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# The development of the cuticle and epicuticular wax of the grape berry

by

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## Développement de la cuticule et de la cire épicuticulaire de la baie de raisin

Résumé : La cuticule et la cire épicuticulaire des baies de raisin du cépage Thompson Seedless se sont développées en couches morphologiquement distinctes couvrant les cellules épidermiques du pistil. La surface de l'ovaire n'avait pas de cuticule ou de cire couvrante jusqu'à approximativement 4 semaines avant floraison. La formation de la cuticule a commencé environ 3 semaines avant floraison sous forme de stries cuticulaires hautement organisées et fermement serrées. Les stries ont continué à se séparer et à s'aplatir, et sont devenu progressivement plus désorganisées pendant la croissance après floraison de la baie de raisin. La formation de la cire épicuticulaire a commencé avec l'apparition de simples plaques de cire en l'espace de quelques jours après floraison. Les plaques de cire ont augmenté en taille, nombre, et complexité pendant la maturation des baies de raisin.

Key words: berry, epidermis, lipid, growth, histology.

## Introduction

Grape berries, like the fruits and leaves of most land plants, are covered by non-living layers of cuticle and epicuticular wax. These protective layers shield the underlying plant tissues from desiccation, infection by pathogenic bacteria and fungi, insect attack, and injuries due to wind, physical abrasion, frost and radiation (MARTIN and JUNIPER 1970). The deposition of pesticides, growth regulators and other agricultural chemicals on the surface of a plant is influenced by the nature of the cuticle and overlying wax layer (MARTIN and JUNIPER 1970). The uptake of water (BONNER 1968) and chemical substances (FLORE and BUKOVAC 1978, 1981; BAKER and HUNT 1981; EL-OTMANI and COGGINS 1985) by the aerial portions of the plant is also affected by the surface waxes.

The epicuticular wax layer of grape berries not only plays an important physiological role during berry development, but also impacts on the economic aspects of all viticultural commodities. The wax bloom scatters light and imparts a frosted appearance to the berry (MARTIN and JUNIPER 1970), which is considered attractive and desirable by consumers of table grapes (NELSON 1979). The structural arrangement of the wax platelets is thought to be the controlling factor in non-stomatal water movement through the berry skin (CHAMBERS and POSSINGHAM 1963; POSSINGHAM *et al.* 1967), and therefore affects water loss from fresh grape berries (YAMAMURA and NAITO 1983) and drying rates of raisin grapes (MARTIN and STOTT 1957). Berry waxes are the primary source of waxes in wines and may contribute to colloidal turbidity of wines (ZHEREBEN and KOLESNIK 1984).

Epicuticular wax may also play an important role in the resistance of grape berries to infection by *Botrytis cinerea* (MAROIS *et al.* 1985, 1986). In tight clusters, the portions of the berry surface that develop in close contact with adjacent berries lack the platelet

structure typical of the normal wax morphology (ROSENQUIST 1986). These contact areas are also more susceptible to *Botrytis* infection under controlled laboratory conditions (MAROIS *et al.* 1986). Small holes or pores, 0.5—2.0 μm in diameter were frequently seen in the wax layer of contact areas, but not in non-contact areas (MAROIS *et al.* 1986; ROSENQUIST 1986). Perforations of similar size were reported in cuticles of *Botrytis*-susceptible varieties from which the wax layer had been removed (BLAICH *et al.* 1984), and it was suggested that the perforations were related to disease susceptibility.

Experimental treatments that alter the amount or structure of the surface wax on a grape berry, including treatment with chloroform or application of many agricultural surfactants, also increase the susceptibility of the berry to infection by *Botrytis* (MAROIS *et al.* 1985). Epicuticular wax production has similarly been shown to be inhibited by application of pesticides in several other agricultural species (PFEIFFER *et al.* 1959; NORRIS and BUKOVAC 1968; FLORE and BUKOVAC 1978, 1981) and by gibberellin application in navel oranges (EL-OTMANI and COGGINS 1985), although in those studies it was not determined if the chemicals themselves or the surfactants used in the spray applications were the cause of the inhibition of wax production.

Environmental variables may also affect the amount or structure of epicuticular wax on many plant surfaces. Morphological differences in wax structure have been observed under conditions of varying light intensity, humidity, and temperature (BANKS and WHITECROSS 1971; REED and TUKEY 1982).

The development of epicuticular wax has been studied in several plant species by the use of scanning electron microscopy (BANKS and WHITECROSS 1971; JARVIS and WARDROP 1974; REED 1982; REED and TUKEY 1982; EL-OTMANI and COGGINS 1985). Scanning electron microscopy was also used in the present study to document the normal morphological development of the epicuticular wax and cuticle of the grape berry.

