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Contribution of incident solar radiation on leaves and pods to soybean seed weight and composition

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ABSTRACT

The weight and composition of soybean seeds (*Glycine Max* L. Merrill) depend on changes in carbon and nitrogen assimilate supply during grain filling. Soybean pods and seeds are green, evidencing their capacity to capture light. However, the current physiological knowledge does not consider any effect of incident solar radiation reaching the pods on seed weight and composition. The objective of this work was to investigate the response of seed weight and composition to changes in assimilate supply from leaves, to the incident solar radiation reaching the pods and to the combination of both, changes in assimilate supply from the leaves and incident solar radiation on pods of soybean plants. Field experiments were performed during two growing seasons at Balcarce, Argentina. Treatments modified the amount of assimilates supplied by the leaves (plant shading, defoliation), the solar radiation reaching the pods (pod shading) or both (defoliation and pod shading) during seed filling. Plant shading and defoliation reduced seed weight, oil concentration and oil and protein content and increased the concentration of saturated and poli-unsaturated fatty acids while reduced oleic acid percentage. Pod shading increased the concentration of stearic acid and reduced the concentration of linolenic acid. When pods were shaded on defoliated plants, seed weight and oil and protein content decreased while fatty acid composition was similar to values obtained under defoliation treatment. Based on these results, a conceptual model that considers photoheterotrophic nature of reproductive structures of soybean is proposed. Seed weight, oil and protein content and oil fatty acid composition depended on assimilate availability for the seeds. The response of oil and protein content to assimilate supply depended on whether leaves were present or not. The effect of solar radiation incident on pods depended on the amount of assimilates available for the seeds: (i) when carbon allocated was low (defoliation treatments), pods contributed to seed carbon economy but solar radiation incident on them did not affect fatty acid composition; (ii) when carbon allocated to the seeds was high (intact plants), contribution of pods to seed carbon economy was not significant, but the amount of solar radiation incident on pods produced significant changes in fatty acid composition.

1. Introduction

The weight and composition of soybean seeds (*Glycine Max* L. Merrill) are the result of complex interactions among genetic characteristics, the maternal plant and the environmental conditions during the seed filling period (e.g. Board and Kahlon, 2011; Gallardo et al., 2008; Hua et al., 2012; Rotundo and Westgate, 2009). Among the environmental factors, incident or intercepted solar radiation have been shown to be the most influential (Andrade and Ferreiro, 1996; Izquierdo et al., 2009; Kane et al., 1997; Zuil et al., 2012).

In actively growing soybean seeds, oil, protein and carbohydrate are synthesized from C and N assimilates supplied by the mother plant. While the main source of N for seed filling is remobilization from vegetative structures, the C source – mostly represented by current C assimilation – depends on the solar radiation intercepted by the plants (ISR; Proulx and Naeve, 2009; Rotundo et al., 2009,

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2011). Several reports in the literature describe how changes in ISR during seed filling impacted the assimilate supply, in turn affecting seed weight and composition. A reduction in incident or intercepted solar radiation resulted in lower seed weight, oil content and percentage of oleic acid (Andrade and Ferreiro, 1996; Board et al., 2010; Borrás et al., 2004; Izquierdo et al., 2009; Kane et al., 1997; Proulx and Naeve, 2009; Zuil et al., 2012). Similar responses were observed in sunflower (Echarte et al., 2010; Izquierdo et al., 2008), a species often used as a model for studying the influence of environmental variables on oil composition (Aguirrezábal et al., 2014). Echarte et al. (2012) reported that the effects of radiation on the final weight and oil composition of sunflower grains could be explained by the availability of assimilates. According to a conceptual model proposed by these authors: (i) grain weight and oil content linearly increase with assimilates allocated to the grains, (ii) when the assimilate supply is limiting for grain growth and oil synthesis, mainly linoleic acid is synthesized and (iii) as the assimilate supply increases, oleic acid desaturation, a rate limiting step in fatty acids biosynthesis, gets saturated and oleic acid accumulates. In soybean, this model could account for the relative increase in seed weight with a relative increase in assimilate availability per seed, as proposed by a meta-analysis performed by Borrás et al. (2004). This model, however did not appropriately describe soybean seed composition responses, since changes in the assimilate availability per seed, estimated as source-sink ratio (ISR/seed number), did not account for differences observed between growing seasons (Izquierdo et al., 2009).

Soybean pods are photosynthetically active organs that host seeds during their development (Aschan and Pfanz, 2003; Bennett et al., 2011; Borisjuk and Rolletschek, 2009). Although soybean seeds are predominantly sink tissues, they also contain the structures and enzymes of typical photosynthetic machinery (Ruuska et al., 2004). Under field conditions, soybean pods are shaded making their contribution to the carbon economy low as compared to leaves (Allen et al., 2009; Quebedeaux and Chollet, 1975; Sambo et al., 1977; Sugimoto et al., 1987). However, a low intensity of light reaching the seed surface has been proposed to be significant for the regulation of biomass accumulation and oil synthesis by providing energy and cofactors (e.g. ATP, reducing power, oxygen; Allen et al., 2009; Bennett et al., 2011; Rolletschek et al., 2005).

With high incident solar radiation, light reaching the seeds could activate the first steps of oil synthesis and desaturation processes. *In vitro* experiments with green oilseeds showed that increasing the light intensity resulted in a higher oil content and a change in the fatty acid composition, leading to lower concentrations of oleic acid and higher concentrations of polyunsaturated fatty acids (Allen et al., 2009; Asokanthan et al., 1997; King et al., 1998; Rolletschek et al., 2005; Ruuska et al., 2004; Vigeolas et al., 2003; Willms et al., 1999). However, a different response was observed *in planta:* the percentage of oleic acid increased while the percentage of polyunsaturated fatty acids decreased with higher solar radiation (Izquierdo et al., 2009; Zuil et al., 2012).

The data available so far suggest that the weight and composition of soybean seeds are the result of a complex process influenced by: (i) effects of solar radiation incident on vegetative organs (leaves) that promote assimilate supply to the seeds and (ii) effects of solar radiation on reproductive structures (pods and seeds) probably regulating the seed metabolism locally. Thus, the objective of this work was to investigate the response of the weight and composition of soybean seeds to changes in assimilate supply from the leaves, the solar radiation reaching the pods and the combination of both.

2. Materials and methods

Two field experiments were conducted at the Instituto Nacional de Tecnología Agropecuaria (INTA) Balcarce Experimental Station, Argentina (37°45′S, 58°18′W) during the 2013–2014 (E1) and 2014–2015 (E2) growing seasons. The cultivar used was SPS 3900 (MG III, indeterminate, oil concentration 235 g kg^{-1}, Syngenta Agro S.A.). The soil was a Typic Argiudoll (USDA taxonomy) with a 5.2% organic matter content. Soil analysis indicated that fertilization was not necessary as nutrient availability was adequate to obtain maximum yield of soybean crops under non limiting water conditions (Andrade et al., 2000). Soybean seeds were inoculated. Rainfall was complemented with irrigations to avoid water deficit. Pests and diseases were adequately controlled. Sowing dates were 11/20/2013 in E1 and 11/28/2014 in E2. Each experiment was designed as randomized complete blocks with three replications. Plots were five rows wide and 6 m long with 0.40 m row distance. Mean plant density was 25 pl m^{-2}. Phenology was recorded according to Fehr and Caviness (1977).

