Tocopherol Oil Concentration in Field-Grown Sunflower Is Accounted for by Oil Weight per Seed

S.M. Nolasco[a,*], L.A.N. Aguirrezábal[b], and G.H. Crapiste[c]

[a]Tecnología de Semillas, Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Olavarría, Argentina, [b]Unidad Integrada Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata (UNMdP)—Estación Experimental Agropecuaria Instituto Nacional de Tecnología Agropecuaria (INTA) Balcarce, Argentina, and [c]Planta Piloto de Ingeniería Química (PLAPIQUI)—Universidad Nacional del Sur (UNS)—Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Bahía Blanca, Argentina

ABSTRACT: Tocopherols are natural antioxidants that increase the stability of fat-containing foods and perform important biological activities. Significant variations (389 to 1873 μg g oil^{-1}) in the total tocopherol concentration of sunflower seed oil have been reported. The main objectives of this work were to determine the influence of intercepted photosynthetically active radiation on tocopherol concentration during seed filling and to establish and validate relationships between tocopherol concentration in oil and other quality variables of the seed. Seven sunflower hybrids were grown under good water and nutritional conditions in two similar experiments carried out in two contrasting environments. Treatments were applied to modify the amount of radiation intercepted per plant during seed filling in order to obtain a range in oil yield per plant and its components. Greater per plant intercepted radiation decreased the tocopherol concentration in oil. Tocopherol concentration decreased when oil weight per seed increased. Tocopherol concentration stabilized for oil weight per seed higher than 23 mg oil seed^{-1}. This exponential relationship accounted for 73% of the variability in tocopherol concentration (507 to 1203 μg g oil^{-1}) despite differences in hull type, locations, hybrids, and radiation treatments. The proposed relationship acceptably predicted independent results. Crop management techniques could lead to seeds with greater concentrations of tocopherols.

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KEY WORDS: Intercepted solar radiation, oil quality, sunflower seed, tocopherol concentration.

Tocopherols are important biological and nutritive components (vitamin E) of human and animal food. They are natural antioxidants that inhibit lipid oxidation in foods and biological systems by stabilizing hydroperoxy and other free radicals (1,2). Vitamin E is only synthesized by higher plants and cyanobacteria. Eight natural compounds possess vitamin E activity: α-, β-, γ-, and δ-tocopherol and four closely related compounds termed tocotrienols. Tocopherols differ in their *in vitro* and *in vivo* antioxidant activities (1). Consequently, the biological and nutritive qualities of oil depend on both the total tocopherol concentrations and the gross composition of the oil.

The total tocopherol concentration in crude oil obtained from whole sunflower seeds typically varies between 447 and 900 μg g oil^{-1} (3) with extreme values varying from 389 to 1873 μg g oil^{-1} (4–7). α-Tocopherol typically represents most of 90% of tocopherol content of sunflower seed oil. Tocopherol levels in a refined, bleached, deodorized oil are affected by tocopherol concentration prior to seed crushing, the length and condition of seed and oil storage, and the extent of processing, especially deodorization (2,8).

Some researchers reported that the genotype (hybrid) could affect the total tocopherol content (9,10). On the other hand, several investigations showed important variation in tocopherol concentration of seed oil of the same sunflower hybrid grown under different environments, i.e., different locations and different sowing dates (4,7,11–13). However, the effect of environmental conditions during seed filling has received little attention. Some attention has been paid to the effect of temperature on tocopherol content (14–16), but the effect of intercepted photosynthetically active radiation (PAR) on total tocopherol content has not been investigated.

Solar radiation intercepted by plants represents the only energy source supporting their growth and metabolism. It affects the grain number (17), the weight per seed and the seed oil concentration (18,19), and the proportion of oil in the kernel (20). No or little effect of intercepted radiation during seed filling on total tocopherol concentration could be expected because the total energy cost needed to obtain this tocopherol level is low compared with the total energy cost required for other compounds in the oil, since the weight of tocopherol in the seed is very low. However, tocopherols are present in the seed in a small quantity (μg g^{-1}), which quantity in its turn depends on intercepted radiation (21). It is possible that changes in intercepted PAR per plant could indirectly affect the tocopherol concentration *via* changes in oil concentration, but the literature disagrees on this point. A negative correlation between oil and tocopherol concentration has been found in sunflower (13) and several mustards (22). However, Velaszco *et al.* (7) found no correlation between seed total tocopherol concentration and seed yield or seed oil concentration in sunflower.

