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Field Crops Research 96 (2006) 151-159



www.elsevier.com/locate/fcr

Growth and development of sunflower fruits under shade during pre and early post-anthesis period

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Received 1 March 2005; accepted 16 June 2005

Abstract

The effect of pre and post-anthesis shading (20% of incident radiation) on pericarp development, cotyledon cell number and seed growth dynamic of fruits from three positions in the capitulum (peripheral, mid and central) of two sunflower (*Helianthus annuus* L.) genotypes were studied at two locations.

Both shading treatments reduced pericarp weight, fruit volume and total yield per plant. Plants shaded during pre-anthesis maintained the number of filled fruits but reduced their individual weight and cotyledon cell number in the three positions on the capitulum. In contrast, post-anthesis shading reduced the number of filled fruits but their individual weight and cotyledon cell number were reduced only in the central fruits.

Sigmoidal functions were fitted to seed growth data to estimate the duration of lag phase, the seed growth rate (SGR) and the effective filling period (EFP). Pre-anthesis shading reduced EFP of peripheral fruits, SGR of mid fruits and SGR and EFP of central ones while post-anthesis shading increased the duration of the lag phase of mid and internal fruits.

The hierarchy of fruit growth between positions within the capitulum was not modified by shading treatments and it was associated with differences, among fruit positions, in cotyledons cells number (except between mid and central fruits in pre-anthesis shading) and SGR (except peripheral and mid fruits in post-anthesis shading).

SGR not only depended on the cotyledons cell number, which was fixed during the cell division phase of seed development, but it was also sensitive to environmental conditions during the linear phase of growth.

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Keywords: Helianthus annuus L.; Cell number; Seed development; Shading; Sunflower; Yield components

1. Introduction

The fruit (grain) of the sunflower is formed by the pericarp (hull), which comprises between 20 and 25% of the fruit weight, and the kernel or seed (mostly embryo) where the largest proportion of lipids and dry matter is stored (Connor and Hall, 1997).

A developmental model of sunflower's seed can be defined by three sequential phases (Lindström et al., 2002). The initial phase (Phase I), up to 10 days after anthesis,

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includes fertilization, the period of active cell division and a slow increase of the dry weight. Here, the anatomy of the embryo structures are defined and the final number of embryo cells is fixed (Prokof'ev et al., 1985; Lindström et al., 2000). Also during this phase the pericarp completes its development (Villalobos et al., 1996; Connor and Hall, 1997; Lindström et al., 2000). Next follows an intermediate phase (Phase II), where there is an increase of cell volume and a rapid accumulation of dry matter in the seed. In the final phase (Phase III) maximum seed weight is achieved and the fruit reaches physiological maturity. A similar seed developmental model has been proposed for wheat (*Triticum aestivum* L.) (Wardlaw, 1970; Gao et al., 1993), corn (*Zea*

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^{0378-4290/\$ –} see front matter \odot 2005 Published by Elsevier B.V. doi:10.1016/j.fcr.2005.06.006

mays L.) (Reddy and Daynard, 1983; Jones et al., 1996) and soybean (*Glycine max* (L.) Merr.) (Bills and Howell, 1963; Egli et al., 1981; Guldan and Brun, 1985).

In the sunflower capitulum, there is a developmental and growth gradient, which progresses from the fruits at the periphery towards those at its center. So, the effect of any environmental stress, while the reproductive development is in progress, will not be similar for the fruits at the different positions on the capitulum. For example, Yegappan et al. (1982) observed that water stress in post-anthesis reduced the weight of the central seeds and not that of the fruits at the capitulum periphery whereas Cantagallo et al. (2004) found that pre-anthesis shading only affected the weight of the fruits at the peripheral and mid positions on the capitulum.

