RESEARCH

Post-Flowering Assimilate Availability Regulates Oil Fatty Acid Composition in Sunflower Grains

María M. Echarte, Ignacio Alberdi, and Luis A.N. Aguirrezábal*

ABSTRACT

Fatty acid composition of *Helianthus annuus* L. (sunflower) oil depends on intercepted solar radiation per plant (ISR) during grain filling. This effect could be accounted for by the assimilate availability of the grains (the source-sink ratio). However, the current physiological-biochemical knowledge does not consider any effect of carbon availability on oil fatty acid composition. The objective of this work was to address the regulation of fatty acid composition by assimilate supply to sunflower grains. A wide range of source-sink ratios was obtained by manipulating either the source or the sink during grain filling. Assimilate supply was also modified by injecting sucrose into the receptacle of sunflower capitula. Grain weight and oil content depended on both ISR and source-sink ratio in a curvilinear manner. When sink size was decreased by grain excision, ISR failed to explain oil fatty acid composition, while source-sink ratio appropriately described it. Sucrose injection significantly increased grain weight, oil content, and oleic acid percentage of shaded plants. It is concluded that effects of ISR on fatty acid composition are a consequence of changes in assimilate availability for grain oil synthesis. To explain these results a conceptual model is proposed: when assimilate supply limits grain growth and oil synthesis, mainly linoleic acid is synthesized. As the assimilate supply increases, oleic acid desaturation process gets saturated and oleic acid accumulates.

Laboratorio de Fisiología Vegetal, Unidad Integrada Facultad de Ciencias Agrarias (UNMdP), Estación Experimental Agropecuaria INTA Balcarce, Ruta 226 km 73.5 C.C. 276, 7620 Balcarce, Argentina. Financial support from the Instituto Nacional de Tecnología Agropecuaria (INTA, PNCER 024022), Agencia Nacional de Promoción Científica y Tecnológica (PICT 08 0941), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP0362) and Universidad Nacional de Mar del Plata (UNMdP). Received 9 Sept. 2011. *Corresponding author (laguirre@mdp.edu.ar).

Abbreviations: ISR, intercepted solar radiation per plant; LD, low plant density; NMR, nuclear magnetic resonance; PAR, photosynthetically active radiation.

HELIANTHUS ANNUUS L. (sunflower) oil is one of the most widely used vegetable oils because of its nutritional and industrial attributes. In this crop, most of the photo-assimilates supplied to the grains (sink) are made by the leaves (source) during grain filling (Hall et al., 1990; López Pereira et al., 2008). Changes in assimilate production at the source during grain filling can be interpreted as changes in carbon availability in developing sinks (Hall et al., 1995), sucrose being the major carbohydrate and the only phloem transport sugar in sunflower plants (Alkio et al., 2002).

The source-sink framework has been widely used for interpretation of plant growth and yield (Alkio et al., 2003; Borrás et al., 2004; Cruz-Aguado et al., 1999; López Pereira et al., 1999; Rotundo et al., 2009). The source of post-flowering assimilates can be indirectly quantified as the post-flowering leaf area index duration or as the intercepted photosynthetically active radiation per plant during grain filling, while the sink of assimilates is represented by the number of grains (for references see Ruiz

Published in Crop Sci. 52:818–829 (2012).
doi: 10.2135/cropsci2011.09.0477

and Maddonni, 2006). Several research works have dealt with source–sink relationships and biomass partitioning in sunflower (e.g., Sadras et al., 1993; Ruiz and Maddonni, 2006). However, the relative role of the source and the sink in controlling the composition of sunflower oil is unclear.

Grain reserve lipids are mainly triacylglycerides (Appelqvist, 1989). The relative abundance of the different fatty acids bound to the glycerol molecules gives specific functional properties to the oil and, therefore, defines its potential use. Sunflower grains of standard varieties contain up to 90% unsaturated fatty acids (oleic and linoleic) and approximately 10% saturated fatty acids (palmitic and stearic; Steer and Seiler, 1990). Fatty acid composition of sunflower oil of standard varieties is closely related to the environmental conditions—particularly temperature and radiation—during grain filling (Echarte et al., 2010; Izquierdo and Aguirrezábal, 2008; Izquierdo et al., 2006; Izquierdo et al., 2009). There is much information about how temperature regulates oleic acid synthesis in sunflower grain by both direct and indirect control of oleate desaturase activity, a key enzyme of fatty acid biosynthesis (Garcés et al., 1992; Kabbaj et al., 1996; Rolletschek et al., 2007). By contrast, light-mediated changes in fatty acids have been less explored, and understanding of how oil synthesis mechanisms are affected is limited.

Oleic acid percentage increases with intercepted solar radiation per plant (Echarte et al., 2010; Izquierdo et al., 2009). Izquierdo et al. (2009) observed differences in oleic acid percentage response to intercepted solar radiation per plant (ISR) between experiments performed in different growing seasons. They argued, although not conclusively, that variations in the source–sink ratio could explain these differences, suggesting that sunflower oil fatty acid composition could be modified by carbon availability for the grains. However, the current physiological-biochemical knowledge does not consider any effect of carbon availability on oil fatty acid composition. Moreover, treatments performed by Izquierdo et al. (2009) only modified the source during grain filling through changes in incident radiation, while small differences in sink size were due to different grain number in two independent experiments. To test their proposal and consider source–sink ratio an explanatory variable (not correlated to ISR), it would be necessary to study the effects of treatments that significantly modify both the source and the sink size (Ruiz and Maddonni, 2006).

In addition to supplying plants with the energy necessary for CO_2 fixation, it has long been known that light also plays indirect roles in controlling assimilates translocation and partitioning (Gifford and Evans, 1981). In heterotrophic grains, such as sunflower grains, a direct effect of light on oil synthesis is quite unlikely. The study reported herein tests the hypothesis that changes in fatty acid composition in response to variations in ISR are mediated by changes in the assimilate supply to the grains. To accomplish this, both the source activity (shading, defoliating, and directly injecting sucrose) and the sink activity (cultivating plants under low density and excising grains) were modified after grain set in a traditional sunflower hybrid. We analyzed the effect of changes of source–sink ratio on grain weight, oil content, and fatty acid composition of sunflower oil and proposed a conceptual model that will help to unravel the mechanism underlying the response of fatty acid composition to solar radiation intercepted by sunflower plants. Understanding the fatty acid desaturation process at the physiological-biochemical level might help to analyze possible ways of genetically modifying the response of grain oil composition to environmental factors. This knowledge also could be useful for improving crop management and modeling of oil quality and oil quality–yield relationships (Martre et al., 2011).

MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L., hybrid MG2, Dow AgroSciences, LLC, Indianapolis, IN) was grown either in the field (source–sink ratio manipulation experiments) or in the greenhouse (sucrose injection experiment) at the Unidad Integrada Balcarce INTA-FCA (37°S, 58°W) Balcarce, Argentina. Soil was Typical Argiudoll with slopes ranging from 0.3 to 3%. These soils have a loam texture at surface layer (0- to 25-cm depth), loam to clay loam at subsurface layers (25- to 110-cm depth), and sandy loam at 110-cm depth (C horizon). Sowing was performed at recommended dates for the location to obtain maximum yield (Andrade and Cirilo, 2002).

Source-Sink Ratio Manipulation Experiments

Experiments were performed during growing seasons 2007–2008 (Exp. 1) and 2008–2009 (Exp. 2). Seeds were planted on 5 Nov. 2007 and 14 Nov. 2008. They were arranged in a randomized complete block design with three replicates. Experimental units were six, 6-m-long rows that were spaced 0.7-m apart. Unless stated otherwise, plant density was 6.5 plants m^{-2}. Plants were grown under optimal nutrient and water conditions. Soil fertility in all experiments was sufficient to attain maximum yields for sunflower crops grown under nonlimiting water conditions (yield >5000 kg ha^{-1}; Andrade et al., 2000; Sosa et al., 1999). Soil water content was measured every 5 to 7 d by the Time Domain Reflectometry method, with a moisture-measuring system (Trase System, Model 6050X1, Soilmoisture Equipment Corp., Santa Barbara, CA). Irrigation was applied to maintain soil water above 40% available water in the first 0.60 m of the soil profile during the entire growing season. Pests, diseases, and weeds were adequately controlled. At flowering, pollination bags (Delnet, Rosario, Argentina) were used to prevent cross-pollination. Flowering of a plant was defined by the appearance of stamens in all florets from the outer whorl of the capitulum (R5.1 stage; Schneiter and Miller, 1981). Flowering of the experimental unit was considered when 95% of the plants had reached R5.1 stage. Plants were self-pollinated manually. Treatments were applied when inner flowers of 95% of the plants had been pollinated (3 d after R6; Schneiter and

Miller, 1981) and ended at physiological maturity. Untreated sunflower plants served as control (C).