*To whom correspondence should be addressed at Tecnología de Semillas, Departamento de Ingeniería Química, Facultad de Ingeniería, UNCPBA, Av. del Valle 5737, B7400JWI Olavarría, Argentina.
E-mail: snolasco@fio.unicen.edu.ar

This work aims, first, to study the effect of intercepted solar radiation per plant during seed filling on the tocopherol concentration of several sunflower hybrids with different potential for oil concentration in the seed and, second, to investigate the relationships between total tocopherol concentration and other quality parameters of the seed, especially the oil concentration.

EXPERIMENTAL PROCEDURES

Cultural details. Seed samples and other data were obtained from two experiments carried out in Argentina (Izquierdo, N., and M. Cantarero, unpublished data): one, hereafter referred to as experiment RB (Radiation Balcarce), at the INTA—Balcarce Experimental Station (37°45′ S, 58°18′ W), and the other, hereafter referred to as experiment RC (Radiation Córdoba), at Universidad Nacional de Córdoba (31°19′ S, 64°18′ W). Sowing dates were 17 November 1997 and 28 October 1997 for experiments RB and RC, respectively. The soil at Balcarce was a Typic Argiudol and at Córdoba, an Entic Haplustoll (23). Three hybrids with black-hull seeds (low oil concentrations, 440 g kg^{-1} or less) and three others with striped-hull seeds (high oil concentrations, 480 g kg^{-1} or more) were sown in the RB and RC experiments. The hybrids common to both experiments were: the striped-hull hybrids Morgan 734 (Mycoyen S.A., Colón, Argentina), Contiflor 3 (Advanta S.A., Balcarce, Argentina), and ACA 884 (Asociación de Cooperativas Argentinas, Pergamino, Argentina), and the black-hull hybrids Dekasol 3881 (Dekalb-Monsanto S.A., Camet, Argentina) and Contiflor 3 (Advanta S.A.). In addition, the black-hull hybrid Paraíso 6 (Nidera S.A., Junín, Argentina) was included in experiment RB, and the black-hull hybrid Orión (Sursem S.A., Pergamino, Argentina) was included in experiment RC. The only difference between black-hull and striped-hull Contiflor 3 was the quantitative trait loci (QTL) conferring the hull type trait in one of their parent lines. This QTL differed around 8.1 centiMorgan between lines (24). Thus, the hybrids differed in oil percentage.

Briefly, the experiments were designed as split-plots with main plots in a randomized complete block with three replicates. The hybrids were assigned to main plots and the radiation treatments to subplots. Each subplot had four rows 0.7 m apart and 6 m long. Final plant density was 60,900 plants ha^{-1} in experiment RB and 52,000 plants ha^{-1} in experiment RC. The mineral nutrition and water availability were not limiting for crop growth and yield. Pests and diseases were adequately controlled. Flowering of a plot (R5 stage; 25) occurred 79 and 75 d after emergence in experiments RB and RC, respectively.

Treatments to vary PAR intercepted per plant during seed filling [from the end of flowering to physiological maturity, R6 to R9 stages (25), respectively] were applied. The treatments were (i) 50% uniform shading with black, synthetic, and neutral mesh cloth (shaded treatment, S), (ii) 50% of the original plant density (thinned treatment, T), and (iii) untreated control (control, C). Further details on the treatments are given in Aguirrezábal *et al.* (21).

Measurements. Air temperatures were measured with copper-constantan thermocouples (Thermoquar, Buenos Aires, Argentina) at the capitulum level and recorded by a data logger (LI-1000; LICOR, Lincoln, NE). Daily mean air temperatures from treatment application to physiological maturity were 18.4 ± 3.3 and 21.1 ± 2.8°C in experiments RB and RC, respectively.

Global daily incident radiation was measured with a pyranometer (LI-200SB, LI-COR) located 400 m from experiment RB and 2000 m from experiment RC. Daily intercepted PAR was obtained according to the procedure described by Dosio *et al*. (19). It was determined on all hybrids in experiment RB and on Dekasol 3881 and ACA 884 in RC. Daily mean incident radiation for end of flowering (R6) to physiological maturity (R9 stage; 25) was greater in experiment RB (19.9 MJ m^{-2} day^{-1}) than in experiment RC (14.7 MJ m^{-2} day^{-1}). Considering both experiments and all treatments, a wide range of variation in intercepted PAR per plant was obtained. In experiment RB, the thinned treatment showed the highest cumulative PAR intercepted per plant during the period R6–R9 (81.5 ± 6.4 MJ $plant^{-1}$), and the shaded treatment showed the lowest (21.3 ± 1.8 MJ plant–1). The untreated control showed an intermediate value (39.2 ± 3.8 MJ $plant^{-1}$). In experiment RC, the mean of ACA 884 and Dekasol 3881 showed a similar trend (12.8 ± 3.3, 33.4 ± 0.1, and 54.0 ± 5.6 MJ $plant^{-1}$ for the shaded, control, and thinned treatment, respectively).