Treatments were applied during the seed filling period (R5.5 to R8; Fehr and Caviness, 1977). They were aimed at modifying: (i) the amount of assimilates supplied by vegetative structures—total plant shading (TS) and total defoliation (TD); (ii) the amount of solar radiation reaching the reproductive structures—pod shading (PS); and (iii) both assimilates from leaves and light on pods—total defoliation and pod shading (TD × PS). In treatment TS, a black, neutral, synthetic mesh cloth was used in order to reduce incident solar radiation by 80%. Treatment TD consisted of leaf removal from all nodes of the canopy. Leaves were removed from branch nodes by pulling leaflets from their respective petioles by hand; petioles were not removed. In the PS treatment, pods at the R5.5 stage (Fehr and Caviness, 1977) were shaded by an opaque plastic hood placed in each node. Total defoliation and pod shading treatments were combined in treatment TD × PS. Untreated plants served as control (C).

2.1. Measurements

The cumulative intercepted solar radiation per plant (ISR) was calculated as the sum of daily intercepted solar radiation values per plant from R5.5 to R8 according to Izquierdo et al. (2009). For this, global daily incident radiation was measured with a pyranometer (LI-200SB; LI-COR, Lincoln, NE) located 800 m away from the experiments. The proportion of photosynthetic active radiation (PAR) intercepted by the canopy at noon (±1 h) was determined every week using a line quantum sensor (LI-191SB, LI-COR, Lincoln, NE). The daily proportion of PAR intercepted between two measurements was calculated by linear interpolation. Daily incident PAR was calculated as 0.48 x global daily incident radiation. Daily intercepted PAR was calculated as the product of daily incident PAR and daily proportion of PAR intercepted.

In experiment E2, incident solar radiation and red/far red (R/FR) ratio on pods were measured at noon (±1 h) on a sunny day once the treatments were applied. For this, a LI-190R quantum sensor (LI-COR, Lincoln, NE) and a 660/730 SKR 110 sensor (SKYE Instruments Ltd., Liandrindod Wells, Powys, Wales, UK) were positioned horizontally close to pods at the mid-height of the plant canopy (canopy height/2). Data were registered with a LI-1000 data-logger (LI-COR, Lincoln, NE).

Air temperature was measured with thermistors placed close to the pods at mid-height of the plant canopy and protected by

homemade shields to prevent absorption of solar and long wave radiation. Seed temperature in each treatment was measured with a 0.7 mm copper-constantan thermocouple inserted 3–4 mm into a pod located at the midpoint of the plant canopy. Data were taken every 60 s, averaged hourly and recorded with a data-logger (Cavadevices, Buenos Aires, Argentina).

2.2. Sample processing and chemical analysis

Determination of seed weight and chemical analysis were performed in samples taken from the mid-section of the canopy. At physiological maturity, when plants reached the R8 stage (95% of pods showed mature pod color), plants from 2 m^2 (in E1) and 1 m^2 (in E2) of the center rows of each plot were harvested. Plant samples were allowed to dry at 60 °C for 1 week. Nonempty seeds were counted and weighted. Seed weight (SW) was obtained as the ratio between sample weight and seed number.

To determine the plant seed number, the seeds of whole plants harvested at physiological maturity (5 plants in E1 and 10 plants in E2) were counted. In the PS and TD × PS treatments of experiment E1 there were not enough plants for this determination.

The oil content was quantified using heptadecanoic acid (17:0) as internal standard as in Echarte et al. (2013). Oil concentration was expressed as percentage of total seed weight. Oil fatty acid composition (FA) was determined by gas chromatography (CGL, Varian 3400) following the technique proposed by Sukhija and Palmquist (1988). Each fatty acid was expressed as percentage of the total fatty acids identified in the oil. Stearic, palmitic, oleic, linoleic and linolenic acids were identified in all the samples.

Nitrogen in the seeds was determined with a TruSpec CN equipment (Leco Corporation, St. Joseph, MI) and the ash content was measured according to AOAC recommendations (AOAC International, 1990). Seed protein content was estimated as nitrogen content × 6.25. The content of carbohydrates was estimated as SW − (oil + protein + ash). Assimilates available for seed biomass production were estimated as carbohydrate equivalents (CE). CE represents the amount of glucose required for the synthesis of one seed, and was calculated as:

$$CE(mg\,seed^{-1}) = PVI(mg\,g^{-1}) \times SW(mg\,seed^{-1})/1000(mg\,g^{-1}).$$

where PVI is the inverse of the production value; it represents the amount of glucose required for the biosynthesis of 1 g of seeds, and considers the costs of synthesis for the different components of the mature seed (Vertregt and Penning de Vries, 1987). PVI was calculated as:

$$PVI(mg\,g^{-1}) = 1.211 \times carbohydrates(mg\,g^{-1}) + 1.793 \times protein(mg\,g^{-1}) + 3.030 \times lipids(mg\,g^{-1}) + 0.906 \times minerals(mg\,g^{-1}).$$

The lignin content was considered negligible and thus it was not included in the PVI.

2.3. Data analysis

The effects of treatments were analyzed by ANOVA using R software (R Core Team, 2014). Mean values, standard deviation and least significant differences were calculated using the Least Squares Fit model. Differences among treatments were evaluated using the studentized range Tukey method ($p < 0.05$). Equation fitting was done by linear simple regression using Sigmaplot software (Sigmaplot 8.0, SPSS Inc., Chicago, IL). The slopes and Y-intercepts of oil and protein content as a function of CE were compared with a dummy variable regression model.

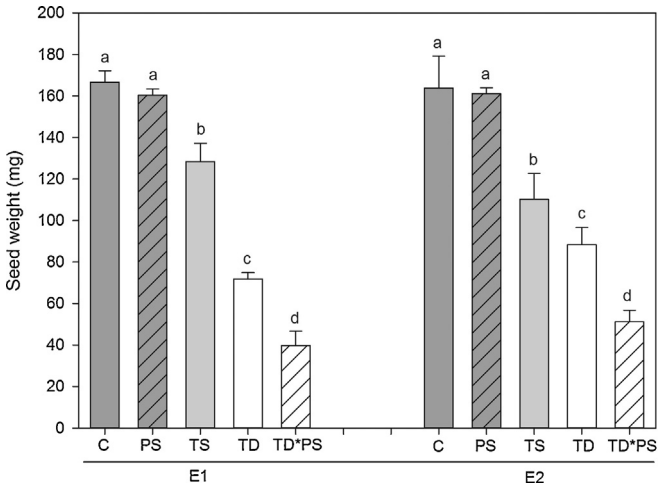

Fig. 1. Grain weight for different treatments in E1 and E2. Values are mean ± standard deviation. Bars with different letters within each experiment are significantly different ($p < 0.05$). C, control; PS, pod shading; TS, plant shading; TD, total defoliation; TD × PS, total defoliation + pod shading.

