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Histological development of the sunflower fruit pericarp as affected by pre- and early post-anthesis canopy shading

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Abstract

The dynamics of pericarp development compared to that of the embryo, as well as the effect of pre-anthesis and post-anthesis shading on pericarp histogenesis and dry weight dynamics of fruits from two sunflower (*Helianthus annuus* L.) hybrid cultivars along three capitulum positions, peripheral, mid and central were studied. During fruit formation, the cell division phase of the carpel takes place before anthesis. Eight days after anthesis the pericarp reached its final size, while its cell wall's sclerification was almost complete 13 days after anthesis. Pre-anthesis shading affected the carpel cell division period reducing (17–33%) the number of pericarp middle layer strata and increased the thickenings of the cell wall of the mid (19%) and central (33–63%) fruits. In central fruits, the dry weight accumulation period was reduced. In contrast, post-anthesis shading reduced both the cell wall thickness (16–64%) and the number (38–58%) of pericarp middle layer sclerified strata of fruits in the three positions of the capitulum. In the mid and central fruits, the dry weight accumulation period extended 11–16 and 3–4 days, respectively, over those of the control. Both shading treatments produced thinner and lighter pericarps, but with different anatomical features that were associated with differences in the efficiency of use of the fruit for industrial oil extraction.
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Keywords: *Helianthus annuus* L.; Fruit development; Pericarp; Shading; Sunflower

1. Introduction

The sunflower fruit consists of the pericarp (hull), which represents about 20–25% of its dry weight, and the seed (kernel) where lipids are synthesized and accumulated.

Transections of mature pericarps show several layers of transversely polygonal fibers interrupted by parenchyma rays of 1–3 cells wide. The sclerenchymatic tissue is limited externally by a several-layered hypodermis, beneath the outer epidermis, and internally by a parenchymatic tissue adjacent to the seed. Between the sclerenchymatic tissue and the hypodermis a layer of phytomelanin, an amorphous dark material, can be seen. Part of the inner parenchyma, the vascular bundles and the inner epidermis are collapsed (Roth, 1977; Knowles, 1978; Seiler, 1997; Lindström et al., 2000).

The pericarp anatomical structure affects the efficiency of industrial processing of fruits as it influences their hulling, which consists of the separation of the pericarp from the seed (Leprince-Bernard, 1990; Beauguillame and Cadeac, 1992). Easy of hulling (or hullability) is determined by pericarp structural properties such as number of parenchymatous rays (Leprince-Bernard, 1990; Beauguillame and Cadeac, 1992; Dennis, 1994; Lindström et al., 2006c), cell wall thickness and lignification (Lindström et al., 2006b,c) as well as moisture content (Wan et al., 1978; Nel, 2001). These properties are also affected by environmental growth conditions (Dennis, 1994) and crop management (Dedio and Dorrell, 1989; Baldini and Vannozzi, 1996). Rondanini et al. (2006) observed that high temperature stress applied during fruit development decreased pericarp cell wall thickness and hullability due to alterations in the pericarp development. This research suggested that further study of the dynamics of pericarp development under different environmental conditions might explain the relationship between pericarp structure and ease of hulling.

Yegappan et al. (1982) established that water stress decreased pericarp weight but this reduction was not related

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to the development stage of the pericarp when the stress was applied. Likewise, Lindström et al. (2006a) found similar pericarp weight reductions with shading treatments: one at pre-anthesis and the other at early post-anthesis. In their work, pericarp weight reduction was associated with fruit volume reduction, but only in the pre-anthesis treatment (Lindström et al., 2006a). In sunflower, the stage of active cell division, where the pericarp basic shape and structure are established, happens during the pre-anthesis period. Cell volume increase and differentiation would start during pre-anthesis and be completed by the early stages of the post-anthesis development (Lindström et al., 2000). As a result, any stress applied at pre-anthesis can affect the cell division in the carpel, reducing the number of cell layers and the final volume of the pericarp, which would become a physical barrier that could prevent the maximum development of the embryo (Lindström et al., 2006a). Mantese et al. (2006) concluded that the ovary size at fertilization as well as pericarp growth rate and duration may impact final embryo size and thus, seed oil concentration. On the contrary, post-anthesis shading (Lindström et al., 2006a) may not have as much effect as it is applied after the completion of pericarp cell volume enlargement. No histogenic study has been previously performed on sunflower pericarp development either over time or by phenologic scale.

In this work, we describe the sunflower fruit pericarp development in relation to that of the embryo. The histogenic changes and the pericarp dry weight accumulation were tested under shading conditions, applied at two stages of crop development: pre-anthesis and early post-anthesis. This research should establish how environmental or management factors that change light conditions within the crop canopy can affect sunflower pericarp development and physiology.

2. Materials and methods

2.1. Pericarp and embryo development in unshaded plants

2.1.1. Plant material

Two commercial sunflower hybrids, Dekasol (DK) 3900 and DK4030 (Monsanto®, Argentina), were sown on 24 October 2001 at the experimental field of the Agronomy Department-UNSur, Bahía Blanca, Argentina (latitude S 38°45′; longitude W 62°11′). The soil was a Typic Ustipsamment (Soil Survey Staff, 1999). Plant density was adjusted to 5.6 plants m^{-2}. The crop was managed according to the recommended conventional agronomical practices (Pereyra and Farizo, 1981). Weeds and insects were adequately controlled. Water was supplied by drip irrigation. The phenological stages referred in this work correspond to that defined by Schneiter and Miller (1981).

2.1.2. Sampling and analysis

The relationship between the pericarp and the embryo development was described, using observations, measurements and drawings of open fresh fruits with half the pericarp removed. A WILD M5 stereoscopic microscope equipped with a drawing tube attached to it was used.

Five flowers and fruits of the mid-position of the capitulum, of five plants of each hybrid, were sampled every 3–5 days from their anthesis (MA; anthesis of mid flowers, i.e. stage R5.5) up to physiological maturity (PM).

In order to describe pericarp histogenesis two ovaries and then fruits from the mid-position of the capitulum of five plants of each hybrid were fixed in FAA solution (Ruzin, 1999). The samples were taken at MA, full anthesis (FA, i.e. stage R5.10), reached in both hybrids 3 days after MA; 5 days after FA (5 DAFA); 10 days after FA (10 DAFA) and PM. The fixed samples were embedded in paraffin, cut (12 μm) and stained (Saffranine–Fast green) according to conventional techniques (Ruzin, 1999).

Observations and drawings of ovary and pericarp cross-sections were performed using a WILD M11 microscope equipped with a drawing tube. Measurements were done with an ocular micrometer.

2.2. Pericarp development in shaded plants

2.2.1. Shading treatments

The same hybrids and experimental field and a similar crop management were used as described in Section 3.1. The sowing was made on 22 October 2002. Two shading periods were applied, one, at pre-anthesis (Pre-A), for 10 days from reproductive stage R3 (when the inflorescence is still surrounded by bracts) to stage R5.0 (the beginning of anthesis of disk flowers); the other, at early post-anthesis (Post-A), for 10 DAFA and a control (Con, unshaded) treatment. In both hybrids FA was achieved 3 and 7 days after the opening of mid and peripheral flowers, respectively. When each shade treatment was started, plants at the appropriate growth stage (R3 and R5.10) were identified and labeled.