To estimate physiological maturity, 15 grains from rows 4 to 19 of three capitula were harvested twice a week during grain filling. Grain removal was repeated on the same plant as long as total removal did not exceed 5% of average final capitulum grain number. Grains were oven-dried at 60°C and weighed. Physiological maturity was determined as the time when average dry weight per grain did not further increase (Aguirrezábal et al., 2003).

Defoliation Treatments

Leaves of plants were cut off along the stem in an alternate way. To establish a gradient of defoliation intensity, leaves were excised by cutting off 25 (D25), 50 (D50), 75 (D75), and 100% (D100) of the total leaf area of all the plants in a plot in Exp. 1 and 60 (D60) and 100% in Exp. 2. The effect of stem wound and defoliation treatment effects other than those of reducing leaf area were evaluated by covering 25, 50, or 100% of leaves of nondefoliated plants in Exp. 1 with paper bags that intercepted 90% of sunlight. Leaves were covered on 10 plants randomly selected from the two central rows of a plot.

Radiation Treatments

Plants were shaded by placing a uniform, black, synthetic, neutral mesh cloth above the canopy of the two central rows of plants to reduce the incident solar radiation by 80% (S80) in Exp. 1 or 50 (S50) and 80% in Exp. 2 (Dosio et al., 2000; Izquierdo et al., 2008). Experiment 2 also included a thinning treatment (Th) meant to increase ISR and achieved by eliminating alternate plants in the row to get 50% of the original plant density (3.3 plant m^{-2}).

Grain Excision

To reduce sink size without affecting the source, grains from one (E25), two (E50), or three quarters (E75) of sunflower capitula were removed after grain set. Plants with remaining grains were grown in the field until physiological maturity and harvested.

Low Plant Density

In Exp. 2, plants were sown in a plot to achieve a target plant density of 3.3 plant m^{-2} (half the density of control plants). Row spacing was similar to control plants (0.7 m). Low plant density (LD) treatment was expected to modify both the source and the sink by increasing leaf area and grain number.

Sucrose Injection Experiment

Sucrose was injected into the receptacle of shaded and unshaded plants. For this, sunflower plants were grown in the greenhouse in 10-L pots filled with soil. Greenhouse roof and walls (polyethylene film 100-μm thick) reduced solar radiation incidence by approximately 30% (17.0 ±5 MJ m^{-2} d^{-1} in the field vs. 12.2 ±6 MJ m^{-2} d^{-1} in the greenhouse).

Five seeds per pot were sown on 3 Nov. 2008. Seven days after seedling emergence, plants were thinned to one plant per pot. Soil was fertilized with N, P, S, and B (Izquierdo et al., 2002) and irrigated every 12 h to avoid water stress. At flowering (95% of plants of the plot at R5.1), capitula were covered with self-pollination bags and cross-pollinated, as described for field experiments.

Injection and shading treatments were applied from R6 to physiological maturity. The experiment was designed as a split-plot, where shading (0 or 80%) was the main plot and injection solution the subplot. The injection system described by Abdin et al. (1998) was used with plants continuously injected in the receptacle with either distilled water or a 150 g sucrose L^{-1} solution. Noninjected plants were also included as controls of injection effects.

Measurements

Global daily incident radiation was measured with pyranometers (LI-200SB, LI-COR, Lincoln, NE) at a weather meteorological station placed 500-m away from the experiment. Daily incident photosynthetically active radiation (PAR) was calculated as 0.48 × global daily incident radiation (Bonhomme, 1993). The proportion of PAR intercepted by the crop at noon (±1 h) was calculated according to Gallo and Daughtry (1986) as (1 − Rb/Ro), where Rb is the radiation measured below the oldest green leaf and Ro is the radiation measured above the canopy. Rb was measured weekly with a line quantum sensor (LI-191SB, LI-COR) positioned across the rows (the length of the sensor was set at a distance equal to row separation: 0.7 m). Three measurements were done per plot. In accordance with Charles-Edwards and Lawn (1984), the daily proportion of intercepted PAR was estimated as the proportion of PAR intercepted at noon × 2/(1 + proportion of PAR intercepted at noon). This correction is a substantial improvement on a single measurement at noon (Trapani et al., 1992). Daily proportion of intercepted PAR between measurements was calculated by linear interpolation. Daily intercepted solar radiation per plant was calculated as the product of daily incident PAR and daily proportion of intercepted PAR divided by the plant density. Intercepted solar radiation accumulated per plant from R6 (Schneiter and Miller, 1981) to physiological maturity (ISR) was calculated as described by Dosio et al. (2000).

Air temperature was measured using shielded thermistors (Cavadevices, Buenos Aires, Argentina) next to the capitulum every 60 s and averaged hourly. Measurements began after flowering and finished at physiological maturity and were recorded by data loggers (Cavadevices, Buenos Aires, Argentina). Mean temperature during grain filling was similar in both field experiments (21.9 and 21.2°C in Exp. 1 and Exp. 2, respectively) but lower than in the greenhouse (24.5°C). Minimum night temperature in the 100 to 300°C day after flowering period (base temperature 6°C), the best temperature predictor of oleic acid percentage in sunflower oil (Izquierdo et al., 2006), was also similar in both field experiments (14.4 and 14.8°C in Exp. 1 and Exp. 2, respectively) and lower than in the greenhouse (15.8°C).

The area of leaves cut off in the defoliation treatment was measured with a LI-COR leaf area meter (LICOR). Defoliation was calculated as the quotient between leaf area of defoliation treatment and D100 treatment.

Sample Processing and Analysis

Once physiological maturity was reached, 10 capitula were randomly sampled from the two central rows of each plot and oven-dried with circulating air at 60°C. Grains of the capitulum were manually separated. Only nonempty grains (kernel occupying at

Table 1. Intercepted solar radiation per plant (ISR), grain number per plant, and source-sink ratio for different treatments performed in both Exp. 1 and Exp. 2. Values are mean ± standard deviation. S80, 80% shading; S50, 50% shading; D25, 25% defoliation; D50, 50% defoliation; D60, 60% defoliation; D75, 75% defoliation; D100, 100% defoliation; E25, 25% grain excision; E50, 50% grain excision; E75, 75% grain excision; Th, thinning; LD, low density.

Experiment	Treatment	ISR	Grain no. per plant	Source-sink ratio
		MJ plant^{-1}		J grain^{-1}
Exp. 1	Control	38.1 ±0.8	1168 ±83	33 ±2
	S80	11.1 ±0.2	811 ±313	15 ±5
	D25	36.9 ±0.9	1170 ±82	32 ±2
	D50	32.9 ±1.7	1228 ±193	27 ±5
	D75	24.7 ±1.1	1171 ±258	22 ± 2
	D100	12.7 ±1.7	837 ±105	16 ±1
	E25	39.1 ±1.6	997 ±317	41 ±14
	E50	40.9 ±1.4	853 ±108	44 ±7
	E75	44.6 ±0.7	360 ±13	124 ±4
Exp. 2	Control	41.0 ±0.6	1461 ±172	28 ±3
	Th	61.3 ±1.4	1554 ±131	40 ±4
	S50	20.8 ±1.2	1483 ±223	14 ±3
	S80	11.3 ±0.7	1269 ±125	9 ±1
	D60	30.2 ±3.8	1443 ±46	21 ±2
	D100	16.8 ±1.8	1252 ±87	15 ±4
	LD	64.9 ±3.9	2144 ±64	30 ±2
	E25	41.7 ±1.1	1252 ±87	32 ±2
	E50	43.6 ±0.8	888 ±148	47 ±8

least 20% of total space in the grain) were recovered. Nonempty grains were counted and weighed. Grain oil concentration was determined by nuclear magnetic resonance (NMR; Spinlock S.R.L., Córdoba, Argentina) technique according to Izquierdo et al. (2008). Oil content (oil weight per grain) was calculated as the product of oil concentration and grain weight.

