SOURCE AND SINK AFFECT PHYTOSTEROL CONCENTRATION AND COMPOSITION OF SUNFLOWER OIL

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ABSTRACT

Phytosterols are oil compounds that help to reduce serum cholesterol. It is unknown if variations in source or sink during grain filling affect these compounds as in other quality traits (tocopherol concentration, fatty acid composition, etc.). The aim of this study was to evaluate the effect of variations in source or sink on phytosterols concentration and composition of the oil in sunflower genotypes with different fatty acid composition. Two field experiments were performed using a traditional, a high oleic (HO) and a high stearic-high oleic (HSHO) hybrid. At the beginning of grain filling treatments were applied to modify the source or sink: 75-80% leaf removal, 50% of grains removal and control. Oil phytosterols concentration and its composition were determined by GLC. In all hybrids, defoliation treatments increased phytosterols concentration in the oil, but reduced the content per grain, compared to the control. Removing grains increased the content of phytosterols per grain but it did not affect its oil concentration because oil per grain was also increased. The most abundant phytosterol was β−sitosterol, followed by Δ^7−stigmastenol, campesterol, stigmasterol and Δ^7−avenasterol. Variations in source or sink only in few cases modified phytosterol composition. In both experiments, the HSHO hybrid had a higher proportion of campesterol and stigmasterol and lower of β−sitosterol than traditional and HO. These results confirm that crop management is important not only for maximizing yield but also for obtaining a good oil quality.

INTRODUCTION

Bioactive compounds are molecules present in several foods that have benefits for health, like tocopherols and phytosterols, among the most important. In plants, phytosterols play an important role in the regulation of membrane fluidity and permeability (Schaller, 2003), embryogenesis (Clouse, 1996), and as precursors of brassinosteroid hormones involved in plant growth and development (Lindsey et al., 2003; Merah et al., 2012). In humans, they have properties as anti−cancer, anti−inflammatory, anti−oxidation activities and prevention of cardiovascular diseases (Hansel et al., 2011; Valerio and Awad, 2011). However, the reduction of total plasma cholesterol and low−density lipoprotein cholesterol (LDL) levels in humans is the best characterized role of phytosterols (Brufau et al., 2008). A meta−analysis of 41 trials showed that a phytosterol intake of 2 g/day reduced LDL cholesterol by 10% (Schwartz et al., 2008). Therefore these compounds help to control plasma cholesterol and prevent cardiovascular diseases (Brufau et al., 2008; Ostlund Jr, 2007; Palou et al., 2005; Roche et al., 2010a).

There is intra− and inter−specific variability in the amount and concentration of phytosterols in oils (Fernández-Cuesta et al., 2014; Fernández-Cuesta et al., 2011; Roche et al., 2010b). Among the vegetable oils, sunflower is characterized for presenting high concentration and good composition of antioxidants. In this species, a variation in oil phytosterols concentration between 3513 and 4936 mg/kg was found due to the environment (Nolasco et al., 2010). Differences up to 118 mg of phytosterols/100 g seed due to changes in sowing date were observed by Roche et al. (2010b). It is known that the amount of source or sink in sunflower partially explains variations in the amount of oil, the fatty acids concentration, and other grain components such as tocopherols (Izquierdo et al., 2008; Izquierdo et al., 2011; Ruiz and Maddonni, 2006). However it is unknown whether they also influence the synthesis of phytosterols, determining their concentration and final composition. So, the objective of this work was to investigate the effect of source or sink on the concentration and composition of phytosterols in sunflower oil. Understanding these effects is important because those management practices that affect the amount of source or sink of the crop (e.g. hybrid choice, sowing date, plant density, etc.) could not only affect yield but also the quality of the oil produced.