The experimental design consisted of complete randomized split plots, with hybrid assigned to main plots and shading treatment to subplots, with three replicates per treatment. Each subplot had four rows 0.70 m apart and 6.0 m long. Shading was achieved with black propylene shade netting (supported by 1.40 m wide × 2.10 m long × 1.55 m high metal structures over the treated plots), which reduced the incident radiation by 80%. The shade cloth extended 0.40 m to the ground so that the plants were essentially in a shade enclosure. Environmental variables, such as mean daily solar radiation (MJ m^{-2} day^{-1}) and temperature (°C) were previously described (Lindström et al., 2006a).

2.2.2. Histological analysis

Ovaries and then fruits from three positions (peripheral, mid and central) of two capitula from each plot were sampled in FA, 10 DAFA and in PM and fixed for histological analysis. In order to obtain cross-sections of the middle portion of the fruits the fixed samples were included in Spurr's low-viscosity resin (Spurr, 1969), cut (1–3 μm) on a LKB ultramicrotome and stained with toluidine blue (Ruzin, 1999). Cross-sections of fruit middle portion were made.

Fresh cross-sections from the middle portion of fruits at PM were made in order to evaluate the sclerification degree of the

pericarp tissues. They were treated with phloroglucinol in acid medium (Ruzin, 1999) and mounted with glycerine:water (50:50).

For photographic observations and records, a Nikon Labophot-2 microscope was used with a Nikon Coolpix 4500 camera and an ocular micrometer attached to it.

Progress in pericarp development was analyzed by comparing the thickness of the hypodermis, middle layer and inner layer (Fig. 1G) between sampling dates on each of the treatments.

2.2.3. Fruit growth dynamics

For the analysis of the dynamics of dry matter accumulation in the pericarp, five fruits of the peripheral, mid and central position of two capitulum of each plot from 2 DAFA in DK4030 and 4 DAFA in DK3900 up to PM were harvested every 3–5 days. The pericarp samples were dried at 60 °C during 72 h and weighted.

2.3. Statistical analysis

Data was subjected to analysis of variance and the differences between the treatments or sampling date means were compared by LSD test. The statistical analysis was performed with SYSTAT statistical software (SPSS, 1997). In the text, means are given in ±S.E.

3. Results

3.1. Relationship between the pericarp and the embryo development in fruits of unshaded plants

The pericarp development compared to that of the embryo was similar in both hybrids, so the observations made on hybrid DK3900 only are presented.

At MA (Fig. 1A), the ovary is 7.7 ± 0.34 mm long and 2.4 ± 0.38 mm wide. The ovule is separated from the carpel wall and occupies almost 50% of the ovary cavity. The mature embryo sac can be observed in the micropilar end. The carpel (Fig. 1F) has an epidermis with a thick cuticle, a single layer hypodermis, a middle layer of 11–12 layers of transversely rounded cells and an inner layer represented by a spongy parenchyma. There are 30–32 amphicribral vascular bundles located at the boundary between the middle and inner layer. In the equatorial area of the pericarp there is a pair of small fiber bundles adjacent to the inner epidermis (Fig. 1F).

At FA (Fig. 1B) fertilization has already occurred; the fruit is about 4.0 ± 0.4 mm wide and 9.0 ± 0.4 mm long. Both the ovule and the embryo sac have grown in length (6.0 ± 0.8 mm and 1.6 ± 0.9 mm, respectively). The embryo sac has an embryo at the heart stage of about 160.0 ± 27.0 μm (long in contact with the endosperm, Fig. 1B). At the chalazal end of the embryo sac a proliferation of the integumentary tapetum cells

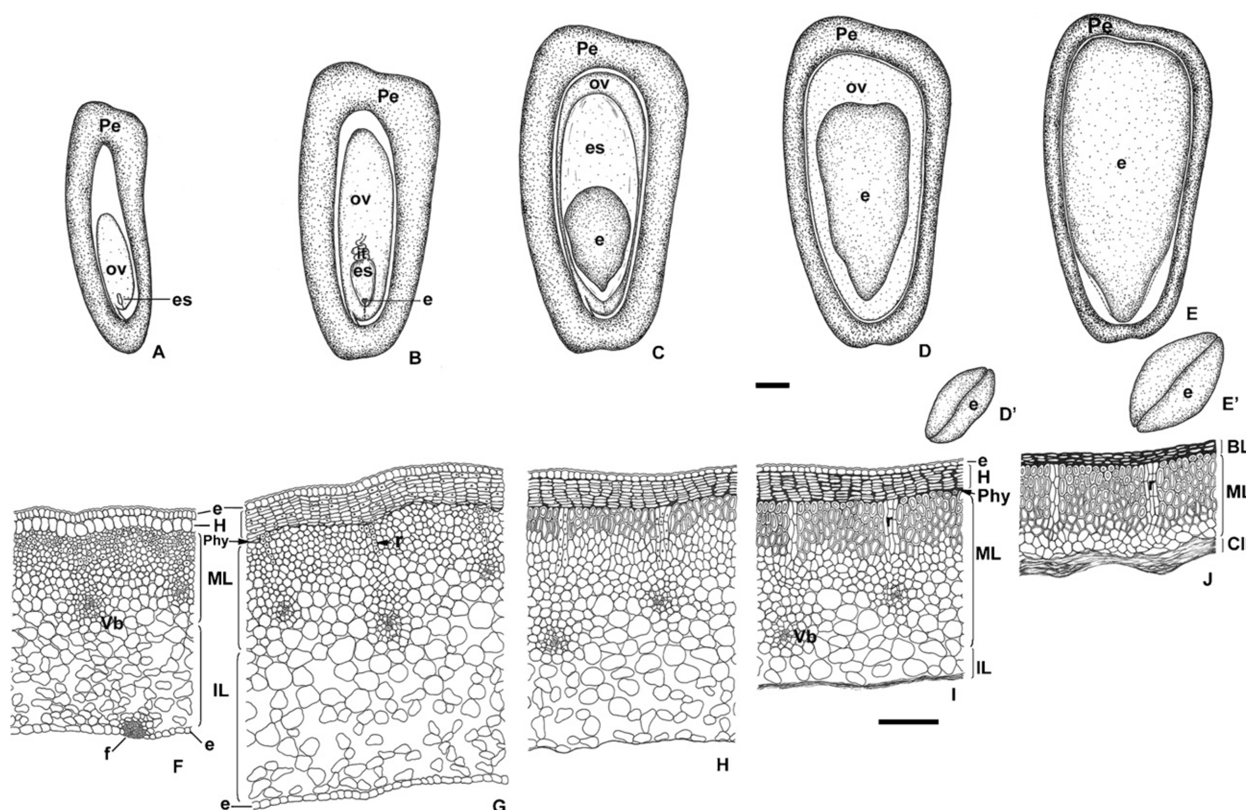

Fig. 1. (A–E′) Relationship between pericarp and embryo development of fruits from the mid position in the capitulum of DK3900 in unshaded plants. (A–E) Open fresh fruits with half the pericarp removed; (D′ and E′) embryo cross-section; (F–J) cross-sections of different stages of the pericarp. (A and F) Anthesis of mid flowers (MA); (B and G) full anthesis (FA); (C and H) 5 days after full anthesis (5 DAFA); (D and I) 10 DAFA; (E and J) physiological maturity (PM). BL, black layer; CIL, compressed internal layers; e, epidermis; em, embryo; es, embryo sac; f, bundle of fibers; H, hypodermis; IL, inner layer; it, integumentary tapetum; ML, middle layer; Ov, ovule; Pe, pericarp; Phy, phytomelanin layer; r, parenchymatic ray; Vb, vascular bundle. Scale bar: A–E = 2 cm; F–J = 100 μm.

