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Dynamics of phytosterols content and concentration in sunflower grains

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Abstract. Phytosterols are allies in the control of plasma cholesterol and in preventing cardiovascular diseases. As vegetable oils are the main source of phytosterols, characterising environmental factors that determine phytosterols accumulation in the oil is an important objective. The present research focuses on evaluating how intercepted solar radiation (ISR, the main environmental factor affecting oil accumulation) can determine phytosterol accumulation in sunflower oil. The aim of this work was to study the dynamics of phytosterols accumulation under different ISR levels and its relationships with the dynamics of oil accumulation. Two field experiments were conducted with hybrids with different fatty acid composition. Treatments applied during grain filling were: two levels of defoliation (75% and 80%) and a control. A 50% grain thinning treatment was also applied. Oil phytosterols concentration increased with defoliation during grain-filling period, whereas phytosterols content per grain decreased. β-sitosterol and campesterol were the most affected sterols. Reduction in ISR did not affect the rates of phytosterols accumulation. The durations of the accumulation period of these components varied in accordance with the duration of oil accumulation period. These results reinforce the importance of environmental factor in determining oil quality in sunflower grains.

Additional keywords: fatty acid composition, *Helianthus annuus*, intercepted solar radiation, oil quality, oil per grain, phytosterols composition.

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Introduction

Plant sterols, also called phytosterols, are steroid alcohols. They resemble cholesterol, the predominant sterol found in animals, both in their chemical structure and their biological function (Piironen *et al*. 2000). Phytosterols have a very important role in maintaining the fluidity and permeability of plant membranes during embryogenesis and as precursors of brassinosteroid hormones, which are involved in plant growth and development (Clouse 1996; Lindsey *et al*. 2003; Schaller 2003; Merah *et al*. 2012). As components of the human diet, phytosterols have been associated with anti-inflammatory properties (Valerio and Awad 2011) and decreased incidence of atherosclerosis and cardiovascular diseases (Hansel *et al*. 2011). However, their effect on decreasing the total amount of cholesterol in blood and, primarily low-density lipoprotein (LDL), is the best characterised and scientifically proven beneficial effect of phytosterols in the human body. A meta-analysis of 41 trials showed that intakes of 2 g of plant sterols/day reduced LDL by 10% (Schwartz *et al*. 2008). Therefore, these compounds are allies in the control of plasma cholesterol and in the prevention of cardiovascular diseases (Palou *et al*. 2005; Ostlund 2007; Brufau *et al*. 2008; Roche *et al*. 2010*b*). In a context where adding value in origin has acquired importance for maintaining and exploring new markets, identifying, characterising and quantifying the variability of these compounds brings knowledge to the oil industry to increase the value of edible oils. Knowing the factors that affect the accumulation of phytosterols and their relationships with other constituents of the grain (e.g. oil content per grain) could serve as a tool to select grain consignment or oils with higher concentration of these bioactive compounds, and to design management strategies in that direction.

Oil phytosterols concentration has inter- and intra-specific variability. There were reported oil phytosterols concentration ranges of 8.1–15.6, 1.4–1.5, 5.1–9.8 and 2.3–4.6 g/kg for corn, olive, rapeseed and soybean, respectively (Piironen *et al*. 2000).

In sunflower, Nolasco *et al*. (2010) observed variations in these compounds between 3513 and 4936 mg/kg oil in hybrids from different environments. These variations are function of the effects of the environment during grain filling on the dynamics of accumulation of oil and phytosterols in the grain. In a genotype of the same species, Roche *et al*. (2010*a*) found differences of up to 118 mg of phytosterols/100 g seed due to changes in sowing date. The reasons that cause such variation in the concentration of phytosterols, even within the same genotype, are unknown. Some of the variability in the concentration of these compounds in sunflower seeds and oil could be explained by the source available for their synthesis during grain filling (e.g. intercepted solar radiation (ISR) by plants) and its relationships with oil synthesis, as previously observed for other minor component (e.g. tocopherol concentration) (González Belo *et al*. 2017). It is known that treatments varying ISR per plant during gran filling modify the amount of oil per grain and other grain components (Aguirrezábal *et al*. 2003; Izquierdo *et al*. 2008), such as tocopherol per grain (Izquierdo *et al*. 2011; González Belo *et al*. 2017) and fatty acid composition within each genotype (Izquierdo *et al*. 2009). For example, lowering the interception of solar radiation reduced the amount of tocopherols and oil in sunflower grains but increased oil tocopherol concentration (Izquierdo *et al*. 2011; González Belo *et al*. 2017), the latter caused by a more pronounced reduction of oil than tocopherol content. There is no evidence in the literature to conclude if the same effect occurs for phytosterols content per grain and oil phytosterols concentration in sunflower. As the effect of the environment on oil accumulation in sunflower has been widely studied, understanding these effects on phytosterols accumulation could help to understand the variability observed in oil phytosterols concentration.

