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NEW PERSPECTIVES OF EUROPEAN OLEOCHEMISTRY
LES NOUVELLES PERSPECTIVES DE L'OLÉOCHIMIE EUROPÉENNE

Effects of technical management on the fatty-acid composition of high-oleic and high-linoleic sunflower cultivars

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Abstract – We investigated the effects of several crop management parameters on the fatty-acid composition of very high-oleic (VHO) and a very high-linoleic (VHL) sunflower cultivar. Experiments were performed on these varieties grown on field plots in 2009 and 2010. Yield and fatty-acid composition were determined and the effect of irrigation was investigated at the flowering stage in VHL cultivars. We also evaluated the effects of three sowing dates studied on Antonil, a VHL cultivar. Yield and fatty-acid content were similar in 2009 and 2010. The best results were obtained for PR65H22 (a hybrid cultivar), for which a mean oleic acid content of 93% was obtained in 2009. For the VHL variety, yields were higher for plots irrigated at flowering. Late sowing was associated with higher linoleic acid content, and irrigation at flowering resulted in higher yields and linoleic contents. These results highlight the effects of different types of technical management on the yield and quality of this crop.

Keywords: Crop management / fatty-acid composition / sowing date / sunflower / irrigation

Résumé – Effets de la conduite culturale sur la composition en acides gras des variétés de tournesol à haute teneur en acide oléique et à haute teneur en acide linoléique. Nous avons étudié les effets de plusieurs paramètres de conduites culturales de tournesol sur la composition en acides gras de variétés de tournesol de très haute teneur en acide oléique (THO) et de très haute teneur en acide linoléique (THL). Des expériences ont été effectuées sur ces variétés cultivées sur 96 parcelles en 2009 et 56 parcelles en 2010. Le rendement et la composition en acides gras ont été déterminés et l'effet de l'irrigation a été étudié au stade de la floraison chez les variétés de tournesol THL. Nous avons également évalué les effets de trois dates de semis étudiés sur Antonil, une variété de tournesol THL. Le rendement et la teneur en acides gras ont été plus élevés en 2010 qu'en 2009 pour toutes les variétés. Les meilleurs résultats ont été obtenus pour PR65H22 (une variété hybride), pour laquelle une teneur moyenne en acide oléique de 93 % a été obtenue en 2009. Pour les variétés de tournesol THL, les rendements étaient plus élevés pour les parcelles irriguées à la floraison. Le semis tardif a été associé à la plus forte teneur en acide linoléique, ainsi que l'irrigation à la floraison a donné des rendements et teneurs plus élevés en acide linoléique. Ces résultats mettent en évidence les effets des différents types de conduite culturale sur le rendement et la qualité de cette culture.

Mots clés : Conduite culturale / composition en acides gras / date de semis / tournesol très haut oléique et très haut linoléique / irrigation

1 Introduction

Sunflower is the fourth most important oilseed species worldwide (Oil World, 2012). It is grown mostly for its oil, but a small proportion of the seeds harvested is destined for direct consumption in food or feed. Sunflower seeds are achenes. They contain about 45% oil, which is harvested mostly for human consumption, but also for use in industry, particularly in

the production of biofuels and biolubricants (Ballerini, 2006; Roche *et al.*, 2010). France is the main consumer and producer of sunflower in the European Union. Oil demand and diversification have increased considerably over the last decade and these trends have promoted breeding for the development of cultivars with different fatty-acid profiles to meet the demands of industry.

The desaturase of sunflower, FAD2, naturally transforms its oleic acid into linoleic acid. The dominant high-oleic mutation underlying the Pervenets population suppresses FAD2