All plants were harvested at physiological maturity (R9 stage; 25). For each subplot, five heads in experiment RB and 10 heads in experiment RC were harvested. The seeds in each head were separated manually. The samples were oven-dried (with air circulating at 60°C) to constant weight and weighed as described by Dosio *et al.* (19). Weight per seed was obtained by dividing the weight of all nonempty seeds by the number of seeds.

Seed oil concentration was measured in duplicate samples by NMR spectroscopy (Analyzer Magnet Type 10; Newport Oxford Instruments, Buckinghamshire, England; 26) and averaged. Weight per seed and oil concentration were expressed on a dry weight basis. Moisture content was determined according to AOAC methods (27).

Seeds were ground in a mill and extracted with *n*-hexane (Soxhlet) at room temperature for 4 h and then at 69°C for the next 4 h. The solvent was evaporated in a rotary vacuum evaporator at 40°C.

Tocopherol concentrations of crude oils were determined by normal-phase HPLC using a Varian chromatography system (HPLC Varian Vista 5500, Varian Associates Inc., Palo Alto, CA) with a LiChrosorb Sil 60 (5 μm, 250 × 4.00 mm; Merck, Darmstadt, Germany) column. Approximately 0.5 g of each oil was dissolved with 5 mL of mobile phase. Twenty-microliter samples were injected for each determination. The column was eluted with 99.5:0.5 vol/vol *n*-hexane/isopropanol (HPLC solvent; J.T. Baker, Phillipsburg, NJ) at a flow rate of 1 mL min^{-1}. The tocopherols were detected, with a fluorescence detector with the excitation wavelength set at

290 nm and the emission wavelength at 330 nm, and quantified using a six-point external standard curve (28).

Data analysis. In each experiment, data on tocopherol concentration in oil and tocopherol concentration in seed were analyzed using an ANOVA procedure (software MSTAT-C Version 2.1, Crop and Soil Sciences Department, Michigan State University). When statistical differences were detected in more than one experiment, the highest *P* value is the only one presented. Differences among treatment means were evaluated with the Tukey test ($P < 0.05$).

Regression analyses were used to determine the relationships between tocopherol concentration in oil and other quality variables of seed.

Validation of relationships between total tocopherol concentration in oil and oil weight per seed. The relationship established between tocopherol concentration in oil and oil weight per seed (mg oil seed^{-1}) was validated by comparison with independent experimental data.

The set of data was obtained from the "Yield Trials Network Buenos Aires Sur and La Pampa" (VE1), which includes four different locations (situated between latitudes 34° and 37° and longitudes 58° and 64°), and the "High Oleic Argentine Yield Trials Network" (VE2), which includes three different locations (situated between latitudes 35° and 38° and longitudes 58° and 60°; 30). Data for validation corresponded to the hybrid ACA 884, the only hybrid common to both yield trial networks and the RB and RC experiments (29).

RESULTS AND DISCUSSION

Intercepted PAR effects during seed filling on total tocopherol concentration in sunflower oil. Total tocopherol concentration in the seed oil varied from 507 to 771 μg g oil^{-1} (mean = 623 ± 18 μg g oil^{-1}) in experiment RB and from 621 to 1203 μg g oil^{-1} (mean = 839 ± 36 μg g oil^{-1}) in experiment RC. The range was similar to that reported by Marquard (480 to 1128 μg g oil^{-1}; 13), and the maximum value was lower than that found by Velasco *et al.* (1872.8 μg g oil^{-1}; 7). Seventeen percent of the samples showed higher tocopherol concentrations than those reported by Gunstone *et al.* (3) as typical values of the species. Total tocopherol concentration in oil varied according to the hybrid. These differences were not related to the color of the hull as shown in Figures 1A and 1B. Total tocopherol concentration in oil was higher in experiment RC than in experiment RB. For similar radiation treatments (shaded, control, and thinned), the incident radiation in experiment RC was different from the incident radiation in experiment RB. The interception proportion was determined only on hybrids Dekasol 3881 and ACA 884 in experiment RC. As a consequence, the effect of radiation on the total tocopherol concentration in oil was analyzed independently in each experiment.