Oil extraction and methylation followed the technique of Sukhija and Palmquist (1988). Fatty acid composition was determined by gas chromatography (GLC, Varian 3400, Varian Inc., Palo Alto, CA) and expressed either as percentage of total fatty acids or as fatty acid content per grain, assuming that fatty acids represent 98% of sunflower oil at physiological maturity (Robertson et al., 1978).

Assimilate availability per grain during grain filling (the source-sink ratio) was estimated as the quotient between ISR and grain number per plant (Ruiz and Maddonni, 2006). Assimilate effectively allocated to the grains was assumed to be represented by carbohydrate equivalents for grain biomass production (Vertregt and Penning de Vries, 1987). For this, carbon and nitrogen in the grains were determined in a TruSpec CN equipment (Leco Corporation, St. Joseph, MI) and ash content, according to AOAC recommendation (AOAC International, 1990).

Data Analysis

Mature grain weight depends not only on carbon substrate but also on the energy required for conversion, polymerization, and other active processes of biosynthesis (Penning de Vries, 1974). Therefore, when comparing grains with different oil concentration, it is convenient to make a correction to grain weight that considers the costs of synthesis of the different compounds (de la Vega et al., 2001). Oil-corrected grain weight was calculated taking into account oil concentration (NMR measurements) and oil synthesis costs according to Hall et al. (1995).

Carbohydrate equivalents for grain biomass production were calculated as described by Vertregt and Penning de Vries (1987). The response of oil and fatty acid content to carbohydrate equivalents was analyzed together in plants grown in the canopy (source-sink ratio manipulation experiments) and isolated plants grown in the greenhouse (sucrose injection experiments).

Results were subjected to analysis of variance (ANOVA) to evaluate the effects of treatments. Mean values of variables, standard errors, and least significant differences were calculated using the Least Squares Fit model. Equation fittings were done by nonlinear simple regressions using the software Sigmaplot 8.0 (1986–2001, SPSS Inc., Chicago, IL). The best fitting equation was defined according to Akaike criterion (Akaike, 1971). Most of the relationships explored were described by an exponential rise up to a maximum (Eq. [1]),

$$y = a\,(1 - e^{-bx}) \qquad [1]$$

where a represents the maximal attainable value and b is the rate at which the function increases.

RESULTS

Effect of the Assimilate Source on Oil-Corrected Grain Weight, Oil Weight per Grain, and Fatty Acid Composition

Two kinds of treatments meant to modify solar radiation interception, and thus assimilate source, without affecting the sink have been imposed on sunflower plants: (i) radiation (shading and thinning) and (ii) defoliation. Accumulated ISR values obtained from R6 to physiological maturity, grain number, and source-sink ratio are detailed in Table 1. These treatments provided ISR values ranging from 11 to 65 MJ plant^{-1} and source-sink ratios from 9 to 40 J grain^{-1}. Since radiation and defoliation treatments have been applied after grain set, grain number was not significantly affected ($P < 0.01$), while ISR was significantly modified, except for D25 ($P < 0.01$). As expected, changes in source-sink ratio in radiation and defoliation treatments were mainly related to changes in ISR.

Measurement of leaf area after defoliation showed that defoliation was 26.6 ±0.5, 51.3 ±5.9, and 75.9 ±1.6% for D25, D50, and D75, respectively, in Exp. 1 and 60.9 ±1.2% for D60 treatment in Exp. 2.

Oil-corrected grain weight and oil weight per grain were significantly reduced with respect to control treatments only by 100% defoliation and S80 treatments in both Exp. 1 and Exp. 2, and by S50 in Exp. 2 ($P < 0.01$, Fig. 1A, B, D, and E). Saturated fatty acid percentage did not significantly change in response to any treatment applied ($P < 0.01$, Fig. 1C and 1F). Oleic acid percentage significantly decreased and linoleic acid significantly increased with shading

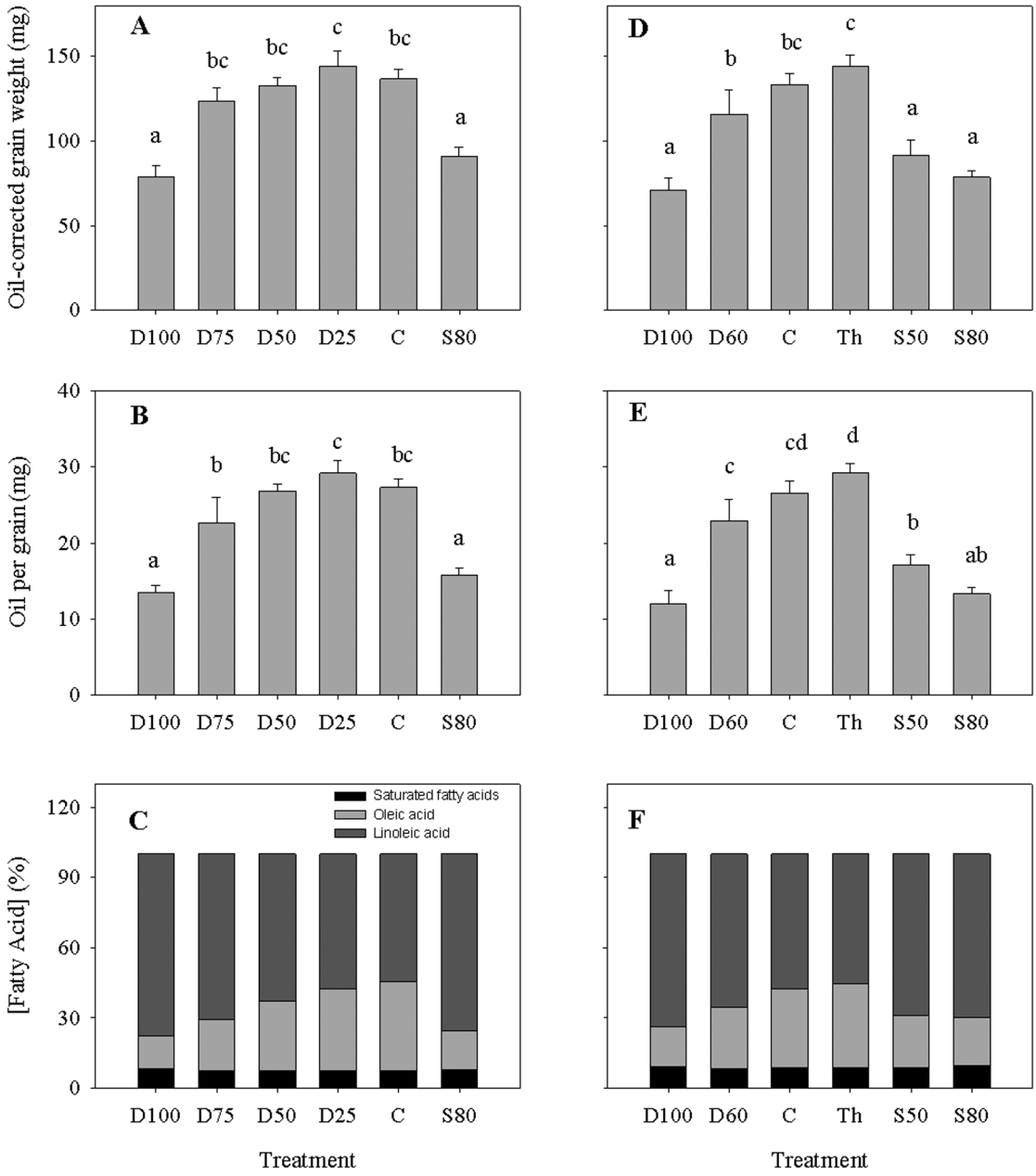

Figure 1. Effects of defoliation, shading, and thinning treatments on (A and D) oil-corrected grain weight, (B and E) oil weight per grain, and (C and F) fatty acid composition of sunflower (*Helianthus annuus* L.). Bars with different letters are significantly different ($P < 0.05$). Left panels: 2007–2008 experiment (Exp. 1); right panels: 2008–2009 experiment (Exp. 2). Bars represent mean values, error bars in panels A, D, B, and E represent standard deviation of mean values.

and defoliation ($P < 0.05$) except for D25 and thinning treatments. Fatty acid composition in thinning treatment did not significantly differ from control plants ($P < 0.01$).