Both oil and phytosterols are synthesised from acetyl-CoA (Merah *et al*. 2012). It is therefore expected that increases in ISR not only increase the amount of oil, but also phytosterols ones. Additionally, it is unknown if all phytosterols are affected in the same way by ISR. This is important because not all phytosterols present the same activity, as β-sitosterol and campesterol are the best allies for reduce cholesterol levels (Brufau *et al*. 2008). Variations on phytosterols composition were reported in the literature. For example, Anastasi *et al*. (2010) observed that Δ^7-stigmastenol was more affected by a water deficit than the other phytosterols, whereas the most abundant phytosterols in sunflower, β-sitosterol was slightly affected. This suggests a differential effect of environmental conditions on the different phytosterols and accordingly a putative effect on their final composition. Consequently, it is important to understand the dynamics of accumulation of phytosterols, as they may explain part of the variability of the amount and concentration of these compounds in sunflower oil.

Intercepted solar radiation affects both the amount of oil per grain (Andrade and Ferreiro 1996; Dosio *et al*. 2000; Aguirrezábal *et al*. 2003; Izquierdo *et al*. 2008), and its degree of unsaturation (Izquierdo *et al*. 2009; Echarte *et al*. 2012). The latter is also affected by the genotype, as there are mutations that modify the biosynthesis of fatty acids and therefore oil fatty acid composition. Genotypes with these mutations present this trait more stable to variations in ISR than Traditional ones (Martínez *et al*. 2012). However, the effect of these mutations on the stability of phytosterol content and composition to ISR is unknown. Accordingly, the aim of this work was to analyse the dynamics of phytosterols accumulation under different ISR levels and its relationship with the dynamics of oil accumulation during grain filling. Different grain-filling conditions were obtained by modifying the interception of radiation (source for grain components synthesis) and the number of grains (sink) to fill with that source. In addition, we included genotypes with different potential grain oil concentration and fatty acid composition. It is known that the mutation in genotypes with modified fatty acid composition affect the response of oil quality to environmental condition (Martínez *et al*. 2012; Zuil *et al*. 2012; Izquierdo *et al*. 2013; Izquierdo *et al*. 2016). So it is important to analyse the synthesis of other quality traits, as phytosterols, in these genotypes.

Materials and methods

We performed two field experiments in order to analyse the dynamics of phytosterols accumulation under different ISR levels. Both experiments included (*i*) genotypes with different potential grain oil concentration and fatty acid composition, and (*ii*) treatments to modify the amount of substrate for oil and phytosterols synthesis during grain filling. The genotypes were a traditional, a high oleic and a high stearic–high oleic, being the first two the most sown by farmers.

The experiments were performed in Balcarce (37°S, 58°W), Argentina. Sowing dates were: 30 October 2012 (Expt A) and 22 October 2014 (Expt B, Table 1). In Expt A, three genotypes were sown: a traditional (Macon, Syngenta), a high oleic (HO, Olisun 2, Advanta Seeds SAIC) and a high stearic–high oleic (HSHO, HS05, Advanta Seeds SAIC, Balcarce, Argentina). The Traditional and HSHO genotypes were also sown in Expt B. The experiments were carried out with a randomised complete block split plots design with three replications. Genotypes were assigned to the main plots and treatments to modify the source (ISR) or sink (number of grains) were assigned to the subplots. Each subplot consisted of six rows, 9 m long and 0.70 m apart, with a plant density of 7 plants/m^2. Source or sink treatments were applied at the beginning of grain filling (R_6, Schneiter and Miller 1981). Treatments were (*i*) removal of 75% ($D_{75\%}$) or 80% ($D_{80\%}$) of the leaves, (*ii*) removal of 50% of the grains ($R_{50\%}$) and (*iii*) control (T) (Table 1). Plants phenology was recorded as Schneiter and Miller (1981). The heads were covered with polyamide bags before flowering to prevent cross-pollination. In all experiments, weeds and pests were controlled and water and nutritional stress was prevented by irrigation and fertilisation. Temperature and ISR were measured. The photosynthetic active radiation (PAR) intercepted per plant was measured according to Izquierdo *et al*. (2011). PAR intercepted per plant was accumulated from R_6 to physiological maturity. A wide range of ISR per plant and oil per grain was explored among treatments and experiments (Table 1).