ANOVA showed a significant interaction between hybrids and treatments for total tocopherol concentration in oil ($P \leq 0.0007$), indicating that the treatment effects were not similar in all the hybrids. In general, a higher intercepted PAR per plant decreased the total tocopherol concentration in oil. However, the Morgan 734 hybrid exhibited the inverse trend in experiment RB. Paraiso 6 hybrid had the highest total tocopherol concentration in the control. Intercepted PAR during seed filling affected total tocopherol concentrations in oil inversely to the effects observed on the weight per seed and the seed oil percentage, which increased when intercepted radiation increased for a wide range of intercepted radiation (18,19,21). Statistically significant differences between treatments were detected in all the hybrids, with the exception of Black Hull Contiflor 3 in experiment RB, and ACA 884 and Striped Hull Contiflor 3 in experiment RC (Figs. 2 and 3). Dekasol 3881 hybrid showed the maximal difference between treatments (439 μg tocopherol g oil^{-1}).

Both α-tocopherol and β-tocopherol were detected in all samples. α-Tocopherol represented the greatest proportion of total tocopherol, ranging from 90.4 to 99.4% (mean = 96.1 ± 0.4%). The predominance of α-tocopherol in sunflower oil is in agreement with previous data in the literature (5,7,10,29). The concentration of α-tocopherol (μg g oil^{-1}) was found to be closely related to the total tocopherol concentration ($R^2 = 0.994$; $P < 0.0001$), indicating that variation in the total tocopherol concentration was almost entirely explained by the variation in α-tocopherol. β-Tocopherol represented only 3.9

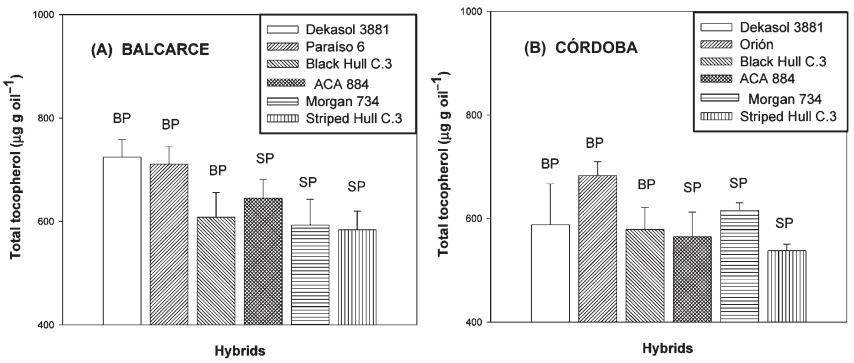

FIG. 1. Total tocopherol concentration of hybrids grown at (A) Balcarce and (B) Córdoba. Vertical bars represent the SD. BP = black-hull hybrid. SP = striped-hull hybrid. Data correspond to control treatment.