The developmental status reached by the pericarp before anthesis could limit the subsequent seed growth and development and, consequently diminish the final fruit weight (Millet, 1986; Egli et al., 1987). Cantagallo et al. (2004) found in sunflower that the carpel weight at anthesis was related to the final weight of the fruit. Likewise, Scott et al. (1983) established, for barley (*Hordeum vulgare* L.) a positive relationship between the carpel size at anthesis and the final size of the caryopses. Also, the final size and shape of rice (*Oryza sativa* L.) (Murata and Matsushima, 1975) and soybean (Egli et al., 1987) seeds decreased when a physical restriction was imposed.

For other crops there is evidence that final seed size is a function of the number of cells present in cotyledons or endosperm (Egli, 1998; Lemontey et al., 2000). During the development of seeds, the increase in cell volume is limited, so the final seed size and the capacity to accumulate dry matter in the seminal tissue will be determined by the number of cells present in the cotyledons or endosperm (Egli, 1998). It has been found that environmental factors such as supra-optimal temperatures in corn (Jones et al., 1985) and water or light stress in wheat (Wardlaw, 1970; Brocklehurst, 1977), which affected the cell division process in the seed by reducing the final number of embryo or endosperm cells, resulted in seeds with a lower weight. In soybean, the reduction in the sourcesink ratio by shading or defoliation during the initial development of the seed resulted in a smaller number of cotyledon cells and a lower final seed weight (Egli et al., 1989).

There is little work relating the effects of environmental factors or agronomic practices on the cell division process in the embryo and subsequent seed growth of sunflower (Karyagina et al., 1999). In the present study, the effect of shade during the pre-anthesis and early post-anthesis periods on the pericarp development, cotyledon cell dynamics and seed growth was analyzed in fruits from three positions in the capitulum.

2. Materials and methods

2.1. Plant material

2.1.1. Experiment I

Two commercial sunflower genotypes, Dekasol (DK) 3900 and DK4030 (Monsanto[®], Argentina), were sown on November 29, 2001 at the Experimental Station INTA Balcarce, Argentina (Lat. S $37^{\circ}45'$; Long. W $58^{\circ}18'$). The soil was a Typic Argiudol (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. Environmental conditions during crop growth kept soil water content above 50% of field capacity.

The phenology referred to here corresponds to that defined by Schneiter and Miller (1981). The treatments consisted of two shading periods: one applied pre-anthesis (Pre-A), for 14 days from reproductive stage R2; the other in early post-anthesis (Post-A), for 14 days from full anthesis (stage R5.10) and a control treatment (Con) (Fig. 1a). When each treatment started, plants at the appropriate growth stage (R2 or R5.10) were identified and marked.

Pre-A shading started at R2 and finished 14 days later at R4 stage. At R2 the inflorescence is surrounded by bracts, the first internode below the base of the inflorescence elongates 0.5–2.0 cm above the nearest leaf (Schneiter and Miller, 1981). At this moment all the disc florets were differentiated. Peripheral, mid and internal ovaries averaged 0.7, 0.4 and 0.2 mm long, respectively. At R4 the inflorescence begins to open and the ray flowers become



Fig. 1. Timing of shading treatments and mean daily solar radiation (MJ $m^{-2} day^{-1} - - -$) and temperature (°C; —) for each location. (a) Experiment I: Balcarce (Lat, S 37°45'; Long, W 58°18'); (b) Experiment II: Bahía Blanca (Lat, S 38°45'; Long, W 62°11'). Horizontal bars over the line graphs indicate the intervals of time when shading was applied. R2, R3 and R5.10 note reproductive stages of sunflower development as described by Schneiter and Miller (1981).

visible (Schneiter and Miller, 1981). Peripheral, mid and internal ovaries were approximately 3.0, 2.0 and 1.2 mm long, respectively.

The experimental design consisted of complete randomized split plots, with genotype 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 $1.40 \text{ m} \times 2.10 \text{ m} \times 1.55 \text{ m}$; wide $\times \text{ long} \times \text{ high metal structures over the treated plots}$), 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.

