

## Effect of maternal environment on sunflower fruit dormancy (*Helianthus annuus*): photoperiod and incident radiation

Pizzorno, L.<sup>1</sup>, Batlla, D.<sup>2,3</sup>, and López Pereira, M.<sup>1</sup>

Cát. de Cultivos Industriales (1) y Cereales (2) FAUBA-CONICET (3)

Av. San Martín 4453. CABA (C1217DSE) Argentina E-mail: [pizzorno@agro.uba.ar](mailto:pizzorno@agro.uba.ar).

### ABSTRACT

- Sunflower fruit (*Helianthus annuus*) usually has a high level of dormancy at harvest time which determines problems in the processing and use of the fruit as seeds. Although this problem is relevant for the seed industry, there is little information in relation to maternal environmental effects (temperature, photoperiod, radiation, water, etc.) on the level of dormancy of sunflower fruit at harvest. Recent evidence strengthens temperature as the factor that modulates dormancy level of sunflower fruit. However, temperature does not explain all the variations that occur in contrasting environmental conditions (normal vs late planting dates), therefore other factors such as radiation and photoperiod may also be affecting fruit dormancy level. In this context, the objectives of this study were to evaluate: i) the effects of day length (Exp<sub>PHOTO</sub>) and incident radiation (Exp<sub>RAD</sub>) on sunflower fruit dormancy level and ii) the physiological basis of the observed responses.

- The Exp<sub>PHOTO</sub> and Exp<sub>RAD</sub> were conducted at the experimental field of the Facultad de Agronomía, Universidad de Buenos Aires (34° 35' S., 58° 29' W.) during the 2010-2011 growing seasons. In Exp<sub>RAD</sub> the incident radiation was managed through the use of black shading cloth mounted on rectangular tubular structures at different times of fruit growth and development from: i) flower bud to first anthesis, ii) first anthesis to last anthesis and iii) last anthesis to 10 days after last anthesis. The Exp<sub>PHOTO</sub> was sown in January to achieve a difference of around 2.5 hours in day length during the grain filling period between control and treatment plots. The photoperiod extension in the treatment plots was achieved through the use of incandescent and low-intensity lights mounted on rectangular tubular structures. Weather conditions were recorded followed during both experiments. In both Exps, fruit was harvested with 11% humidity. Then, the fruit was dried at 40 ° C to reach 6% humidity and the grain was stored in chambers at 15 ° C. Dormancy level was determined from harvest and every 20-25 days until the proportion of fruit that germinated was equal to or greater than 90%. Germination experiments were performed at two temperatures (12 and 25°C) and included two treatments: fruit and embryo (fruit without pericarp and seed coat).

- A smaller amount of incident radiation during the period of growth and grain development diminished fruit dormancy level. Five months after harvest, when the fruits were incubated at 25 °C, the germination percentage was 75% in the radiation treatments while it was 30% in control. In contrast, when fruits were incubated at 12 °C, there were no differences between treatments. The embryos showed more than 80% germination after 30 days from harvest for control and radiation treatments, suggesting that dormancy is imposed by fruit covers. The day length during the filling period also affected fruit dormancy level. After 34 days from harvest, fruit germination percentage was 50 and 10% for treatments of 14 and 12 hrs day length during the grain filling period, respectively. However, in both treatments 90% of fruit germinated 74 days after harvest.

- Obtained results showed that incident radiation and photoperiod explored for sunflower fruit during development and maturation can affect the dormancy level of harvested fruit. In the case of incidence radiation, observed effects were mainly explained by difference in fruit covers imposed dormancy. On the other hand, the expression of the dormancy level imposed by fruit covers depended on incubation temperature (dormancy was expressed at higher incubation temperatures). Regarding the effect of photoperiod, obtained results showed that this factor can affect fruit dormancy level; however dormancy disappeared after a short period of storage, suggesting that photoperiod may be affecting embryo dormancy which has been proven to be less lasting than the dormancy imposed by fruit covers.

- The results presented in this abstract, are to the best of our knowledge, the first documented evidence that the incident radiation and photoperiod affect sunflower fruit dormancy. Therefore, the variation of these environmental factors, in addition with temperature, should be considered for designing management strategies (sowing dates, planting locations etc.) to obtain lower levels of dormancy of sunflower fruit at harvest.

**Key words:** dormancy, fruit cover imposed dormancy, incident radiation, maternal environment, photoperiod, sunflower fruits.