3. Results

Mean values of daily air temperature and daily incident photosynthetic active radiation during seed filling were lower in E1 than in E2 (17.3 °C and 4.5 MJ $m^{-2}nd^{-1}$ vs 20.1 °C and 7.7 MJ m^{-2} d^{-1}, respectively). Micrometeorological conditions and seed number for the different treatments are shown in Table 1. The seed number for any treatment was also lower in E1 than in E2. Mean seed temperature was similar among treatments, except for total shading in E2. Treatments effectively changed the amount of incident solar radiation reaching the pods. In particular, shading the pods of intact plants (PS) reduced the incident solar radiation without causing significant changes in seed temperature, intercepted solar radiation accumulated per plant (ISR) or seed number. Note that, as defined above, ISR represents the cumulative solar radiation intercepted by the whole plant during seed filling and not the solar radiation actually incident on the pods. Incident solar radiation reaching the pods at noon (IR) was significantly reduced when the whole canopy or just the pods were shaded, while it increased up to values similar to solar radiation incident on the top of the canopy of the C treatment (1403 ± 40.9 μmol s^{-1} m^{-2}) when defoliation was performed. The R/FR ratio incident on the pods was modified by treatments in the same fashion as the IR. Solar radiation intercepted by defoliated plants (TD) gave rise to ISR values between those of control and shaded plants. In this treatment, most of the solar radiation was intercepted by stems, petioles and pods, as leaf unfolding after treatment application was negligible. The seed number was significantly reduced by total plant shading and defoliation, being most affected by the combination of pod shading and defoliation. Pods in plants under TS and TD treatments received different IR intensities with contrasting R/FR. However, the effects of the two treatments on seed number and most of the traits reported here were similar.

3.1. Response of seed weight and composition to assimilates availability and solar radiation on pods

Mean seed weight across treatments was similar in the two experiments (113.4 mg $seed^{-1}$ vs. 115.0 mg $seed^{-1}$ in E1 vs. E2, respectively). The response of seed weight (SW) to treatments

Table 1
Micrometeorological measurements and seed number per plant for different treatments.

Treatment	MST (°C)		IR(μmol s^{-1} m^{-2})		R/FR		ISR (MJ plant^{-1})		SN (seed plant^{-1})	
	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
Control	16.0	20.0	–	15.0	–	0.13	7.6 ± 0.1	14.1 ± 0.3	70 ± 13	113 ± 15
Pod shading	16.5	20.3	–	0.3	–	0.06	7.6 ± 0.2	14.0 ± 0.3	–	107 ± 2
Total shading	16.0	18.0	–	1.5	–	0.06	1.4 ± 0.1	2.1 ± 0.1	31 ± 3	45 ± 10
Total defoliation	16.1	19.9	–	1431.7	–	1.38	5.6 ± 0.7	9.2 ± 0.4	24 ± 7	56 ± 8
Total defoliation × Pod shading	15.9	20.7	–	0.4	–	0.06	6.0 ± 0.2	9.2 ± 0.4	–	43 ± 8

MST: Mean seed temperature. IR: incident solar radiation. R/FR: red/far red ratio (R/FR). Sensors for MST, IR and R/FR measurements were placed by the pods. ISR: intercepted solar radiation per plant accumulated during grain filling. SN: seed number per plant.

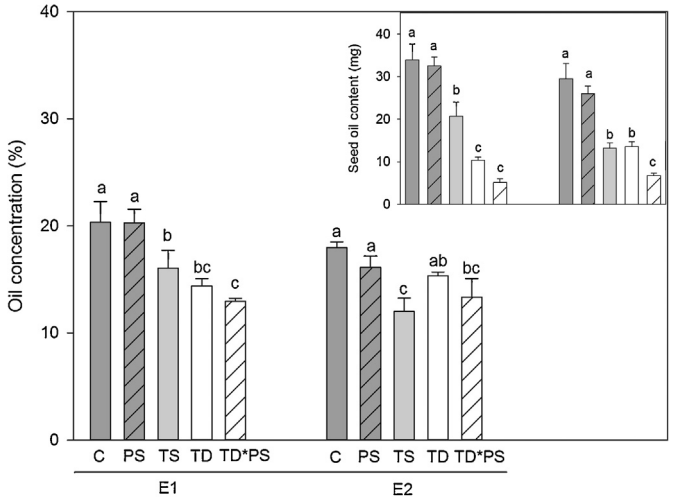

Fig. 2. Grain oil content for different treatments in E1 and E2 experiments. Values are mean ± standard deviation. Bars with different letters within each experiment are significantly different ($p < 0.05$). C, control; PS, pod shading; TS, total plant shading; TD, total defoliation; TD × PS, total defoliation + pod shading.

showed the same pattern in E1 and E2 (Fig. 1), despite different environmental conditions during the filling period (i.e. temperature and solar radiation, see above). Shading and defoliation treatments reduced SW compared to control plants. Reduction of leaf area by defoliation produced lower SW than shading treatments. Pod shading of intact plants (PS) did not modify SW. Interestingly, in the two experiments, when pods were shaded on defoliated plants (TD × PS), the observed reduction in SW was maximal in both experiments.

The effects of treatments on oil content (mg of oil per seed) and concentration (oil percentage) are presented in Fig. 2. Plant shading (TS) reduced the oil concentration in the two experiments, while total defoliation (TD) only affected it significantly in E1. Shading the pods, whether it was done in intact or defoliated plants, did not have a significant impact on oil concentration (compare PS to C or TD × PS to TD). As to the oil content, treatment TD significantly reduced it in the two experiments (inset to Fig. 2). In E2, this was explained by a strong effect of TD on seed weight. In the two experiments, when pods of defoliated plants were shaded, the lowest oil contents were obtained (around 20% of control values) due to both lower seed weight and oil concentration.

Protein weight per seed decreased with defoliation, whether the pods were shaded or not – TD and TD × PS – while no significant effects were detected when the plants or the pods were shaded – TS and PS – (data not shown). The sum of protein and oil percentages (P + O) was 58.0 ± 1.6% for all the treatments, except for TD*PS, where it decreased to 52.0 ± 0.5%. This drop was due to lower percentages of both oil and protein. Since the percentage of ash was not significantly modified by the treatments (5.4 ± 0.6%), the lower P + O found in treatment TD × PS was attributed to a higher percentage of carbohydrates.

The concentration of each individual fatty acid was expressed as percentage of total fatty acids in the oil. Saturated fatty acids responses to treatments are shown in Fig. 3. The response of palmitic acid depended on the experiment: in E1 the concentration of this acid increased as result of total shading and defoliation while in E2 the concentration did not change (total shading) or decreased (defoliation) with respect to control plants. In the two experiments the concentration of stearic acid significantly increased with respect to the control when the pods were shaded (PS).