can be seen. The two antipodes are still inside this tapetum. In the pericarp (Fig. 1G) the hypodermis divide periclinally in a centrifugal direction (i.e. the youngest cells lie directly beneath the outer epidermis) to form several-celled radial rows. Schizogenic spaces are generated in the areas where the phytomelanin, which can already be seen between the hypodermis and the middle layer, will collect. This phytomelanin deposit progresses basipetally inside the fruit. There are cells in active division in the areas where the future parenchyma rays will be located. Pericarp thickness continues to increase as a consequence of cell volume increase (Fig. 1G).

At 5 DAFA (Fig. 1C), the fruit reaches its final dimensions, about 5.0 ± 0.4 mm wide and 10.0 ± 0.2 mm long. The ovary cavity is fully occupied by the ovule that contains the endosperm and the developing embryo, of about 2.56 ± 0.9 mm long. These structures start to compress the inner layer of the pericarp (Fig. 1C). The pericarp has reached its maximum thickness (Fig. 1H). The hypodermis consists of 8–10 cell layers and the phytomelanin deposit appears as a continuous layer. The two outermost cell layers of the middle layer start to sclerify, the cells become polygonal in cross-section and slightly increase their length radially.

At 10 DAFA (Fig. 1D), the ovule and developing embryo, the last one 6.0 ± 0.44 mm long and 3.0 ± 0.3 mm wide and 1.5 ± 0.14 mm in cross-section, compress part of the cell layers of the inner layer. Between the cotyledons the remains of endosperm can be seen. The pericarp sclerification reaches the four outer layers of the middle layer. There are parenchyma rays (from uniseriated to triseriated) interrupting the middle layer (Fig. 1I).

When the fruit reaches PM (about 33 DAFA; Fig. 1E and J) the seed has completed its development and is approximately 8.4 ± 0.66 mm long and 4.0 ± 0.25 mm wide by 2.5 ± 0.06 mm in cross-section. The epidermal cells, the compressed hypodermis cells and the phytomelanin layer form a black layer (BL; Fig. 1J). The compression exerted by the growing embryo on the inner tissues of the pericarp results in the generation of the compressed inner layer (CIL). It includes the crushed vascular bundles, the inner layer and some of the most internal layers of the middle layer (Fig. 1J). The number of layers that remain uncompressed in the middle layer is about eight. Six of them are sclerified but sclerification is significantly higher in the four outermost cell layers (Fig. 1J).

3.2. *Effect of shading*

3.2.1. *Pericarp dry weight dynamics*

As the sampling dates of fruits (Fig. 2) were different among hybrids, the comparison of the pericarp dry weight between dates was made individually for each hybrid and treatment.

A pericarp dry weight reduction was observed in both shading treatment from the first sampling date in all fruit positions of the capitulum in both hybrids (Fig. 2). In the peripheral fruits of DK3900, no differences in the pericarp weight were detected between sampling dates in any of the three treatments from 4 DAFA (Fig. 2A) onward. In DK4030, the pericarp weight increased from 2 DAFA to 5 DAFA in the Con and Post-A treatment while it did not change between sampling dates in the Pre-A treatment (Fig. 2D).

In mid fruits of DK3900, the maximum dry weight was achieved 7 DAFA in the Con and Pre-A treatments (Fig. 2B). In the Post-A treatment, the pericarp registered a loss of 30% in dry weight between 4 DAFA and 14 DAFA then reaching its maximum value similar to the first sampling date, 18 DAFA (Fig. 2B). The pericarp weight of mid fruits of DK4030 increased from 2 DAFA to 9 DAFA in the Con and Pre-A treatment (Fig. 2E). In Post-A treatment, this parameter remained constant up to 16 DAFA, to reach then its maximum value at 23 DAFA (Fig. 2E).

In central fruits of DK3900, the pericarp weight reached its maximum value 18 DAFA in the Con and 10 DAFA in the Pre-A treatment (Fig. 2C). In Post-A treatment it decreased 25% between 4 DAFA and 14 DAFA to then reach it maximum value 18 DAFA (Fig. 2C). In DK4030, the pericarp weight increased up to 23 DAFA and 12 DAFA in the Con and Pre-A treatments, respectively, while in Post-A it decreased 17–33% between 2 DAFA and 23 DAFA, finally reaching its maximum value, similar to the carpel initial weight, at 26 DAFA (Fig. 2F).

3.2.2. *Pericarp anatomy*

Since there was no hybrid by treatment interactions ($P > 0.05$) for all anatomical variables analyzed, only the average results for both hybrids are presented (Table 1 and Fig. 3).

In FA and as a consequence of the Pre-A treatment, we observed a reduction of the number of layers (25–30%; Fig. 3A–C) and of the thickness (21–33%; Fig. 3D–F) of the middle layer in fruits of the three positions in the capitulum with respect to the Con and Post-A treatments, which showed no difference between them (Fig. 3). In the Pre-A treatment the inner layer thickness decreased significantly in the peripheral (16%) and mid (26%) fruits of the capitulum compared to the Con and Post-A treatments which showed no differences between them (Table 1). A similar trend was observed in the central fruits ($P = 0.08$; Table 1). In FA, one or two sclerified layers were observed in the middle layer of peripheral fruits.