2.1.2. Experiment II

The same genotypes and experimental design were used as in Experiment I. The sowing was made in an experimental field of the Agronomy Department-UNSur, Bahía Blanca, Argentina (Lat. S $38^{\circ}45'$; Long. W $62^{\circ}11'$) on October 22, 2002. The soil was a Typic Ustipsamment (Soil Survey Staff, 1999). Plant density was adjusted to 5.6 plants m⁻². Crop management and light stress protocols were similar to those applied in Experiment I. Water was supplied by drip irrigation. The treatments consisted of two shading periods, one applied in Pre-A, for 10 days beginning at stage R3, and the other in early Post-A, for 10 days from full anthesis (stage R5.10) and a control treatment (Con) (Fig. 1b).

At R3 the inflorescence is still surrounded by bracts and the first internode below its base is longer than 2 cm (Schneiter and Miller, 1981). Peripheral, mid and central ovaries were approximately 1.4, 0.8 and 0.7 mm long, respectively. At R5.0 (beginning of anthesis of disk florets) peripheral, mid and central ovaries averaged 8.0, 2.2 and 1.7 mm long, respectively.

In both experiments, both genotypes achieved full anthesis (anthesis of central florets) 3 and 7 days after the opening of the peripheral and the mid-section florets, respectively. These intervals also correspond to the time elapsed from the beginning of the initial phase of seed development and post anthesis pericarp growth. At this moment peripheral, mid and central ovaries were approximately 10.0, 10.0 and 8.0 mm long, respectively.

When Post-A treatment concluded, pericarp growth had finished while the embryos of the three positions, in Experiment I, and only those of the peripheral and mid section in Experiment II, were in the linear phase of growth.

Daily records of temperature and solar radiation were obtained from a meteorological station located 400 m from the experimental field in Experiment I (Fig. 1a) and 800 m from the experimental field in Experiment II (Fig. 1b).

2.2. Measurements and observations

2.2.1. Yield components

At physiological maturity (stage R9), 3 and 2 capitula per plot in Experiment I and Experiment II, respectively, were harvested. For each capitulum, total filled fruit weight (yield per plant) and the number of filled and empty fruits were determined. The total number of florets was calculated as the total of filled and empty fruits. Empty fruits included aborted florets and incompletely developed fruits (Lindström et al., 2004). Receptacle diameter after removal of the bracts, was measured to calculate the receptacle area.

2.2.2. Pericarp and seed weight

At physiological maturity seed and pericarp dry weight of five fruits from the peripheral, mid and central position on two capitula per plot was determined in both experiments, after drying at 60 °C for 72 h. The fruits were harvested evenly around the circumference of the capitulum at the three locations.

2.2.3. Fruit and seed volume

In Experiment II, for each treatment and position on the capitulum, the volume of mature fruits and their corresponding seeds (20 per replicate) were determined using the technique described by Wessel-Beaver et al. (1984).

2.2.4. Cotyledon cell number

Cotyledon cell number was determined in seeds of two fruits from the peripheral, mid and central position on two capitula per plot of the three treatments. In Experiment I the determination was made on physiologically mature seeds. In Experiment II the determination was made on seeds sampled 14 days after full anthesis. At this date the cotyledon cell number has reached its highest value (Lindström, unpublished data).

Samples were processed by adapting the technique described by Rijven and Wardlaw (1966). The cotyledons were hydrolyzed with 5 N HCl for 30 min in an ice bath followed by 60 min in water bath at 20 °C. The hydrolyzed material was then stained with Feulgen reagent (Ruzin, 1999) followed by the enzymatic separation of cells with cellulase 0.5% (w/v) (Onozuka, Yakult Onza Co. Ltd., Japan) at pH 5 in a water bath at 40 °C for 2 h. In mature seeds it was necessary to double the enzymatic digestion time to achieve separation of the cotyledon cells. For the cell counts, diluted samples of the digested material were observed using a hemocytometer. Dilutions were made in order to achieve approximately 100 cells per quadrant. Average cotyledon cell volume was estimated by dividing the seed volume by the corresponding number of cells in the cotyledon.

