Proc. 17th International Sunflower Conference p. 249-253
Córdoba, Spain, June 8 to 12, 2008

Epicuticular wax content in the pericarp of sunflower fruits (*Helianthus annuus* L.) grown under moderate water deficit

María Clara Franchini [1], Lilia I. Lindström [1], Luis F. Hernández [1,2]
[1] Laboratorio de Morfología Vegetal. Depto. de Agronomía, UNSur. Bahía Blanca, 8000, Argentina.
[2] Comisión de Investigaciones Científicas de la Pcia. de Buenos Aires (CIC). La Plata, 1900, Argentina

ABSTRACT

In the present work the effect of a moderate water deficit (MWD), imposed to field grown plants in two sunflower hybrids from early anthesis (reproductive stage 6 or R6) to harvest maturity (HM), on the development of epicuticular waxes (**epw**; mg/g) of the fruit's pericarp, was studied. The experiment was repeated during two consecutive years.

In both hybrids and experiments, plants grown under MWD showed an **epw** content higher than controls. A decrease in the **epw** from stage R6 to HM was observed. This could be attributed to the erosive action on the surface of the pericarp by particulate solids carried by wind or rain.

These results constitute valuable information for sunflower breeders to further investigate about the mechanisms that regulate wax content in the fruit's pericarp.

Keys words: Epicuticular wax - *Helianthus annuus* - pericarp - sunflower - water deficit.

INTRODUCTION

After the sunflower oil has been industrially obtained and cooled, a crystalline sediment can be observed which affect its commercial quality (Rivarola *et al*., 1988). This sediment is mainly composed by waxes of epicuticular origin (epicuticular waxes or **epw**). They come from the fruit's pericarp (hull; 83 %) (Martin and Juniper, 1970; Morrison, 1983), from the seed teguments (16 %) and the embryo (1 %) (Morrison *et al*., 1994).

The amount of waxes passing to the oil during the extraction process depends on the relative hull content of the fruit and the amount of wax its carries. In modern hybrids with high oil content, a thin pericarp is strongly adhered to the seed increasing **epw** transfer to the oil (Morrison *et al*., 1984). In these hybrids, fruit's hull content is inversely correlated with oil wax content (Morrison, 1983).

Although waxes constitute a problem for the oil industry, to date, no studies on the development of **epw** in the sunflower hull are available. So, there is not information about the variability in the **epw** content among hybrids or the effect that different environmental factors and agronomical practices could produce on the **epw** genesis.

It is known that thermal and water stress can trigger an enhance epicuticular wax synthesis in several plant organs (Premachandra *et al*., 1992) and that the level of response is phenotipically sensitive and genetically controlled (Koornneef et al, 1989; Jenks *et al*., 2002). So in this work we have analyzed the evolution of **epw** content in the pericarp through different developmental stages of two sunflower hybrids grown under two water regimes.

MATERIALS AND METHODS

- *Plant material*. Two sunflower hybrids, Dekasol (DK) 3900 and DK4030, were sown at the Department of Agronomy, UNS, experimental field (Bahía Blanca. Argentina, Lat. S., 38° 45'; Long. W, 62°11') during two consecutive growing seasons (Experiment I: 2003/2004; Experiment II: 2004/2005). The crop was grown under drip irrigation and managed according to recommended conventional agronomical practices (Pereyra and Farizo, 1981). Plant density was adjusted at 5.6 plants/m^2. Fruit samples taken from the capitulum's periphery during reproductive stages R6, R9 and harvest maturity (HM) (Schneiter and Miller, 1981) were analyzed (Table 1).
- *Treatments*. During the reproductive stages R4 to R6 a moderate water deficit (MWD) was generated by interrupting irrigation. It was monitored by measuring the relative water content of plant leaves (RWC$_{\text{leaf}}$) in each treatment at different crop developmental stages.

- *Determination of* **epw** *content*. **Epw** content was measured in the pericarp of the fruits at each sampling stage, for each hybrid and experiment, following the technique described by Franchini and Hernández (2006) using carbon tetrachloride as extracting agent. The **epw** content was expressed in mass of **epw** by mass of pericarp dry weight (mg/g).
- *Experimental design and statistical analysis*: Both experiments consisted of complete randomized split plots, with water status assigned to main plots and hybrid to subplots. To determine differences between treatments and hybrids, experimental results were processed by ANOVA and differences between means were evaluated with LSD test.