## INTRODUCTION

Sunflower (*Helianthus annuus*) seeds usually have a variable dormancy at harvest. In some cases, dormancy decreases in a relatively short time without interfering with the use of grain as seed. However, in some years, the level of dormancy remains high for a long time difficult tasks of processing of seeds. The high level of seed dormancy at harvest is associated with the coexistence of a dormancy imposed by the covers and some remaining embryo dormancy (Corbineau et al, 1990). Embryo dormancy usually decreases soon after harvest, but the dormancy imposed by the covers can be stored for much longer (Rodriguez et al., 2010), the latter being responsible for the problems mentioned above. Despite the relevance of this problem, there is insufficient information globally and locally in relation to the effects of environment (temperature, photoperiod, radiation, water, etc.) in determining the level of dormancy during the seeds development. The only factor that has so far been evaluated is temperature. In this sense, Fonseca et al., (2000) observed differences in final germination percentage between fruit harvested from plants that had been exposed to two contrasting temperatures (12.9°C y 31.6°C) during fruit filling period. Within two months of storage, the fruits that developed at high temperature during grain filling period had a lower percentage germination in relation to those from plants exposed to lower temperatures, especially when they were incubated at low temperatures (ie 12 ° C ). Bodrone et. al., (2010) found that brief periods of high temperatures during seed growth mostly favor dormancy imposed by grain covers. Bodrone et al. (2010) evaluated the effect of two contrasting sowing dates on the dormancy level in sunflower seeds. The seeds of the late sowing planting date were developed with an average temperature lower than normal planting date (approx. 4 ° C difference). The fruit germination percentage (after 60 days of storage) of seeds that developed at lower temperatures (ie late planting date) was 50%, while fruit early planting date was 0%. These results taken together strengthen temperature as a factor that would modulate the level of dormancy of sunflower fruit during its development. But, it is necessary to consider a potential role that could have other environmental factors. In this sense, the approach (ie planting dates) used by Bodrone et al. (2010) not only changed the thermal environment under which the fruit is developed, but radiation and photoperiod. Additionally it is known that high temperatures during grain filling in sunflower affect the structure of grain covers (Rondanini et al., 2006). In this sense, Lindström et al., (2007) determined that radiation stress (80% less than the incident radiation) in preanthesis (flower bud to anthesis) and early post anthesis causes a reduction in the number of cell layers of pericarp. Additionally, there is association between the thickness of the seed coat and level of dormancy in seeds of *Chenopodium album* and *Abutilon theophrasti* m, both attributes are strongly modified by photoperiod and light quality (Guterman 1973 and 1978, Kigel et al. al., 1977, Pourrat & Jacques, 1975). The aims of this research were i) to evaluate and quantify the effect of photoperiod and radiation on the dormancy level of sunflower seeds ii) identify periods of high sensitivity to the effect of radiation during grain growth.

## MATERIALS AND METHODS

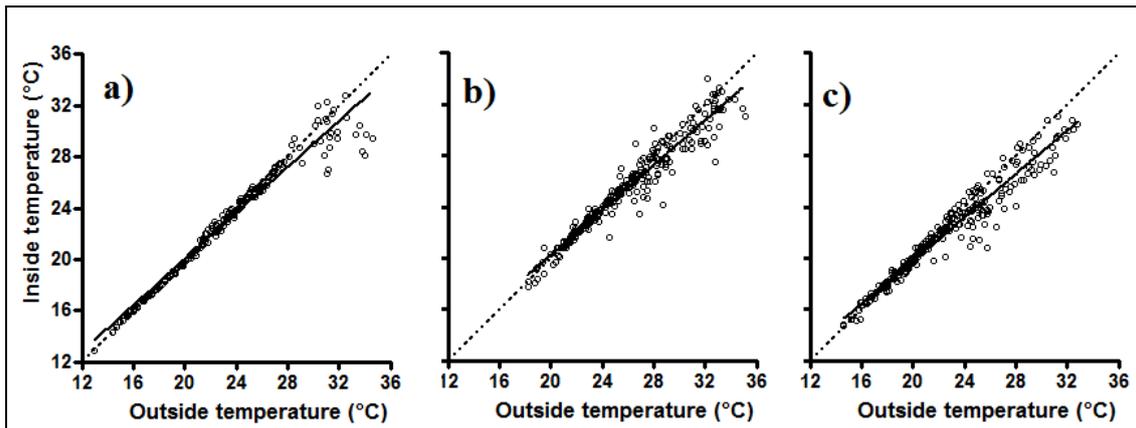
Two experiments were conducted at the experimental field of the Facultad de Agronomía, Universidad de Buenos Aires (34° 35' S., 58° 29' W.) during the 2010-2011 growing season. The incident radiation (Exp<sub>RAD</sub>) was managed through the use of shelters that were constructed with black shading cloth (-80% incident radiation) mounted on rectangular tubular structures at different times of fruit growth and development: i) from flower bud to first anthesis, ii) during 10 days prior to first anthesis and iii) during 10 days after the end of anthesis. The Exp<sub>PHOTO</sub> was sown in January to achieve a difference of around 2.5 hours in day length during the grain filling period between control and treatment plots. The photoperiod extension in the treatment plots was achieved through the use of incandescent and low-intensity lights mounted on rectangular tubular structures. A female inbred line, which presents high dormancy, was sown. In both experiments crops were over-sown and desired crop density (5 pl m<sup>-2</sup>) was established by removing plants from the over-sown stand at the two-leaf stage. Both experiments were laid out in a completely randomized design with three replications. Each plot was 4m<sup>2</sup> (3 rows of 2 m with rows at 0.70 m. The plots were fertilized (60 kg N ha<sup>-1</sup>) and irrigated. Diseases, insects and lodging were controlled with pesticides and fungicides. In both Exps, fruit was harvested with 11% humidity. Then, the fruit was dried at 40 ° C to reach 6% humidity and the grain was stored in chambers at 15 ° C. Dormancy level was determined from harvest and every 20-25 days until the proportion of fruit that germinated was equal to or greater than 90%. Germination experiments were performed at two temperatures (12 and 25°C) and included two treatments: fruit and embryo (fruit without pericarp and seed coat). Weather conditions were recorded followed during both experiments. In radiation experiment hourly average temperature was measured during the course of radiation treatments inside and outside