2.2.5. Seed growth

The accumulation of dry matter by seeds over time was analyzed in samples taken from the three treatments of Experiment II. Five fruits were extracted from the peripheral, mid and central position of two capitula per plot at 3-day interval from full anthesis to physiological maturity. The seeds were separated from the pericarp and dry weight determined after drying at 60 °C for 72 h.

2.2.6. Statistical analysis

The growth dynamics of the seeds was analyzed by fitting the dry weight data from each replication to a sigmoidal function, using non-linear regression algorithms incorporated in the software Sigma Plot, v. 8.0 (Logistic, four parameters; Systat Software Inc., Richmond, CA, USA). From the values estimated by the sigmoid equation and following the criteria proposed by Johnson and Tanner (1972) the following phases of the seed growth were defined: a lag phase, considered as the period elapsed until the seed reached 5% of its final weight; seed growth rate (SGR, mg seed⁻¹ day⁻¹), estimated by linear regression analysis from the values between 5 and 90% of the final seed weight, and the effective filling period (EFP) defined as the quotient between the final seed weight and SGR (Egli, 1998).

To determine the differences between treatments, the experimental results of each variable were processed by analysis of variance and differences between treatment means were evaluated with LSD test.

3. Results

Since there was no genotype \times treatment interaction (P > 0.05) for any of the variables (Tables 1 and 2; Fig. 2), only the average results for both genotypes in each experiment (Experiment I and Experiment II) are presented.

3.1. Yield components

Shading treatments reduced yield per plant, in both experiments. This was associated with a reduction of 26–29% in Pre-A and 23–29% in Post-A in the receptacle area (Table 1). Floret number per capitulum did not vary among treatments. The Pre-A treatment produced the same number of filled fruits as Con but their individual weight was reduced by 19% in both experiments (Table 1). Conversely, Post-A treatment produced a significant reduction (15% in Experiment I and 30% in Experiment II) in the total number of filled fruits per capitulum in both experiments, whereas the individual fruit weight was similar to Con (Table 1). In

Post-A the number of empty fruits produced per capitulum increased by 53% in Experiment I and by 113% in Experiment II (Table 1).

3.2. Pericarp weight and fruit volume

Shading treatments affected pericarp growth in both experiments. The largest dry weight reduction of the pericarp occurred in fruits from the central position of the capitulum of Post-A (43% in Experiment I and 52% in Experiment II), while in the fruits of other positions, the reduction fluctuated between 16 and 29% in both treatments (Fig. 2a and b).

In Experiment II the reduction in the pericarp weight in Pre-A was associated with a reduction of 25% in fruit volume from the peripheral and mid positions on the capitulum, and a similar tendency occurred in the central fruits of both shading treatments (Table 2).

3.3. Seed weight and volume

In Experiment I the seed weight of the fruits from the peripheral and mid position of the capitulum in the Pre-A decreased approximately 17% with respect to that of Con, while seed weight in Post-A was intermediate between Con and Pre-A (Fig. 2c). The seed weight of fruits in the central position decreased 21 and 25% in Pre-A and Post-A, respectively (Fig. 2c).

In Experiment II seed weight of fruits from the peripheral and mid-position of the capitulum in Pre-A decreased about 29% with respect to the seed weight of the fruits of the same position in Con and Post-A plants (Fig. 2d). There was no significant differences between the seed weight of these last two treatments. In fruits from the central position on the capitulum, seed weight decreased 41 and 49%, in Pre-A and Post-A plants, respectively (Fig. 2d).

In Experiment II the seed volume decreased by 17 and 21% in the fruits in the peripheral and central positions, respectively, in Pre-A, while Post-A treatment only affected the seed volume in the central position on the capitulum (Table 2).

