REDUCTION IN THE NUMBER OF FILLED SEED IN SUNFLOWER (*Helianthus annuus* L.) BY LIGHT STRESS

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SUMMMARY

Light stress reduces seed number (SN) in sunflower crops. The goals of this research were: (*i*) to evaluate whether a reduction in SN is caused by a reduction in the number of florets, and/or fertilization difficulties and/or seed setting failures; (*ii*) to identify the processes (i.e. pollen development and viability; stigma receptivity; style functionality; ovary viability; embryo growth) affected by light stress that cause SN reduction in the period of floret growth and development; and (*iii)* to rank the processes and phenophases involved in SN responses to light stress. Artificial shading (80 % shading for periods of 10 days between 30 before and 20 days after anthesis) was applied during different phenophases of crop development. Shading from floral stages (FS) 5 though 8 affected the integral of the generative area and the number of florets, without altering the duration of the floret differentiation period. In the FS9-end of seed setting phenophase, all shaded crops suffered significant ($P<0.05$) reductions in SN. Sensitivity of SN to shading varied between positions on the head (higher in the center) and developmental phase at which shading took place (higher in the beginning of embryo growth). All aborted flowers, including those from pre-anthesis shading treatments, had been successfully fertilized. Therefore, light stress produced delayed effects on seed setting. Pollen viability was affected by light stress although a high percentage of pollen grains successfully germinated *in vivo*.

INTRODUCTION

Light stress reduces seed number (SN) in sunflower crops when artificial shading is applied between floral initiation (approximately the 30 days before anthesis) and 20 days after 50% first anthesis (Cantagallo *et al*, 1997, Chimenti and Hall, 1992). These results show that the critical window for seed number determination in sunflower is broader than in other crops. Cantagallo *et al*. found a slightly greater sensitivity of SN to shading in the post-anthesis as against the pre-anthesis phase. The variable sensitivity during this broad response window suggests that several different processes involved in SN determination are affected by light stress in sunflower.

The number of differentiated florets per head in sunflower is a function of size and duration of a meristematic area (generative area, GA) during the floral stages (FS) 5 to FS8 (Marc and Palmer, 1981; Palmer and Steer, 1985). Radiation stress may affect the number of florets by affecting the activity of this meristematic area.

From FS8 to the beginning of anthesis, florets develop and grow completing macro- and micro-sporogenesis. Later, pollination and ovule fertilization occur and the resultant embryo may or may not persist and generate a normal seed. Thus there are a number of candidate processes that may be involved in the responses of SN to shading.

The goals of this research were: (*i*) to evaluate whether a reduction in SN is caused by a reduction in the number of florets, and/or fertilization difficulties and/or seed setting failures; (*ii*) to identify the processes (i.e. pollen development and viability; stigma receptivity; style functionality; ovary viability; embryo growth) affected by light stress that cause SN reduction in the period of floret growth and development; and (*iii)* to rank the processes and phenophases involved in SN responses to light stress.

MATERIALS AND METHODS

Seed number dependence on radiation stress was examined in three experiments (Exp.1 to 3) conducted at the Facultad de Agronomía, Universidad de Buenos Aires (34° 35'S 58° 29'W). Sunflower crops of were grown under field conditions with adequate levels of water and nutrition and were kept free from weeds, insects and diseases.

Shading effects during the floret differentiation period

Experiments 1 and 2

Two experiments were conducted to examine SN responses to shading during floret differentiation Crops sown on 16 Nov. 1995 (Exp.1, hybrid G-100, Dekalb) and 20 Nov. 1996 (Exp.2, hybrid CF21, Zeneca) were exposed to high (100%) or low (20%) levels of short-wave radiation during FS 5 to FS 8. Low radiation level treatment (LR) plots were shaded with black shade netting. Plant density was 5.1 plant m^{-1} and the dimension of plots (experimental units) was 9 rows by 6 plants per row. The experiment was laid out as complete randomized blocks with three (Exp. 1) or four (Exp. 2) replicates. At one (Exp. 2) or two-day (Exp. 1) intervals plants were harvested and apices dissected. The dissected apex of each plant was micro-photographed and image analysis (Harris and Campbell, 1989) was used to determine the generative area. In all harvests the number of florets per apex was also counted. At physiological maturity (R9, Schneiter and Miller, 1981) 4 heads per plot were harvested and the number of filled and unfilled (including dead flowers) seeds per head determined.