MATERIALS AND METHODS

Two field experiments were carried out in Balcarce (37ºS, 58ºO Argentina) during 2012/2013 (Exp 1) and 2014/2015 (Exp 2) growing seasons. A traditional (Macon), a high oleic (Olisun 2) and a high stearic−high oleic (HS05) genotype were used in Exp 1. The traditional and the high stearic−high oleic genotypes were used in Exp 2. The experiments were carried out with a split-plot design with three blocks, where the genotype was assigned to main plots and treatment to modify the amount of source or sink of the plant (F−D) to subplots. Treatments to modify the source or sink consisted of removing leaves or grains. The size of the subplots was six rows 0.70 m apart and 9 m long, at densities of 7 pl/m^2. Treatments were applied in early grain filling (R_6). The sowing date and applied treatments of each trail are present in Table 1.

Table 1: Sowing date and applied treatments of each trail.

	Sowing date	Treatments
Exp 1	30−oct	75% leaf removal ($D_{75\%}$),
		50% grain removal ($R_{50\%}$)
		Control (T)
Exp 2	22−oct	80% leaf removal ($D_{80\%}$),
		Control (T)

In both experiments, weed and pest were controlled and water and nutritional stress were prevented by irrigation and fertilization. Phenology was recorded as Schneiter and Miller (1981). Before flowering, capitula were covered with nylon pollination bags to prevent cross−pollination to preserve the fatty acid composition of each genotype. Plants were harvested after physiological maturity. Oil content was determined by nuclear magnetic resonance according to Robertson and Morrison (1979). Oil was extracted from grounded grain using n−hexane as solvent by percolation−immersion for 3 h at room temperature and 3h at 80ºC (Izquierdo et al., 2011). The amount and type of phytosterol were analyzed by gas chromatography (Fernández-Cuesta et al., 2012). The comparison of phytosterols

concentration, amount per grain and composition between genotypes and F−D treatments were performed by analysis of variance using R package (R CORE TEAM, 2012).

RESULTS AND DISCUSSION

Total phytosterols concentration

Total phytosterols concentration varied between 3157−5139 µg/g of oil, among experiments, genotypes and F−D treatments. These concentrations are higher than those observed by Nolasco et al. (2010), but were in the same range reported by CODEX STAN 210 (1999), that was between 1700−5300 µg/g of oil for traditional and high oleic sunflower. In both experiments, the interaction between genotype and F−D treatment on total phytosterols concentration were no significant ($p>0.35$).

Total phytosterols concentration was increased by leaf removal treatment ($D_{75\%}$ and $D_{80\%}$) compared to controls ($p<0.0022$,

Figure **1**). In Exp 1 grain removal did not affect total phytosterols concentration. Among the three genotypes, Macon presented the lowest phytosterols concentration (3674 vs >3900 µg/g). No significant variations were observed between hybrids in Exp 2. There are no reports in the literature related to the effect of changes in source and sink on phytosterols concentration. An increase of total phytosterols concentration with a decrease in available water was observed by Anastasi et al. (2010). But it is unknown whether the results reported by these authors are mediated by direct effects of water, or an effect on the source or sink of the plant.