Table 1

Receptacle area and yield components of plants averaged across shading treatment for each experiment

Treatment	Experiment I			Experiment II					
	Con	Pre-A	Post-A	S.E.	Con	Pre-A	Post-A	S.E.	
Receptacle area (cm ²)	283.6 a ^a	202.0 b	219.1 b	16.0	255.1 a	188.1 b	184.8 b	4.0	
Fruit yield per plant (g)	79.5 a	62.5 b	63.3 b	3.8	81.8 a	53.0 b	55.6 b	6.2	
Florets per capitulum	1638 a	1590 a	1660 a	131.1	1828 a	1504 a	1771 a	121.1	
Filled fruits per capitulum	1238 a	1213 a	1047 b	40.3	1484 a	1179 ab	1038 b	98.2	
Empty fruits per capitulum	400 a	377 a	613 b	38.1	344 a	325 a	733 b	39.8	
Fruit weight (mg fruit ⁻¹)	63.6 a	51.5 b	59.9 a	2.3	54.9 a	45.5 b	53.5 a	1.7	

Con: control; Pre-A: shade from pre-anthesis; Post-A: shade from post-anthesis. Experiment I: Balcarce (Lat. S 37°45'; Long. W 58°18'); Experiment II: Bahía Blanca (Lat. S 38°45'; Long. W 62°11').

^a In a row, between shading treatments within each experiment, means followed by the same letter are not significantly different at P < 0.05. S.E. = standard error.

Table 2

Effect of a 10 day pre-anthesis (Pre-A) and post-anthesis (Post-A) shading on final fruit and seed volume, seed cell volume, and seed growth dynamics (lag phase duration, seed growth rate and effective filling period) for fruits at three positions in the capitulum averaged over genotype in Experiment II

Fruit position	Periphery				Mid-section				Central			
Treatment	Con	Pre-A	Post-A	S.E.	Con	Pre-A	Post-A	S.E.	Con	Pre-A	Post-A	S.E.
Fruit volume (μ L fruit ⁻¹)	93.3 a ^a	69.8 b	82.3 a	4.4	71.3 a	53.9 b	64.1 ab	4.3	56.6 a	40.6 a	43.2 a	6.8
Seed volume (μ L seed ⁻¹)	52.3 a	43.5 b	51.2 a	1.4	40.6 a	36.6 a	40.4 a	1.9	33.6 a	26.6 b	28.2 b	1.2
Cotyledon cell volume (μ L cell ⁻¹ 10 ⁵)	2.0 a	1.7 b	1.9 ab	0.1	2.2 a	2.6 a	2.3 a	0.2	2.0 a	1.6 b	2.0 a	0.1
Lag phase duration (days)	10.2 a	10.8 a	11.0 a	0.8	10.2 a	10.0 a	15.2 b	0.8	10.2 a	8.5 a	18.8 b	0.8
Seed growth rate (mg seed ^{-1} day ^{-1})	2.1 a	2.1 a	1.9 a	0.2	1.8 a	1.5 b	1.9 a	0.1	1.4 a	1.1 b	_	0.1
Efective filling period (days)	24.5 a	17.5 b	26.2 a	1.5	22.3 a	19.2 a	20.9 a	1.4	22.0 a	16.9 b	-	1.3

Con: control.

^a In a row, between shading treatments within each capitulum's position, means followed by the same letter are not significantly different at P > 0.05. S.E. = standard error.

3.4. Cotyledon cell number

In Experiment I cotyledon cell number of fruits in the mid and peripheral position of Pre-A decreased 18 and 25%, respectively, with respect to Con and Post-A, which showed no differences between them (Fig. 2e). Both shading treatments reduced the cotyledon cell number in central fruits (about 22%).



Fig. 2. Pericarp and embryo weight and cotyledon cell number for fruits in three positions of the capitulum (periphery, mid and central) of control (\square), Pre-A (\square) and Post-A (\square) shaded plants averaged over two genotypes. a, c and e, Experiment I; b, d and f, Experiment II. Within each set, bars topped by different letters are significantly (P < 0.05) different.