Shading effects during the floret growth, anthesis, fertilization, and seed setting period

Experiment 3

Crops of sunflower cv. CF 21 (Zeneca) sown on 27 Nov. were exposed to 100% (control) and 20% (shading treatment) levels of short-wave radiation for 10-d intervals at different times during FS 9 (Marc and Palmer, 1981) and end of seed setting period. Each plot (experimental unit) consisted in 3 rows by 6 plants per row (18 plants) using the same shading net as in Exp.

1. The first 10-d shading treatment (S1) began at FS 9. Five days later, S2 was applied, followed by S3 and S4 at further five days intervals from each other. The two last treatments (S5 and S6) were applied commencing 10 days after the start of the preceding treatment. Two heads per experimental unit were bagged to avoid cross-pollination. The goal of this was to evaluate the shading effects on pollen viability and performance. Samples of florets and young seeds taken from control and shaded plants at the beginning and at the end of each 10-d shading treatment were dissected, measured and photographed. Samples were taken from three different sectors of the head: the periphery, the middle, and the center. The goals here were *i*) to develop a scale of phenological stages from FS9 to the end of seed setting, and *ii*) to evaluate the effects of radiation stress on the growth and development of floret structures.
During anthesis of controls and treatments S1 to S4 (i.e. shades applied before or up until anthesis), sequential samples were taken of florets reaching anthesis on each particular day. At 12, 24, 48, 72, 96, 120, and 144 hs from pollination florets or young fruit were harvested (10 per sample and position on the head). These samples were analyzed to follow up pollen germination, fertilization, and early embryo growth. An indirect method (epifluorescence) was also used to analyze the effect of low radiation on pollen viability in samples taken at four times during anthesis. An andro-sterile hybrid (GV331076, Zeneca) was also used to evaluate pollen viability *in vivo*. The number of filled seed, empty seed, and florets per head was determined at physiological maturity (R9, Schneiter and Miller, 1981) in the cross-pollinated and auto-pollinated (bagged heads) plants.

RESULTS AND DISCUSSION

Shade effects during the floret differentiation period

Dynamics of the generative area in control treatments differed somewhat between Experiments 1 and 2 but responses to shading effects were analogous. The integral of the generative area in the shaded treatment was 41% (Exp. 1) and 32% (Exp 2) less than the corresponding control ($P<0.005$; Student's t, Fig. 1). The duration of the period of floret generation was not affected by the treatment in either experiment, although these differed somewhat in the size of the generative area.