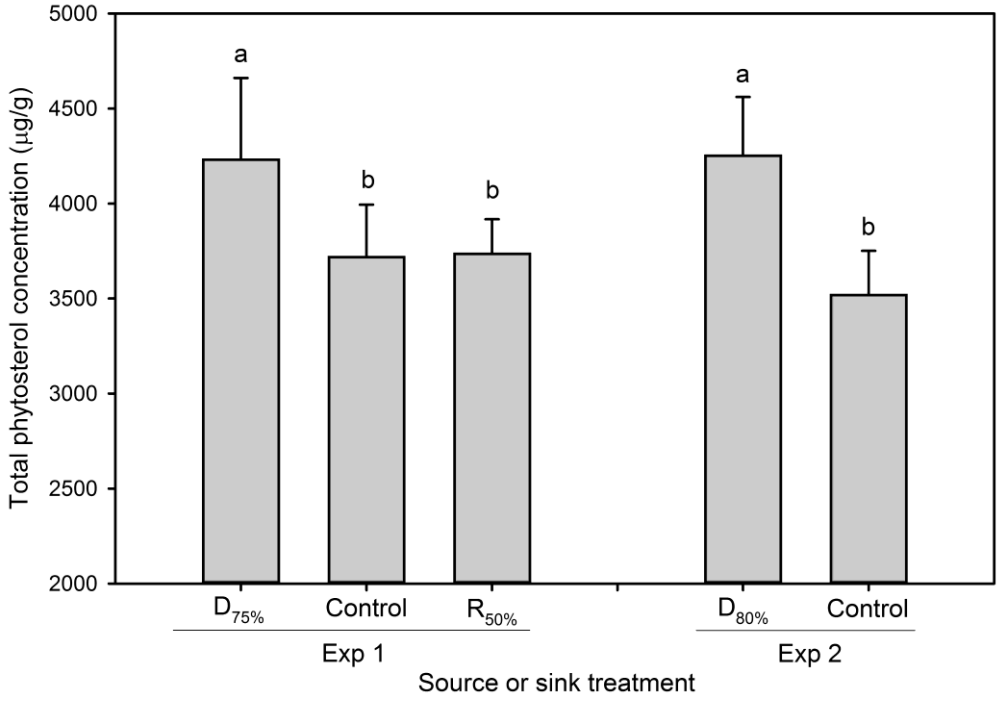

Figure 1: Total phytosterol concentration for each F−D treatment for Exp 1 and Exp 2. Means with the same letter are not significantly different in each experiment.

Total phytosterols per grain

The amount of phytosterols per grain varied between 57 and 118 µg/grain. Those values are similar to those reported by other authors (Anastasi et al., 2010; Fernández-Cuesta et al., 2014; Roche et al., 2010b). There was no significant variation between genotypes and F−D treatments in Exp 1 ($p>0.07$). $D_{75\%}$ presented the lowest value of total phytosterols per grain, followed by control and $R_{50\%}$ (

Figure **2**). Macon and Olisun 2 presented the greatest differences between F−D treatments (data not shown). In Exp 2, total phytosterols per grain were not modified by F−D treatments ($p>0.2688$,

Figure **2**). Higher total phytosterols per grain were observed in a high oleic genotype than traditional ones by Anastasi et al. (2010). However, traditional and high oleic genotypes presented similar total phytosterols per grain in our experiments. In both trials, the amount of phytosterols per grain was directly related to the weight of the grains. Thus, HS05 presented less total phytosterols per grain than Macon and Olisun 2 (63 vs >93 µg/ grain Exp 1, $p<0.0010$), due to lower grain weight.