Grain samples were collected every 3−4 days during grain filling. Yield components (weight per grain and grain number), oil content (Robertson and Morrison 1979) and phytosterol

Table 1. Description of hybrid, sowing and flowering date, treatment and range of intercepted solar radiation (ISR) from R_6 to R_9 and oil per grain on each experiment

Expt	Sowing date	Hybrid		Flowering date	Treatments	ISR (MJ/plant)	Oil per grain (mg)
A	30 October 2012	Macon	Traditional	8 January 2013	$D_{75\%}$	9.9–12.9	19.5–23.7
					Control	23.1–31.3	24.8–26.7
					$R_{50\%}$	23.4–27.7	28.7–31.6
	30 October 2012	Olisun 2	HO	15 January 2013	$D_{75\%}$	8.3–12.3	18.9–19.2
					Control	18.0–25.5	19.7–27.9
					$R_{50\%}$	17.7–29.5	28.0–29.5
	30 October 2012	HS05	HSHO	8 January 2013	$D_{75\%}$	5.0–7.3	12.4–13.9
					Control	20.8–21.5	16.4–18.7
					$R_{50\%}$	17.5–21.8	14.7–19.6
B	22 October 2014	Macon	Traditional	5 January 2015	$D_{80\%}$	7.8–8.0	24.0–26.9
					Control	14.9–20.8	29.1–34.5
	22 October 2014	HS05	HSHO	4 January 2015	$D_{80\%}$	7.7–10.6	14.7–15.3
					Control	18.3–21.6	18.6–20.9

content and composition (Fernández-Cuesta *et al.* 2012) were determined in all samples. Data were analysed with analysis of variance (ANOVA). Differences among treatments were evaluated with Tukey's test ($P < 0.05$). Linear-plateau adjustments were performed to compare the rates of phytosterols accumulation and the duration of the accumulation period among treatments. Analysis was carried out with the R statistical package (R CORE TEAM 2012). The dynamics of oil accumulation were taken from González Belo *et al.* (2017).

Results

Phytosterol per grain

The phytosterols content per grain varied between 60.1−108.9 µg and between 66.3−107.5 µg in Expt A and B, respectively. The interactions genotypes × treatments were not statistically significant for phytosterols content per grain ($P > 0.0766$). The HO hybrid, evaluated only in Expt A, presented similar total phytosterols per grain than the Traditional one. In both experiments, the HSHO hybrid presented lower content of phytosterols per grain than the Traditional one, partially explained by its lower grain weight (53.8 vs 38.8 mg for Traditional and HSHO genotypes, respectively). Treatments affected the phytosterols per grain in Expt A, where $D_{75\%}$ and $R_{50\%}$ were those with the lowest and highest contents, respectively (Fig. 1). In Expt B, a similar trend was observed between control and $D_{80\%}$ but differences were not statistically significant ($P > 0.7071$).

The most abundant phytosterol was β-sitosterol, which represented more than 50% of total phytosterols. Campesterol, stigmasterol and Δ^7-stigmastenol represented each between 7% and 16% of total phytosterols. Minor concentrations of Δ^7-avenasterol and other phytosterols were detected. Some differences for phytosterol composition were observed among genotypes (Fig. 1), mainly between the HSHO and the Traditional one. In both experiments, the HSHO hybrid presented the smallest percentage of β-sitosterol and the highest concentrations of campesterol and stigmasterol. The HO genotype presented a phytosterol composition similar to that of the Traditional one.

The phytosterol composition was scarcely affected by treatment. The percentage of the main phytosterol, β-sitosterol, did not vary statistically among treatments ($P > 0.0551$, Fig. 1) and only minor variations among treatments were observed in the percentage of the other phytosterols. Thus, the effect of treatments on the amount of each phytosterol per grain, mainly β-itosterol and campesterol, are similar to those described for total phytosterols per grain (Fig. 1).

Dynamics of phytosterol accumulation

The accumulation of phytosterols in the grain presented a bilinear response, with a linear increase during all the period of accumulation and a final plateau. So, data of phytosterols content per grain during grain filling were fitted to linear−plateau models to compare accumulation rates and durations of the accumulation periods among genotypes and treatments. As an example, the dynamics of oil and phytosterol content per grain for the control treatment of the Traditional and HO genotypes from Expt A are shown in Fig. 2. The evolution of oil phytosterols concentration during grain filling was also plotted, with a high initial concentration followed by a sharply decrease and then kept constant until physiological maturity.

