic tents covering the plants in the experimental plot to increase temperature artificially during the whole period of achene development, and iii) installation of polyethylene plastic tents during each of three intervals of achene development: AT1 (from 13 to 28 days after anthesis, DAA), AT2 (from 29 to 46 DAA) and AT12 (from 13 to 46 DAA). Control treatment consisted in plots without plastic tents. At different intervals after harvest, germination of sunflower achenes (experiment 1, 2 and 3) and embryos (without pericarp and seed coat) (experiment 3) were scored.

In experiments 2 and 3, the tents were placed at 13 days after first anthesis to prevent high temperatures to cause abortion of flowers and/or newly fertilized seeds. The tents could be opened at the bottom and top. During the period of application of the treatments, each lateral was kept opened 50 cm at the bottom, and the roofs were open daily for 1 hour, from 8 am to 9 am, to prevent accumulation of excessive moisture. In addition, the roofs were partially opened whenever the internal temperature reached 42°C. During the seed development period that did not correspond to the high temperature treatments, plots remained covered only by the plastic roofs with a small polyethylene lateral superior coverage. Control plots remained covered under these same roofs until the application of the treatments ended. Temperature and humidity were recorded in each cage on an hourly basis using dataloggers (Hobo U10).

Treatments finalized after physiological maturity. To establish physiological maturity, 20 achenes per replication and experiment were dried in an oven at 80°C for 72 hs and weighed. This procedure was repeated every five days after first anthesis until at least three points with similar dry weight values were observed. Harvest time was determined when grain moisture content was lower than 11%. After harvest, plant heads were divided into three concentrical regions and grains from the middle third region of the heads with similar first anthesis moment (less than two days difference) were dried at 40°C until reaching 6% grain moisture.

Germination tests:

Grains from the middle third region of the plant heads of each experiment and thermal environment treatment were utilized in the germination tests. At different intervals after harvest, four replicates of 25 achenes and naked embryos 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°C for a period of 20 days. Achenes were considered to have germinated when radicle was visible outside the envelopes, and embryos, when radicle elongated 3 mm.

RESULTS

Experiment 1: The major differences in the thermal environment between sowing dates took place between 10 AM and 6 PM hours. During this period the thermal environment recorded (in a nearby meteorological station) during the grain filling period was similar for the first two sowing dates (Sept 22nd and Oct 22nd) with a similar mean temperature value around 26 °C. Lower temperatures were recorded for the third sowing date (Dec. 2nd) for which a mean temperature of 23.8 °C was recorded. A comparison of mean temperature values recorded for both earliest and latest sowing dates during the grain filling period is presented on figure 1. Final grain dry weight of the latest sowing date was about 39 mg, while the value observed for the earliest sowing dates was ca. 67 mg.

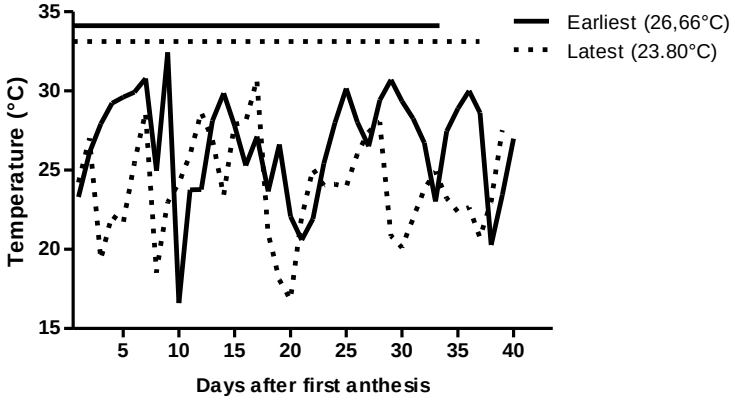


Fig. 1. Mean daily temperature between 10 AM and 6 PM for the earliest (filled line) and the latest (dashed line) sowing dates after first anthesis (Experiment 1). The lines above the picture indicate duration of the grain filling period for both sowing dates.

Significantly higher levels of dormancy were observed in achenes from the earliest sowing dates (first and second) in which higher temperatures occurred during fruit development, as compared to the third sowing date (Fig 2). At harvest achenes from earliest sowing dates showed almost no germination, while achenes from the third sowing date showed germination values close to 50%. In addition, while achenes from the third sowing date reached full germination (100 %) 30 days after harvest, those from earliest sowing dates reached full germination 60-75 days after harvest.