At 10 DAFA, the difference in the number of cells layers of the middle layer of the pericarp in Pre-A, with respect to Con and Post-A, was similar to that at FA (Fig. 3A–C). Both shading treatments significantly reduced the middle layer thickness of the pericarp (Pre-A: 18–33%; Post-A: 19–44%) in the three capitulum positions (Fig. 3D–F). The pericarp of the peripheral fruits of the Con and Pre-A treatments showed five to six sclerified layers (Table 1) and, in mid fruits, only four to five sclerified layers were counted (Table 1 and Fig. 4A and B). In the Post-A treatment, the number of sclerified layers of the pericarp middle layer of peripheral and mid fruits decreased 39 and 60%, respectively (Table 1; Fig. 4C) in comparison to the Con treatment. In the mid (Fig. 4C) and central fruits of Post-A, one to three compressed cell layers were observed in the non-sclerified middle portion of the middle layer. In the central fruits, there was hybrid by treatment interaction for the middle layer number of sclerified layers (Table 1). In DK3900, the number of sclerified layers of the middle layer decreased

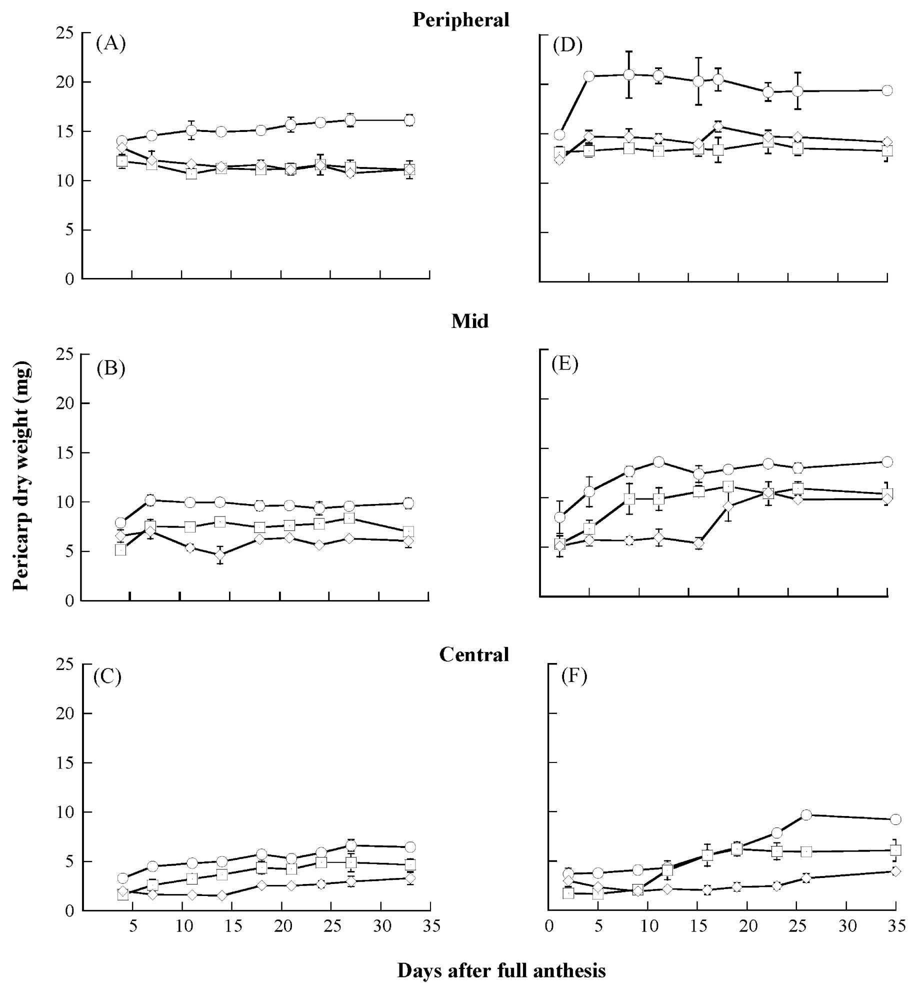

Fig. 2. Changes with time of pericarp dry weight for fruits in three positions of the capitulum (peripheral, mid and central) from the control (Con; ○); pre-anthesis (Pre-A; □) and post-anthesis (Post-A; ◇) shaded plants of DK3900 (A–C) and DK4030 (D–F).

Table 1
Effect of pre-anthesis (Pre-A) and post-anthesis (Post-A) shading on some parameters of the pericarp measured at full anthesis (FA), 10 days after full anthesis (10 DAFA) and physiological maturity (PM), in fruits at three positions in the capitulum averaged over two hybrids. Con: control (unshaded), Pre-A: R3 + 10 days of shading, Post-A: R5.10 + 10 days of shading

	Fruit position											
	Peripheral				Mid				Central			
	Con[a]	Pre-A[a]	Post-A[a]	S.E.[a]	Con[a]	Pre-A[a]	Post-A[a]	S.E.[a]	Con[a]	Pre-A[a]	Post-A[a]	S.E.[a]
FA												
Inner layer width (μm)	370 a	311 b	411 a	10.0	353 a	262 b	343 a	18.0	313 a	250 a	285 a	17.0
10 DAFA												
Number of sclerified layers in the middle layer	5.3 a	6.2 a	3.6 a	0.3	4.5 a	5.0 a	2.1 b	0.3	4.2* a 0* a	4.7* a 0* a	0.3* a 0* a	0.3 0.3
PM												
Number of sclerified layers in the middle layer	7.2 a	7.4 a	4.5 b	0.3	6.4 a	6.4 a	3.2 b	0.2	5.3 a	6.1 b	2.2 c	0.33
Middle layer cell wall thickness (μm)												
First layer	9.3 a	10.2 a	7.8 b	0.4	8.4 a	9.1 a	5.8 b	0.8	5.6 a	8.3 b	2.9 c	0.6
Fourth layer	4.7 a	5.0 a	2.3 b	0.4	4.8 a	5.7 b	1.7 c	1.0	2.4 a	3.9 b	1.0 c	0.4
Parenchyma rays frequency (r/mm)	3.5 a	5.1 a	4.5 a	1.0	5.3 a	5.5 a	3.9 a	0.9	4.2 a	6.9 b	5.4 a	0.8

Values followed by different letters indicate significant differences ($P < 0.05$) between shading treatments within each fruit position within the sunflower head. S.E., standard error; *hybrid × treatment interaction; upper row: DK3900; lower row: DK4030 in central 10 DAFA.

[a] Treatment.