The rates of total phytosterols accumulation varied from 2.45 to 4.74 µg phytosterols/day among treatments, genotypes and experiments (Table 2). This wide range was mainly due to differences among genotypes. Traditional and HSHO genotypes presented the highest and lowest accumulation rates for total and each phytosterols. In general, no effects of treatments were observed on the accumulation rates of phytosterols. The variability in phytosterols accumulation rate observed among treatments was not accounted for by ISR ($P > 0.7027$).

The duration of the period of phytosterols accumulation varied between 28 and 38 days (Table 2). This range of variation was mainly caused by treatments, as in general the duration of this period was similar in all genotypes. In most cases, this period decreased when defoliation was applied in Expt A. This effect was more evident for total phytosterols and β-sitosterol contents, for which defoliation decreased up to 6 days the duration of the accumulation period compared with the control. $R_{50\%}$ treatment did not increase the duration of this period compared with the control. In Expt B, treatments did not affect the duration of the accumulation period. An increase in ISR linearly increased the duration of the period of phytosterols

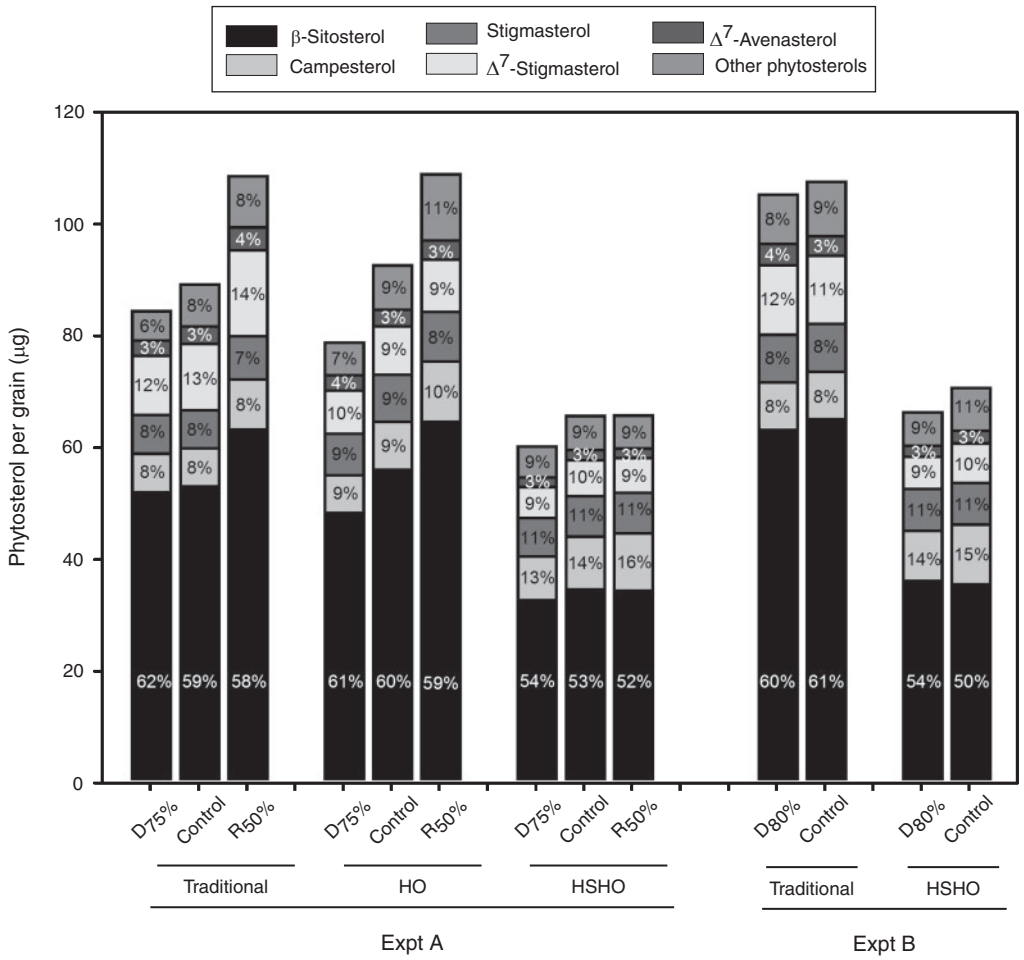

Fig. 1. Genotype and treatments effects on phytosterols content per grain for Expt A and B. Numbers within each bar are the percentage of each phytosterol. Genotypes are: a traditional, a high oleic (HO) and a high stearic–high oleic (HSHO). Treatments are: defoliation of 75% ($D_{75\%}$) or 80% ($D_{80\%}$), grain thinning of 50% ($R_{50\%}$) and a control.

accumulation, accounting for 56% of the variability in this period.