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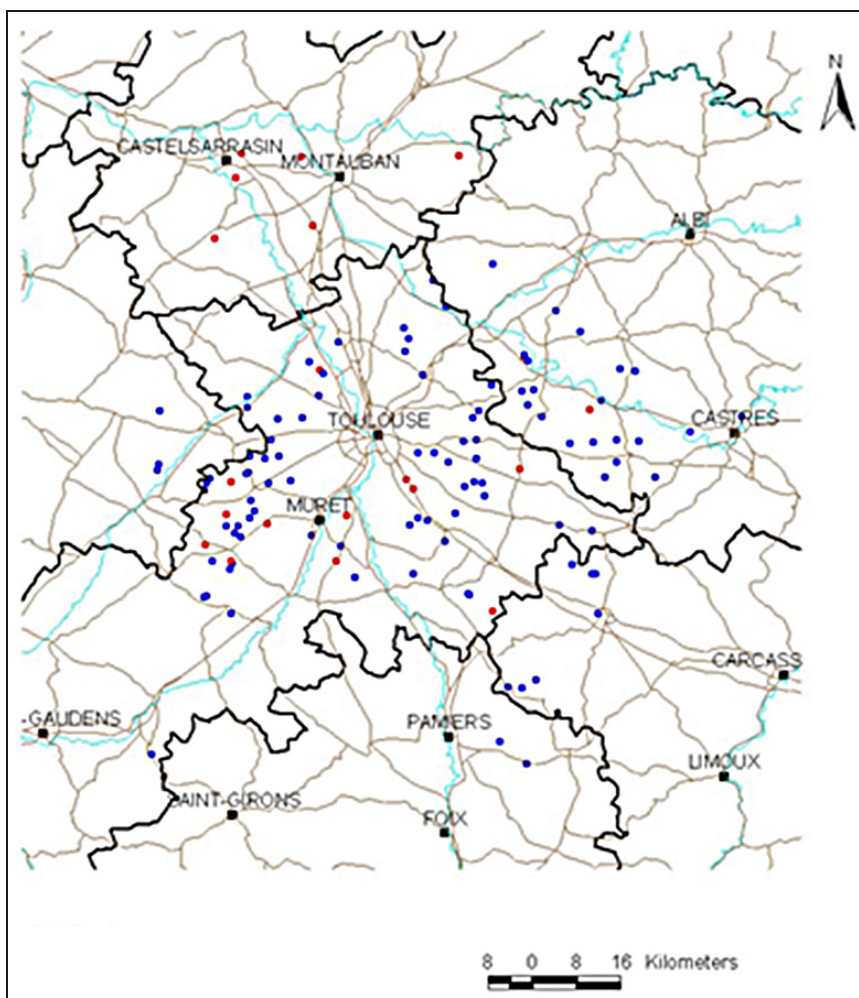


Fig. 1. Map locating the plots used in 2009. All the plots were located around Toulouse (South West France). Red points: VHL sunflower. Blue points VHO sunflower (online version in color).

activity through a gene duplication event leading to silencing of the FAD2 gene (Lacombe *et al.*, 2009). This silencing phenomenon is not complete, so even high-oleic sunflower (HO) varieties produce seeds containing at least 5% linoleic acid (Lagravere *et al.*, 2004). FAD2 is encoded by a family of three genes in sunflower. However, the expression of only one of these genes is blocked by the Pervenets mutation. This dominant mutation is expressed exclusively in the seed and is strongly influenced by genetic background. Other fatty-acid profiles exist in sunflower and have been developed for specific industrial needs. Cultivars with “high” stearic or palmitic acid levels have been produced, for example (Perez-Vich *et al.*, 2002; Fernandez-Moya *et al.*, 2002; Salas *et al.*, 2007; Serrano-Vega *et al.*, 2005). Ongoing plant breeding efforts are continuing to generate sunflower varieties with very high linoleic acid or very high oleic acid contents.

FAD2 activity is limited by temperature. Indeed, the fluidity of the fatty acids in cell membranes depends on the rate of lipid desaturation: the maintenance of correct fluidity requires lower rates of desaturation at higher temperatures than at lower temperatures. Several studies have shown that oleic acid content increases with cumulative temperature during peak lipo-

genesis activity (Roche *et al.*, 2006, 2010; Baud and Lepiniec, 2010; Merah *et al.*, 2012). It may, therefore, be necessary to modify cropping practices when growing “technical” varieties.

Many studies have highlighted the impact of high temperatures on stearic and oleic acid contents (Fernandez-Moya *et al.*, 2002, Perez-Vich *et al.*, 2002; Roche *et al.*, 2006; Merah *et al.*, 2012). Few studies have investigated the effect of crop management on yields and fatty acid contents in VHO or VHL cultivars.

The objective of this study was to investigate the impact of agro-environmental factors on VHO and VHL varieties of sunflower, to determine the most appropriate technical management practices (sowing time, irrigation, etc.) for the varieties tested.

2 Materials and methods

The experiments were performed in the Toulouse region in 2009 and 2010, with field plots of very high-oleic acid content (VHO) cultivars and one very high-linoleic acid content (VHL) cultivar (Fig. 1).

Table 1. The prevailing weather conditions during the plant growth cycle in 2009 and 2010, and the mean values for the last 50 years.