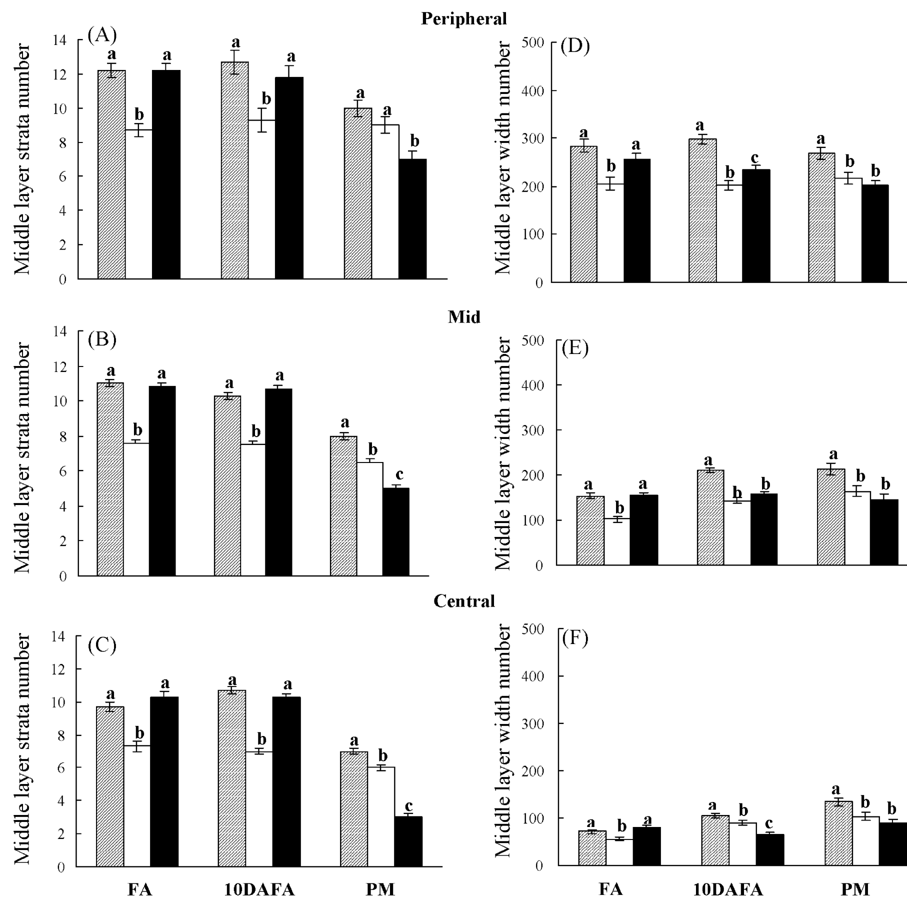

Fig. 3. Number of cell layers (A–C) and width (μm; D–F) of the middle layer (ML) at full anthesis (FA), 10 days after full anthesis (10 DAFA) and physiological maturity (PM) for fruits from three positions of the capitulum (peripheral, mid and central) of the control (Con, ▨) and pre-anthesis (Pre-A, □) and post-anthesis (Post-A, ■) shaded plants averaged over two hybrids. Within each set bars topped by different letters are significantly ($P < 0.05$) different.

approximately 92–94% in the Post-A treatment as compared to the Con and Pre-A treatments, which showed no difference between them (Table 1). In DK4030, no sclerification was observed in fruits of any treatment (Table 1).

At PM, both shading treatments showed a lower number of cells layers in the pericarp middle layer of mid and central fruits, as compared to the Con treatment (Pre-A: 17–25%; Post-A: 38–53%; Fig. 3A–C), while in peripheral fruits, this reduction was only observed in the Post-A treatment (Fig. 3A–C). The middle layer thickness decreased 20–23% in the Pre-A (Fig. 5D–F) treatment and 23–33% in the Post-A (Fig. 5G–I) with respect to the Con (Fig. 5A–C) in fruits of all capitulum positions (Fig. 3D–F). In the Pre-A treatment, cell wall thickness of the fourth layer of the middle layer increased

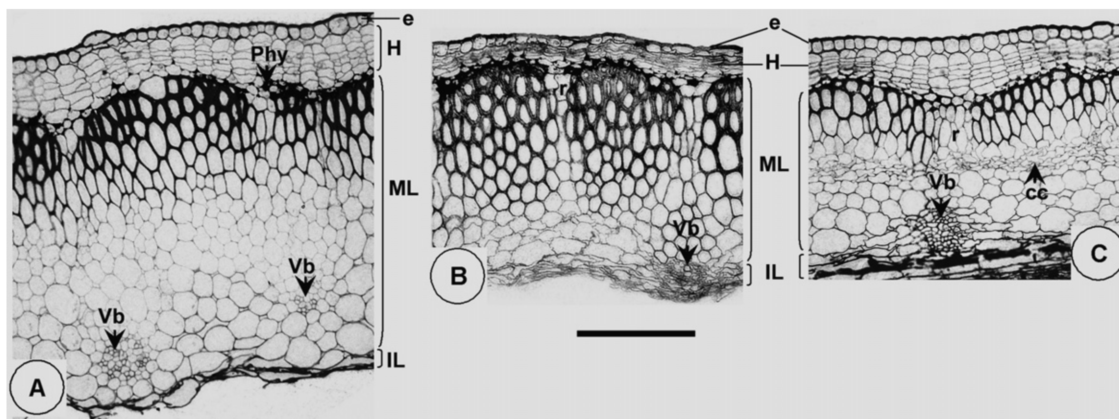

Fig. 4. Pericarp cross-sections 10 days after full anthesis (10 DAFA) of the control (Con; A) and pre-anthesis (Pre-A; B) and post-anthesis (Post-A; C) shaded plants of DK3900. Sections correspond to fruits of the mid-positions of the capitulum. cc, compressed cells; e, epidermis; H, hypodermis; IL, inner layer; ML, middle layer; Phy, phytomelanin layer; r, parenchyma ray; Vb, vascular bundle. Scale bar = 100 μm.