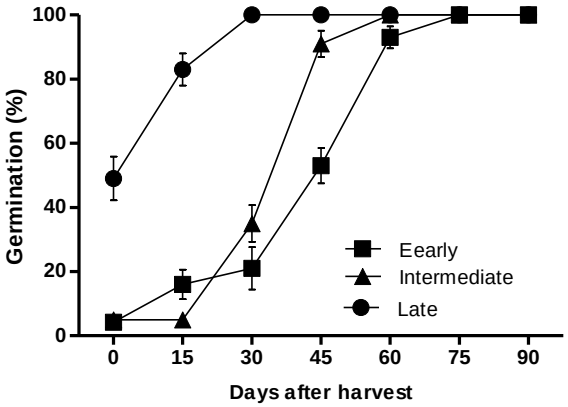
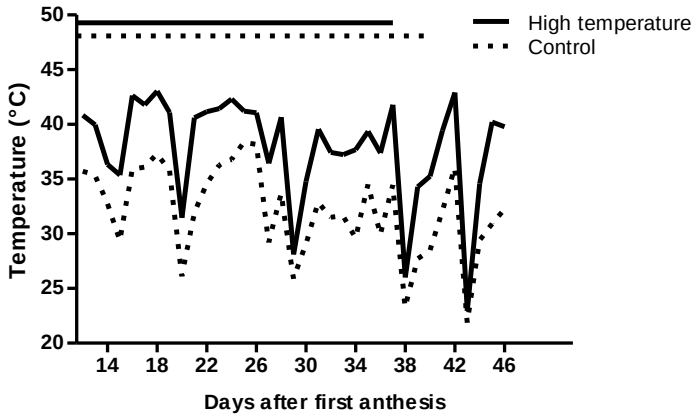


Fig. 2. Final germination percent of achenes from the first (■), second (▲) and third (●) sowing dates (Experiment 1). Germination assays were repeated at different times after harvest. Incubation temperature was 25°C. Vertical bars indicate standard error.

Experiment 2: The placement of polyethylene tents raised the temperature of the air surrounding the plants during the grain filling period (Fig. 3), showing a mean temperature value between 10 AM and 6 PM for the whole period of 37.7 °C for the high temperature treatment and 32.1 °C for the control. No significant differences in relative humidity were recorded between treatments (data not shown). As

expected, final dry weight of fruits exposed to high temperature treatments was lower (47 mg) than that of fruits harvested from control treatments (61 mg).
Achenes from plants exposed to both treatments (high temperature and control) showed very low germination levels at harvest, denoting a very high dormancy level. However, dormancy release was significantly delayed in fruits from plants in which temperature was increased artificially with plastic



tents as compared to achenes from control plots (Fig. 4).

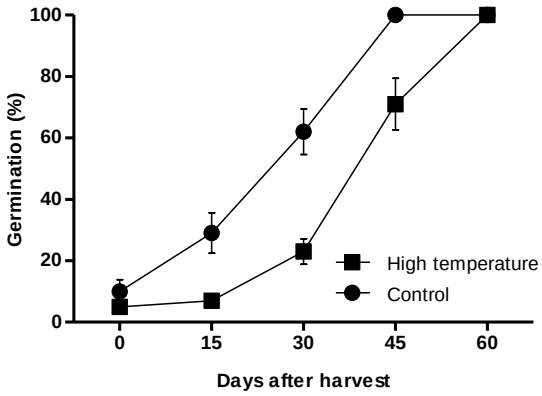


Fig. 3. Mean temperature between 10 AM to 6 PM for plots that received high temperature treatment (plastic tent; solid line) and Control plots (plastic roof only; dashed line) during the treatment period (Experiment 2). The lines above the picture indicate duration of the grain filling period in each treatment.
Fig. 4. Evolution of final germination percent during post-maturation of achenes from the high temperature (■) and Control (●) treatments, incubated at 25°C. Vertical bars indicate standard error.

Experiment 3: The disposition of polyethylene tents generated four different thermal environments during the grain filling period (Fig. 5). Mean temperature values between 10 AM and 6 PM for the whole period was 31 °C for control and 35 °C for AT12, while for AT1 and AT2 the mean temperature value the period when tents were present was ca. 35 °C. Final dry grain eight was 40 mg for AT1, 54 mg for AT2, 46 mg for AT12 and 61 mg for control treatment.