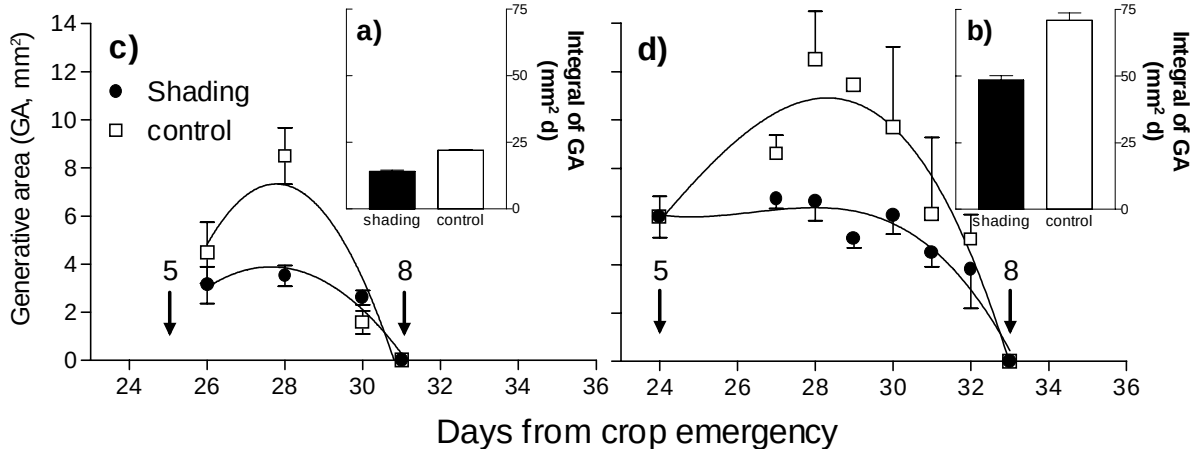

Fig. 1. (a and b) Shade effect on integral of generative area (GA). (c and d) Dynamics of GA during the period of primordia differentiation.

Reductions in the integral of the generative area (GA) were reflected in significant reductions in the total number of florets (Fig. 2, Table I). The rate of primordia differentiation was significantly affected in the shading treatment (Table I). Both the shading effect and the time from the beginning of treatment effect were highly significant. The interaction between time and treatment (Table I), is attributable to an increasing effect of shading with time from the beginning of the treatment.

Table I. Two way ANOVA for the number of primordia per apex during the differentiation period (Exps. 1 and 2). No significant differences between cultivars were found.

Variance source	% of the variance total	P value
Treatment	6,6	<0,0001
Days from EF 5	79,0	<0,0001
Interaction	9,2	<0,0001

Table II. Number of filled seeds per head at physiological maturity.

	Control			Shading			
	mean	S. E. †	n	mean	S. E. †	n	P ‡
Exp. 1	1740	92	4	1087	96	4	< 0,01
Exp. 2	2643	145	3	1461	76	3	< 0,01

† S.E.: standard error of the mean; ‡ Student´s t for comparison between treatments

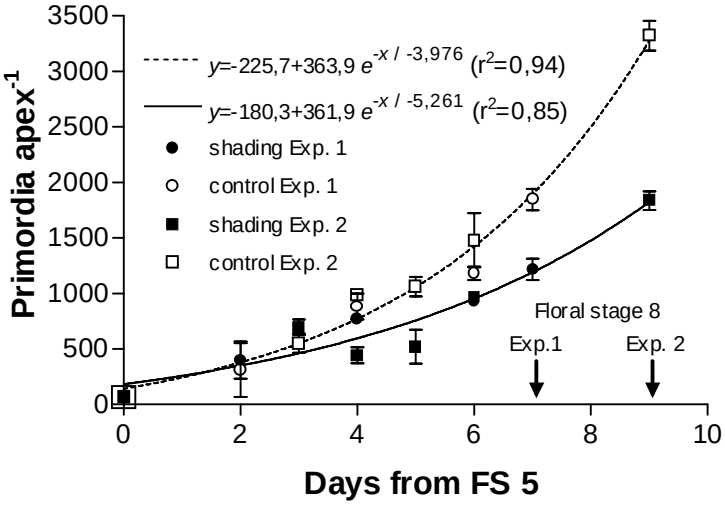

Fig. 2. Relationship between number of primordia per apex and days from floral stage (FS) 5. See Table I.

Percentage of seed set (filled seed number/ florets + empty seed) for both cultivars was not affected by the treatment (P>0.1; Student's t, data not shown). In consequence, the reduction in number of filled seed in shaded plants (Table II) was attributable to the effects of shade on the number of florets, linked, in turn, to the effects of shade on the integral of the generative area. These results are consistent with the notion (Palmer and Steer, 1985) that the integral of the generative area defines potential seed number in sunflower.

Shade effects on floret growth up to anthesis, fertilization, and seed setting

An ontogenetic index was developed using microscopic observations of dissected flowers and fruit obtained between FS10 and the early embryo development stage (Table III).

Table III: Ontogenic Index of reproductive organ development in sunflower.