Oil-corrected grain weight, oil weight per grain, and oleic acid percentage responded to ISR in a curvilinear manner regardless of the method employed to modify light interception (Fig. 2). Saturated fatty acids did not show any trend with ISR, while oleic acid percentage was linearly and negatively related to linoleic acid (data not shown). The slope of this relationship was 1.01 ($R^2 = 0.96$, $P < 0.0001$), indicating that an increase in oleic acid percentage corresponded to a decrease of similar magnitude in the relative concentration of linoleic acid. Cumulated ISR accounted for most of the effect of both radiation and defoliation treatments. No differences in trait response to ISR between defoliation or radiation treatments were detected.

Possible effects of stem wound on sunflower oil fatty acids profile were tested in Exp. 1 by covering 25, 50, or 75% of plant leaves with paper bags that intercepted approximately 90% of sunlight. There were no significant differences between plants with covered leaves and the same degree of defoliation (data not shown). Effects of defoliation on grain growth and composition other than

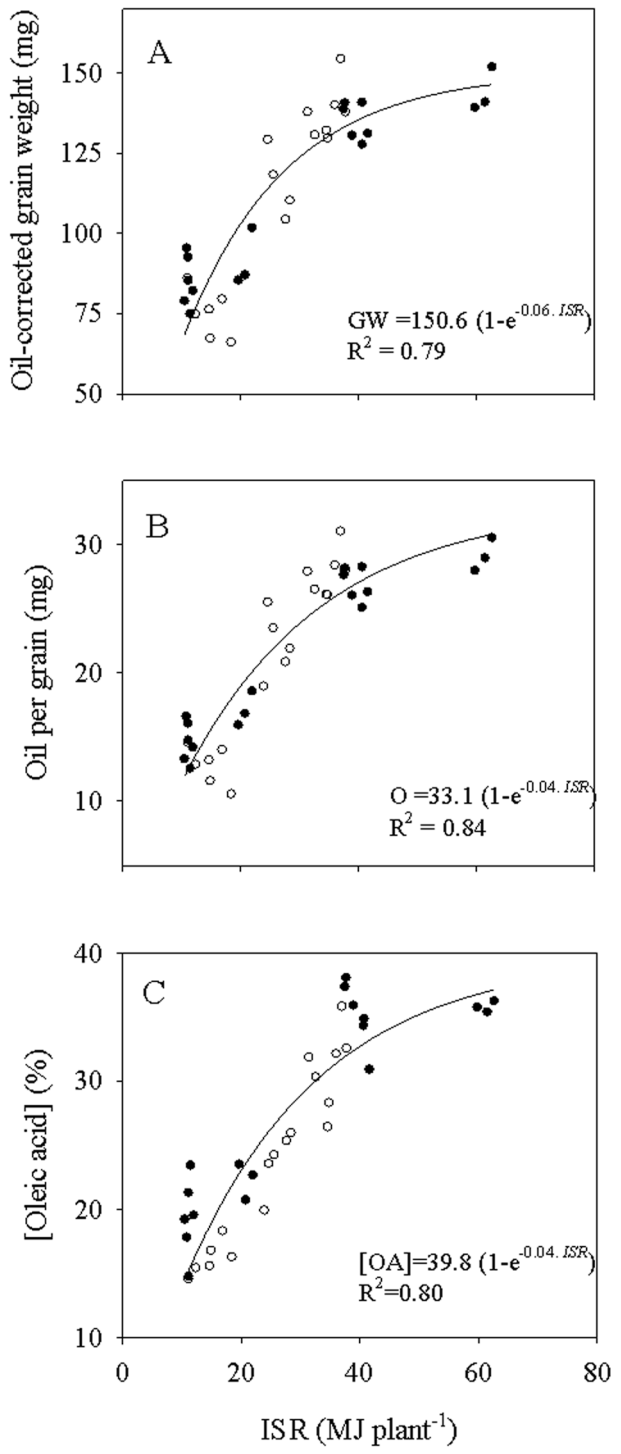

Figure 2. Relationship between (A) oil-corrected grain weight, (B) oil weight per grain, and (C) oleic acid percentage and intercepted solar radiation per plant (ISR) accumulated from R6 to physiological maturity of sunflower (*Helianthus annuus* L.) under radiation and defoliation treatments. Filled symbols represent control plants, shading, and thinning treatments. Empty symbols correspond to defoliation treatments. Continuous line represents the fitting of Eq. [1] to experimental data. GW, oil-corrected grain weight; O, oil weight per grain; OA, oleic acid percentage.

via radiation interception reduction (e.g., wounds) were, therefore, unlikely.

Sink Effect on Oil-Corrected Grain Weight, Oil Weight per Grain, and Fatty Acid Composition

Grain excision did not affect grain weight or oil content but increased oleic acid percentage (Table 2), which was linearly and negatively related to changes in linoleic acid percentage ($R^2 = 0.98$, $P < 0.0001$). This response was significant when up to 50% of grains had been removed; further excision did not increase oleic acid percentage (Table 2). Intercepted solar radiation per plant slightly increased (less than 10%) when more grains were excised (Table 1), suggesting a negative feedback regulation of the source by the sink.

Figures 3A and 3B show that ISR accounted for most of the effect of excision treatments on both oil-corrected grain weight and oil weight per grain at physiological maturity. For both traits, RMSE of data coming from defoliation, radiation, excision, and LD did not differ (data not shown), indicating that error magnitude was similar in different treatments. Interestingly, when oleic acid percentage was represented as a function of ISR, data deviated from the previous observed pattern and had to be excluded from the fitting (rounded symbols, Fig. 3C). Once again, saturated fatty acids did not significantly change, while linoleic acid was inversely related to oleic acid and, therefore, was lower than expected according to ISR response (data not shown).

Plant Density Effect on Oil-Corrected Grain Weight, Oil Weight per Grain, and Fatty Acid Composition

Low density treatment produced not only more grains per plant but also a larger leaf area per plant (8896 ±990 cm^2 vs. 6105 ±906 cm^2) and, therefore, higher values of accumulated ISR. As a consequence of both source and sink increase, source–sink ratios obtained were similar to those of control plants (Table 1). This treatment showed grain weight, oil content, and oleic acid percentage values similar to those obtained in control conditions and a similar pattern of response to ISR (Fig. 3A to 3C).

Fatty Acid Composition Dependence on Source-Sink Ratio

Oil-corrected grain weight, oil weight per grain, and oleic acid percentage increased with source–sink ratio until they reached a plateau (Fig. 3D to 3F). Value of source–sink ratio at which plateau was attained was higher for oleic acid percentage than for oil-corrected grain weight or oil weight per grain. It is noteworthy that some data that could not be accounted for ISR (e.g., oleic acid of grains from excision treatments—rounded data in Fig. 3C) were appropriately described by the source–sink ratio (compare Fig. 3C and 3F).

Sucrose Injection

Sucrose injection significantly increased oil-corrected grain weight and grain weight per grain of shaded plants (Fig. 4A

Table 2. Oil-corrected grain weight, oil weight per grain, and fatty acid composition of excision treatments in Exp. 1 and Exp. 2. E25, 25% grain excision; E50, 50% grain excision; E75, 75% grain excision. Means followed by the same letter are not statistically different ($P < 0.05$).

Experiment	Treatment	Oil-corrected grain weight	Oil weight per grain	Fatty acid		
				Saturated	Oleic	Linoleic
		mg		%		
Exp. 1	Control	137 ±5a	27.3 ±1.1a	7.7 ±0.2a	38.2 ±0.7a	55.2 ±1.2a
	E25	136 ±11a	27.1 ±2.4a	8.1 ±0.2a	42.8 ±1.0b	49.0 ±0.8b
	E50	141 ±10a	27.7 ±2.0a	8.2 ±0.3a	45.2 ±0.4bc	45.9 ±0.4c
	E75	130 ±4a	26.2 ±2.4a	8.9 ±0.1b	46.3 ±1.7c	44.6 ±1.2c
Exp. 2	Control	133 ±7a	28.8 ±1.3a	9.0 ±0.3a	33.4 ±2.1a	57.6 ±2.4a
	E25	140 ±6a	27.6 ±1.8a	9.5 ±0.1a	39.0 ±1.0b	51.8 ±0.9b
	E50	143 ±9a	28.4 ±1.7a	9.6 ±0.3a	41.2 ±0.5b	49.2 ±0.6b

and 4B) but had no effect on unshaded plants. Shading reduced oil-corrected grain weight and grain weight per grain both in water-injected and noninjected plants. In sucrose-injected plants, shading effect on oil-corrected grain weight and oil weight per grain was not significant. Water injection did not produce any noticeable effect on any of these variables.