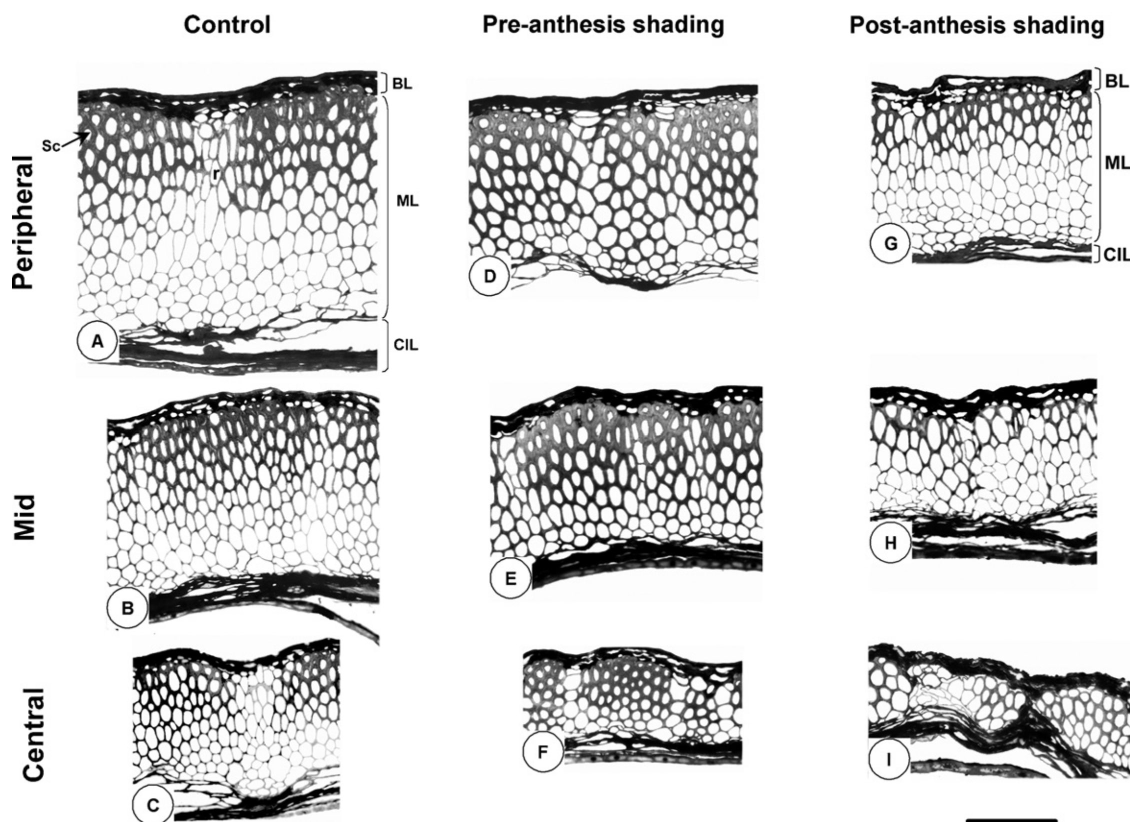

Fig. 5. Pericarp cross-sections of DK3900 fruits from the peripheral (A, D and G); mid (B, E and H) and central (C, F and I) positions of the capitulum at physiological maturity (PM). Control (Con, A–C), pre-anthesis (Pre-A, D–F) and post-anthesis (Post-A, G–I) shaded plants. BL, black layer (formed by the epidermis, hypodermis and phytomelanin layer); ML, middle layer; CIL, compressed inner layer, including the seed tegument, the crushed vascular bundles, the inner layer and some of the most internal layers of the ML; r: parenchyma ray; Sc, sclerified cells. Scale bar for all figures = 100 μm.

(19%) in mid fruits (Table 1; Fig. 5E) and in the first layer (33%) and fourth layer (63%) of central fruits, as compared to the Con treatment (Table 1; Fig. 5F). On the other hand, in the Post-A treatment the number of sclerified layers (37–58%) and the thickness of the cell wall of the first (16–48%) and fourth (51–64%) layers of middle layer decreased in the three capitulum positions (Table 1; Fig. 5G–I).

The parenchyma ray frequency showed no differences between treatments in fruits of any of the capitulum positions (Table 1).

3.2.3. Thickening of the different pericarp layers

The hypodermis thickness remained similar from FA to 10 DAFA. It was compressed in PM across all capitulum positions, without differences ($P > 0.05$) among treatments and hybrids.

The inner layer thickness of the pericarp decreased from FA to PM in every treatment and capitulum position as a consequence of the compression exerted by the developing embryo.

In the peripheral fruits, the pericarp middle layer thickness did not change ($P > 0.05$) between sampling dates in any of the treatments (Fig. 3D). In mid fruits, it increased up to 10 DAFA in the Con and Pre-A treatments, without showing differences ($P > 0.05$) between sampling dates in the Post-A treatment (Fig. 2E). Nevertheless, in central fruits the middle layer thickness of the Con and Pre-A treatments was different ($P < 0.05$) between sampling dates, in fact increasing from FA to PM. In the Post-A treatment, the middle layer thickness remained constant ($P > 0.05$) between sampling dates (Fig. 2F).

4. Discussion

4.1. Fruit development

Until now, studies of the histogenic development of the sunflower fruit partially describe some developmental steps of the pericarp (Hanausek, 1902; Roth, 1977; Mantese et al., 2006) or embryo (Newcomb, 1973a,b; Cantagallo, 2000) separately. The results reported here provide the first complete description of the histological development of the carpel in relation to the ovule and/or embryo, in a phenological sequence from anthesis to fruit physiological maturity.

At anthesis (MA), the ovule shows an unfertilized mature embryo sac. In full anthesis (FA; Fig. 1B), the embryo attains a heart shape stage and is in contact with the endosperm, which would be used by the embryo from this stage onwards (Newcomb, 1973b). Newcomb (1973b) suggested that the loose arrangement of the central cells of the integument enable the diffusion of translocated substances from the vascular tissue to the developing embryo. The presence of antipodes in the area of integumentary tapetum cell proliferation reinforces the concept

about their possible haustorial role during seed formation (Akhalkatsi and Wagner, 1997; Newcomb, 1973a).

After 5 DAFA, the embryo cells are still in active division. This lasts up to 10 DAFA (Lindström, unpublished data), when the linear phase of embryo growth starts (Lindström et al., 2006a).

During pericarp development, the active cell division phase, when the basic structure of the pericarp is fixed, takes place during the Pre-A period. Thus, at anthesis the number of cell layers of the carpel was already fixed and there were only cell divisions in the areas where the rays and the hypodermis will be located (Fig. 1F).

At full anthesis (FA) sclerification of the middle layer external layers is still pending. The phytomelanin can already be observed as a continuous deposit. Pandey and Dhakal (2001) state that phytomelanin layer development has a synergistic effect on early lignification and hardening of sclerenchyma cell walls.

At 5 DAFA, the fruit dimensions are already fixed (Fig. 1C) and there is no more variation in the pericarp cell volume, while sclerification is already evident (Fig. 1H). The ovule and the embryo have significantly increased their size and starts compressing the inner layer.

At physiological maturity, the final structure of the pericarp is represented by the epidermis and the hypodermis, which form a dark layer of flattened cells of 10–15 μm thick (Figs. 1J and 5) and six to nine layers of middle layer. The inner layer, the vascular bundles and the two or three innermost layers of the middle layer are fully collapsed (Figs. 1J and 5).

Even though there is a small increase in the secondary wall deposit after 10 DAFA (Fig. 1I and J), this process mainly occurs prior to this time. From then onwards the dry weight fast accumulation phase of the embryo starts (Lindström et al., 2006a).

4.2. Effect of shading

4.2.1. Pericarp dry weight dynamics

In the Con treatment, the pericarp of peripheral and mid fruits reached maximum weight about 4–5 DAFA and 7–9 DAFA, respectively. In fruits of the central position, such a period extended to 18 DAFA in DK3900 and 23 DAFA in DK4030. This delay in the maturation process of the central fruits with respect to those from the periphery confirms a fruit timming and developmental hierarchy in the sunflower capitulum (Alkio et al., 2003; Lindström et al., 2006a).

Pre-A treatment significantly reduced the carpel final weight. The pericarp presented dry weight accumulation dynamics similar to that of the Con in fruits in the mid and peripheral position of the capitulum. In central fruits, the pericarp dry weight accumulation period was shortened by about 8–11 days as compared to Con treatment (Fig. 2C and F). The significant reduction, in both carpel weight and ovary dimensions (length and width) beginning at FA (Lindström, unpublished data) translated into fruits with lighter and smaller volume pericarps in PM (Lindström et al., 2006a). A stress applied in Pre-A always generates comparatively smaller fruits at harvest than in controls, even when the conditions are re-established for normal development of the embryo (Connor and Hall, 1997; Lindström et al., 2006a). This would confirm the hypothesis that the smaller volume achieved by the seed as a result of a Pre-A stress (Lindström et al., 2006a) would be a consequence of physical restrictions imposed by the reduced development of the pericarp.