Stage	**General**	**Female**	**Male**
0	Floret of aprox. 0.25 X 0.25 mm. Corolla not fused yet.		Staminal primordia (aprox. 0.05 X 0.05 mm)
1	Floret of aprox. 0.50 X 0.40 mm. Corolla completed.	Ovary cavity present, early stage of ovular primordia.	Anther primordia. (aprox. 0.175 X 0.1 mm)
2	All floral cycles present. 0.65 mm height X 0.45 mm width.	Symmetric ovular primordia. 0.025 mm height X 0.050 mm width.	Anther lobes begin developing. Epidermis and endothecium differentiated.
3	Floret of aprox. 2.30 X 0.80 mm.	Ovular primordia begin curving. 0.12 mm height X 0.08 mm width.	Pollen mother cell (microsporocyte) and tapetum differentiated.
4	Floret of aprox. 3.00 X 1.00 mm.	Ovular inclination 180°. Ovular tegument appear.	Meiosis complete and tetrads formed.
5	Floret of aprox. 3.60 X 1.00 mm.	Ovule twist complete.	Micropores released. Tapetal cell shrinking.
6		Linear tetrad of four magaspores.	Tapetal protoplasm extruded into the locule. Pollen grain developed.
7		Two-nucleated embryo sac.	Pollen grains mature.
8		Complete embryo sac (egg, antipodal cell, central cell, two synergid cells)	
9	Anthesis		Anthesis
10		Embryo sac containing a globular proembyo. Endosperm growing.	
11		Early heart-stage of the embryo	
12		Visibly plumule aprox. 0.02 mm height X 0.15 mm width. Cotyledon growing.	
13	Black and hard pericarp	Embryo of 1.80 mm width. Plumule of aprox. 0.02 mm height X 0.15 mm width. Developed cotyledons.	

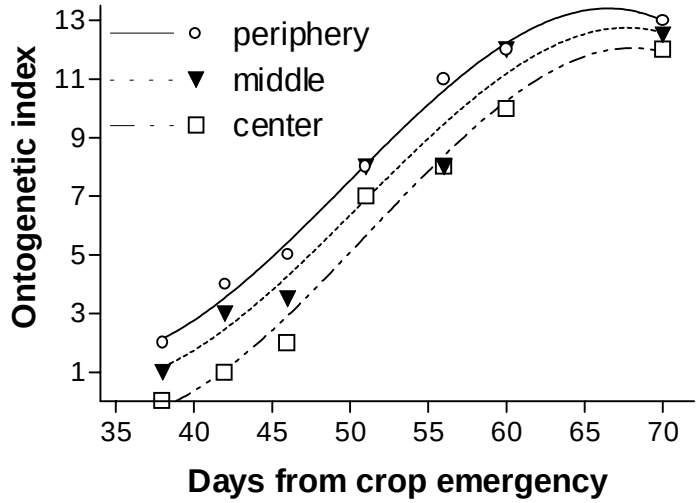

Fig. 3. Floral development in different sectors of the head (control treatment).

Progress of floral ontogeny differed according to the floret position (periphery, middle or center of the head; Fig. 3). Shading delayed floret development in some of the treatments; this delay was more important in the center of the head (data not shown).

Shading after FS 9 significantly ($P<0.05$) reduced the number of filled seed compared with the controls irrespective of timing of the shading. Sensitivity of seed number to stress differed between sectors of the head and with timing of shade application (Fig. 4). Both factors had a highly significant effect ($P<0.0001$) and interaction between them was significant ($P<0.055$; Fig. 4).

