# DEVELOPMENT OF CUTICLE AND EPICUTICULAR WAXES IN THE PERICARP OF THE SUNFLOWER (*HELIANTHUS ANNUUS)* FRUITS

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**Abstract**

The waxes present in the sunflower oil, when it is cooled down and stored after industrial processing, are part of organic compounds that form the cuticle and its outermost deposits, called epicuticular layers of the pericarp and the seed teguments. This work presents a preliminary description of the ontogenesis of the cuticle and the epicuticular waxes in the pericarp of sunflower fruits of a modern hybrid genotype, grown under field conditions. It was seen that between reproductive stages R4 and R8, the cuticle thickness increased from 1.0-2.4 µm to 4.5-8.8 µm. At plant maturity and harvesting time the deposits of epicuticular waxes were distributed in a decreasing gradient in the basipetal direction of the fruits. These results shown here are the starting point to define the critical moment of wax accumulation in the pericarp of the sunflower fruit.

## Introduction

When the sunflower oil is cooled down and stored after industrial processing, a white undesirable precipitate is produced. This comes mainly from the waxes present in the pericarp and in the seed teguments. They are part of organic compounds that form the cuticle and its outermost deposits, called epicuticular layers (Martin and Juniper, 1970; Cutter, 1971).

In recent years there has been reported an increment of waxes in the oil together with the remarkable increase of oil content in the fruits of new commercial genotypes (Morrison et al., 1984; Aguirrezábal and Pereyra, 1998). This would suggest a positive correlation between fruits of "high oil" genotypes and wax content in the seed teguments or the pericarp.

Considering the potential need to modify this morphological characteristic of sunflower fruits in order to reduce the amount of waxes obtained in the oil and since the epicuticular wax development a genetically manageable character (Jenks et al., 2002), it is relevant to get information on aspects related to its genesis, its variability amongst genotypes and its response to different crop growth conditions and management.

The studies on waxes in the fruits of Angiosperms are mainly performed in fleshy fruits, in which its presence is important for quality and conservation (Cutler, 1982). The studies made on wax development in the pericarp of dry fruits such as the sunflower cypselas are scarce (Kerstiens, 1996). There is no information available about cuticular morphology, nor about epicuticular waxes occurred during the maturation of its fruits. The most detailed description for the cuticle of sunflower fruits was made by Hanausek (1902), Putt (1944) and more recently by Roth (1977). Nevertheless this information is not quantitative and was obtained from pericarps of “low oil” fruits obtained from genotypes quite different from the modern ones.

In this work, a preliminary description of the ontogenesis of the cuticle and the epicuticular waxes in the pericarp of fruits of a modern sunflower hybrid genotype, grown under field conditions, is presented.

## Materials and Methods

Sunflower plants of the hybrid Dekasol 3900 (Monsanto Argentina), were grown in the experimental field of the Agronomy Department-UNS (Lat. S., 38º45’; Long. W, 62º11’) at a density of 5.6 plants per m sq. under appropriate irrigation and fertilization.

Ovaries were sampled starting from the appearance of the phyllaries (R4 stage, Schneiter and Miller, 1981) and at 3-day intervals until physiological maturity (R9) and harvesting maturity (15% RH, dry weight basis).

For the study of cuticle ontogenesis, the ovaries were fixed in FAA and processed for histological analysis (Jensen, 1962). Sections 10 µm thick were cut, stained with safranin-fast green (Jensen, 1962) and observed with light microscopy. Sampled ovaries fixed in glutaraldehyde 8% in phosphate buffer were processed for transmission electron microscopy (TEM, Ruzin, 1999). For the ontogenic study of the epicuticular waxes deposit, ovaries collected at each sampling date were air-dried. These ovaries were processed for observation with scanning electron microscopy (SEM, Ruzin, 1999).

## Results

The preliminary results obtained in this experiment are summarized in Figure 1. The main reproductive developmental stages are described in a time line. During these stages, significant changes were observed in cuticle development and in the epicuticular wax accumulation on the pericarp surface.

It was seen that, between stages R4 and R8 (Figures 1E-H), the cuticle thickness increased from 1.0-2.4 µm to 4.5-8.8 µm (Figure 2; Figures 1A1, B1). Also, there was an important accumulation of epicuticular waxes (Figures 1A2-C2), acquiring a globular appearance (Figure 1B2).

At plant maturity and harvesting time (Figure 1I) it was seen that the deposits of epicuticular waxes were distributed in a decreasing gradient in the basipetal direction of the fruits (Figures 1C2-E2).

The results presented herein are the starting point to define the critical moment of wax accumulation in the pericarp of the sunflower fruit. Further microstructural and sequential research, in plant fruits of different genotypes growing under contrasting environmental conditions, will allow determining in more detail their accumulation dynamics and the factors able to modify it.