The responses of unsaturated fatty acids to treatment are shown in Fig. 4. The concentrations of oleic acid varied among treatments between 13.8 and 22.6%. Plant shading (TS) and defoliation (TD) reduced the concentration of oleic acid in the two experiments. This fatty acid was most sensitive to the TD and TD × PS treatments, where it decreased six percentage points in average. Pod shading (PS) did not affect the concentration of oleic acid. Polyunsaturated fatty acids (i.e., linoleic + linolenic) ranged from 59.2 to 68.6%. TS and TD treatments increased their concentrations compared to control plants. Upon defoliation, higher concentrations of both linoleic and linolenic acids were observed, whereas plant shading mostly caused an increase in the concentration of linolenic acid. Pod shading reduced the concentration of polyunsaturated fatty acids, mainly by reducing the concentration of linolenic acid. Interestingly, this effect was found only when pod shading was applied on intact plants; in the TD × PS treatment, the prevailing effect was similar to that produced by defoliation (i.e., an increase of polyunsaturated fatty acids).

3.2. *Response of seed weight and composition to assimilates allocated to the seeds*

Seed weight and composition were analyzed as a function of assimilates allocated to the seeds, expressed as carbohydrate equivalents (CE), as reported by Echarte et al. (2012) (see Section 2). Seed weight linearly increased with assimilate availability indicating the universe of treatments here applied had a major impact on seed weight and a comparatively much less significant effect on the seed composition (Fig. 5A). Conversely, oil weight per seed depended on CE in a biphasic way: when assimilate availability was reduced by defoliation, both the slope ($p = 0.0023$) and ordinate ($p = 0.0001$) of the curve changed with respect to non-defoliated plants (Fig. 5B). When leaves were present (intact plants), the amount of oil synthesized per unit of CE was almost twice the one obtained in defoliated plants (compare slopes in Fig. 5B). The response of protein weight per seed to CE was also described by two different lines according to the treatments, depending on whether the plants were defoliated or not (Fig. 5B). Differences in slope ($p = 0.0003$) and ordinate ($p = 0.0001$) were significant. At the low assimilate availability conditions produced by defoliation, proteins accumulated rather than oil with increasing CE (0.26 mg protein/mg CE vs. 0.10 mg oil/mg CE, $p = 3.60e\text{-}15$). As assimilate availability increased in intact plants, oil

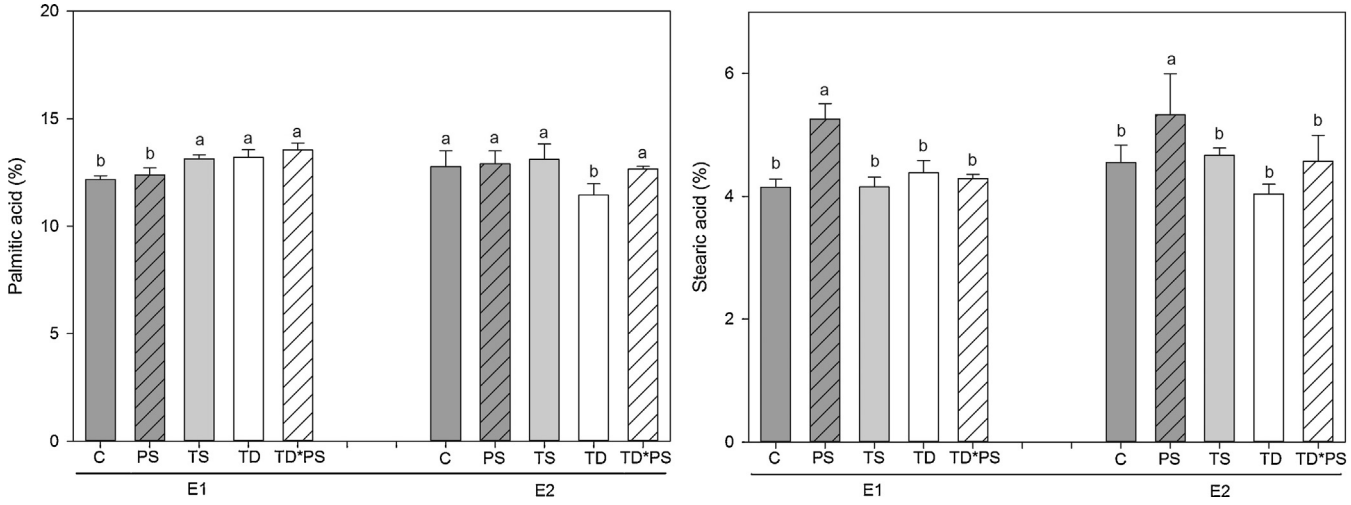

Fig. 3. Saturated fatty acids of soybean oil for different treatments in E1 and E2 experiments. Left panel: palmitic acid (%); right panel: stearic acid (%). Values are mean ± standard deviation. Bars with different letters within each experiment are significantly different ($p < 0.05$). C, control; PS, pod shading; TS, plant shading; TD, total defoliation; TD × PS, total defoliation + pod shading.