Oleic acid percentage increased in response to sucrose injection in both shaded and unshaded plants (Fig. 4C). The injection of sucrose in shaded plants increased oleic acid percentage to unshaded noninjected control levels. By comparing Fig. 4 and 3, it can be observed that oil-corrected grain weight and grain weight per grain of untreated plants grown in the greenhouse are near maximal attainable values, and therefore, sucrose injection did not produce any effect on these trials. By contrast, unshaded noninjected plants showed oleic acid percentages far below potential values, and consequently, sucrose injection increased oleic acid percentage in the oil of these plants. Water injection produced a slight decrease in oleic acid percentage, suggesting a carbon dilution effect on oil fatty acid composition.

Response of Oil and Fatty Acids Content to Assimilate Allocated to the Grain

The fact that oil-corrected grain weight reaches a plateau value (potential weight) at high source-sink ratios implies that either assimilate translocation from the aerial vegetative parts of the plant to the grain or the assimilate utilization by the grain gets saturated. Therefore, to model how oil accumulation and fatty acid composition depend on carbon substrate in sunflower grains, carbohydrate equivalents for grain biomass production were calculated and assumed to represent assimilates effectively allocated to the grains and, thus, available for oil synthesis.

Figure 5 shows oil weight per grain, saturated fatty acids, oleic acid, and linoleic acid content as a function of carbohydrate equivalents for both experiments performed in the field and the greenhouse. Oil as well as saturated fatty acid content linearly increase (Fig. 5A and 5B) while oleic acid exponentially increases (Fig. 5C) with carbohydrate equivalents. Linoleic acid steeply increases with carbohydrate equivalents when they are less than approximately 120 mg. At higher values, linoleic acid shows a slower response up to 93% of the maximal value estimated by equation fitting (Fig. 5D). More than 90% of changes in oil content as a consequence of differences in assimilate allocated to the grains can be attributed to oleic and linoleic acid content: at low carbohydrate equivalent values, oil accumulation is mainly due to linoleic acid synthesis, while at high assimilate supply conditions, oil increase is mainly a consequence of oleic acid accumulation (Fig. 5 E).

It is important to note that although in sucrose-injection experiments the grain weights were lower (compare Fig. 3 and 4), data show that grain composition as a function of carbohydrate equivalents shows the same behavior as that observed in the field (filled symbols in Fig. 5).

DISCUSSION

Defoliation and radiation treatments are reliable methods of manipulating the source-sink ratio of sunflower plants (Alkio et al., 2003). In the present work, a wide range of source-sink ratios was obtained from invasive (grain and leaf removal) and noninvasive treatments (shading of 80% of the sunlight, thinning of 50% of the plants, low density stand). These treatments established a range of relative source-sink ratios wider than the one explored by Ruiz and Maddonni (2006), who analyzed a database that provided values of −0.66 to 1.27 (vs. −0.78 to 2.3 in this work) to quantify source-sink ratio effects on grain weight and oil concentration in sunflower.

Plant sensitivity to leaf removal changes according to when they are defoliated (Schneiter and Johnson, 1994). In sunflower it has been shown that defoliation before anthesis promotes growth and photosynthetic efficiency of the remaining leaves (Alkio et al., 2003; Rodrigues Pereira, 1978). By contrast, when defoliation treatments are applied after anthesis they do not produce any significant effect on the remaining plant (Moriondo et al., 2003). Response to ISR of traits explored here (oil-corrected grain weight, oil weight per grain, and fatty acid composition) showed the same pattern regardless of whether they were obtained by applying radiation or defoliation treatments after grain set. This suggests that no significant effect of defoliation on

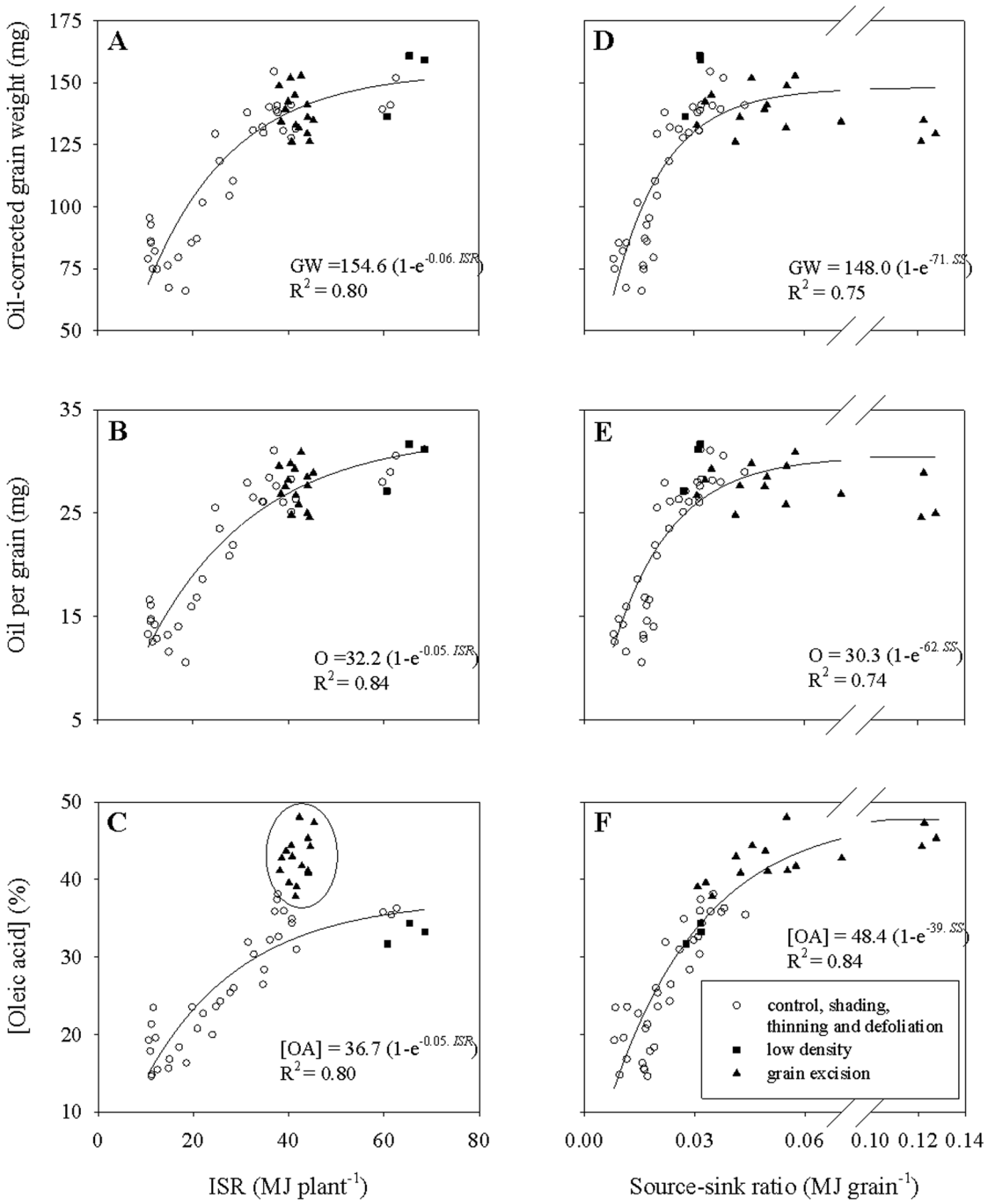

Figure 3. Left panels: relationship between (A) oil-corrected grain weight, (B) oil weight per grain, and (C) oleic acid percentage and intercepted solar radiation per plant (ISR) accumulated from R6 to physiological maturity. Right panels: response of (D) oil-corrected grain weight, (E) oil weight per grain, and (F) oleic acid percentage to source-sink ratio. Continuous line represents the fitting of Eq. [1] to experimental data. Rounded data have not been included in equation fitting. GW, oil-corrected grain weight; O, oil weight per grain; OA, oleic acid percentage.

grain metabolism further than lowering down radiation interception was produced.