Month	Rainfall (mm)			Temperature (°C)		
	2009	2010	50-year mean	2009	2010	50-year mean
January	95.4	45.8	65.0	4.9	3.8	5.7
February	40.4	43.6	55.9	5.8	5.1	6.9
March	23.6	42.6	57.1	8.7	8.3	9.2
April	105.4	48.9	66.6	11.7	12.5	11.5
May	44.4	118.1	77.2	16.8	14.3	15.3
June	39.4	52.6	61.1	20.1	18.8	18.9
July	41.2	62.3	47.1	21.7	22.6	21.5
August	20.0	23.2	58.3	22.7	20.9	21.3
September	46.8	31.0	55.9	18.9	18.0	18.5
October	35.6	104.8	56.8	15.0	13.4	14.4
November	102.2	91.5	57.2	10.9	9.0	9.0
December	34.6	21.1	68.1	5.9	4.1	6.2
Mean				13.6	12.6	13.2
Annual total	629	685.5	726.3			
Mean Mar-Sept				17.2	16.5	16.6
Sum Mar-Sept	320.8	378.7	423.3			
Mean May-July				19.5	18.6	18.6
Sum May-July	125.0	233.0	185.4			

The cultivars were provided by Pioneer Cie. PR65H22, PR64H41 and PR64H32 are hybrids with high yields and oleic acid contents. Antonil (developed by Pioneer) has very high linoleic acid content. This cultivar flowers very early and was developed for use as a cover crop. In 2009, all three VHO varieties were sown (over 98 plots), whereas, in 2010, only PR65H22 was sown, on 96 plots. Antonil (VHL) was sown in 2009 (21 plots) and 2010 (35 plots). We also tested three sowing dates for this variety: before June 1st, between June 10th and June 30th and after July 1st, in 2010 only.

Temperatures and rainfall were recorded during the two crop cycles (Tab. 1). For the period of the year corresponding to the cropping cycle, 2009 was the hottest and driest year, and there was more rainfall in 2010. During the grain-filling period, 2009 was less rainy and hotter than 2010.

Flowering began between the end of June and the middle of July, depending on the year. Seed maturity was achieved at the end of July or the start of August.

Grain yield and oleic and linoleic acid contents were determined. The fatty acid profile of the seeds was determined by gas chromatography (GC) analysis of the methyl esters of fatty acids. This procedure includes two preliminary steps: the extraction and esterification of fatty acids. The results for each fatty acid are expressed as a percentage of total fatty-acid composition. The four major fatty acids – oleic, linoleic, stearic and palmitic acid – were determined.

Fatty acids were determined by a method based the solubility of oils in tertbutylmethyl ether (TBME; ME0552, Scharlau) and the transformation of fatty acids into their methyl esters (ISO 5509 standard: 1990). Seeds were ground in 5 ml TBME. A 2 ml was then passed through a GHP filter with 0.45 µm pores. We then mixed 50 µl of 0.2 M trimethylsulfonium hydroxide in methanol (Macherey-Nagel) with 100 µl of the filtrate according to the NF EN ISO 12966-3 standard. The resulting mixture (1 µl) was injected, via an automatic sampler, into a GC-3800 chromatograph (Varian) equipped with a FID

Table 2. Yield and fatty-acid contents of sunflower seeds during the two years of experiments in the Toulouse region.

Type	Trait	Water condition	2009	2010
VHO	Yield (t/ha)	Rain-fed	2.1	2.3
	Oleate content (%)	Rain-fed	90.9	91.5
VHL	Yield (t/ha)	Irrigation (flowering)	1.9	–
	Yield (t/ha)	Rain-fed	1.4	1.2
	Linoleate (%)	Rain-fed	69.1	73.2

Table 3. Fatty-acid contents for the cultivars studied during two years of experiments in the Toulouse region.

Year	Type	Cultivar	Fatty acid content (%)
2009	VHO	PR64H32	90.2
2009	VHO	PR64H41	89.5
2009	VHO	PR65H22	93.0
2010	VHO	PR65H22	91.5
2009	VHL	Antonil	69.1
2010	VHL	Antonil	73.0

detector. The GC was also equipped with a CP Select CB 50 m capillary column (D: 0.25 mm). The initial oven temperature was maintained at 185 °C for 40 min, then increased at a rate of 15 °C/min to 250 °C, this temperature then being maintained for 10 min. The injector and detector temperatures were 250 °C. The fatty acid methyl ester (FAME) proportions were determined by comparison with the retention times of a known standard mixture of FAMES (a fatty acid methyl ester mixture from oilseed rape, Supelco, USA), used as an external standard.

3 Results and discussion

Tables 2 and 3 present seed yields and oleic acid contents for the three VHO varieties in 2009 and PR65H22 in 2010.