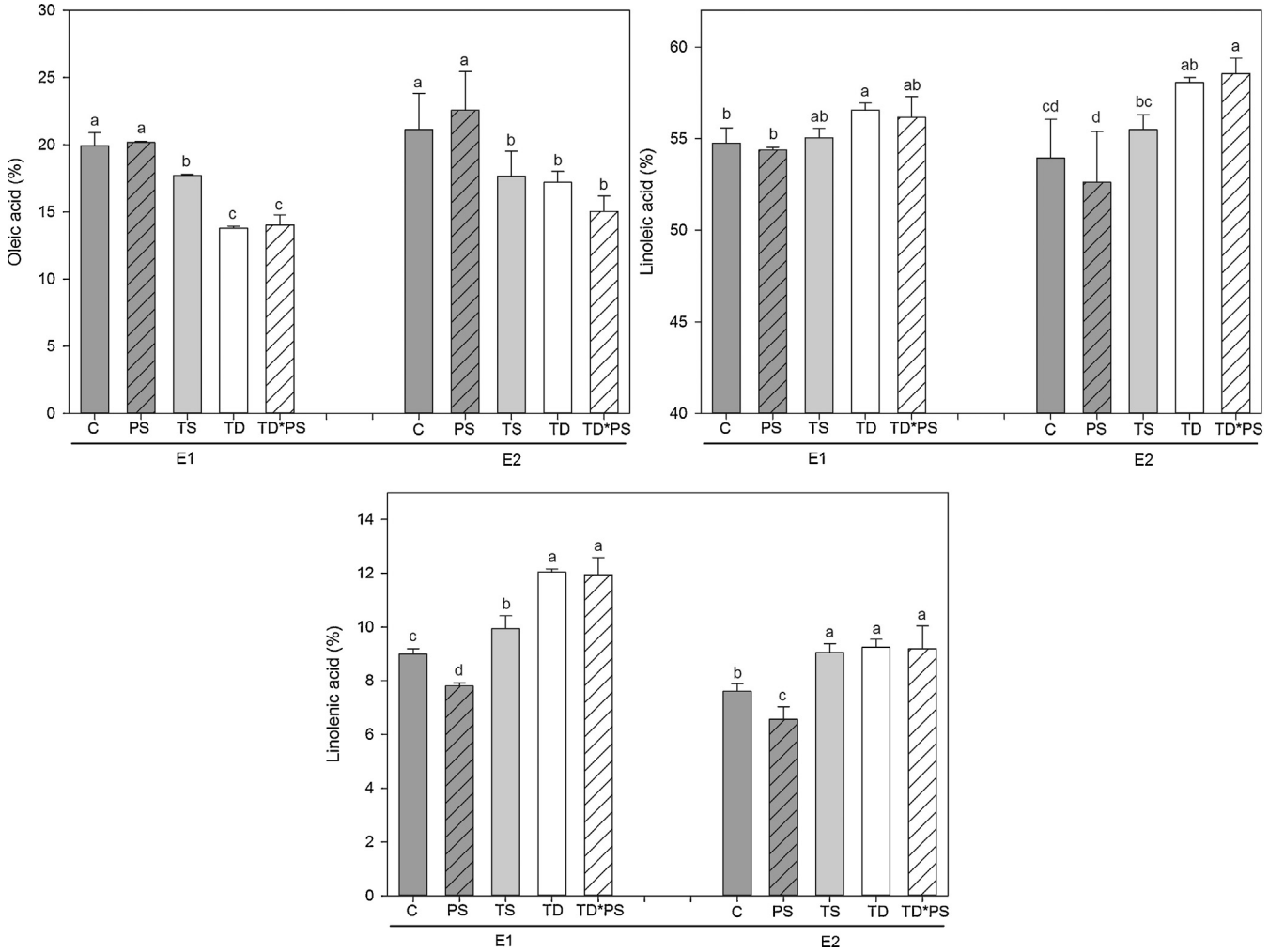

Fig. 4. Unsaturated fatty acids of soybean oil for different treatments in E1 and E2 experiments. Values are mean ± standard deviation. Bars with different letters within each experiment are significantly different ($p < 0.05$). C, control; PS, pod shading; TS, plant shading; TD, total defoliation; TD × PS, total defoliation + pod shading.

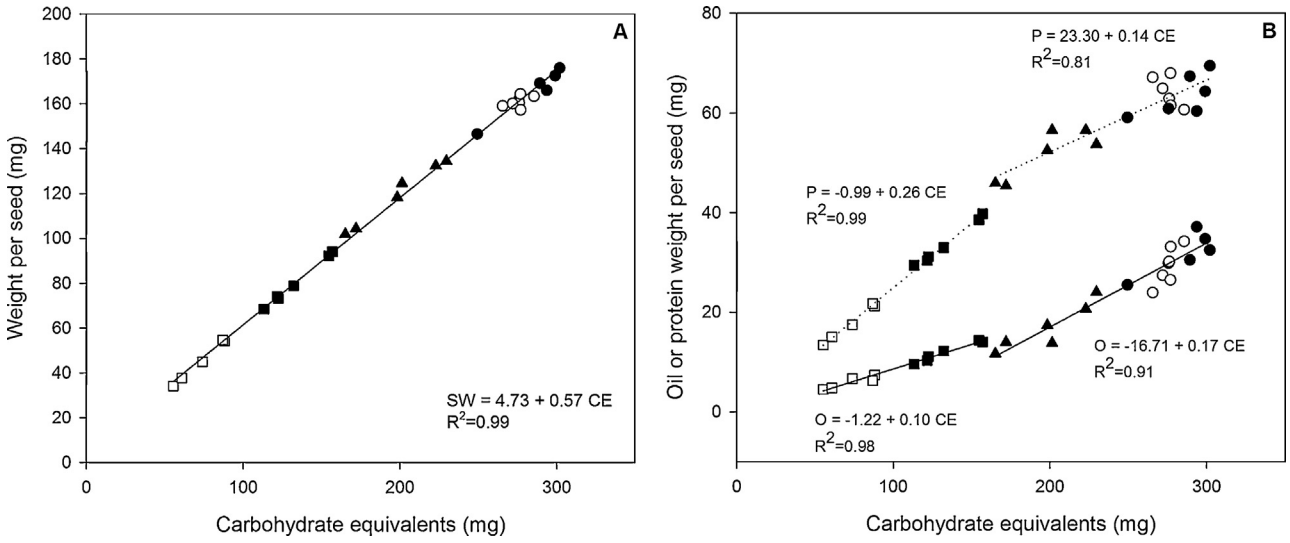

Fig. 5. Grain weight and composition dependence on carbohydrates equivalents. Weight per grain (A), and oil (continue line) and protein (dashed line) weight per grain (B) are plotted as a function of carbohydrate equivalents. Control: closed circles; pod shading: open circles; plant shading: triangles; total defoliation: closed squares; total defoliation + pod shading: open squares. Continuous lines represent the fitting of equations in the corresponding inserts to experimental data. GW, grain weight; O oil weight per grain; P protein weight per grain; CE, carbohydrate equivalent.

and protein increased with CE at similar rates (0.17 mg oil/mg CE vs. 0.14 mg protein/mg CE, $p = 0.2819$). Since in intact plants the ordinates for both oil and protein content were different ($p = 5.36e-08$) and the slopes were similar, the higher the CE, the higher the oil percentage (and the lower the protein percentage).

For the sake of clarity, fatty acids were analyzed as a function of carbohydrate equivalents in terms of fatty acid percentage. Fig. 6 shows that: (i) the concentrations of palmitic and stearic acid did not respond to CE (Fig. 6A and B); (ii) the concentration of oleic acid linearly increased with CE and was negatively correlated to the polyunsaturated fatty acids (Fig. 6C), (iii) both linoleic and linolenic acids responded to CE with similar slopes (1.5×10^{-2} and 1.4×10^{-2}%/mg, respectively; Fig. 6D). Linear fitting was performed excluding data from the treatments where the pods were shaded (PS and TD × PS). In the case of stearic and linolenic acid, data corresponding to pod shading treatment (PS) deviated from the general behavior at high CE values.

4. Discussion

In this research we studied how the final weight and composition of soybean seeds respond to the availability of assimilates, the incident solar radiation reaching the reproductive structures (pods and seeds) and their combination. Assimilate availability for the seeds was manipulated through plant shading and defoliation treatments while incident solar radiation reaching the pods was modified by locally shading them. The treatments affected the quality of solar radiation incident on pods and leaves in terms of the R/FR ratio. The differences in light quality were not related to changes in any trait here analyzed. Results found in this work make it unlikely that changes in seed weight and composition were mediated by the quality of incident solar radiation. Although the quality of incident solar radiation has been shown to affect internode elongation, branching and biomass allocation (Ballaré et al., 1990; Kasperbauer, 1987), no significant effects on seed weight and composition have been reported to our knowledge. By shading and/or illuminating individual soybean nodes, Heindl and Brun (1983) found that seed number but not seed weight depended on the intensity of light reaching the pods. In agreement with our results, these authors found that the photomorphogenic response of pods was related to light quantity but not to light quality.