## Materials and methods

Grape berries (*Vitis vinifera* L. cv. Thompson Seedless) were collected at frequent intervals from 4 weeks pre-anthesis through maturity from vines grown in the University vineyard, Davis, California. Gibberellins or other agricultural chemicals containing surfactants were not used during the growing season, but sulfur dust was applied bi-weekly as a fungicide. Pre-anthesis pistils and young berry specimens were viewed whole; older specimens consisted of small pieces of berries, 5 mm in diameter, which included the wax layer, cuticle and epidermis. The fresh specimens were frozen in liquid nitrogen and packed in dry ice, then dried in an FTS Systems Dura-Dry + Dura Top freeze drier unit at a shelf temperature of −50 °C, and a condensor temperature of −95 °C. Dried specimens were mounted on aluminum stubs with copper paint (GC Electronics) and coated with gold in a Technics Hummer sputter coater. Samples were viewed with an International Scientific Instruments DS 130 scanning electron microscope and photographed with type 55 Polaroid film. Post-anthesis berries to be viewed for cuticle development were rinsed in 3 one-minute changes of chloroform before dissection to remove epicuticular wax.

## Results and discussion

The cuticle and epicuticular wax of Thompson Seedless grape berries developed as morphologically and developmentally distinct layers. Formation of the cuticle began on the pre-anthesis pistil approximately 3 weeks before bloom. Prior to this time, the epi-

dermal cell walls were clearly visible (Fig. 1 A). The outer tangential cell walls of the epidermal cells were thicker than interior walls, but the outer wall surface was smooth and showed no signs of cuticular deposition (Fig. 1 B).

Cuticle development began with the formation of ridges at the perimeters of the epidermal cells (Fig. 1 C). The cuticle formed as a distinct layer easily distinguishable from the epidermal cell wall when seen in cross section (Fig. 1 D). Deposition of cuticular ridges proceded rapidly in the week following cuticle initiation, and within 2 weeks

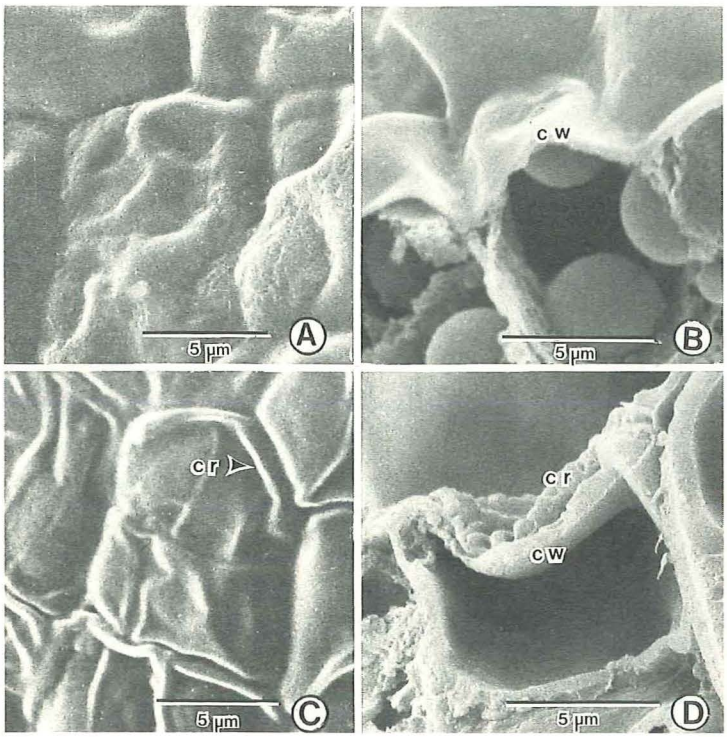


Fig. 1: Scanning electron micrographs of cuticle formation on the pre-anthesis grape pistil. — A) The surface of the ovary 4 weeks before anthesis. B) A cross section through the ovary approximately 3 weeks pre-anthesis, showing smooth outer epidermal cell walls. C) The surface of the ovary 3 weeks pre-anthesis, showing formation of cuticular ridges at the perimeters of the cells. D) Cross section through the pistil approximately 2 weeks pre-anthesis, showing cuticular ridges covering the outer epidermal cell walls. All samples were freeze-dried without fixation. — cw = cell wall; cr = cuticular ridge.

Microscopie électronique à balayage de la formation de cuticules sur le pistil de la baie de raisin avant floraison. — A) Surface de l'ovaire 4 semaines avant floraison. B) Coupe du pistil de la baie de raisin environ 3 semaines avant floraison, montrant les parois lisses cellulaires extérieures épidermiques. C) Surface de l'ovaire 3 semaines avant floraison, montrant la formation de stries cuticulaires sur les périmètres des parois des cellules. D) Coupe d'une baie de raisin environ 2 semaines avant floraison, montrant des stries cuticulaires couvrant les parois cellulaires épidermiques extérieures. Tous les échantillons ont été lyophilisés sans fixation. — cw = parois cellulaire; cr = strie cuticulaire.