In Experiment II cotyledon cell number in peripheral fruits showed no differences between treatments (Fig. 2f). In fruits in the mid position of the capitulum, this parameter was reduced by 25% in plants from Pre-A, with respect to Con and Post-A. In the fruits in the central position on the capitulum, cotyledon cell number decreased 7% in the Pre-A and 9% in Post-A.

3.5. Cotyledon cell volume

In Experiment II cotyledon cell volume of peripheral and central fruits of Pre-A decreased between 11 and 16% with respect to Con and Post-A, which showed no differences between them. No differences were found between treatments in cotyledon cell volume of mid fruits with respect to Con (Table 2).

3.6. Seed growth dynamics

Dry weight dynamics of seeds of most combinations of treatment and fruit position on the capitulum fitted a sigmoidal function, showing in every case, coefficients of determination greater than 0.94. Seed growth curves of fruits of the central position of the capitulum in Post-A did not resolve to a sigmoidal function, since the dry weight data described a long lag phase and the last portion of the growth linear phase did not reach a plateau. In this case neither the SGR nor the duration of its EFP could be determined (Table 2). Otherwise, seed growth in every treatment in the three positions on the capitulum was consistent with the seed developmental model suggested for other crops (Egli, 1998) and described previously in this paper. Differences appeared, when compared to Con, in the relative duration of each stage in some of the combinations of treatment and fruit position (Table 2).

The lag phase duration, SGR and seed EFP duration showed no genotype \times treatment interaction so the comparison between treatments was made with the average values of the genotypes.

The lag phase duration and the SGR of the peripheral fruits in both shading treatments were not different from Con while the EFP of the Pre-A was between 7 and 8.7 days shorter, respectively, with respect to Con and Post-A, which showed no differences between them (Table 2).

The mid fruits of Pre-A had the same duration of the lag phase and the EFP as the Con, even though the SGR was decreased by 15%. Post-A increased the duration of the lag phase to 15 days, without changing the SGR or the EFP duration with respect to Con (Table 2).

In central fruits, the Pre-A treatment decreased the SGR by 23% and shortened the EFP 5 days as compared to Con (Table 2). In Post-A, the lag phase lasted nearly 19 days after anthesis before start in the linear growth phase. In the Post-A treatment the plateau of the curve was not reached for central fruits in either genotype, thus the maximum seed weight under this treatment corresponds to that observed at harvest (Fig. 2c).

4. Discussion

Yield per plant decreased in both shading treatments (Pre-A and Post-A), with respect to Con (Table 1), even though it was associated with different yield components.

None of the shading treatments affected the total number of florets produced per capitulum since they were applied after this variable was already fixed (Marc and Palmer, 1981). In the Pre-A shading the most affected yield component was the individual weight of the fruits while in the Post-A shading, it was the number of filled fruits. These results are consistent with previous observations made by Cantagallo et al. (2004).

4.1. Pre-anthesis shading

In Pre-A treatment the pericarp weight and fruit volume decreased in all three capitulum positions. This response may reflect changes in the available space per disc floret as a consequence of the reduction of the receptacle area without changes in the number of filled fruits (Table 1) and/or direct effect on ovary growth (Cantagallo et al., 2004). Preliminary observations showed also a greater pericarp sclerification (Lindström, unpublished data). Seed weight reduction was also observed, which was associated with a lower cotyledon cell number, except in the peripheral fruits in Experiment II. This observation could be the result of the longer duration of shading in Experiment I (14 days) with respect to Experiment II (10 days), which caused the cell division process of the peripheral fruits to be affected only in Experiment I (Fig. 2e and f).