Seed weight (SW) was modified by plant shading or defoliation in agreement with data available in the literature (Andrade and Ferreiro, 1996; Borrás et al., 2004; Board et al., 2010; Proulx and Naeve, 2009). Pod shading of intact plants did not affect the number or the weight of the seeds, despite the low radiation values reaching the pods (Table 1). Consistent with this, Allen et al. (2009) found that biomass accumulated by excised seeds did not increase under high light (similar to *in planta* light intensities in a canopy) compared to moderate light intensity, suggesting embryos adaptation to shading. Interestingly, of all the treatments applied in this study, shading the pods of defoliated plants produced the lowest SW (Fig. 1). *In vitro* experiments have shown that the main role of whole pods, seeds and cotyledons photosynthesis, is the re-assimilation of their respiratory CO_2 without a significant net photosynthetic capacity (Quebedeaux and Chollet, 1975; Sambo et al., 1977; Sugimoto et al., 1987). However, the results of this work suggest that carbon derived from pod photosynthesis could substantially contribute to the seed carbon economy when assimilates from the leaves are scarce or null. It is important to note that in defoliated plants, radiation reaching the pods was higher than in control plants (Table 1) and thus it is possible that the role of reproductive organs in C assimilation is enhanced in this situation (square symbols in Fig. 5A). This contribution could be important in field conditions when leaves are lost during the seed filling period as a consequence of several biotic and/or abiotic stresses (e.g. insect leaf feeders, diseases, hail, etc.). To the best of our knowledge, this research is the first one to show the contribution of soybean pods *in planta* to the seed carbon economy.

In the present work, a reduction in assimilate supply from the leaves during the seed filling period, reduced the oil concentration (Fig. 2). While plant shading clearly affected this trait, the effect of defoliation depended on the experiment. In agreement with this, Proulx and Naeve (2009) observed that the oil concentration was less affected by defoliation than by plant shading. In this study different effects on oil concentration were observed when the assimilate supply was modified by either shading or defoliation. However, the oil content showed the same response to both treatments, mainly driven by changes in seed weight.

In vitro experiments have highlighted the role of incident light on photosynthetic embryos in their oil synthesis. In this sense, light increased oil synthesis in soybean and rape through the provision of

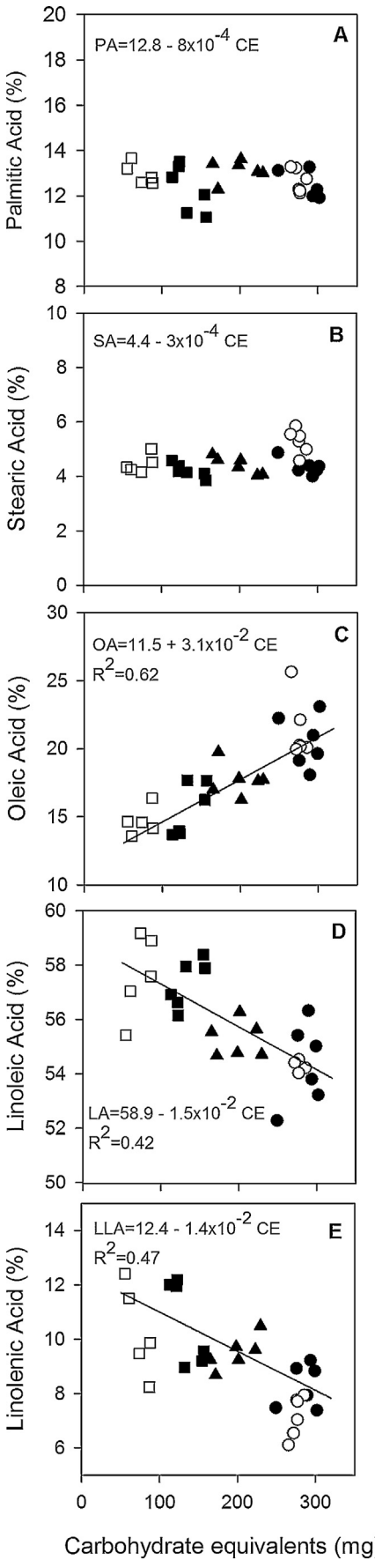

Fig. 6. Fatty acids concentration dependence on carbohydrates equivalents. (A) palmitic acid, (B) stearic acid, (C) oleic acid, (D) linoleic acid and (E) linolenic acid per grain are plotted as a function of carbohydrate equivalents. Control: closed circles; pod shading: open circles; plant shading: triangles; total defoliation: closed squares; total defoliation + pod shading: open squares. Continuous lines represent the fitting of equations in the corresponding inserts to experimental data. PA, palmitic acid per grain; SA, stearic acid per grain; OA, oleic acid per grain; LA, linoleic acid per grain; LLA, linolenic acid per grain; CE, carbohydrate equivalent.

ATP, reducing power and photosynthetic oxygen (Allen et al., 2009; Asokanthan et al., 1997; Rolletschek et al., 2005; Ruuska et al., 2004; Vigeolas et al., 2003). In this research, shading the pods of a whole plant within a canopy did not significantly affect the oil concentration although a decreasing trend with shading could be observed (Fig. 2). This suggests that the assimilates contributed by the mother plant to seed filling mainly control the oil concentration, while the direct effect of light on pods can be disregarded. Shading the pods of defoliated plants (TD × PS) yielded the lowest oil content in the seeds (inset to Fig. 2). Like the results found in this work for SW, the carbon contribution of pods for oil synthesis was significant when assimilates coming from the leaves were low and pods were fully illuminated. It is well known that the photosynthetic activity of leaves is regulated by the sink activity (Gifford and Evans, 1981). A question that remains is whether pod photosynthesis, in turn, is affected by leaf removal.

The metabolism of carbon and nitrogen are intricately related and any effect on the availability of one of them is bound to have an influence on the other (Paul and Foyer, 2001). Proulx and Naeve (2009) reported that the changes in the main seed components produced by defoliating soybean plants were different than those produced by shading, presumably due to changes in the N metabolism. In the present work, both oil and protein responses to the assimilates available for the seeds were different in plants where leaves had been removed compared to intact plants (Fig. 5B). In intact plants, both the oil and protein content increased with assimilate availability with similar slopes but different ordinates. Consequently, the oil percentage increased with CE (and the percentage of protein decreased). In agreement with this, ^{13}C labeling experiments showed that C assimilated during effective seed filling was partitioned preferentially to lipids rather than protein (Yamagata et al., 1987). By contrast, when the leaves were removed, the oil content increased with CE with a lower slope than in intact plants. Similarly, protein weight per seed in defoliated plants increased with CE with a higher slope than in intact plants. One possible explanation is that in the absence of leaves, N can still be provided by other organs (stems or petioles) or contemporarily absorbed from the soil, while photosynthesis form vegetative organs is mostly hindered. In addition, in pea and vetch, N uptake by the pods has been shown to be maximal during the early stages of grain filling (see references in Weber et al., 2005). It is possible that most of the N metabolized by the seeds during the filling period had already been allocated to the pods by the time the treatments were applied. However, since the lowest values of CE (lower than 170 mg CE) were achieved by defoliating plants while intact plants rendered the highest ones, it is difficult to assess whether this effect was due to a different range of CE values or due to the presence or absence of leaves. Further research on N remobilization dynamics is necessary to clarify this point.