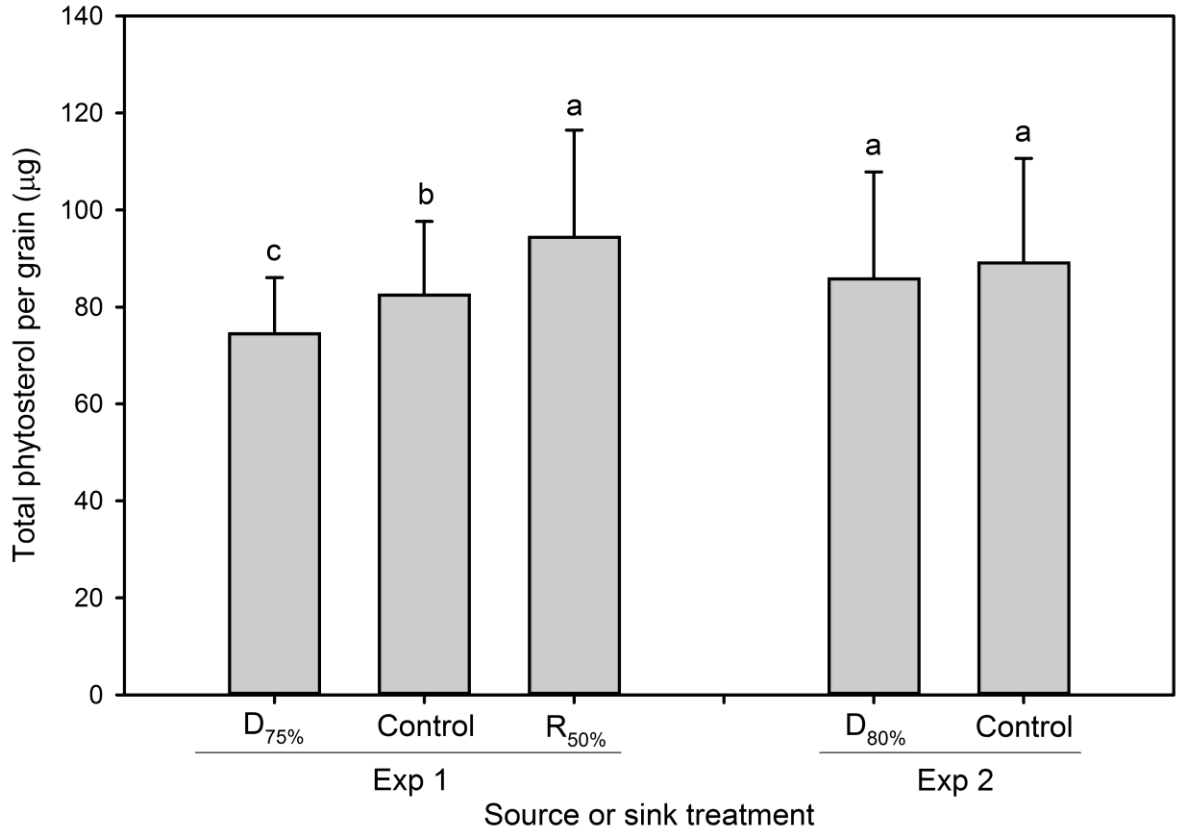

Figure 2: Total phytosterols per grain for each F−D treatment for Exp 1 and Exp 2. Means with the same letter are not significantly different in each experiment.

Phytosterols composition

The most abundant phytosterol was □−sitosterol (57% in average), followed by campesterol, □^{7}−stigmastenol and stigmasterol representing around 10% each of total phyosterols (

Figure **3**). The Δ7−avenasterol was found in concentrations close to 3% of total phytosterols identified. This composition of phytosterols is similar to that reported by other authors for the same species (A.O.C.S., 2014; Velasco et al., 2013).

Genetic variability for phytosterol composition was reported in sunflower (Merah et al., 2012; Roche et al., 2010b; Velasco et al., 2013). This variability was observed in the three hybrids used in our experiments. In both experiment, HS05 presented a lower β−sitoesterol and higher stigmasterol proportion than Macon. No significant variation in phytosterol composition was observed between Macon and Olisun 2.

The proportion of β−sitoesterol was not modified by F−D treatments ($p>0.0551$). $R_{50\%}$ increased 1.1 percentage points the campesterol proportion compared to control and $D_{75\%}$ in Exp 1. $D_{80\%}$ decreased 1.6 percentage points the proportion of campesterol compared to control in the HS05 hybrid, while this percentage did not change in Macon, in Exp 2.

Stigmasterol proportion increased in the order $D_{75\%}$, control and $R_{50\%}$ in Exp 1, whereas there was no difference between treatments in Exp 2. Interaction between genotype and F−D treatment was found for Δ7−stigmastenol and Δ7−avensterol proportion. $R_{50\%}$ increased Δ7−stigmastenol proportion than control and $D_{75\%}$ for Macon, whereas the opposite effects for Olisun 2 were observed. No significant variation in Δ7−stigmastenol and Δ7−avensterol proportion between F−D treatments in HS05 was observed. $R_{50\%}$ and $D_{75\%}$ were those with the highest and lowest Δ7−avensterol proportion, respectively in Macon, whereas the opposite effect was found in Olisun 2.