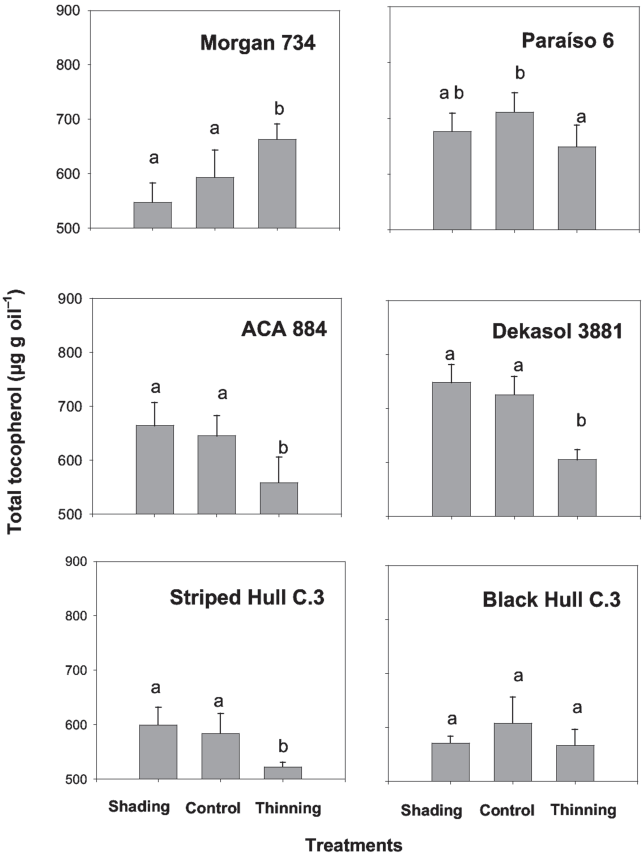

FIG. 2. Total tocopherol concentration of different hybrids grown at Balcarce for each of the treatments applied. Means accompanied by the same letter for each hybrid are not significantly different at $P < 0.05$ using Tukey's method. Vertical bars represent the SD.

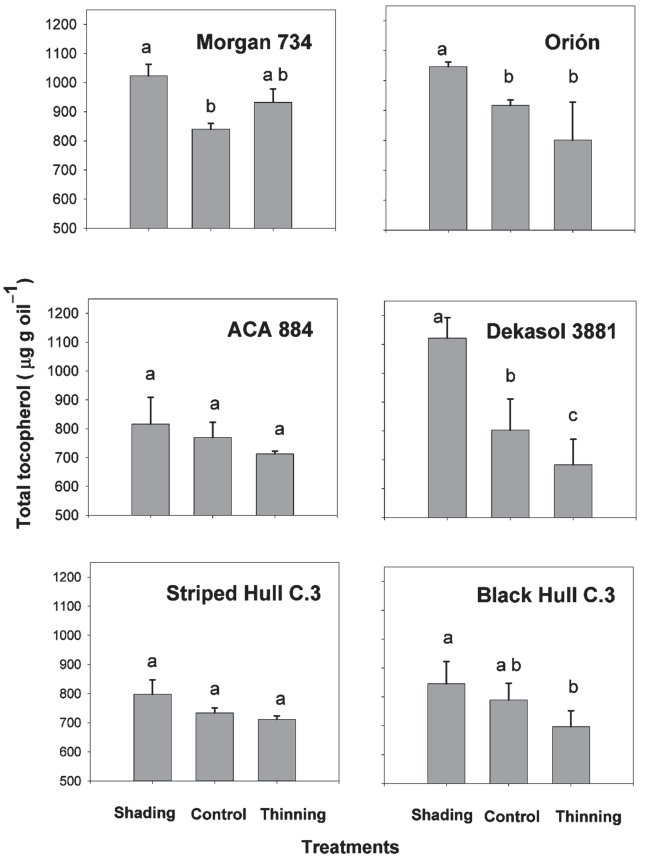

FIG. 3. Total tocopherol concentration of different hybrids grown at Córdoba for each of the treatments applied. Means accompanied by the same letter for each hybrid are not significantly different at $P < 0.05$ using Tukey's method. Vertical bars represent the SD.

± 1.8% of the total tocopherol and varied from 4 to 59 µg g oil^{-1}. No relation between β-tocopherol concentration and the total tocopherol concentration ($R^2 = 0.019$; $P = 0.163$) was found in our experiments. This result disagrees with that of Velasco *et al.* (7), who determined a positive relationship between both concentrations.

Significant interaction between hybrids and treatments ($P \leq 0.0001$) was detected for the α-tocopherol. The effect of the treatment on the α-tocopherol concentration in oil was similar to that detected for the total tocopherol oil concentration in all the hybrids. However, hybrid per treatment interaction was not found for β-tocopherol ($P > 0.05$). The treatments did not affect significantly the β-tocopherol concentration ($P \geq 0.216$) in experiment RB, whereas the concentration was considerably lower in the thinned treatment compared with the shaded and control treatments in experiment RC.

Relationships between total tocopherol concentration in the oil and other quality variables of the seed. The treatments applied and the study of different genotypes provided samples that covered a wide range of total tocopherol concentration in oil and in different quality characteristics of the seed (oil percentage, hull color, weight per seed, yield per plant, and the number of seeds per plant). Mean weight per seed and oil concentration showed significant variations considering all the samples in a whole obtained in each experiment. Mean weight per seed ranged from 35.4 to 71.2 mg in experiment RB and from 22.4 to 54.3 mg in experiment RC. Oil concentration varied from 41.4 to 56.8% in experiment RB and from 34.9 to 50.7% in experiment RC. Both mean weight per seed and oil concentration were affected by intercepted PAR, so they were higher in the thinned treatment and lower in the shaded treatment (Izquierdo, N., unpublished data).