Previous works have shown that the saturated fatty acids of soybean oil did not respond to radiation treatments (Izquierdo et al., 2009; Zuil et al., 2012). Additionally, the palmitic/stearic acid ratio has been shown to be stable across species and environments (Harwood et al., 2013). In the present work, saturated fatty acids did not change with treatments or in response to CE (Figs. 3, 6 A and B) except when pods were shaded in intact plants. In this case, the percentage of stearic acid deviated from the fitted equation (open circles in Fig. 6B), changing the palmitic/stearic acid ratio. Changes in pool sizes of intermediates in a pathway provide *in situ* evidence for points of metabolic regulation (Post-Beittenmiller et al., 1992).

Similar palmitic acid concentration with higher accumulation of stearic acid in shaded pods suggest that the stearic desaturation process, where stearic acid desaturase enzyme (SAD) is involved, is regulated by incident light reaching the pods. It is possible that cofactors produced by local photosynthesis (e.g. oxygen, ATP and reducing equivalents) affect SAD activity as has been previously shown for oleate desaturase in *in vitro* experiments (Rolletschek et al., 2005).

Shading and defoliation treatments reduced the percentage of oleic acid and increased polyunsaturated fatty acids whether pods were shaded or not (Fig. 4). The differences in fatty acid composition between these treatments could not be explained based only on the radiation intercepted by the plants. We observed that the defoliation treatment, which intercepted higher amounts of radiation per plant (and also per seed) than plant shading (Table 1), led to similar or lower oleic acid levels. The lack of correlation between the percentage of oleic acid and ISR per seed agree with the results reported by Izquierdo et al. (2009). When individual data were plotted against CE, it was observed that oleic acid increased (Fig. 6C) while polyunsaturated fatty acids decreased with increasing CE (Fig. 6D and E). According to these results and in agreement with a conceptual model developed for sunflower, the desaturation process (driven by the ODS enzyme) that turns oleic acid into polyunsaturated fatty acids would get saturated at higher CE availability (Echarte et al., 2012).

Shading the pods of intact plants significantly reduced the percentage of linolenic acid (Fig. 4), the most oxidized species of polyunsaturated fatty acids, in spite of minor changes in carbohydrate allocation. This effect deviates from the general data behavior when the relationship between linolenic acid and CE is analyzed (open circles in Fig. 6E). The negative correlation between stearic and linolenic acid found when assimilates produced by the leaves were sufficient and pods were shaded, evidenced local effects of light on the stearate desaturation step, as mentioned above. Therefore, when assimilate availability is non-limiting, this variable is not enough to explain the composition of fatty acids in soybean, but rather mixed effects of incident solar radiation on the leaves and pods must be considered. The effects of solar radiation on soybean seed weight and composition differed from those reported for sunflower (Echarte et al., 2012). As the oil synthesis progresses, sunflower grains remain covered by a thick envelope that prevents any direct effects of light on the synthesis of fatty acids and oil assembly. In contrast, in green oilseeds like soybean, light plays a role in the regulation of these processes.

Based on the model proposed by Echarte et al. (2012) and the results presented in this paper, we propose the following conceptual model for soybean seed filling: seed weight and oil content increase as more assimilates are allocated to the seeds. These assimilates are usually provided by the leaves but, in their absence, they can be provided by the pods. The fatty acid composition is also dependent on the assimilates available for the seeds. When plenty of assimilates are available, oleic acid is accumulated, presumably because the oleic desaturation machinery is not able to process all the substrate reaching that step. The direct effects of light on the pods depend on the amount of carbohydrates available: i) when assimilates allocated to the seeds are low (defoliation treatments), pods contribute to the seed carbon economy but do not affect the fatty acid composition; ii) when assimilates allocated to the seeds are high (intact plants), the contribution of the pods to the seed carbon economy is not significant, but local effects of light on the pods produce significant changes in the fatty acid composition: in the absence of light, the stearic desaturation process is slowed down, and stearic acid accumulates with a concomitant decrease of linolenic acid. Further validation of the conceptual model proposed above can be achieved assessing whether the same behavior reported herein for soybean is also observed in other species carrying photo-heterotrophic reproductive organs (e.g. rapeseed).

5. Conclusion

The final weight and composition of soybean seeds depend on the combination of the assimilates available to them and local effects of light reaching the pods. The local effects of light reaching the pods depend on the amount of assimilates available during the seed filling period. When assimilates are low, light promotes seed weight and oil synthesis. As the assimilate availability increase, light reaching the pods does not affect seed weight and oil content, but plays a role in regulating the fatty acid composition.

The results reported in this paper will contribute to unraveling the mechanism underlying the effect of solar radiation on the weight and composition of green seeds or grains of different oilseed species.

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References

Aguirrezábal, L.A.N., Matre, P., Pereyra Irujo, G., Izquierdo, N.G., Echarte, M.M., Allard, V., 2014. Improving grain quality. Ecophysiological and modelling tools to develop management and breeding strategies. In: Sadras, V.O., Calderini, D. (Eds.), Crop Physiology: Applications for Genetic Improvement and Agronomy. , 2nd edition. London Academic Press, pp. 423–465.

Allen, D.K., Ohlrogge, J.B., Shachar-Hill, Y., 2009. The role of light in soybean seed filling metabolism. Plant J. 58 (2), 220–234.

Andrade, F.H., Ferreiro, M.A., 1996. Reproductive growth of maize: sunflower and soybean at different source levels during grain filling. Field Crops Res. 48, 155–165.

Andrade, F.H., Echeverría, H., González, N., Uhart, S., 2000. Requerimientos de nutrientes minerales. In: Andrade, F., Sadras, V. (Eds.), Bases Para el Manejo del Maíz, el Girasol y la Soja. , 443 pp.

Aschan, G., Pfanz, H., 2003. Non-foliar photosynthesis—a strategy of additional carbon acquisition. Flora 198, 81–97.

Asokanthan, P.S., Johnson, R.W., Griffith, M., Krol, M., 1997. The photosynthetic potential of canola embryos. Physiol. Plant. 101 (2), 353–360.

Ballaré, C.L., Scopel, A.L., Sánchez, R.A., 1990. Far red radiation reflected from adjacent leaves: an early signal of competition in plant canopies. Science 247 (4940), 329.

Bennett, E.J., Roberts, J.A., Wagstaff, C., 2011. The role of the pod in seed development: strategies for manipulating yield. New Phytol. 190 (4), 838–853.

Board, J.E., Kumudini, S., Omielan, J., Prior, E., Kahlon, C.S., 2010. Yield response of soybean to partial and total defoliation during the seed-filling period. Crop Sci. 50 (2), 703–712.

Board, J.E., Kahlon, C.S., 2011. Soybean yield formation: what controls it and how it can be improved, soybean physiology and biochemistry. In: El-Shemy, Hany (Ed.), ISBN: 978-953-307-534-1, InTech. INTECH Open Access Publisher, Available from: http://www.intechopen.com/books/soybean-physiology-and-biochemistry/soybean-yield-formation-what-controls-it-and-how-it-can-be-improved.

Borisjuk, L., Rolletschek, H., 2009. The oxygen status of the developing seed. New Phytol. 182 (1), 17–30.

Borrás, L., Slafer, G.A., Otegui, M.E., 2004. Seed dry weight response to source–sink manipulations in wheat, maize and soybean: a quantitative reappraisal. Field Crops Res. 86 (2–3), 131–146.