Table 1. Days from first anthesis to attain reproductive stages **R6**, **R9** and **HM** (Schneiter and Miller, 1981) in each of the hybrids and experiments **HM:** harvest maturity

	Experiment I		**Experiment II**	
	Hybrid		**Hybrid**	
Stage	**DK3900**	**DK4030**	**DK3900**	**DK4030**
R6	8	12	13	12
R9	58	48	48	44
HM	71	68	60	56

RESULTS

- *Plant water status.* In both experiments and at different sampling times, an overall decrease of RWC_{leaf} was observed in plants under MWD comparing to control plants (Figs. 1A and 1B). Nevertheless a significant reduction (Fig. 1B; $p<0.05$) of the RWC_{leaf} was only observed 79 days after crop emergency in Experiment II accompanied by a temporary leaf wilting. After irrigation was reestablished, leaves recovered their normal turgidity.
- **Epw** *content in the pericarp.* In both hybrids and treatments a reduction in **epw** content was observed from R6 to HM (Figs. 2A and 2B). In fruits of DK3900, during Experiment I, the observed reduction was 28 % ($p<0,05$) from stage R6 to HM (Fig. 2A), while during the Experiment II, the observed reduction was not significant ($p = 0,09$; Fig. 2B).

  Although a continuous reduction in the **epw** content of DK4030 fruits was observed from stage R6 to HM, such observation resulted not significant in Experiment I ($p>0,05$; Fig. 2A). In Experiment II, **epw** content was significantly reduced to 14% ($p<0,05$) from R6 to R9, with no significant differences detected between the latter stage and HM (Fig. 2B).
- *MWD and* **epw** *content.* Since there was no hybrid x water regime interaction ($p>0,05$) for the variable **epw** content, only the average results for both hybrids (Table 2) in each experiment are presented. In both experiments and in each reproductive stage studied, **epw** of fruits from plants under MWD showed a 33% **epw** increase compared to control plants (Table 2). Nevertheless it must be mentioned that during Experiment I water deficit was not as high as expected so the differences between treatments could be not so evident.

Table 2. Average content of **epw** (mg/g) of the pericarp of the sunflower hybrids DK3900 and DK4030 both in control and under moderate water deficit (MWD). **R6**, **R9**: Reproductive stages as described by Schneiter and Miller (1981). **HM**: harvest maturity.

	Experiment I			**Experiment II**		
Stage	**Control**	**MWD**	**S.E.**	**Control**	**MWD**	**S.E.**
R6	5,08 **a***	6,24 **a**	0,3	5,25 **a**	7,08 **b**	0,3
R9	4,72 **a**	5,59 **b**	0,4	4,46 **a**	6,53 **b**	0,3
HM	3,64 **a**	4,44 **a**	0,4	4,24 **a**	6,22 **b**	0,3

* In a row, within each assay, means followed by the same letter are not significantly different at $p>0,05$. **MDW**: Moderate Water Deficit. **S.E.**:Standard error.

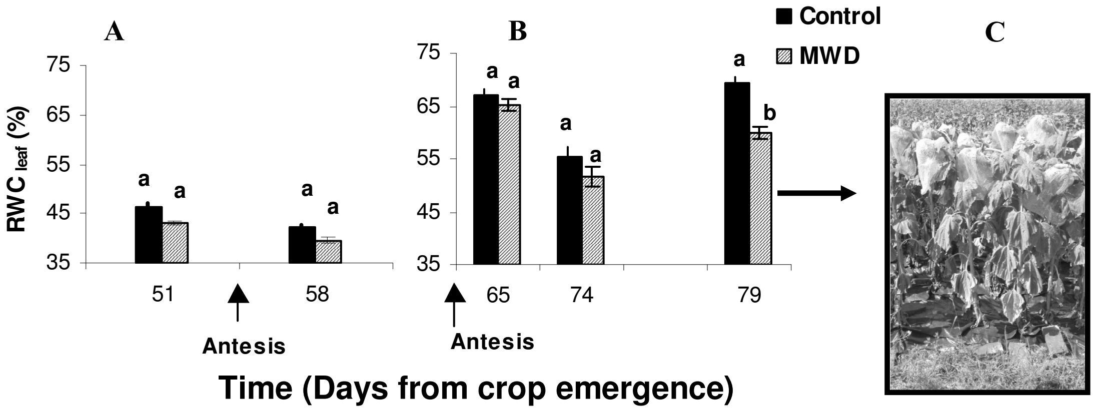

Figure 1. Leaf relative water content ($\mathbf{RWC}_{\mathrm{leaf}}\%$) in the sunflower hybrids DK3900 and DK4030 during experiment I (**A**) and II (**B**). **C.** Temporary wilting of leaves of plants under MWD during experiment II, 79 days after crop emergence (24 days after anthesis). Leaves become turgent once irrigation was reestablished. **MDW**: Moderate Water Deficit. Within each set, bars topped by the same letter are not significantly different at p>0,05.