shelters. Because of the black shading cloth the temperature inside the shelters could be reduced. Analysis of variance and regression analysis were used to establish significance of differences among treatments and associations between variables.

## RESULTS

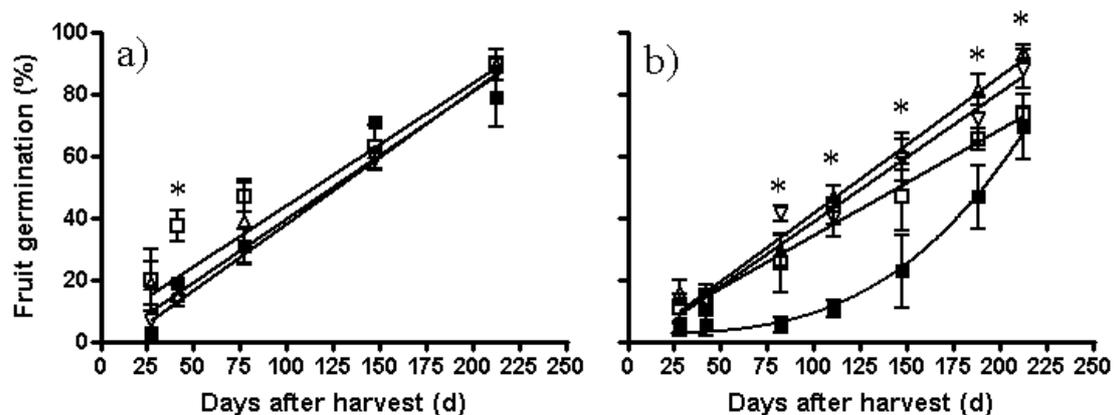
### Radiation experiment

Hourly average temperature for all treatments within and outside of shelters was similar (Fig. 1). The points that are separated from the 1:1 ratio correspond to the time zone of 11 to 14 hours; on average the temperature inside the shelters was 2°C lower than the temperature outside shelters.



**Fig. 1:** Relation between hourly media temperature (C) inside and outside shelters during radiation stress treatments: flower bud to first anthesis (a), first anthesis to last anthesis (b), last anthesis to 10 days after last anthesis (c). The dotted line is the relation 1:1 between both variables.