The total tocopherol concentration in oil diminished as the seed yield per plant and the oil yield per plant ($P < 0.0001$, $n = 104$) increased. Curvilinear relationships accounted for the variation of total tocopherol to only a low extent ($R^2 = 0.372$ and $R^2 = 0.437$, respectively). This could be partly explained by the fact that both yields closely depend on the number of seeds per plant. Determination of this yield component began approximately 30 d before anthesis. The seed number per plant varied significantly between experiments but only slightly with treatment effects.

Oil weight per seed was computed, and the relationship between total tocopherol concentration in oil and oil weight per seed was tested. The latter showed an exponential decay that can be represented as $Y = C_1 + C_2 * \exp(-C_3 * X)$ with $C_1 = 562.8 \pm 32.5$; $C_2 = 1451.3 \pm 291.8$; and $C_3 = 0.1249 \pm 0.03$, where Y = µg of total tocopherols g oil^{-1} and X = mg of oil

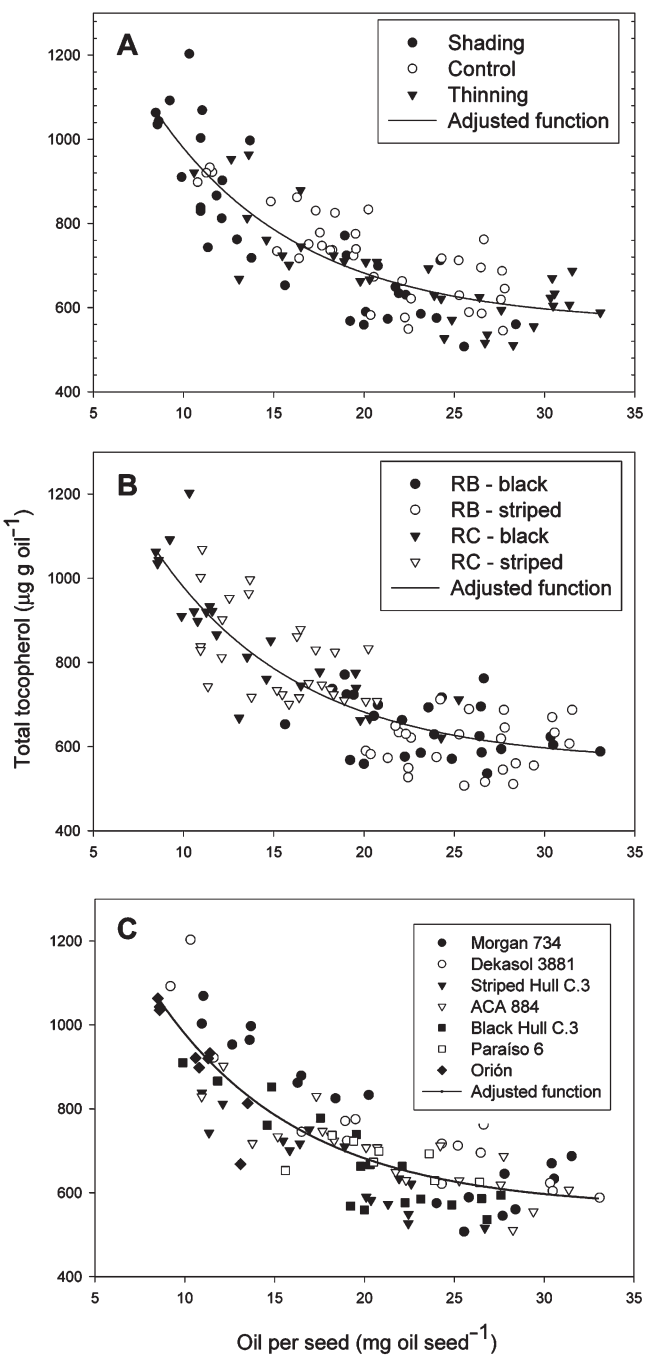

FIG. 4. Total tocopherol concentration as a function of oil weight per seed, over all hybrids and treatments (experiments RB and RC) differentiated between (A) treatments, (B) location and hull color, and (C) hybrids. The symbols correspond to values obtained experimentally. The line represents the established relationship.

seed^{-1} ($P < 0.0001$; $n = 104$). This function accounted for 73% of the variability in total tocopherol concentration in the oil, regardless of the treatment applied (Fig. 4A), hull color (Fig. 4B), the location of the experiment (Fig. 4B) or the hybrid studied (Fig. 4C).