As in the Pre-A treatment, a pericarp weight reduction under Post-A treatment was observed from the first sampling date in the fruits in all capitulum positions (Fig. 2A and F). In the mid and central fruits, the pericarp dry weight accumulation started from 4 to 13 days after the end of shade treatment and lasted for 3–4 days (Fig. 2B, C, E and F). The sudden pericarp weight reduction at the beginning of Post-A shading and its later stability during treatment show that fruit growth is closely related to the current photo-assimilates produced by the leaves. García-Luis et al. (2002) showed that *Citrus* fruit growth was related to leaf area, thus indicating it would be held back by limits on photosynthesis. The embryo of the shaded fruit remained in the growth lag phase 5–10 days after the Post-A shading treatment was finished (Lindström et al., 2006a). Post-A treatment did not affect final seed weight but significantly decreased the number of filled fruits indicating that a yield compensation mechanism between seed size and number come into play (Connor and Hall, 1997; Lindström et al., 2006a). Similarly, Steer et al. (1988) found that partial defoliation at FA decreased seed number and pericarp more than seed weight.

4.2.2. Pericarp structure

Potential fruit size depends on the cell number within the developing ovary, while final fruit size depends on the further expansion of these cells (Ho, 1992). One of the strategies that plants adopt under stress conditions is to slow down growth. The ability to reduce cell division under unfavorable conditions may not only allow conservation of energy for defense purposes but may also limit the risk of heritable damage (May et al., 1998).

During this work, it was observed that Pre-A shading affected the carpel cell division phase from the first sampling date, reducing the number of cell layers in the middle layer compared to the Con and Post-A treatments in the fruits of the three capitulum positions (Fig. 3A–C). The difference in the number of layers of the middle layer was evident until 10 DAFA. Later these differences were not detected in peripheral fruits as a result of the compression exerted by the developing embryo on the pericarp internal cell layers (Fig. 3A–C). In PM, the pericarp thickness is fixed by the number of cell layers of the middle layer not compressed by the embryo. A reduction in the number of layers could result in thinner pericarps, as happened in the Pre-A treatment (Table 1). However, a middle layer with a lower number of layers at PM, does not necessarily mean that less layers has been fixed in the carpel. It could also be the consequence of compression of a higher number of layers by the embryo. Reductions in the number of layers in the pericarp middle layer from plants under thermal stress were observed at PM by Rondanini et al. (2006). In their case it could be a consequence of compression or disorganization of the

innermost layers of the middle layer, since at the time of the thermal stress treatments (10, 18 and 26 days after anthesis), the cell division phase in the carpel had finished (Rondanini et al., 2006).

Our work shows that the deposit of secondary walls in the cells of the middle layer started after the Pre-A treatment was completed. An increase of pericarp cell wall thickness was observed in Pre-A-treated plants, in the mid and central fruits of the capitulum (Table 1; Fig. 5B, C, E and F). Other authors have also established a negative correlation between the cell wall thickness of xylem elements and plant growth rate (Dickison, 2000; Lundgren, 2004). Fertilization and irrigation treatments that increased the plant growth rate decreased the cell wall thickness in *Picea abies* (L.) Karst wood (Lundgren, 2004). Likewise, Refrégier et al. (2004) observed, in *Arabidopsis* hypocotyl, thick walls in slowly growing cells and thin walls in rapidly growing cells, an indication that cell wall synthesis is not coupled with cell elongation. If indeed the growth rate of a fruit depends on the number of cells fixed during ovary development (Andrews et al., 2002; Baldet et al., 2006), a lower number of cells fixed in the carpel due to shading during the Pre-A treatment (Fig. 2A–C) would decrease the subsequent growth rate of the pericarp. This in turn would contribute to an increase in the cell walls thickness and to a premature visualization of this thickening (Fig. 4B). This effect was more noticeable in central fruits than in mid and peripheral ones (Fig. 5E and F), probably contributing to the shortening of the pericarp growth period (Fig. 2C and F).

The effect of the Post-A treatment on pericarp growth became evident through the unchanging thickness of the middle layer between sampling dates in the mid and central fruits (Fig. 2E and F). Although a cell volume increase occurred in the middle layer (Fig. 2E and F) between FA and 10 DAFA, this did not translate into an increment of the total thickness of this layer, as some layers in its middle region, the walls of which had not thickened, where slightly compressed (Fig. 4C). Possibly this compression was a consequence of the pressure exerted externally by the outermost layers of the middle layer once they started to sclerify and, internally, by the developing embryo. Between 10 DAFA and PM a slight increase in the secondary wall deposit of the outermost cell layer of the middle layer was observed. At the same time, some of the earlier compressed layers regained their initial shape once normal development conditions were restored after the shading treatment was completed (Fig. 5H and I). The middle layer thickness remained constant due to the significant reduction of non-compressed layers of the middle layer in PM (Fig. 5H and I). The pressure exerted over the internal layers by the developing embryo would have kept the middle layer thickness unchanged. The embryo volume in this shading treatment and that of the Con fruits remained similar (Lindström et al., 2006a).

Even though secondary wall deposit continued after the shading was removed (Figs. 4C and 5H), the sclerification level reached by the pericarp was significantly lower in PM than in Con and Pre-A (Table 1; Fig. 5G–I). Post-A treatment decreased both the number of sclerified layers and cell wall thickness (Table 1; Fig. 5G–I). Probably, the start of the embryo linear phase of growth (15–19 DAFA; Lindström et al., 2006a) determined the end of pericarp growth. The pericarp and the embryo are not necessarily structures that develop independently. In some species growth has been demonstrated to stop alternatively in the pericarp and the embryo, thus showing some competition in development between these two structures (Roth, 1977).

In conclusion, even though both shading treatments produced a similar reduction in the pericarp weight (Lindström et al., 2006a) the development status of the carpel or of the pericarp, at the moment at which shading treatments were applied, generated pericarps with very different structure. These differences affected ease of hulling. Lindström et al. (2006b,c) observed that the fruits coming from Post-A treatment presented lower hullability (57%) than Con and Pre-A treatments, which showed no significant difference between them (74–76%). Thicker walled pericarps in the Con and Pre-A treatments produce more rigid and brittle structures that favor dehulling as compared to the pericarps with thinner and more flexible walls as seen from Post-A treatment.

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References

Akhalkatsi, M., Wagner, J., 1997. Comparative embryology of three *Gentianaceae* species form the Cental Caucasus and the European Alps. Plant Syst. Evol. 204, 39–48.

Alkio, A., Schubert, W., Diepenbrock, W., Grimm, E., 2003. Effect of source–sink ratio on seed set and filling in sunflower (*Helianthus annuus* L.). Plant Cell Environ. 26, 1609–1619.

Andrews, J., Adams, S.R., Burton, K.S., Edmondson, R.N., 2002. Partial purification of tomato fruit peroxidase and its effect on the mechanical properties of tomato fruit skin. J. Exp. Bot. 53, 2393–2399.

Baldini, M., Vannozzi, G.P., 1996. Crop management practice and environmental effects on hullability in sunflower hybrids. Helia 19, 47–62.

Baldet, P., Hernould, M., Laporte, F., Mounet, F., Just, D., Mouras, A., Chevalier, C., Rothan, C., 2006. The expression of cell proliferation-related genes in early developing flowers is affected by fruit load reduction in tomato plants. J. Exp. Bot. 57, 961–970.

