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Official URL: https://doi.org/10.1051/ocl/2016039

To cite this version:

Gouzy, Adeline and Paulhe-Massol, Anne and Mouloungui, Zéphirin and Merah, Othmane. Effects of technical management on the fatty-acid composition of high-oleic and high-linoleic sunflower cultivars. (2016) Oilseeds & fats Crops and Lipids, 23 (5). D502. ISSN 2272-6977

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

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Received 21 June 2016 – Accepted 31 August 2016

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 Antolin, 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

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
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 | 76.8          | 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         |
| Annual total | 629  | 685.5 | 726.3        |       |       | 13.2         |
| Mean Mar-Sept | 320.8 | 378.7 | 423.3        |       |       | 16.6         |
| Sum Mar-Sept | 320.8 | 378.7 | 423.3        |       |       | 16.6         |
| Mean May-July | 125.0 | 233.0 | 185.4        |       |       | 18.6         |
| Sum May-July | 125.0 | 233.0 | 185.4        |       |       | 18.6         |

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 at 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 on the solubility of oils in terbutylmethyl 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 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.

### 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                   |

### 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.
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 in sunflower seeds.

Acknowledgements. Funds for this study were provided by the FUI OLEVISON program (FUI-AAP7-OLEOVISION 36520), and the Midi-Pyrenees regional authority. This work was also supported by the EFDR and the French Ministry of Industry (DGSI). We thank the Arterris team and farmers, the Pioneer Company and researchers from the Ecole d’Ingénieurs de Purpan at Toulouse for their practical input. The authors would like to thank Félicity VEAR for the revision of the manuscript.

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Cite this article as: Adeline Gouzy, Anne Paulhe-Massol, Zéphirin Mouloungui, Othmane Merah. Effects of technical management on the fatty-acid composition of high-oleic and high-linoleic sunflower cultivars. OCL 2016, 23(5) D502.