Analysis of the seed growth variables in Experiment II indicated that cause of the seed weight reduction differed depending on the position of the fruits in the capitulum. The lower seed weight of the fruits in the peripheral position on the capitulum was a result of a shorter EFP and a smaller cell volume, without changes in cotyledon cell number (Fig. 2df; Table 2). The shorter EFP could be explained by the reduction of the available space for seed development, as a consequence of the smaller volume of the fruit (Table 2), and/or restrictions in assimilate supply after shading (Cantagallo et al., 2004). Sinsawat and Steer (1993) observed in sunflower that shading altered the distribution pattern of assimilates increasing the proportion destined to the leaves, with detriment to those destined for the stem and capitulum. Conversely, the lower weight of the mid fruit seeds was associated with the establishment of a smaller number of cells in the cotyledons with the consequent reduction of the SGR without affecting, in this case, the EFP nor the seed volume (Fig. 2d-f; Table 2). These results agree with the findings of Egli et al. (1989) in soybean, who stated that, in some cases, variations in seed size were not directly related to cotyledon cell number variations. Finally, weight reduction of the central seeds was associated with a reduction of the cotyledon cell number, the SGR, the EFP and cell volume (Fig. 2d-f; Table 2).

4.2. Post-anthesis shading

Post-A treatment reduced the pericarp weight of the fruits in all three positions of the capitulum, but the pericarp of the central fruits had the largest weight reduction (Fig. 2a and b). The pericarp grew for 10 days after anthesis so the Post-A shading included 3 days, 7 days and the total period of pericarp growth in the peripheral, mid and central fruits, respectively. This would explain the greater effect of this treatment on the pericarp weight of the central fruits (Fig. 2a and b).

Post-A treatment reduced the number of filled fruits and at the same time, it modified the growth dynamics of the seeds in the mid and central fruits by extending the duration of the lag phase in these fruits to 15 and 18 days, respectively. Boyle et al. (1991) showed that the first signal of seed abortion in corn, was the reduction of the sucrose supply as a result of the photosynthesis reduction in stressed plants. The marked increase in the number of empty fruits in Post-A, as a consequence of the above mentioned reason (Table 1), would have maintained the level of photoassimilates allocated to each seed similar to that of Con in most parts of the capitulum, except in the central fruits. This could be the reason why shade only affected the central fruit's growth, resulting in cotyledon cell number, SGR, EFP duration and seed weight of the external and mid fruits similar to Con.

4.3. Fruit growth hierarchy between positions within the capitulum

The centripetal progression of fruit maturation in the sunflower capitulum sets a hierarchy in the development and competitive ability of the peripheral fruits with respect to those in the central position, generating a positional gradient in the demand of photoassimilates (Hernández and Orioli, 1991; Alkio et al., 2003). Steer et al. (1988) found that outer fruits control the growth of inner ones, possibly by competition for space on the receptacle and preferential sequestration of organic and inorganic nutrients. Our work shows that peripheral fruits are characterized by a higher pericarp and seed weight than mid fruits, and these, in turn, than central ones (P < 0.05; Fig. 2a–d). In agreement with previous findings (Sinsawat and Steer, 1993), we observed that this hierarchy did not change under lowered levels of incident irradiance (i.e. shading). The gradient of seed weight between positions was associated with differences, in a similar trend, in cotyledon cell number (except between mid and central positions in Pre-A; Fig. 2f) and SGR (except between peripheral and mid positions in Post-A; Table 2). The difference in cell number between fruits of different positions on the inflorescence can be attributed to differences in resource availability during the cell division phase related to the developmental lag among fruits.

Hormonal disbalance in the developing seeds could also be involved. Evidences indicate a correlation between