Previous work shows that oleic acid percentage increases with ISR (Echarte et al., 2010; Izquierdo et al., 2009). Effects of changes in ISR obtained by shading, thinning, and defoliation treatments agree with this observation. Oleic acid increases up to a maximum that would represent the potential concentration. However, grain excision significantly increased oleic acid percentage without changing ISR, showing that the plateau value observed when increasing ISR was not the potential value and that oleic acid percentage could further increase. Although the behavior of oleic acid percentage in these treatments could not be explained by ISR variations, it was appropriately described when considered as a function of source-sink ratio, indicating that oil fatty acid

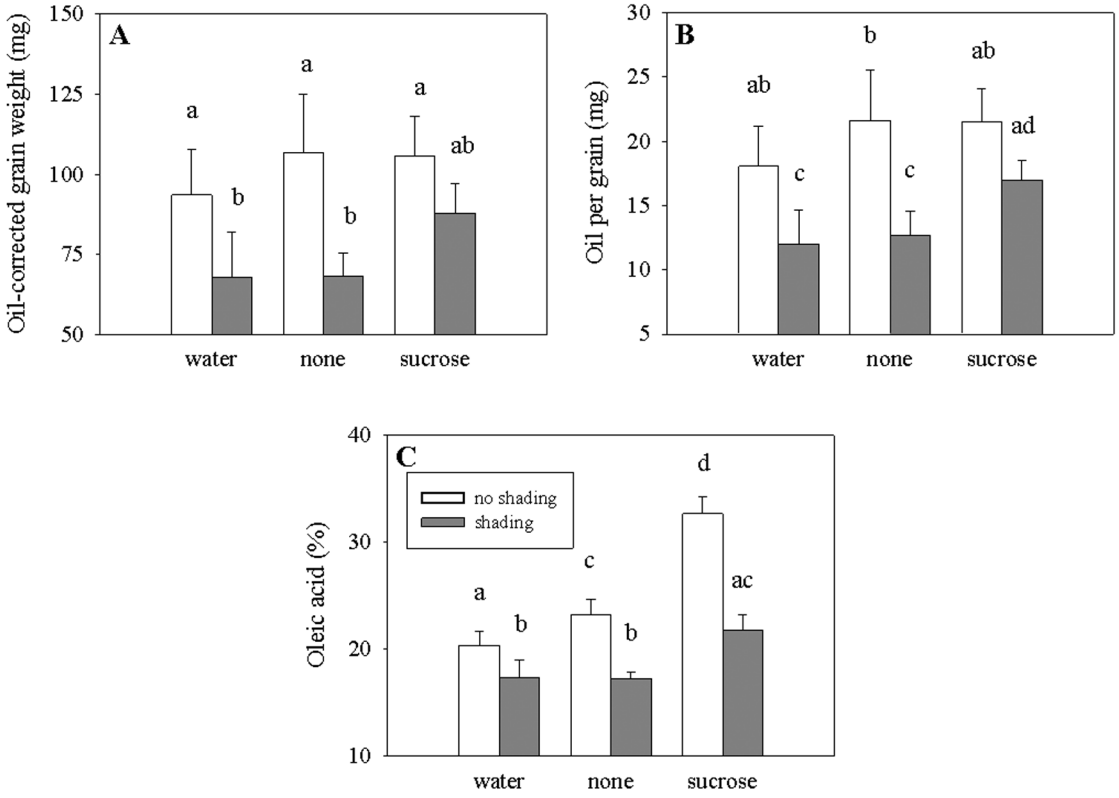

Figure 4. (A) Oil-corrected grain weight, (B) oil weight per grain, and (C) oleic acid percentage for different injection treatments. White bars represent unshaded plants and black bars, shaded plants. Bars with different letters are significantly different ($P < 0.01$). Values are mean ± standard deviation.

composition does not depend directly on ISR but on the assimilate availability for the grains.

It is well known that plant density has a significant effect not only on grain number per plant but on leaf area index and leaf area duration and, hence, in assimilate supply (Ruiz and Maddonni, 2006; Sadras and Hall, 1988; Steer et al., 1986). In LD treatments, ISR and grain number counterbalance leads to source–sink ratios similar to those obtained for control plants. The fact that data obtained from this treatment are also similar to those of control plants reinforces the idea of the source–sink ratio governing the synthesis of oil and its chemical composition.

In the present work, it is proposed that radiation effects are produced through the regulation of assimilate availability for the grains. If this were the case, supply of large amounts of sucrose to shaded plants should overcome the effects of reduced carbon that would have been supplied by photosynthesis (Begna et al., 2002). Thus, the impact of a manipulative increase in assimilate supply on lipid synthesis by directly injecting sucrose into the capitulum receptacle was tested. In the greenhouse, control conditions—without any injection or shading—would represent potential conditions for grain weight and oil content, and therefore, sucrose injection did not produce any effect on them. Under severe shading, both variables increased in response to sucrose injection. Sucrose injection increased oleic acid percentage both under full sunlight and shading. In agreement with results obtained by manipulating the source–sink ratio, this shows that although growth conditions were potential for seed weight and oil content, they were not for oleic acid relative concentration. The increase in this fatty acid in response to sucrose injection of unshaded plants could be explained by considering that interception of radiation in greenhouse conditions was lower than in full sunlight field ones. This is a consequence of the lower incident solar radiation (polyethylene film intercepted approximately 30% of incident radiation) and lower radiation interception owing to a smaller leaf area developed by plants grown in pots compared with plants grown in the field. Although performed under different experimental conditions, sucrose-injection experiment results agree with those of source–sink manipulation experiments and confirm the observation that effects of ISR on fatty acid composition of sunflower oil are mediated by assimilate availability for the grains.

Fatty acid synthesis is a complex process that involves several enzymes placed in different subcellular compartments of the grains (Garcés and Mancha, 1991; Gray and Kekwick, 1996; Harwood, 1996). The main products from the intraplastidial *de novo* fatty acid biosynthesis are palmitoyl-ACP, stearoyl-ACP, and oleoyl-ACP. These acyl-ACPs are hydrolyzed to free fatty acids, activated to the corresponding acyl-CoAs, and exported to the cytosol to be incorporated into glycerolipids. The enzyme responsible for the desaturation of oleic acid into linoleic acid is oleoylphosphatidylcholine desaturase (FAD2),