Total phytosterol per grain increased when the durations of the accumulation periods increased in Traditional and HO genotypes, accounting for 52% of these variations ($P < 0.0419$). There was no relationship between these variables in HSHO genotype. The accumulation rate did not account for the variability in total phytosterols per grain ($P > 0.081$).

Phytosterols accumulation followed similar dynamics as oil accumulation. For Expt A and B, oil per grain varied from 13.0 to 31.7 mg via variations in rate and duration of the accumulation period (González Belo *et al*. 2017). A close relationship was observed between the durations of these periods (Fig. 3), where the slope did not differ from 1 ($P > 0.7384$). This association is explained because the duration of both periods were related to ISR during grain filling ($P < 0.0033$ and $R^2 = 0.559$ for phytosterols and $P < 0.00289$ and $R^2 = 0.569$ for oil, data not shown).

Oil phytosterols concentration

Oil phytosterols concentration decreased up to 29 days after flowering and remained constant until physiological maturity (Fig. 2). The variations in oil and phytosterols per grain were reflected in the final oil phytosterols concentration, which varied from 3463 to 4624 µg/g (Expt A) and from 3400 to 4403 µg/g (Expt B). There were no interactions between genotype and treatments for oil phytosterols concentration in both experiments ($P > 0.3523$). In Expt A, the Traditional hybrid presented lower oil phytosterols concentration than HO and HSHO ones, whereas in Expt B, differences between genotypes were not statistically significant (Fig. 4*a*). In both experiments, oil phytosterols concentration was 17% higher in defoliation treatments than in the controls (Fig. 4*b*). Thinned treatment did not modify oil phytosterols concentration compared with the control.

Discussion

The dynamics of phytosterols accumulation in sunflower grains were affected by the ISR levels. These compounds linearly increased during grain filling and reached a final plateau. In general the rate of accumulation was constant, but the duration of the accumulation period depended on ISR, being longer when the ISR increased. The bi-linear behaviour for phytosterols accumulation was reported by Roche *et al*.