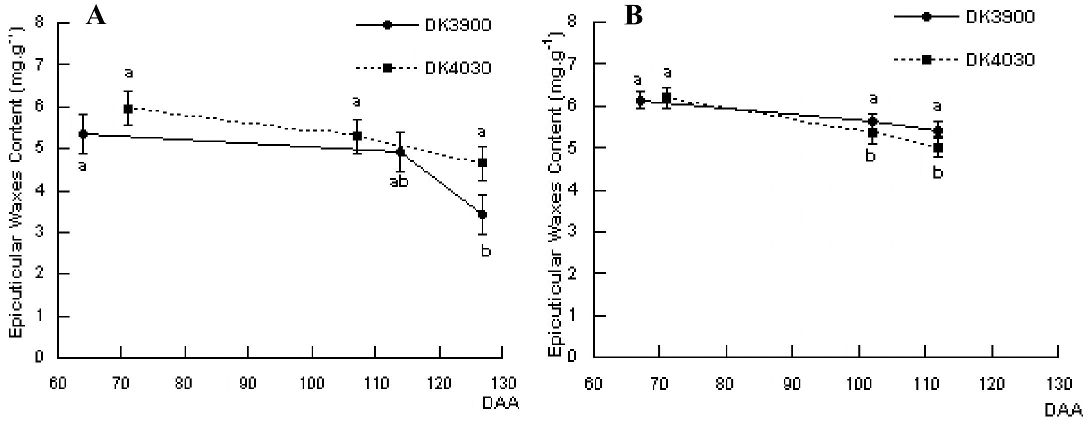

Figure 2. Changes with time of **epw** (mg/g) in the pericarp of fruits of the sunflower hybrids DK3900 and DK4030, averaged across water treatment, from R6 to HM. **A)** Experiment I. **B)** Experiment II. **DAA**: Days after anthesis. For each hybrid, values followed by different letters indicate significant differences among sampling dates (p<0,05).

DISCUSSION

- *Plant water status.* The observed RWC_{leaf} magnitudes (Fig. 1) show that in both experiments, the procedure of irrigation shortage was sufficient to generate a suboptimal water status in the critical developmental stages of the formation of pericarp (stages R5 and R6; Lindström *et al*., 2000).

- **Epw** *content in the pericarp.* The observed reduction in **epw** content from R6 to HM in both hybrids and experiments, could be attributed to the erosive action produced by several environmental factors, among those rainfall and wind are particularly common. They can transport abrasive particulate material removing wax crystals from the pericarp surface. The same effect has been observed in leaves of *Eucalyptus* sp. (Baker and Hunt, 1986), *Brassica* sp. and *Fraggaria* sp. (Neinhuis and Barthlott, 1997). Also, in both hybrids and experiments, the highest content of **epw** measured in R6, when the pericarp is still young and contains high water concentration (Rondanini *et al*., 2007), agrees with the phenomenon observed by Neinhuis *et al*. (2001). These authors demonstrated that cuticular transpiration allows the waxes attached to water molecules, to move from the inner regions of the leaf to its outer surface. So, in young epidermis with a thin cuticle, such as that present in undeveloped fruits, with lower resistance for the passage of waxes through it compared with mature ones, a higher **epw** content can be expected.

- *MWD and* **epw** *content*. In both experiments and in the three fruit developmental stages (Table 2), the imposed leaf water deficit induced to a comparatively higher **epw** than controls. Similar results can be found in leaves of weeping lovegrass (*Eragrostis curvula* Schrad) (Echenique *et al*., 1986) and sorghum (*Sorghum bicolor* L.) (Premachandra *et al*., 1992), where a constant water stress led to an increase in the content of **epw** and a reduction in the cuticular transpiration rate.

CONCLUSIONS

A moderate plant water deficit during fruit development led to an increase of 33 % of the **epw** content in the pericarp, compared with those of the control plants.