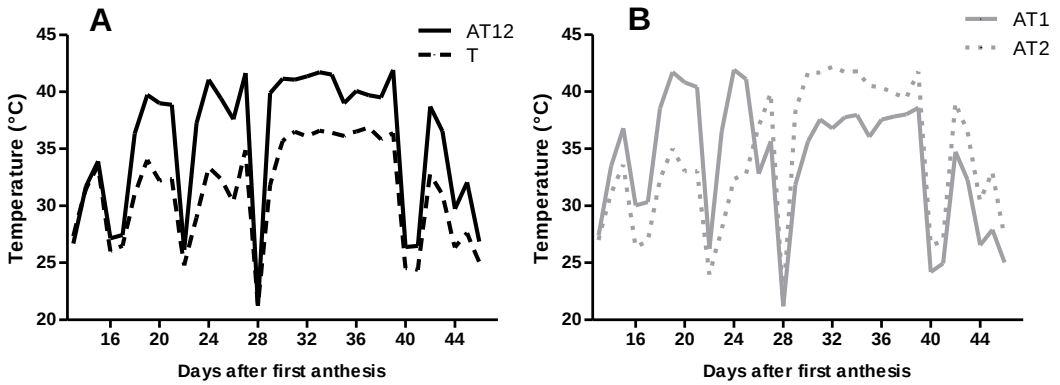


Fig. 5. A. Mean temperature between 10 AM and 6 PM recorded for the high temperature treatment applied during the whole period of seed development (filled line; AT12) and for the Control treatment (dashed line; T). **B**. Mean temperature between 10 AM and 6 PM recorded for the treatment of high temperature during the first period of seed development (filled line; AT1) and the treatment of high temperature during the last period of seed development (dashed line; AT2).

For experiment 3, germination was tested once twelve days after harvest. As observed in experiment 2, achenes from plants exposed to high temperature during the whole grain filling period (AT12) were more dormant than those from the control treatment. However, the high temperature treatment imposed during the first half of the grain filling period (AT1) showed no significant differences with the control treatment (T). A significantly lower germination level (increased dormancy) as compared to the control (T) was observed when the high temperature treatment was imposed during the second half of the grain filling period (AT2) (Fig. 6).
The differences in germination observed in achenes between T and AT12 was not observed when isolated embryos were incubated at the same temperature (Fig. 7). Embryos excised from fruits from both temperature treatments reached almost 100 % germination, evidencing no embryo dormancy under these conditions.

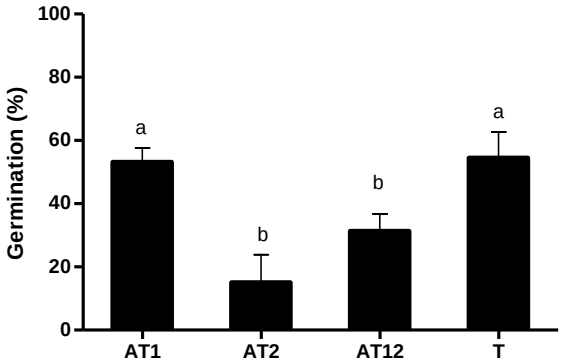


Fig. 6. Final germination percent of achenes from plants exposed to high temperature during the first (AT1), last (AT2), whole (AT12) period of seed development, and from Control plants (T), incubated at 25°C. Error bars indicate standard error. Different letters indicate significant differences between treatments (Mixed models, DGC test, α=0.05

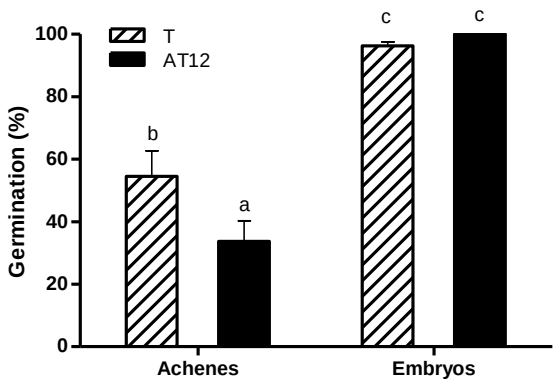


Fig. 7. Final germination percent for achenes and isolated embryos coming from plants exposed to high temperature during the whole period of seed development (black bars) and Control plants (dashed bars). (Experiment 3). Incubation temperature was 25°C. Different letters indicate significant differences between treatments (Mixed models, DGC test, α=0.05).