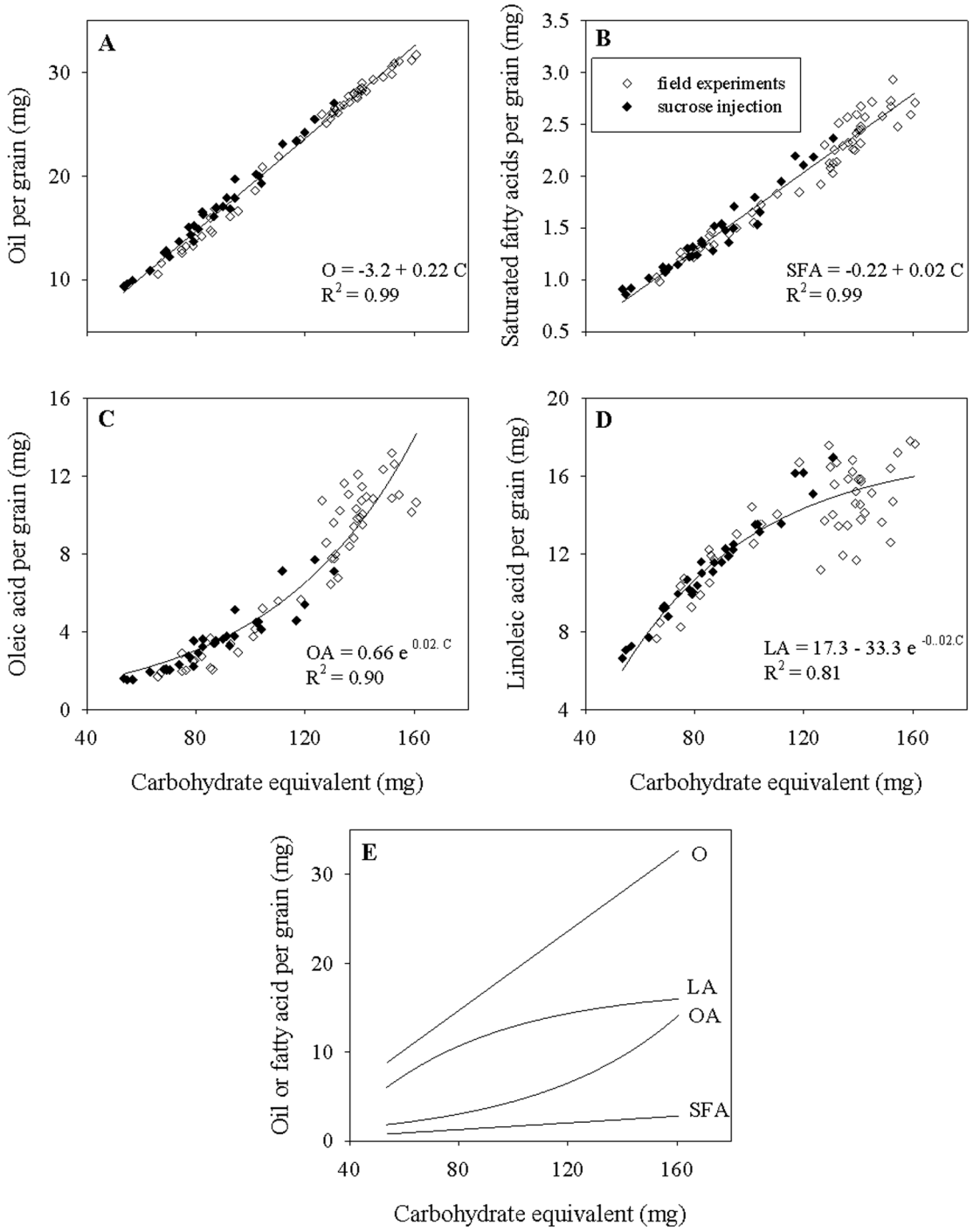

Figure 5. Oil and fatty acid content dependence on carbohydrate equivalents. (A) Oil, (B) saturated fatty acids, (C) oleic acid, and (D) linoleic acid per grain are plotted as a function of carbohydrate equivalents. (◊) field experiments; (♦) sucrose injection experiment. Continuous lines represent the fitting of equations in the corresponding inserts to experimental data. Panel E shows the fittings represented in panels A to D all together. O, oil weight per grain; LA, linoleic acid per grain; OA, oleic acid per grain; SFA, saturated fatty acids per grain; C, carbohydrate equivalent.

which has been shown to be highly regulated by temperature. Previous knowledge was not sufficient for understanding how oil synthesis depends on the availability of carbon substrates for these enzymes. Here carbon allocated to the grain was estimated as carbohydrate equivalents for grain biomass synthesis. Carbohydrate equivalents provided a framework that allowed analyzing together data coming from experiments performed under very different experimental conditions (field and greenhouse experiments). Saturated fatty acids linearly increased with carbon allocated to the grain, while oleic acid exponentially increased and linoleic acid increased up to a maximum with increasing carbon. The analysis of oil and fatty acids content as a function of carbohydrate equivalents (Fig. 5) led us to propose the following conceptual model: at very low

assimilate allocated to the grains, both first steps of oil synthesis and oleic acid desaturation, a key control step in fatty acids biosynthesis pathway (Garcés et al., 1992), are subsaturated. In these conditions, oil accumulates and most oleic acid can be transformed into linoleic acid. When assimilate allocated to the grain further increases, oleic acid desaturation gets saturated and oleic acid begins to accumulate. Thus, oleic acid and oil content increase in the grain at the same rate. Linear responses of oil and saturated fatty acids content to carbohydrate equivalent suggest that no saturation of first steps of oil synthesis by carbon exist. This model explains results previously reported (Izquierdo et al., 2009; Echarte et al., 2010), where oleic acid of sunflower oil has been shown to increase in response to ISR.

Grain composition is the result of complex interactions among grain genetic characteristics, the maternal plant, and the environment (Aguirrezábal et al., 2009; Andrade et al., 2005). It results from several processes controlled at different organizational levels, from the subcellular to the crop level. Ecophysiological process-based simulation models, built on evidence-based hypotheses about interrelationships among processes and how they are affected by environmental variations, help unravel this complexity (Martre et al., 2011). In this work, an ecophysiological approach allowed us to interpret the mechanism underlying ISR effect on fatty acid composition at the biochemical level. In this sense, the effects of ISR reported until now on fatty acid composition of sunflower oil can be interpreted as a consequence of changes in substrate availability for grain oil synthesis. Moreover, these results could be useful for building a simulation model for the biosynthesis of lipid reserves with parameters that are relatively independent of the environment and that could explain the fatty acid composition response to stress produced by different factors (e.g., low radiation, crop damage, water or nutrient deficits, etc.).

CONCLUSIONS

Knowing the mechanism underlying plant response to an environmental factor is important not only for understanding biochemical and physiological processes and their regulation but for assisting crop management, genetic analysis, and breeding. Changes in assimilate availability for the grains explain the response of fatty acid composition to ISR previously reported. Furthermore, assimilate allocated to the grains emerge as a robust variable for understanding changes in fatty acid composition in response to changes in assimilate availability, while the source-sink ratio provides a useful and easy-to-measure or simulate trait to estimate this effect.

Acknowledgments

The authors wish to thank Dr. Jorge Tognetti for valuable suggestions. This work was supported by Instituto Nacional de Tecnología Agropecuaria (INTA, PNCER 024022), Agencia Nacional de Promoción Científica y Tecnológica (PICT 08 0941), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP0362), and Universidad Nacional de Mar del Plata (UNMdP). M.M. Echarte and L.A.N. Aguirrezábal are members of CONICET. I. Alberdi has a scholarship from the Agencia Nacional de Promoción Científica y Tecnológica.

References

Abdin, O.A., X. Zhou, B.E. Coulman, D. Cloutier, M.A. Faris, and D.L. Smith. 1998. Effect of sucrose supplementation by stem injection on the development of soybean plants. J. Exp. Bot. 49:2013–2018.

Aguirrezábal, L., P. Martre, G. Pereyra Irujo, N. Izquierdo, and V. Allard. 2009. Management and breeding strategies for the improvement of grain and oil quality, p. 387–421. *In* V. Sadras and D. Calderini (ed.) Crop physiology. Applications for genetic improvement and agronomy. Vol. 16. Academic Press, San Diego, CA.

Aguirrezábal, L.A.N., Y. Lavaud, G.A.A. Dosio, N.G. Izquierdo, F.H. Andrade, and L.M. González. 2003. Intercepted solar radiation during seed filling determines sunflower weight per seed and oil concentration. Crop Sci. 43:152–161.

Akaike, H. 1971. Autoregressive model fitting for control. Ann. Inst. Stat. Math. 23:163–180.

Alkio, M., W. Diepenbrock, and E. Grimm. 2002. Evidence for sectorial photoassimilate supply in the capitulum of sunflower (*Helianthus annuus*). New Phytol. 156:445–456.

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

Andrade, F.H., and A.G. Cirilo. 2002. Fecha de siembra y rendimiento de los cultivos. Bases para el manejo del maíz, el girasol y la soja:137–156.

Andrade, F.H., L.A.N. Aguirrezábal, and R.H. Rizzali. 2000. Crecimiento y rendimiento comparados. Bases para el manejo del maíz, el girasol y la soja:61–96.

Andrade, F.H., V.O. Sadras, C.R.C. Vega, and L. Echarte. 2005. Physiological determinants of crop growth and yield in maize, sunflower and soybean: Their application to crop management, modeling and breeding. J. Crop Improv. 14:51–101.

AOAC International (Association of Official Analytical Chemists). 1990. Official methods of analysis. Arlington, VA: AOAC International, 942.05.

Appelqvist, L.A. 1989. The chemical nature of vegetable oils. Oil Crops of the World:22–37.