As the oil weight in seed increased (mg oil seed^{-1}), the tocopherol weight per seed also increased (µg total tocopherol seed^{-1}), but generally in lower proportion to the oil weight increase (Fig. 5). These results suggest a nonstoichiometric relationship between the accumulation of tocopherol and the oil, as has been suggested by other authors (2,15). Although both substances are synthesized during grain filling, their synthetic pathways are independent (2,15).

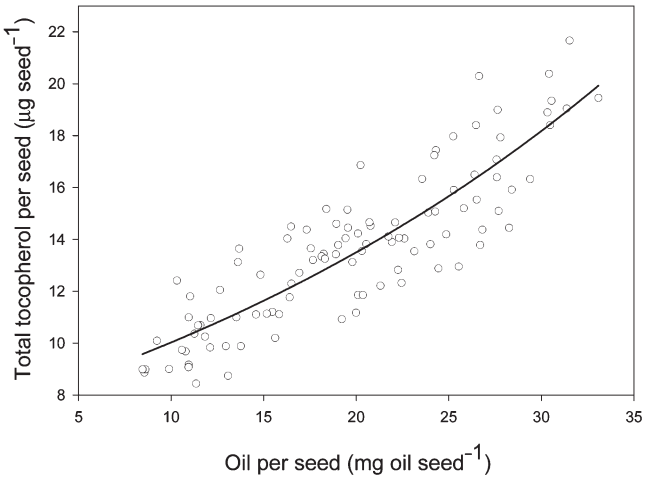

FIG. 5. Total tocopherol weight per seed as a function of oil weight per seed. The adjusted function is $Y = 7.45 * \exp(0.0297 * X)$, where Y = total tocopherol weight per seed and X = oil weight per seed ($R^2 = 0.764$; $P < 0.0001$; $n = 104$). Data correspond to values obtained in experiments RB and RC for different hybrids and treatment of the solar radiation.

For a wide range of total tocopherol weights per seed (approximately 8.4–14.7 µg seed^{-1}, which corresponds to lower levels of intercepted radiation), the effect of intercepted radiation on oil weight per seed was higher than the effect on tocopherol concentration. The oil accumulated in a greater proportion, thus causing a decrease in the tocopherol concentration (Fig. 4). At an oil weight per seed higher than 23 mg seed^{-1} (Fig. 5), the effect of solar radiation was approximately proportional to the oil and tocopherol accumulation, since their relationship tends to be constant (Fig. 4). The oil weight per seed (mg oil seed^{-1}) and the tocopherol weight per seed (µg of total tocopherols seed^{-1}) varied proportionally in this range. The explanation for this behavior could be found in tocopherol biosynthesis and/or in the tocopherol location in the seed structure and cotyledon cells. Further research on these items is necessary to understand the underlying mechanisms that explain the results of our regression analysis.

The relationship obtained suggests that PAR during seed filling would affect the tocopherol content in seed (µg of tocopherols seed^{-1}). In both experiments (experiment RB and experiment RC), differences between treatments were statistically significant for the total tocopherol per seed (µg of tocopherols seed^{-1}). The hybrid × treatment interaction was not statistically significant ($P > 0.100$). In general, the shaded treatment, which showed the smallest seeds, presented the lowest total tocopherol weight per seed.

The intercepted PAR during seed filling affected the oil percentage, the tocopherol concentration in oil, and the total

tocopherol weight per seed. Nevertheless, the results obtained suggest that for an important range of variation in the oil weight per seed, the effect of the radiation intercepted on the tocopherol concentration in oil originates mainly from variations in oil concentration rather than from a direct effect on the total quantity of tocopherol per seed.

Significant relationships between the total tocopherol concentration (μg g oil^{-1}) and the oil concentration (%) (R^2 = 0.290; $P < 0.0001$; $n = 108$) as well as with the weight per seed ($R^2 = 0.704$; $P < 0.0001$; $n = 104$) were found. These relationships were expected since the oil weight per seed (mg oil seed^{-1}) is the product of both variables. However, oil weight per seed better explained total tocopherol concentration than its components. Moreover, tocopherol concentration as a function of oil weight per seed of near isohybrids Black Hull Contiflor 3 and Striped Hull Contiflor 3, which differed in oil percentage (46.5 ± 3.8 vs. 41.5 ± 2.7%), highly overlapped (Fig. 4C).