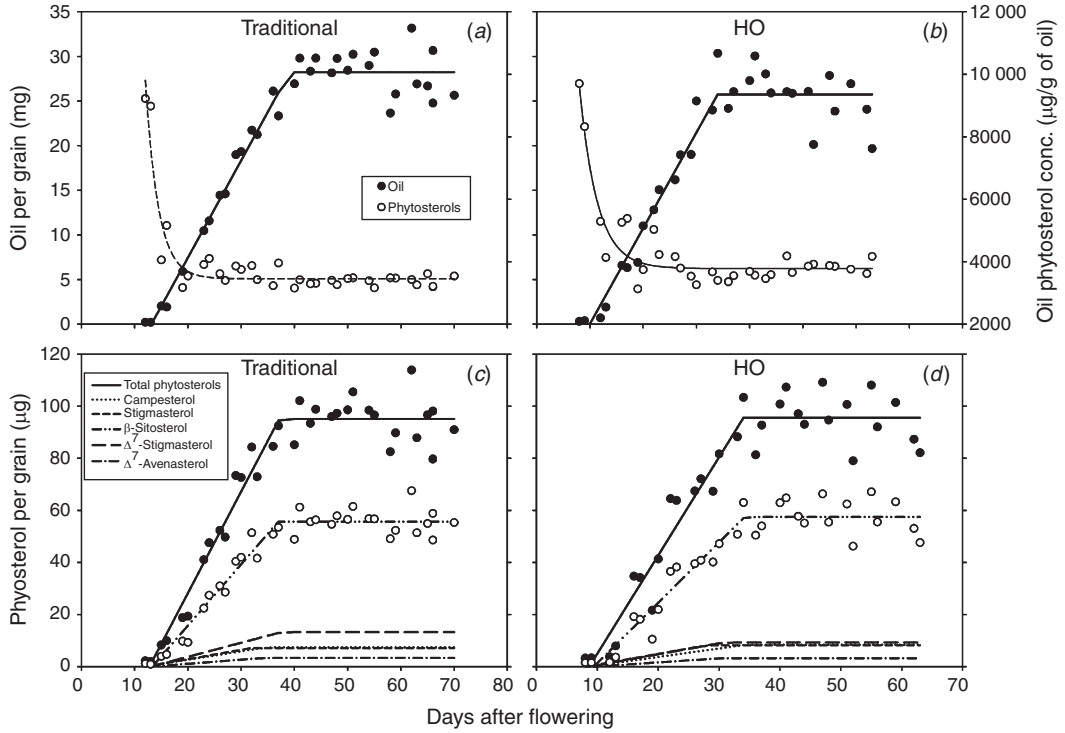

Fig. 2. Dynamics of oil and oil phytosterols concentration (*a–b*) and the contents of total phytosterols and the most abundant phytosterols per grain (*c–d*) during grain filling for control treatment of the traditional and high oleic (HO) genotypes from Expt A. In all figures, each data point corresponds to an observation. For minor sterols, only the adjusted functions are presented. R^2 of adjustments were between 0.85 and 0.96.

Table 2. Rates of accumulation (µg/day) and durations of the accumulation periods (days) of total phytosterols per grain during grain filling for genotypes and treatments in Expt A and B

Values followed by same lowercase letters indicate no significant differences among treatments. Values followed by same uppercase letters indicate no significant differences among genotypes

	Expt	Treatment	Rate						Duration					
			Traditional		HO		HSHO		Traditional		HO		HSHO	
Total phytosterols	A	$D_{75\%}$	4.74	aA	4.41	aAB	3.34	aB	31	bA	28	bB	29	bAB
		Control	3.90	aA	3.77	aA	2.45	bB	37	aA	34	aA	35	aA
		$R_{50\%}$	4.13	aA	4.35	aA	2.80	bB	38	aA	34	aA	34	aA
	B	$D_{80\%}$	3.30	aA	–	–	2.78	aA	34	aA	–	–	29	aB
		Control	3.37	aA	–	–	3.50	aA	38	aA	–	–	32	aB

(2016). These authors observed a slight decrease in seeds phytosterols concentration at the end of grain filling, similar to that observed in our work. This decrease could be associated to the conversion of sterols to vitamin and hormones that regulate plant growth and development (Vriet *et al*. 2013). However, Roche *et al*. (2010*a*) hypothesise that the slight decrease of phytosterols at the end of this period could be explained by a decrease in β-sitosterol content due to its antioxidant capacity and its role in protecting the embryo during seed desiccation. Zlatanov *et al*. (2009), studying high oleic sunflower genotypes reported that 74% of the free sterols are formed in the first 15 days and their content increases to 86% by the 90th day after flowering. However, these authors explored a period much longer than the typical grain-filling duration in sunflower (30–45 days, Mantese *et al.* 2006; Andrianasolo *et al.* 2016) as was observed in our work. Similar results to those observed in our experiments were reported for sunflower genotypes by Roche *et al*. (2010*a*) and Roche *et al*. (2016). Although the general trend is in accordance with previous works, we showed that ISR modifies these dynamics, mainly via variations on the accumulation period of these compounds.

As ISR levels modified the dynamics of phytosterols accumulation, a wide range of final phytosterols per grain was observed among treatments and experiments (from 60.1 to 108.9 µg). These amounts of phytosterols were similar to those reported in the literature for this species (Nolasco *et al.* 2009). The composition of the phytosterols observed in our experiments was also similar to those reported in the literature (e.g. Roche *et al*. 2010*b*). Fernández-Cuesta *et al*. (2014)

observed variations between 44.8% and 75.5% for β-sitosterol, from 4.1% to 12.8% for stigmasterol, from 3.2% to 19.7% for campesterol, from 0.8% to 27.4% for Δ^7-stigmastenol and 0.4% to 6.6% for Δ^7-avenasterol in the evaluation of a collection of 985 sunflower genotypes. In addition to sunflower, it has been reported that β-sitosterol is the main phytosterol in cultivated crops, such as soybean, rapeseed, cottonseed and corn (Piironen *et al*. 2000). It is important to notice that both high oleic genotypes (HO and HSHO) had higher percentage of stigmasterol and campesterol and lower percentage of Δ^7-stigmastenol than the Traditional genotype. Similar results were observed by Anastasi *et al*. (2010), where a HO genotype presented lower Δ^7-stigmastenol percentage than a Traditional one.