Beauguillame, A., Cadeac, F., 1992. Elements of explication of variability of hulling ability in sunflower. In: Proceedings of the 13th International Sunflower Conference, Pisa, Italy, pp. 1000–1004.

Cantagallo, J., 2000. Reducción del número de frutos llenos en girasol (*Helianthus annuus* L.) por estrés lumínico. Tesis de Magíster UBA. Bs. As, Argentina, p. 102.

Connor, D.J., Hall, A.J., 1997. Sunflower physiology. In: Schneiter, A.A. (Ed.), Sunflower Technology and Production. Amer. Soc. Agron., Madison, WI, USA, pp. 113–182.

Dedio, W., Dorrell, D.G., 1989. Factors affecting the hullability and physical characteristic of sunflower achenes. Can. Inst. Food Sci. Technol. J. 21, 143–146.

Dennis, L., 1994. Étude génetique de l'aptitude au decorticage des akénes de tournesol (*Helianthus annuus* L.). Tesis doctoral. École Nationale Superierure Agronomique de Rennes, France, p. 117.

Dickison, W.C., 2000. Integrative Plant Anatomy. Academic Press, San Diego, CA, USA, p. 533.

García-Luis, A., Oliveira, M.E.M., Bordón, Y., Siqueira, D.L., Tominaga, S., Guardiola, J.L., 2002. Dry matter accumulation in *Citrus* fruit is not limited by transport capacity of the pedicel. Ann. Bot. 90, 755–764.

Hanausek, T.F., 1902. Development of the pericarp of *Helianthus annuus* L. Ver. Dtsch. Bot. Ges. 20, 449–454.

Ho, L.C., 1992. Fruit growth and sink strength. In: Marshal, C. (Ed.), Fruit and Seed Production. Aspects of Development, Environmental Physiology and Ecology. Cambridge University Press, pp. 101–124.

Knowles, P.F., 1978. Morphology and anatomy. In: Schneiter, A.A. (Ed.), Sunflower Technology and Production. Am. Soc. Agron., Madison, WI, USA, pp. 55–85.

Leprince-Bernard, M.N. 1990. Aptitude au décorticage de la graine de tournesol. Ëtudes structurale, physico-chimique et comportementale, en fonction des variétés, des lieux de culture, de l'étal de l'eau et de traitements préalables. Doctoral Thesis, Institut. Nat. de la Recherche, Agronomique, Nantes, France. p. 136.

Lindström, L.I., Pellegrini, C.N., Hernández, L.F., 2000. Anatomía y desarrollo del pericarpio de distintos genotipos de girasol (*Helianthus annuus* L.). In: Proceedings of the 15th International Sunflower Conference, Toulouse, France, June 12–15, pp. D13–D18.

Lindström, L.I., Pellegrini, C.N., Aguirrezábal, L.A.N., Hernández, L.F., 2006a. Growth and development of sunflower fruits under shade during early pre and post-anthesis period. Field Crops Res. 96, 151–159.

Lindström, L.I., Pellegrini, C.N., Torrea, M.B., Hernández, L.F., 2006b. Composición química y estructural del pericarpio de frutos girasol desarrollados bajo condiciones de estrés lumínico. In: Proceedings of XXVI Argentine Meeting of the Argentine Society of Plant Physiologists, Chascomús, Argentina, Octubre 4–6, p. 161.

Lindström, L.I., García, M.E., Pellegrini, C.N., Hernández, L.F., 2006c. Aptitud al descascarado de frutos de girasol desarrollados bajo condiciones de estrés lumínico. In: Proceedings of XXVI Argentine Meeting of the Argentine Society of Plant Physiologists, Chascomús, Argentina, Octubre 4–6, p. 162.

Lundgren, C., 2004. Cell wall thickness and tangential and radial cell diameter of fertilized and irrigated Norway spruce. Silva Fennica 38, 95–106.

Mantese, A.J., Medan, D., Hall, A.J., 2006. Achene structure, development and lipid accumulation in sunflower cultivars differing in oil content at maturity. Ann. Bot. 97, 999–1010.

May, M.J., Vernoux, T., Leaver, C., Van Montagu, M., Inzé, D., 1998. Glutathione homeostasis in plants: implications for environmental sensing and plant development. J. Exp. Bot. 49, 649–667.

Nel, A.A., 2001. Determinants of sunflower seed quality for processing. Doctoral Thesis, Faculty Natural and Agricultural Sciences, University of Pretoria, Pretoria, South Africa.

Newcomb, W., 1973a. The development of the embryo sac of sunflower *Helianthus annuus* before fertilization. Can. J. Bot. 51, 863–878.

Newcomb, W., 1973b. The development of the embryo sac of sunflower *Helianthus annuus* after fertilization. Can. J. Bot. 51, 879–890.

Pandey, A.K., Dhakal, M.R., 2001. Phytomelanin in compositae. Curr. Sci. 80 (8), 933–940.

Pereyra, V.R., Farizo, C.L., 1981. Girasol. Técnicas de producción, INTA Balcarce.

Refrégier, G., Pelletier, S., Jaillard, D., Höfte, H., 2004. Interaction between wall deposition and cell elongation in dark-grown hypocotyls cells in *Arabidopsis*. Plant Physiol. 135, 959–968.

Rondanini, D.A., Mantese, A., Savin, R., Hall, A.J., 2006. Responses of sunflower yield and grain quality to alternating day/night temperature regimes during grain filling: effects of timing, duration and intensity of exposure to stress. Field Crops Res. 95, 305–315.

Roth, I., 1977. Fruits of Angiosperms. Gebrüder Borntraeger, Berlín, p. 675.

Ruzin, S.E., 1999. Plant Microtechnique and Microscopy. Oxford University Press, Oxford, New York, p. 322.

Schneiter, A.A., Miller, J.F., 1981. Description of sunflower growth stages. Crop Sci. 21, 901–903.

Seiler, G.J., 1997. Anatomy and morphology of sunflower. In: Schneiter, A.A. (Ed.), Sunflower Technology and Production. Am. Soc. Agron., Madison, WI, USA, pp. 67–111.

Soil Survey Staff, USDA, 1999. Soil Taxonomy: A Basic System for Classifying Soils. Soil Survey Staff, Agriculture, Homework, p. 436.

Spurr, A.R., 1969. A low viscosity resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26, 31–43.

Steer, B.T., Hocking, P.J., Low, A., 1988. Dry matter, minerals and carbohydrates in the capitulum of sunflower (*Helianthus annuus*): effects of competition between seeds, and defoliation. Field Crops Res. 18, 71–85.

SPSS, 1997. SYSTAT for Windows®, Release 7.0. SPSS Inc., Chicago, IL.

Wan, P.J., Baker, G.W., Clark, S.P., Matlock, S.W., 1978. Factors influencing the decortication of high oil content sunflower seed. In: Proceedings of the 8th International Sunflower Conference, Minneapolis, MN, July 23–27, pp. 533–543.

Yegappan, T.M., Paton, D.M., Gates, C.T., Muller, W.J., 1982. Water stress in sunflower (*Helianthus annuus* L.): responses of cypsela size. Ann. Bot. 49, 69–75.