DISCUSSION

In the present study we report evidence from field experiments with sunflower plants indicating that temperature during fruit development can significantly affect dormancy level of the harvested achenes. In our experiments, higher temperature environments during fruit development in the mother plant were followed by higher levels of dormancy at harvest and afterwards during post-maturation (Figs. 2, 4 and 6). These results obtained from field experiments are consistent with those obtained in tests carried out in pots in greenhouse by Fonseca (2000) and Rodriguez et al (2003).

Obtained results also suggest that regulation of dormancy level by the thermal environment is mainly exerted during the final stages of the grain filling period. Thus, different thermal environments towards the final stages of the grain filling period appear to be associated with differences in dormancy level at harvest, while different thermal environments in the early stages of this period would not lead to differences in grain dormancy level (Fig. 6). A similar response pattern was reported in barley, where high temperatures by the end of grain filling (ie 300-350 ° Cd from the beginning of grain filling) were those that exhibited the highest correlation with dormancy level observed at harvest in this species (Rodriguez et al., 2001). These results are of practical interest for the sunflower seed production industry in order to avoid high levels of dormancy that are frequently observed in seed lots at harvest for certain hybrids. 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 the grain filling period.

In relation to grain structures responsible for the level of dormancy (embryo and/or coat), obtained results show that differences in dormancy due to temperature effects during grain filling were explained by changes in coat imposed dormancy, while embryo dormancy was not affected (Fig. 7). Similar results were previously reported by our group in this species (Bodrone et al., 2009), and by Ceccato et al. (2010) in Quinoa (*Chenopodium quinoa*). In Quinoa, high temperature environments during grain filling were also associated with higher levels of dormancy imposed by the fruit envelopes.

Finally, obtained results suggest that temperature was not the only environmental factor regulating the level of dormancy in this species. In this sense, different sowing dates involved changes not only in the thermal environment, but also in other environmental factors such as photoperiod and radiation. Changes in the level of dormancy have been reported in several species associated with differences in radiation and/or photoperiod (Fenner, 1991). According to the results obtained in this work, the delay in sowing date (Fig. 2) generated a greater difference in the final germination percent at harvest (and consequently on the level of dormancy) than the difference in the percentage germination resulting from the application of differential thermal environments through the implementation of plastic tents (Figs. 4 and 6).

REFERENCES

Batlla, D. 2004. Regulación de los cambios cíclicos en el nivel de dormición de semillas de *Polygonum aviculare* por efecto de la disponibilidad hídrica y la temperatura del suelo. Un modelo de simulación. Tesis Doctoral. Facultad de Agronomía, Universidad de Buenos Aires, Argentina.

Bodrone, P.; Batlla, D.; Arisnabarreta S.; Rodríguez, V. (2009). Efectos del ambiente térmico explorado por aquenios de girasol durante la etapa de llenado sobre el nivel de dormición de los granos a cosecha. Poster presented at 5to Congreso Argentino de Girasol, Buenos Aires, Argentina, 1-2 de Junio de 2010.

Ceccato, D. 2010. Efecto de las condiciones ambientales durante el desarrollo, maduración y almacenamiento sobre la dormición en semillas de quinoa (*Chenopodium quinoa* Willd) con tolerancia potencial al brotado pre-cosecha. Tesis de Maestría. Facultad de Agronomía, Universidad de Buenos Aires, Argentina.

Corbineau, F., Bagniol S., Côme D. 1990. Sunflower (*Helianthus annuus* L.) seed dormancy and its regulation by ethylene. Israel Journal of Botany 39: 313-325.

Fenner, M. 1991. The effects of the parent environment on seed germinability. Seed Science Research 1: 75-84.

Fonseca, A. 2000. Efecto de la temperatura durante el llenado de grano sobre el comportamiento de semillas de girasol (*Helianthus annuus L.*). Trabajo de intensificación para optar por el título de Ingeniero Agrónomo. Facultad de Agronomía, Universidad de Buenos Aires, Argentina.

Rodríguez, M. V., Rondanini D., Líbenson S. and Sánchez R. A. 2003. Efectos contrastantes de altas temperaturas durante todo o parte del período de crecimiento de los frutos sobre la germinación en girasol. Poster presented at: XXIX Jornadas Argentinas de Botánica & XV Reunión Anual de la Sociedad Botánica de Chile. 19-21 de Octubre de 2003. San Luis, Argentina. Boletín de la SAB 38: 195.