The relative weight of the effect of oil percentage and weight per seed on total tocopherol content was assessed by multiple linear regression. All variables were normalized with their highest values. The relationship obtained was: $Z = -0.50\ W_1 + 0.80\ W_2$ ($R^2 = 0.990$; $n = 108$), where Z, W_1, and W_2 represent total tocopherols concentration in oil, seed oil concentration (%), and seed weight, respectively. This shows that the changes in the concentration were mainly affected by changes in weight per seed and, in a lower proportion, by modifications in the oil percentage. This result partially coincides with the data obtained by Marquard *et al.* (22), who worked with different species of the Cruciferae family and showed an important inverse relationship between the tocopherol concentration and the oil percentage. However, in sunflowers, Velaszco *et al*. (7) found no correlation between seed total tocopherol content and seed yield or kernel oil content. The relationships between oil weight per seed and tocopherol concentration in oil has not been investigated in previous work, so it is not possible to verify whether the relation found in this work is similar in other oil crop species.

Validation of the relationship between total tocopherol concentration in oil and oil content per seed. In VE1 and VE2, the hybrid ACA 884 showed a variation of oil percentage between 40.7 and 50.1%, a weight per seed between 29.3 and 81.1 mg seed^{-1}, and a total tocopherol concentration between 680 and 997 μg g oil^{-1}. The relationship between the total tocopherol concentration in oil and oil weight per seed in these experiments showed the same trend as the one observed in experiments RC and RB (Fig. 6). This trend was confirmed by the linear relationship between the experimental values of the tocopherol concentration in the hybrid ACA 884 and those predicted from the function established using data from experiment RC and RB ($R^2 = 0.847$; $P < 0.0001$; $n = 18$), whose slope was close to one (0.951 ± 0.10; $P < 0.0001$). Its intercept was greater than zero (156 ± 76, $P = 0.0328$), thus showing a systematic underestimation. The slight underestimation observed in the predictions suggests that tocopherol concentration may be affected by other variables not studied in this work.

A unique exponential relationship accounted for 73% of the important variation obtained in the tocopherol concentration in oil. This relationship obtained between the total tocopherol concentration in oil and the oil weight per seed could be useful for predicting the tocopherol concentration in sunflower hybrids grown in different environments, which produce weights in oil per seed between 8.5 and 33 mg seed^{-1}. The relationship established and validated could be useful to obtain seeds with a greater quantity of tocopherols. It could be used to improve crop management and as a simulation tool coupled to models of sunflower growth and yield (30) or to other simple tools of simulation of the oil weight per seed (21). In addition, the use of this relationship could be useful in the commercialization field and/or seed processing. The tocopherol concentration of a seed lot could be estimated by this relationship from the mean weight per seed and the oil percentage. These two determinations are simpler, faster, and less expensive than the determination of the total tocopherol concentration in oil.

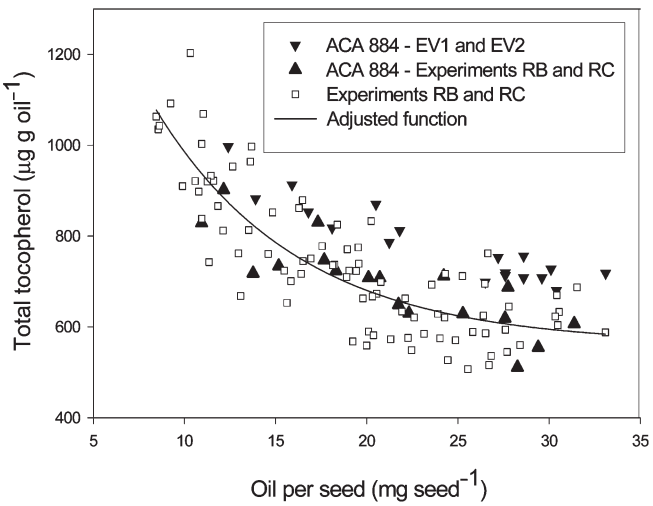

FIG. 6. Total tocopherol concentration in oil as a function of oil weight per seed, including all the values obtained in experiments RB and RC (data of the ACA 884 hybrids are distinct), and the values of the ACA 884 hybrids obtained in VE1 and VE2.

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