From R6 to HM, **epw** content decreased possibly due to the erosive action produced by wind and rain on the fruit surface.

The results shown here can be used as a physiological tool to define the dynamics of wax accumulation in the sunflower fruit pericarp, a variable that can be genetically modified (Jenks *et al*., 2002). Thus, breeders would be able to manipulate two characters, which are currently antagonists in the sunflower fruit: seed oil and pericarp wax content.

ACKNOWLEDGMENTS

This work was funded by grants to L.F.H. of the Agencia Nacional de Promoción Científica y Tecnológica and the Argentine Sunflower Association (ANPCyT-ASAGIR, PICTOS-13151), the Secretaría Gral. de Ciencia Tecnología (SeGCyT-UNS) and the Comisión de Investigaciones Científicas (CIC, La Plata) Argentina. Sunflower seed was kindly provided by Monsanto of Argentina.

REFERENCES

Baker, E.A. and G.M. Hunt. 1986. Erosion of waxes from leaf surfaces by simulated rain. New Phytol. **102**: 161-173.

Echenique, C.V., M. Poverene and N.R. Curvetto. 1986. Epicuticular wax and water stress in *Eragrostis curvula* (Schrad) cultivars. Effects of water stress on the quantity and morphology of epicuticular wax. Microscopía Electrónica y Biol. Cel. **10**: 91-95.

Franchini, M.C and L.F. Hernández. 2006. Contenido de ceras epicuticulares del pericarpio de frutos de girasol. Distribución espacial en el capítulo. XXVI RAFV. Chascomús, Buenos Aires, Argentina.

Jenks, M.A. 2002. In: The Arabidopsis Book. Somerville, C., E. Meyerowitz (Eds). ASPB. pp, 1-24. http://www.aspb.org/publications/arabidopsis.

Koornneef, M., C.J. Hanhart and F. Thiel. 1989. A genetic and phenotypic description of *eceriferum* (cer) mutants in *Arabidopsis thaliana.* J. Heredity **80**: 118-122.

Lindström, L.I., C.N. Pellegrini and L.F. Hernández. 2000. Anatomía y desarrollo del pericarpio de distintos genotipos de girasol (*Helianthus annuus* L.). In: Proc. 15th Int. Sunfl. Conf., Toulouse, France. pp. D13-D18.

Martin, J.T. and B.E. Juniper. 1970. The cuticles of plants. Edward Arnold, London, p. 347.

Morrison III, W.H. 1983. Variation in wax content of sunflower seeds with location and hibrids. J. Am. Oil Chem. Soc. **61**: 1013-1015.

Morrison III, W.H., R.E. Sojka and P. Unger. 1984. Effects of planting date and irrigation on wax content of sunflower-seed oil. J. Am. Oil Chem. Soc **61**: 1242-1245.

Morrison III, W.H., R.J. Hamilton and C. Kalu. 1994. Sunflower Oil. In: R.J. Hamilton (ed.), Development in Oils and Fats. Liverpool John Moores University, Liverpool, UK, pp. 133-152.

Neinhuis, C. and W. Barthlott. 1997. Characterization and distribution of water-repelent, self-cleaning plant surfaces. Ann. Bot. **79**: 667-677.

Neinhuis, C., K. Koch and W. Barthlott, 2001. Movement and regeneration of epicuticular waxes through plant cuticles. Planta **213**: 427-434.

Pereyra, V.R. and C.L. Farizo. 1981. Girasol. Técnicas de Producción. INTA, Balcarce, 27 p.

Premachandra, G.S., H. Saneoka, K. Fujita and S. Ogata. 1992. Leaf Water Relation, Osmotic Adjustment, Cell Membrane Stability, Epicuticular Wax Load and Growth as Affected by Increasing Water Deficits in Sorghum. J. Exp. Bot. **43**: 1569-1576.

Rivarola, G., M.C. Añon and A. Calvelo. 1988. Chrystalization of waxes during sunflower seed oil refining. J. Am. Oil Chem. Soc. **62**: 1508-1513.

Rondanini, D.P., R. Savin and A. Hall. 2007. Estimation of physiological maturity in sunflower as a function of fruit water concentration. Europ. J. Agronomy **26**: 295-309.

Schneiter, A.A. and J.F. Miller. 1981. Description of sunflower Growth Stages. Crop Sci. **21**: 902-904.