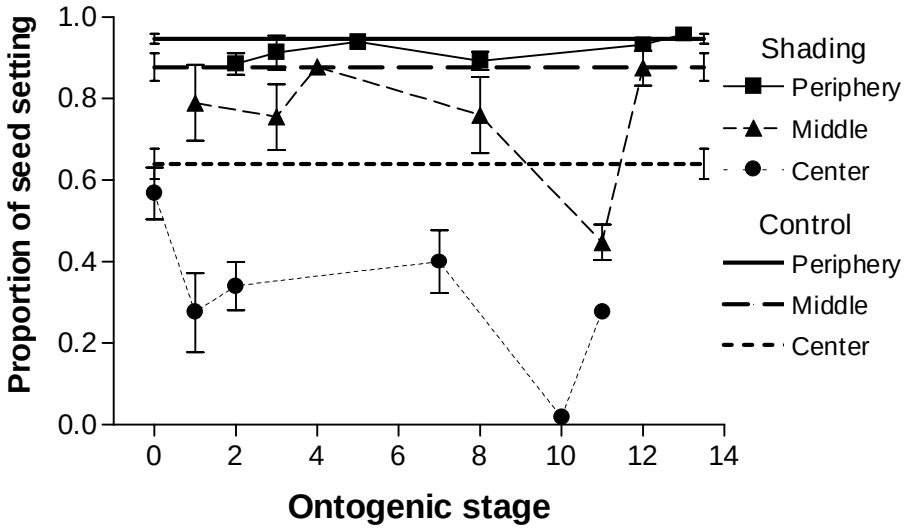

Fig. 4. Proportion of seed setting, in each sector of the head, in function of the ontogenetic stage at the beginning of the shading treatment.

Greater effects of light stress on seed setting occurred in the floret ontogenic stages close to Stage 10 (Table III) in the middle and central parts of the head (Fig. 4). However, in the central sector there was also a reduction in seed setting with shading applications between stages 1 and 7. This suggests there might be delayed stress effects from shading immediately after FS10. Analysis of dissected florets/fruits from these treatments showed that fertilization was successful and that the process affected is embryo setting. Pollen viability was also affected by light stress but, nevertheless, a high percentage of pollen grains successfully germinated *in vivo* (data not shown).

CONCLUSIONS

Two processes proved particularly sensitive to light stress within the critical window for seed number: primordia differentiation and the early embryo growth (Fig. 5). This information considerably enriches our comprehension of how seed number responds to light stress and shows the need to weight light stress effects according to the crop ontogeny and floret position on the head. Future work should focus on the control of primordia differentiation and seed set. The ontogenic index of reproductive organ development is a useful additional product of this research, providing a reference for floret development in the interval between FS10 (Marc and Palmer, 1981) and early embryo growth. No time-related ontogenetic scale had been developed to cover this phase in previous work (Newcomb, 1973; Marc and Palmer, 1981).

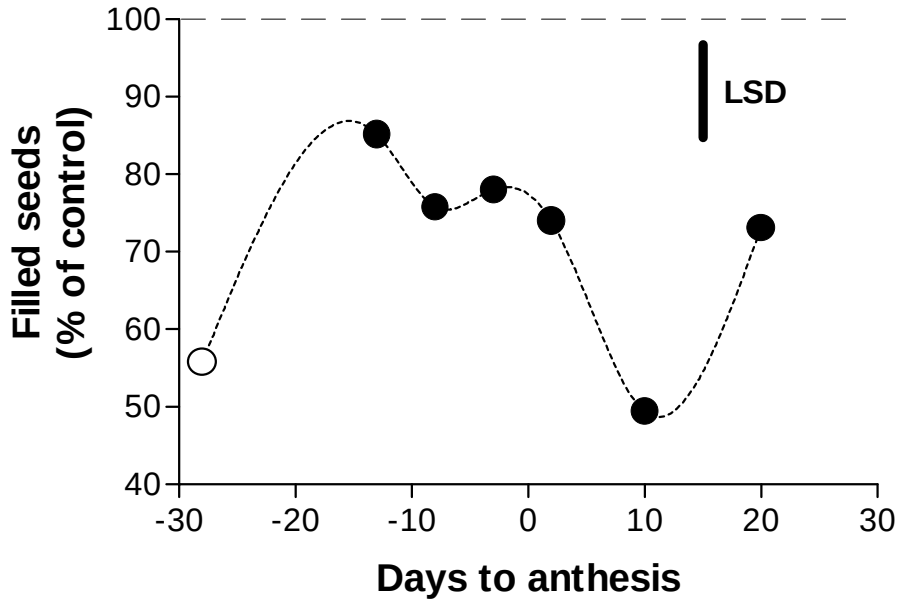

Fig. 5. Number of filled seed expressed as percentage of the Control treatment in function of the middle of shading period. Open circle: shading treatment from Exp. 2; filled circles: shading treatment from Exp. 3. LSD: least significant difference.

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