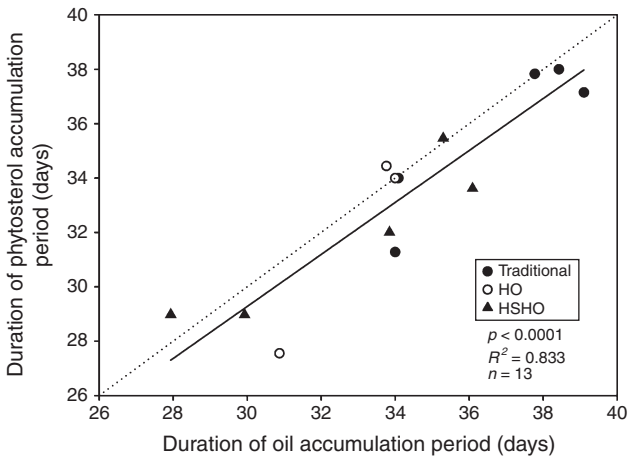

Fig. 3. Relationship between the durations of the accumulation periods of oil and phytosterols for the genotypes evaluated in all experiments: a traditional, a high oleic (HO) and a high stearic–high oleic (HSHO). The dotted line represents the theoretical slope of 1. Data of duration of oil accumulation period were taken from (González Belo *et al*. 2017).

The dynamics of phytosterols accumulation were linked to the dynamics of oil accumulation. It is known that the applied treatments modify the source available to fill grains and else the final oil accumulated (Ruiz and Maddonni 2006; Echarte *et al*. 2012). In fact, for Expt A and B, oil per grain varied from 13.0 to 31.7 mg via variations in the rate of accumulation and the duration of the accumulation period. For phytosterols, no relationship between rate of accumulation and ISR was observed. The phytosterols synthesis seems not to be limited by the substrate, as the rate of synthesis did not increase when source increased. So, the variations in phytosterols per grain are mainly a consequence of the longer duration of the accumulation period when source available to fill grains is enough. For tocopherols, an increase in the accumulation period was also reported when ISR per plant increased, but that increase was lower than the one observed for the duration of oil accumulation period (González Belo *et al*. 2017). In the present research, the period of phytosterols accumulation was similar to that of oil accumulation period in any grain-filling conditions. Additionally, another important concern is that phytosterols content per grain variations were accounted mainly by the duration of the accumulation period. This is a novel result, because it is contrary to what happens with the oil and tocopherols per grain, where the content of these compounds are mainly explained by changes in the rate of accumulation than the duration of the accumulation period (González Belo *et al*. 2017).

The oil phytosterols concentration was high at the beginning of grain filling and steeply decreased, when oil is being accumulated, to reach its final concentration at an early stage. The high concentration at the beginning of grain filling may be related to high cell division (Lindström *et al*. 2006), as these

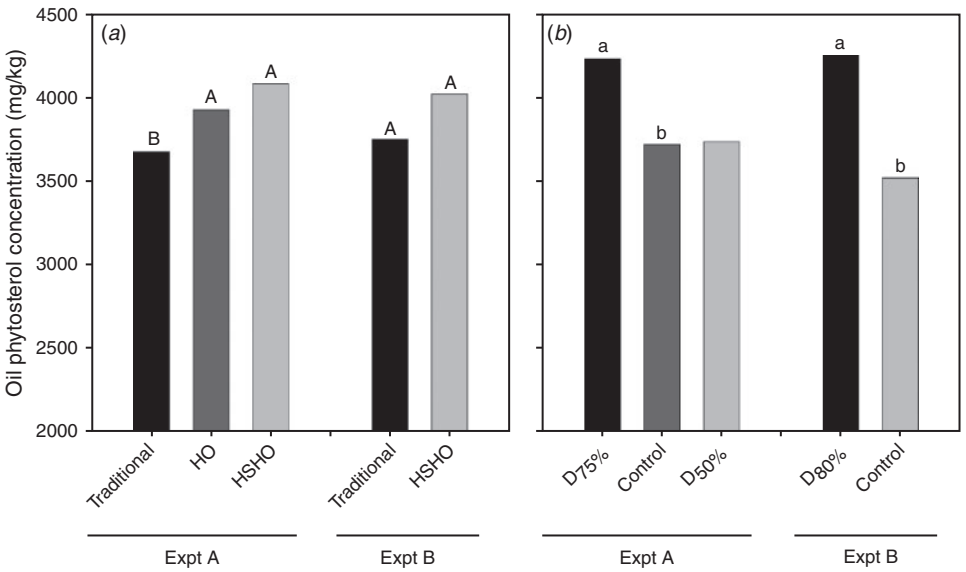

Fig. 4. (*a*) Genotype and (*b*) treatments effects on oil phytosterol concentration for Expt A and B. Genotypes are: a traditional, a high oleic (HO) and a high stearic–high oleic (HSHO). Treatments are: defoliation of 75% ($D_{75\%}$) or 80% ($D_{80\%}$), grain thinning of 50% ($R_{50\%}$) and a control. Same letters indicate no significant differences among (*a*) genotypes or (*b*) treatments.