high cytokinin level in developing seeds and cell division activity (Abdel-Rahman, 1977; Bohner and Bangerth, 1988a,b) and also that cytokinins located in the embryo at early stages of seed development are imported from the parent plant together with phloem unloaded sucrose (Bewley and Black, 1994; Emery et al., 2000). On the other hand, it is known that the flow of assimilates to sunflower seeds was accompanied by an increase in the level of IAA in the seed during the first days of development which, in turn, accelerated cell divisions in cotyledons (Karyagina et al., 1999). Munier-Jolain and Salon (2003) suggested that sucrose together with endogenous phytohormone levels (mainly cytokinins and auxins), derived from the phloem during the embryo cell division phase, would determine its mitotic activity by modulating the expression of genes that participate in the cell cycle regulation. So, if this is the case, when cell division starts in peripheral fruits of the capitulum, competition for assimilates is low as few fruits are undergoing cell division. During the cell division phase of internal fruits, peripheral and mid-section fruits complete their cell division phase and begin with the phase of rapid dry matter accumulation (linear phase of growth). Hence, competition for assimilate supply increases in the reproductive tissues of peripheral and mid sections of the capitulum, generating a restriction of assimilates and/or growth regulators in the inner developing seeds and, consequently, limiting their cell division rate and growth.

SGR is a function of cotyledon cell number, as indicated by the positive and significant correlation (P < 0.01) found between both variables (Fig. 3). Nevertheless in Pre-A, mid and central fruits showed different SGR (P < 0.05; Table 2) associated with similar cotyledon cell number (P > 0.05; Fig. 2f). Conversely, in Post-A, peripheral and mid fruits presented similar SGR (P > 0.05: Table 2) even when the cotyledon cell number were different (P < 0.05; Fig. 2f).

3

2.5

2

1.5

1



rate in Experiment II (Bahía Blanca; Lat, S 38°45'; Long, W 62°11'). Seed growth rate = 0.50 + 0.58 cells per two cotyledons $\times 10^{-6}$; ($r^2 = 0.45$).

The above mentioned, together with the low determination coefficient ($R^2 = 0.45$) found between cotyledon cell number and SGR (Fig. 3) suggest that differences in SGR are not only correlated with variations in cotyledons cell number. Besides the dependance of a genetic control (Egli, 1998) this parameter, also responds to altered environmental conditions (i.e. shading) that affect the ability of the parent plant to supply assimilates to the seed during the linear phase of growth (Egli et al., 1989).

5. Conclusions

Both shading treatments affected the fruit's pericarp development by modifying their weight and/or volume. The effect of Pre-A shading was uniform throughout the fruits in three capitulum positions, while the effect of Post-A shading increased from the peripheral fruits inwards to those at the capitulum center. In some cases, the lower volume reached by the pericarp could have limited the development of the seeds, as probably occurred with the external fruits in Pre-A.

Within each position on the capitulum the shading treatment that reduced the cotyledon cell number decreased the SGR and their final weight. SGR not only depended on the cotyledon cell number, which was fixed during the cell division phase of seed development, but also on environmental conditions during the linear phase of seed growth.

The assimilate requirements of all the seeds in the capitulum must be balanced with the actual assimilate supply by the leaves. Therefore, the growth rate of a single seed will play an important role in the determination of the total number of filled fruits produced by the plant (Egli, 1998). The Pre-A treatment reduced the cotyledon cell number and the SGR, thus establishing a lower requirement of photoassimilates per seed. This could be the mechanism by which the plants in Pre-A produced no adjustments in the number of filled fruits produced, in spite the reduction in photoassimilates, but they adjust their individual fruit weight.

On the contrary, the generation of empty fruits in Post-A allowed maintaining the seed growth variables at levels similar to Con, producing, in this case, adjustments in the number of filled fruits and not in their individual weight.

Acknowledgements

This work was supported by a grant of the Argentine Sunflower Association (ANPCyT—ASAGIR, PICTOS No. 13151) to L.F.H., the Secretaría Gral. de Ciencia y Tecnología (SeGCyT) UNS and the Comisión de Investigaciones Científicas (CIC, La Plata) Argentina. We want to thank Drs. Barbara W. Pennypacker (Pennsylvania State University) and Hugh J. Earl (University of Guelph) for their valuable comments on the manuscript. We also thank Ms. Andrea Flemmer for collaborating in the field experiments. Sunflower genotypes kindly provided by Monsanto of Argentina are greatly appreciated.

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