Anastasi et al. (2010) observed increases in the proportion of β−sitoesterol or campesterol when water availability was increased. On the other hand, Roche et al. (2010b) reported variations in phytosterols composition when sowing date was delayed, effect attributed to the variations in temperature during grain filling. Variations in phytosterols composition observed in our work cannot be attributed to water availability or temperature since all treatments were conducted under similar conditions and sowing date. So, these effects are explained by the variations in source or sink during grain filling.

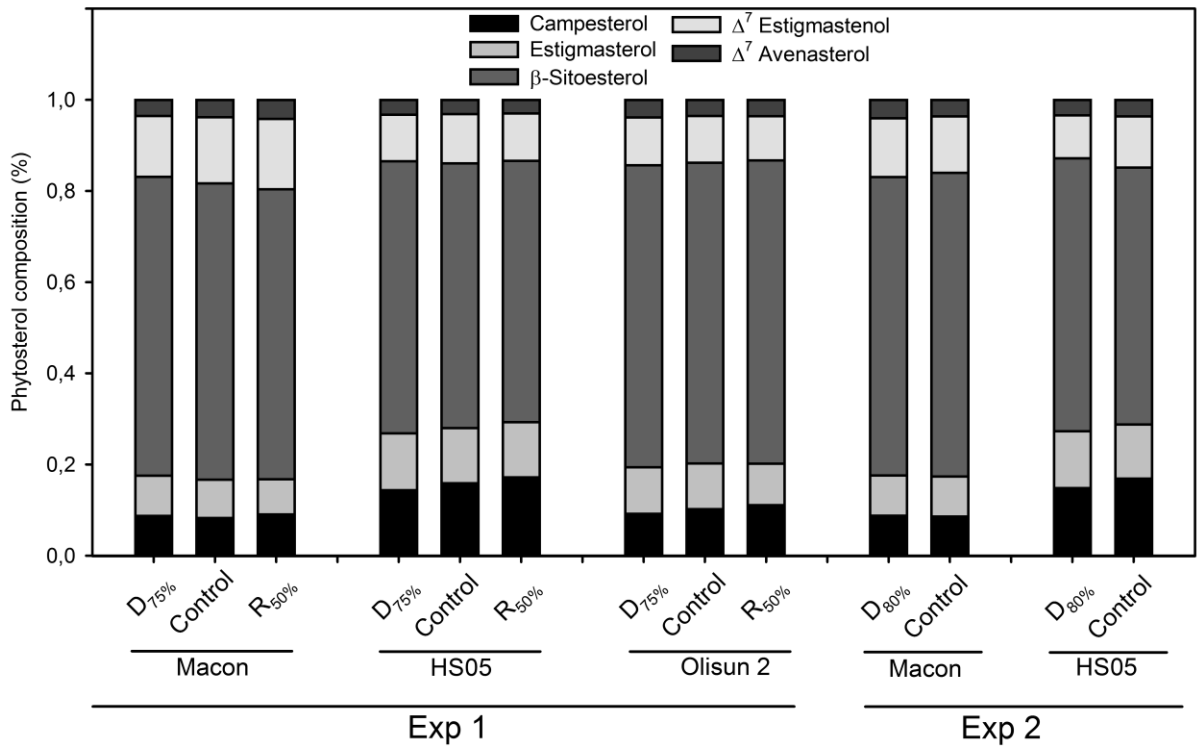

Figure 3: Phytosterol composition for each F−D treatment and genotype in Exp 1 and Exp 2.

CONCLUSIONS

In this work we observed that reductions in source of plants reduce the amount of phytosterols per grain and increase its concentration in the oil. These effects were similar in genotypes with different oil fatty acid composition. These results agree with those reported for other minor oil constituents such as tocopherols. More research is needed to understand the relation between minor oil constituents and oil biosynthesis and how their final concentration in the oil is determined in sunflower crops grown under different source or sink conditions.

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