compounds are structural components of membranes (Piironen *et al*. 2000). In contrast to phytosterols, oil tocopherol concentration increases gradually during grain filling to reach its maximum concentration and remain constant until physiological maturity (Falk *et al*. 2004). The variations in the dynamics of oil and phytosterols accumulation were reflected in variations in final oil phytosterols concentration, which varied between 3400 and 4624 µg/g. These concentrations of phytosterols in sunflower oil were similar to those reported in the literature (Velasco *et al*. 2013; Velasco and Ruiz-Méndez 2015) and those defined by the Codex Alimentarius for sunflower oil from Traditional and high oleic genotypes (2400–5000 µg/g and 1700–5200 µg/g, respectively) (CODEX STAN 2005).

For the same Traditional and HO genotypes used in our experiments, Nolasco *et al*. (2009) reported phytosterol concentration between 3513–4432 and 3720–4454 µg/g, respectively. In canola, it has been observed that genotypes with modified fatty acid composition had lower oil phytosterols concentration than Traditional ones (Abidi *et al*. 1999). In our experiments, the HSHO genotype presented higher phytosterols concentration compared with the HO and the Traditional hybrids, explained by its lower grain weight and oil concentration. Anyway, in all tested genotypes ISR similarly affected oil phytosterols concentration, showing that mutations in HO and HSHO genotypes did not modify the response of oil phytosterols concentration to ISR. In both experiments, defoliated treatments increased the concentration of these compounds in the oil. There are no reports in the literature about the effects of grain-filling conditions on oil phytosterols concentration in sunflower genotypes. This effect is in accordance with those reported for tocopherols concentration in sunflower, where reducing ISR per plant increased the concentration of these compounds in the oil (Nolasco *et al*. 2004; Izquierdo *et al*. 2011; González Belo *et al*. 2017).

Although phytosterols represent only a minor fraction of vegetable oils, high concentrations are desired by consumers for their reported benefits to human health (Palou *et al*. 2005; Ostlund 2007; Brufau *et al*. 2008; Schwartz *et al*. 2008; Hansel *et al*. 2011; Valerio and Awad 2011). The results presented in this work bring new insights related to the accumulation and final concentration of these compounds in sunflower oils. During the oil refining, between 10% and 70% of the phytosterols are lost, especially in the neutralisation and deodorisation phases, depending on the number of steps performed and the conditions (Verleyen *et al*. 2002; Verhé *et al*. 2006). However, these compounds can be recovered for being used in the formulation of foods and nutraceutical or pharmaceutical phytosterols enriched products. Therefore, the information here is useful both for the industry and to understand the mechanisms involved in the variability of phytosterols concentration in sunflower oil. One of the strategies to boost the greater consumption of bioactive compounds for health care, like phytosterols, could be through increasing the concentration in foods that naturally contain them, by genetic improvement and agronomic management. In this sense, these results could be included in sunflower crop models to simulate and predict the best combination of environment and genetic to obtain high oil phytosterols concentration (Villalobos *et al*. 1996; Pereyra-Irujo and Aguirrezábal 2007). This would improve the nutritional value of the diet and reduce the prevalence of chronic non-inherited diseases, integrating knowledge in nutrition, health, agriculture and environment.

Conclusions

The dynamics of phytosterols accumulation in sunflower were affected by ISR. Reduction in ISR decreased phytosterols accumulation mainly via reductions in the duration of the accumulation period and not via variations in the rate of accumulation. The durations of the accumulation period of these components varied in accordance with the duration of oil accumulation period. By improving environmental conditions, the increase in oil accumulation rate was reflected in a decrease in the final concentration of phytosterols in the oil. These results reinforce the importance of management on grain quality, as variations in sowing date, plant density, and other practices can modify the status of plants, the interception of solar radiation and else grain and oil quality.

Conflicts of Interest

The authors declare no conflicts of interest.

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