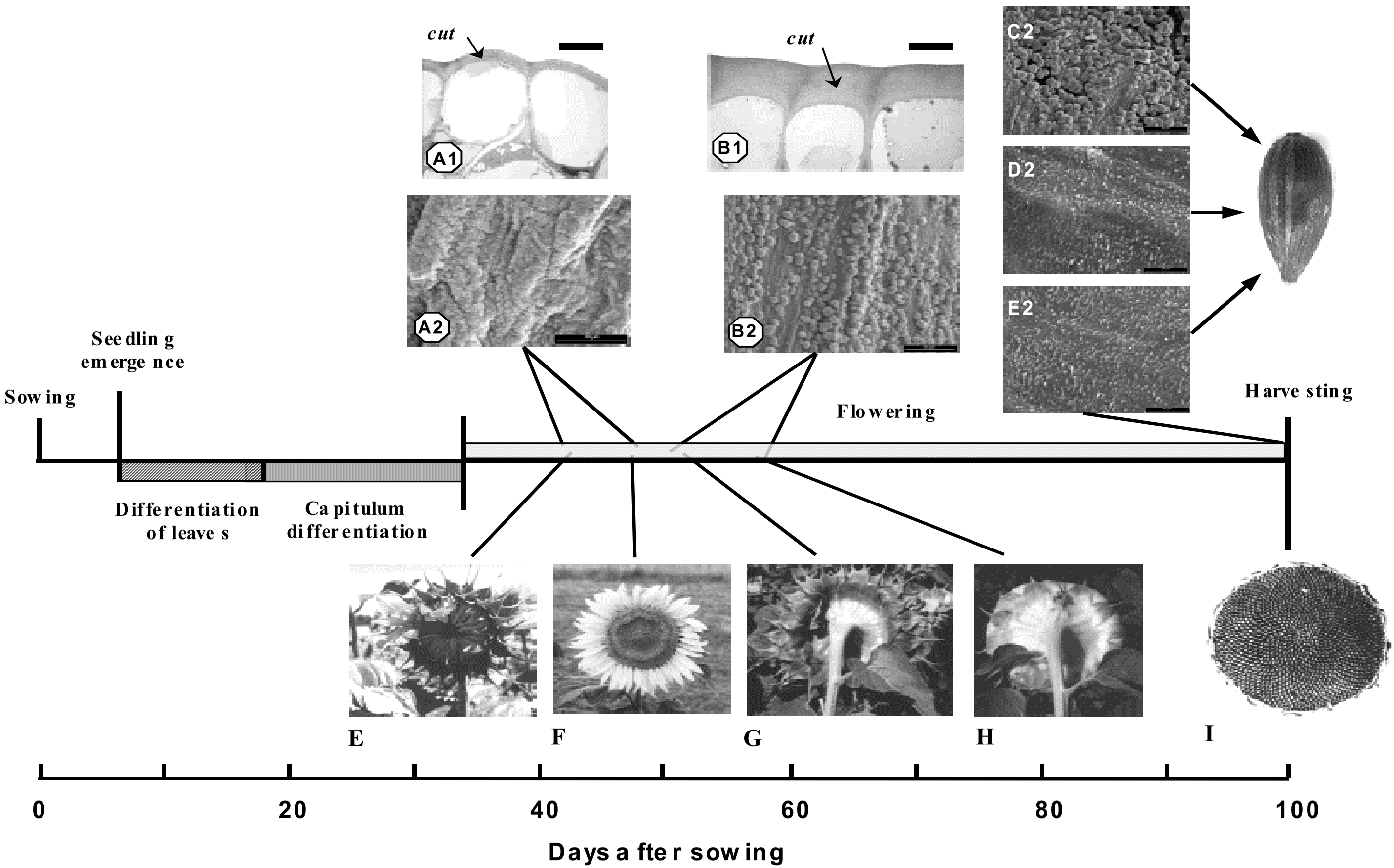


Figure 1. Development of the cuticle and deposit of epicuticular waxes in the pericarp. A1-B1: TEM of transverse sections of the pericarp showing the ultrastructure of the cuticle (cut). Bar=10 µm. A2-E2; SEM of the pericarp surface showing the morphology of its epicuticular waxes. Bar=20µm. E-I: External capitulum morphology.

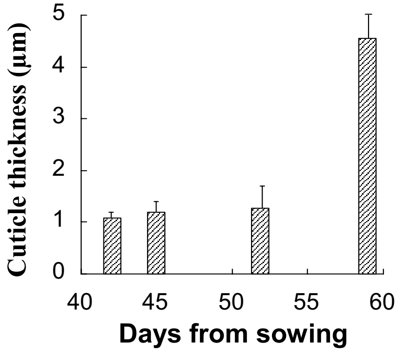


Figure 2. *Cuticle thickness in the pericarp of the sunflower fruit at four sampling times. Bars = ± 1 s.e.*


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