Echarte, M.M., Angeloni, P., Jaimes, F., Tognetti, J., Izquierdo, N.G., Valentinuz, O., Aguirrezábal, L.A.N., 2010. Night temperature and intercepted solar radiation additively contribute to oleic acid percentage in sunflower oil. Field Crops Res. 119 (1), 27–35.

Echarte, M.M., Alberdi, I., Aguirrezábal, L.A.N., 2012. Post-flowering assimilate availability regulates oil fatty acid composition in sunflower grains. Crop Sci. 52 (2), 818–829.
Echarte, M.M., Puntel, L.A., Aguirrezábal, L.A.N., 2013. Assessment of the critical period for the effect of intercepted solar radiation on sunflower oil fatty acid composition. Field Crop Res. 149, 213–222.
Fehr, W.R., Caviness, C.E., 1977. Stages of soybean development. In: Spec. Rep. 80. Iowa State Univ. Agric. Exp. Stn., Ames.
Gallardo, K., Thompson, R., Burstin, J., 2008. Reserve accumulation in legume seeds. C. R. Biol. 331 (10), 755–762.
Gifford, R.M., Evans, L.T., 1981. Photosynthesis carbon partitioning, and yield. Annu. Rev. Plant Physiol. 32, 485–509.
Harwood, J.L., Ramli, U.S., Tang, M., Quant, P.A., Weselake, R.J., Fawcett, T., Guschina, I.A., 2013. Regulation and enhancement of lipid accumulation in oil crops: the use of metabolic control analysis for informed genetic manipulation. Eur. J. Lipid Sci. Technol. 115 (11), 1239–1246.
Heindl, J.C., Brun, W.A., 1983. Light and shade effects on abscission and ^{14}C-photoassimilate partitioning among reproductive structures in soybean. Plant Physiol. 73 (2), 434–439.
Hua, W., Li, R.J., Zhan, G.M., Liu, J., Li, J., Wang, X.F., Liu, G.H., Wang, H.Z., 2012. Maternal control of seed oil content in Brassica napus: the role of silique wall photosynthesis. Plant J. 69 (3), 432–444.
Izquierdo, N.G., Dosio, G.A.A., Cantarero, M., Luján, J., Aguirrezábal, L.A.N., 2008. Weight per grain, oil concentration, and solar radiation intercepted during grain filling in black hull and striped hull sunflower hybrids. Crop Sci. 48 (2), 688–699.
Izquierdo, N.G., Aguirrezábal, L.A.N., Andrade, F.H., Geroudet, C., Valentinuz, O., Pereyra Iraola, M., 2009. Intercepted solar radiation affects oil fatty acid composition in crop species. Field Crops Res. 114 (1), 66–74.
Kane, M.V., Steele, C.C., Grabau, L.J., Mackown, C.T., Hildebrand, D.F., 1997. Early-maturing soybean cropping system: III: protein and oil contents and oil composition. Agron. J. 89, 464–469.
Kasperbauer, M.J., 1987. Far-red light reflection from green leaves and effects on phytochrome-mediated assimilate partitioning under field conditions. Plant Physiol. 85 (2), 350–354.
King, S.P., Badger, M.R., Furbank, R.T., 1998. CO_2 refixation characteristics of developing canola seeds and silique wall. Aust. J. Plant Physiol. 25 (3), 377.
Paul, M.J., Foyer, C.H., 2001. Sink regulation of photosynthesis. J. Exp. B 52, 1383–1400.
Post-Beittenmiller, D., Roughan, G., Ohlrogge, J.B., 1992. Regulation of plant fatty acid synthesis: analysis of acyl-coenzyme A and acyl-acyl carrier protein substrate pools in spinach and pea chloroplasts. Plant Physiol. 100, 923–930.
Proulx, R.A., Naeve, S.L., 2009. Pod removal, shade, and defoliation effects on soybean yield, protein, and oil. Agron. J. 101 (4), 971.
Quebedeaux, B., Chollet, R., 1975. Growth and development of soybean (*Glycine max* [L] Merr:) pods–CO_2 exchange and enzyme studies. Plant Physiol. 55, 745–748.
Rolletschek, H., Radchuk, R., Klukas, C., Schreiber, F., Wobus, U., Borisjuk, L., 2005. Evidence of a key role for photosynthetic oxygen release in oil storage in developing soybean seeds. New Phytol. 167 (3), 777–786.
Rotundo, J.L., Westgate, M.E., 2009. Meta-analysis of environmental effects on soybean seed composition. Field Crops Res. 110 (2), 147–156.
Rotundo, J.L., Borrás, L., Westgate, M.E., Orf, J.H., 2009. Relationship between assimilate supply per seed during seed filling and soybean seed composition. Field Crops Res. 112 (1), 90–96.
Rotundo, J.L., Borrás, L., Westgate, M.E., 2011. Linking assimilate supply and seed developmental processes that determine soybean seed composition. Eur. J. Agron. 35 (3), 184–191.
Ruuska, S.A., Schwender, J., Ohlrogge, J.B., 2004. The capacity of green oilseeds to utilize photosynthesis to drive biosynthetic processes. Plant Physiol. 136, 2700–2709.
Sambo, E.Y., Moorby, J., Milthorpe, F.L., 1977. Photosynthesis and respiration of developing soybean pods. Aust. J. Plant Physiol. 4, 713–721.
Sukhija, P.S., Palmquist, D.L., 1988. Rapid method for determination of total fatty acid content and composition of feedstuff s and feces. J. Agric. Food Chem. 36 (6), 1202–1206.
Sugimoto, T., Tanaka, K., Momma, M., Saio, K., 1987. Photosynthetic activity in the developing cotyledon of soybean seeds. Agric. Biol. Chem. 51 (5), 1227–1230.
Vertregt, N., Penning de Vries, F.W.T., 1987. A rapid method for determining the efficiency of biosynthesis of plant biomass. J. Theor. Biol. 128 (1), 109–119.
Vigeolas, H., van Dongen, J.T., Waldeck, P., Huhn, D., Geigenberger, P., 2003. Lipid storage metabolism is limited by the prevailing low oxygen concentrations within developing seeds of oilseed rape. Plant Physiol. 133 (4), 2048–2060.
Weber, H., Borisjuk, L., Wobus, U., 2005. Molecular physiology of legume seed development. Annu. Rev. Plant Biol. 56, 253–279.
Willms, J.R., Salon, C., Layzell, D.B., 1999. Evidence for light-stimulated fatty acid synthesis in soybean fruit1. Plant Physiol. 120 (4), 1117–1128.
Yamagata, M., Kouchi, H., Yoneyama, T., 1987. Partitioning and utilization of photosynthate produced at different growth stages after anthesis in soybean (*Glycine max* L. Merr.): analysis by long-term 13C-labeliing experiment. J. Exp. Bot. 38 (193), 1247–1259.
Zuil, S.G., Izquierdo, N.G., Luján, J., Cantarero, M., Aguirrezábal, L.A.N., 2012. Oil quality of maize and soybean genotypes with increased oleic acid percentage as affected by intercepted solar radiation and temperature. Field Crops Res. 127, 203–214.