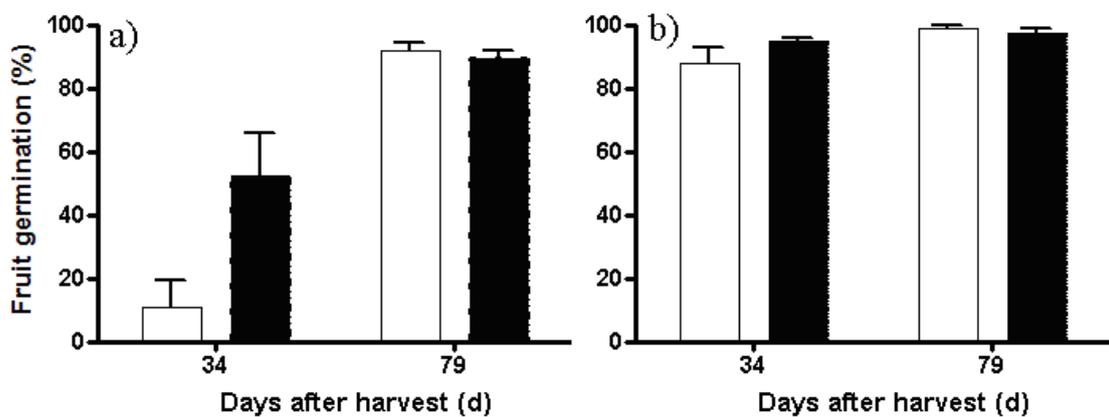
The expression of dormancy is associated with incubation temperature in radiation incident experiments (Exp<sub>RAD</sub>). At 25 °C the germination percentage was lower than 12 °C in Control treatments (Fig 2 a y b). When the fruit was incubated at 12 °C there were no significant differences between treatments, except 50 days after fruit harvest. The germination percentage in preanthesis radiation stress treatment ( $37, 33 \pm 8.33\%$ ) was significantly higher than in the rest of treatments ( $15, 56 \pm 1, 54 \%$ ) (Fig. 2a). At 25°C the differences among radiation stress and control treatments were more evident than 12°C. The germination percentage was significantly higher in radiation stress treatments than in control at 25°C from 75 days to 200 days after harvest. But there were no significant differences among different radiation stress treatments (Fig. 2b). 80% of embryos of all radiation and control treatments germinated 30 days after harvest at both incubation temperatures.



**Fig. 2:** Evolution of fruit germination in radiation stress treatments: flower bud to first anthesis (open squares), first anthesis to last anthesis (open triangles), last anthesis to 10 days after last anthesis, post-anthesis (open inverted triangles) and control (squares) at two incubation temperatures 12° C (figure a) and 25° C (figure b). Error bars are  $2 \pm$  SEM and are drawn when larger than symbols. Asterisks (\*) indicate significant differences among treatments ( $p < 0.05$ ).

### Photoperiodic Experiment

Duration of the pre-anthesis phase was 60 days from emergence. Photoperiod did not affect the duration of grain filling period. The post-anthesis phase was completed in 40 days in both treatments; control and photoperiod extension treatment. The photoperiod affected the dormancy level of sunflower fruits even though dormancy disappeared after a short period of storage. At 12° C of incubating temperature, the fruit germination percentage was  $52 \pm 2$ , 33 % and  $11 \pm 15$ , 14 % for 14 and 12 hours day length ( $p < 0, 06$ ). These differences disappeared 80 days after harvest, the fruit germination percentage was  $92 \pm 4$  and  $89 \pm 4.6$  % for 14 and 12 hours of day length ( $p > 0,49$ ) (Fig.3).



**Fig. 3:** Fruit germination percentage in photoperiod treatments (white bars, day length duration =12hours; black bars; day length duration=14hours,) at two incubation temperatures 12 °C (a) and 25 °C (b). Error bars are  $2 \pm$ SEM and are drawn when larger than symbols.

### DISCUSSION

The results obtained in this work showed that incident radiation explored by sunflower fruit during development and maturation affected the dormancy level of harvested fruits. But there were no differences among different times of fruit growth and development (i.e., flower bud to first anthesis, first anthesis to last anthesis and last anthesis to 10 days after last anthesis). The dormancy level is expressed only at 25C of incubation temperature, not at 12 C, suggesting that the observed effects were mainly explained by difference in fruit covers. In sunflower seeds, dormancy expression at low temperatures is attributed to embryo dormancy which is not expressed at high temperatures (Corbineau, Bagniol and Côme, 1990); conversely, dormancy expressed at high temperatures results from coat-imposed dormancy (Corbineau, Bagniol and Côme, 1990). Hence, the high germination percentage of embryo a few days after harvest (i.e., 42 days) reaffirms the idea that the fruit dormancy in this experiment is imposed by coats. The photoperiod also affected seed dormancy level; however dormancy disappeared after a short period of storage (between 35 and 75 days from harvest) suggesting that photoperiod may be affecting embryo dormancy which is lost a brief time after harvest.

Our results highlight that the incident radiation and photoperiod affect sunflower fruit dormancy. Therefore, the variation of these environmental factors, in addition to temperature, should be considered for designing management strategies (sowing dates, planting locations etc.) to obtain lower dormancy levels of sunflower fruit at harvest.

### ACKNOWLEDGMENTS

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