Begna, S.H., L.M. Dwyer, D. Cloutier, L. Assemat, A. DiTommaso, X. Zhou, B. Prithiviraj, and D.L. Smith. 2002. Decoupling of light intensity effects on the growth and development of C3 and C4 weed species through sucrose supplementation. J. Exp. Bot. 53:1935–1940.

Bonhomme, R. 1993. The solar radiation: Characterization and distribution in the canopy. Crop Structure and Light Microclimate:17–28.

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

Charles-Edwards, D.A., and R.J. Lawn. 1984. Light interception by grain legume row crops. Plant Cell Environ. 7:247–251.

Cruz-Aguado, J.A., F. Reyes, R. Rodes, I. Perez, and M. Dorado. 1999. Effect of source-to-sink ratio on partitioning of dry matter and 14C-photoassimilates in wheat during grain filling. Ann. Bot. (Lond.) 83:655–665.

de la Vega, A., S. Chapman, and A. Hall. 2001. Genotype by environment interaction and indirect selection for yield in sunflower I. Two-mode pattern analysis of oil and biomass yield across environments in Argentina. Field Crops Res. 72:17–38.

Dosio, G.A.A., L.A.N. Aguirrezábal, F.H. Andrade, and V.R. Pereyra. 2000. Solar radiation intercepted during seed filling and oil production in two sunflower hybrids. Crop Sci. 40:1637–1644.

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

Gallo, K.P., and C.S.T. Daughtry. 1986. Techniques for measuring intercepted and absorbed photosynthetically active radiation in corn canopies. Agron. J. 78:752–756.

Garcés, R., and M. Mancha. 1991. In vitro oleate desaturase in developing sunflower seeds. Phytochemistry 30:2127–2130.

Garcés, R., C. Sarmiento, and M. Mancha. 1992. Temperature regulation of oleate desaturase in sunflower (*Helianthus annuus* L.) seeds. Planta 186:461–465.

Gifford, R.M., and L.T. Evans. 1981. Photosynthesis, carbon partitioning, and yield. Annu. Rev. Plant Physiol. 32:485–509.

Gray, D.A., and R.G.O. Kekwick. 1996. Oleate desaturase activity in sunflower (*Helianthus annuus*) seeds and its relation to associated constituents during seed development. Plant Sci. 115:39–47.

Hall, A., D. Whitfield, and D. Connor. 1990. Contribution of preanthesis assimilates to grain-filling in irrigated and water-stressed sunflower crops II. Estimates from a carbon budget. Field Crops Res. 24:273–294.

Hall, A.J., D.J. Connor, and V.O. Sadras. 1995. Radiation-use efficiency of sunflower crops: Effects of specific leaf nitrogen and ontogeny. Field Crops Res. 41:65–77.

Harwood, J.L. 1996. Recent advances in the biosynthesis of plant fatty acids. Biochimica et Biophysica Acta—Lipids and Lipid Metabolism 1301:7–56.

Izquierdo, N., L. Aguirrezábal, F. Andrade, and V. Pereyra. 2002. Night temperature affects fatty acid composition in sunflower oil depending on the hybrid and the phenological stage. Field Crops Res. 77:115–126.

Izquierdo, N.G., and L.A.N. Aguirrezábal. 2008. Genetic variability in the response of fatty acid composition to minimum night temperature during grain filling in sunflower. Field Crops Res. 106:116–125.

Izquierdo, N.G., L.A.N. Aguirrezábal, F.H. Andrade, and M.G. Cantarero. 2006. Modeling the response of fatty acid composition to temperature in a traditional sunflower hybrid. Agron. J. 98:451–461.

Izquierdo, N.G., G.A.A. Dosio, M. Cantarero, J. Lujan, and L.A.N. Aguirrezabal. 2008. Weight per grain, oil concentration, and solar radiation intercepted during grain filling in black hull and striped hull sunflower hybrids. Crop Sci. 48:688–699.

Izquierdo, N.G., L.A.N. Aguirrezábal, F.H. Andrade, C. Geroudet, O. Valentinuz, and M. Pereyra Iraola. 2009. Intercepted solar radiation affects oil fatty acid composition in crop species. Field Crops Res. 114:66–74.

Kabbaj, A., V. Vervoort, A. Abbott, M. Tersac, and A. Bervillé. 1996. Expression of stearate oleate and linoleate desaturase genes in sunflower with normal and high oleic contents. Helia 19:1–17.

López Pereira, M., V. Sadras, and N. Trápani. 1999. Genetic improvement of sunflower in Argentina between 1930 and 1995. I. Yield and its components. Field Crops Res. 62:157–166.

López Pereira, M., A. Berney, A.J. Hall, and N. Trápani. 2008. Contribution of preanthesis photoassimilates to grain yield: Its relationship with yield in Argentine sunflower cultivars released between 1930 and 1995. Field Crops Res. 105:88–96.

Martre, P., N. Bertin, C. Salon, and M. Génard. 2011. Modelling the size and composition of fruit, grain and seed by process-based simulation models. New Phytol. 191:601–618.

Moriondo, M., S. Orlandini, and F.J. Villalobos. 2003. Modelling compensatory effects of defoliation on leaf area growth and biomass of sunflower (*Helianthus annuus* L.). Eur. J. Agron. 19:161–171.

Penning de Vries, F.W.T. 1974. Substrate utilization and respiration in relation to growth and maintenance in higher plants. Neth. J. Agric. Sci. 22:40–44.

Robertson, J.A., G.W. Chapman, and R.L. Wilson. 1978. Relation of days after flowering to chemical composition and physiological maturity of sunflower seed. Journal of the American Oil Chemists' Society 55:266–269.

Rodrigues Pereira, A.S. 1978. Effect of leaf removal on yield components of sunflower. Neth. J. Agric. Sci. 26:133–144.

Rolletschek, H., L. Borisjuk, A. Sánchez-García, C. Gotor, L.C. Romero, J.M. Martínez-Rivas, and M. Mancha. 2007. Temperature-dependent endogenous oxygen concentration regulates microsomal oleate desaturase in developing sunflower seeds. J. Exp. Bot. 58:3171–3181.

Rotundo, J.L., L. Borrás, M.E. Westgate, and J.H. Orf. 2009. Relationship between assimilate supply per seed during seed filling and soybean seed composition. Field Crops Res. 112:90–96.

Ruiz, R.A., and G.A. Maddonni. 2006. Sunflower seed weight and oil concentration under different post-flowering source-sink ratios. Crop Sci. 46:671–680.

Sadras, V.O., and A.J. Hall. 1988. Quantification of temperature, photoperiod and population effects on plant leaf area in sunflower crops. Field Crops Res. 18:185–196.

Sadras, V.O., D.J. Connor, and D.M. Whitfield. 1993. Yield, yield components and source-sink relationships in water-stressed sunflower. Field Crops Res. 31:27–39.

Schneiter, A., and J. Miller. 1981. Description of sunflower growth stages. Crop Sci. 21:901.

Schneiter, A.A., and B.L. Johnson. 1994. Response of sunflower plants to physical injury. Can. J. Plant Sci. 74:763–766.

Sosa, L., H. Echeverría, G. Dosio, and L. Aguirrezábal. 1999. Evaluación de la nutrición nitrogenada de girasol cultivado en Balcarce (Buenos Aires, Argentina). Cienc. Suelo 17:20–26.

Steer, B., and G. Seiler. 1990. Changes in fatty acid composition of sunflower (*Helianthus annuus*) seeds in response to time of nitrogen application, supply rates and defoliation. J. Sci. Food Agric. 51:11–26.

Steer, B.T., P.D. Coaldrake, C.J. Pearson, and C.P. Canty. 1986. Effects of nitrogen supply and population density on plant development and yield components of irrigated sunflower (*Helianthus annuus* L.). Field Crops Res. 13:99–115.

Sukhija, P.S., and D.L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36:1202–1206.

Trapani, N., A.J. Hall, V.O. Sadras, and F. Vilella. 1992. Ontogenetic changes in radiation use efficiency of sunflower (*Helianthus annuus* L.) crops. Field Crops Res. 29:301–316.

Vertregt, N., and F.W.T. Penning de Vries. 1987. A rapid method for determining the efficiency of biosynthesis of plant biomass. J. Theor. Biol. 128:109–119.