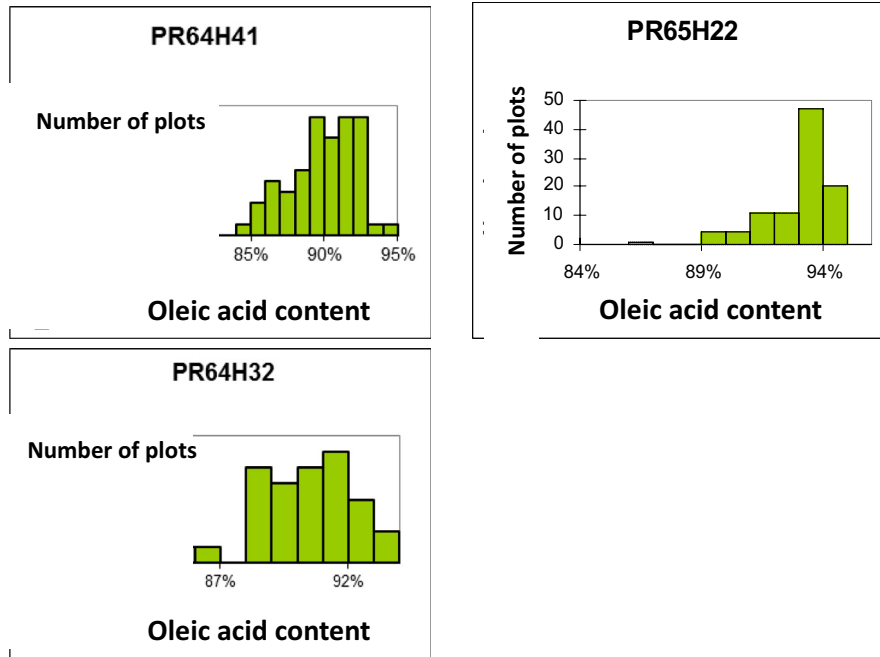


Fig. 2. Distribution of oleic acid content for VHO sunflower harvested in 2009.

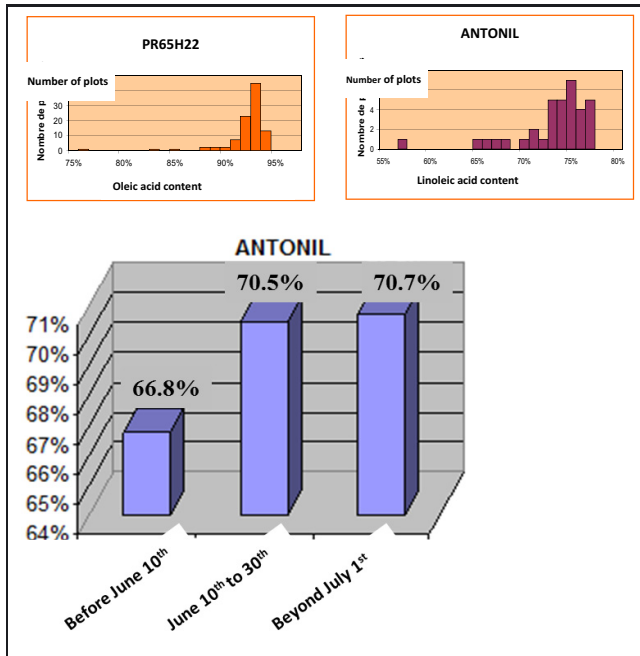


Fig. 3. Impact of sowing date on linoleic acid content in Antonil (VHL sunflower cultivar) in 2010.

Seed yields were similar the two years and in the normal range for non-irrigated crops in south west France.

In 2009, the oleic acid content of PR65H22 was slightly higher than those of the other two VHO varieties, and showed the least variation between fields (Fig. 2). In 2010, results were very similar, the slightly lower mean for this variety probably being due to a few plots with lower oleic acid contents (Fig. 3),

which could result from some pollination by other sunflower varieties.

For Antonil (VHL sunflower), linoleic acid content was higher in 2010 than in 2009, but yield was slightly higher in 2009 than in 2010. These findings may reflect the differences in climatic conditions between 2009 and 2010 (Tab. 2). Crop management practices were also more appropriate in 2010 than in 2009.

In 2009, we compared rain-fed and irrigated conditions (at sowing and flowering) for the VHL cultivar. Higher yields were obtained for this cultivar if the plots were irrigated at flowering (1.9 t/ha), the mean yield obtained for the rain-fed crop being only 1.4 t/ha.

Sowing date affected linoleic acid content, which was higher if the seeds were sown after June 10th (Fig. 3). Linolenic acid content seemed to be increased by lower temperatures at flowering or perhaps by a short photoperiod. It was increased by irrigation at flowering. Our results highlight the importance of careful technical management to get the most out of these crops.

4 Conclusion

Our results show that yield and oleic acid content depend on the cultivar used and on crop management practices. Oleic acid levels are stable over a large number of plots and 2 years with a slightly different weather conditions for VHO varieties. In contrast, these studies confirm that a low temperature at flowering promotes linoleic acid content of conventional varieties and therefore that such VHL variety is useful if it is sown just to complete its cycle when a harvest can be performed under favorable conditions. Irrigation at flowering stage of sunflower development increased yield and fatty-acid content. These results highlight the potential of crop management

practices for manipulating yield and oleic or linoleic acid content. Further studies are required to confirm these results and to optimize seed production and the levels of specific fatty acids in sunflower seeds.

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