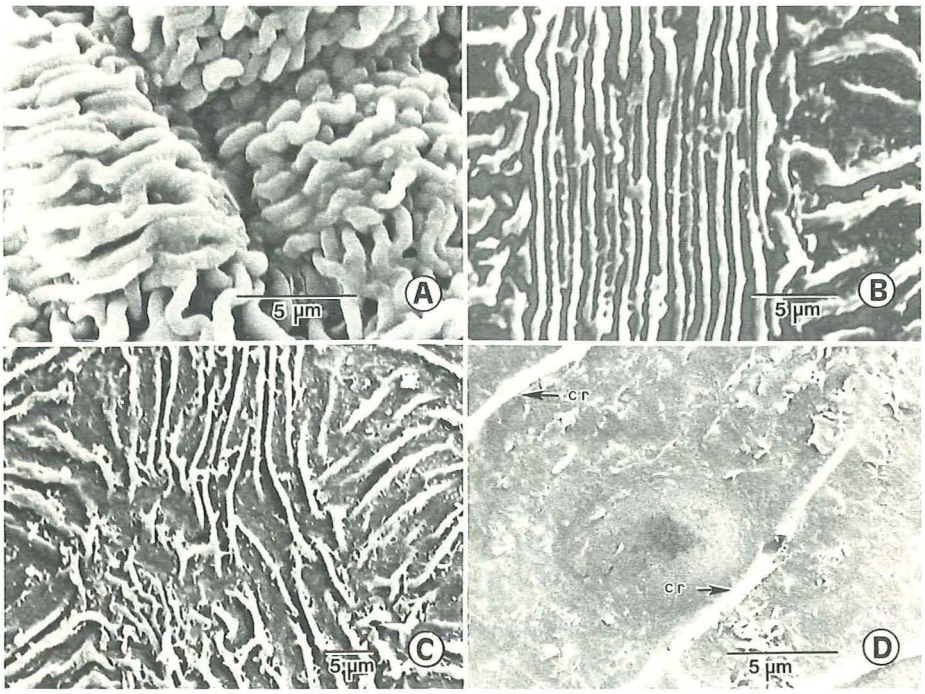


Fig. 2: Development of cuticular ridges. The epicuticular wax was removed with chloroform, and the samples were fixed in glutaraldehyde before freeze drying. — A) The surface of the ovary approximatively 2 weeks pre-anthesis. B) The berry surface shortly after anthesis. C) The berry surface at berry set, 11 d after bloom. D) The berry surface at maturity, showing remnants of cuticular ridges.

Développement de la strie cuticulaire. La cire épicuticulaire a été enlevée au chloroforme et les échantillons ont été fixés au glutaraldéhyde avant lyophilisation. — A) Surface de l'ovaire environ 2 semaines avant floraison. B) Surface de la baie de raisin peu après floraison. C) Surface de la baie de raisin à nouaison, 11 d après floraison. D) Surface de la baie de raisin à maturité, montrant les restes des stries cuticulaires.

the entire surface of the ovary was covered with tightly appressed, convoluted cuticular ridges (Fig. 2 A). The ridges were oriented in rows parallel to the longitudinal axis of the pistil over the epidermal cells, and perpendicular to the longitudinal axis between cells. Similarly oriented cuticular ridges have been reported for the cultivar Gordo (CONSIDINE and KNOX 1979 a and b, 1981). Approximately 10—15 ridges formed over each epidermal cell, and a similar number between adjacent cells.

At anthesis, epicuticular wax began to form over the cuticle. The wax was removed with chloroform to allow the continued observation of cuticular development. By anthesis, the cuticular ridges were less tightly appressed than immediately after their formation; they appeared to have spread apart and flattened somewhat during the pre-anthesis period of pistil expansion (Fig. 2 B). The number of cuticular ridges covering each cell remained constant after their initial formation, however, indicating that deposition of cuticle in the form of ridges is not continuous during berry development.

Deposition of cuticle in a less highly ordered form may continue, however. This is suggested by the observations of CONSIDINE and KNOX (1979 b), who reported that cuticle thickness increased during early berry growth, then remained constant during the later stages of grape berry development.

The highly ordered pattern of ridges seen on pre-anthesis pistils became progressively more disorganized as the cuticular ridges continued to flatten and spread apart during the period of rapid berry growth after fertilization (Fig. 2 C). CONSIDINE and KNOX (1979 b) likewise found that the pattern of cuticular ridges in Gordo became disorganized, which they attributed to continued cell division. At maturity, the berry cuticle was a thin, continuous, relatively smooth layer underlying the outer layer of epicuticular wax, with only scattered remnants of cuticular ridges still visible (Fig. 2 D). The berries used in this study were seedless grapes that had not been treated with gibberellin, and thus were relatively small. It seems likely that the remnants of ridges would disappear entirely on larger berries. Although previous workers considered the purpose of the cuticular ridges to be obscure, it was noted that ridging brings about an increase in cuticular surface area (CONSIDINE and KNOX 1979 a, b). Because of the spreading and flattening of the ridges during berry growth seen in this study, we suggest that the ridges function as a form of stored cuticular material which later spreads out, giving a continuous protective layer over the developing berry during periods of rapid berry expansion. The almost complete disappearance of the ridges at maturity,

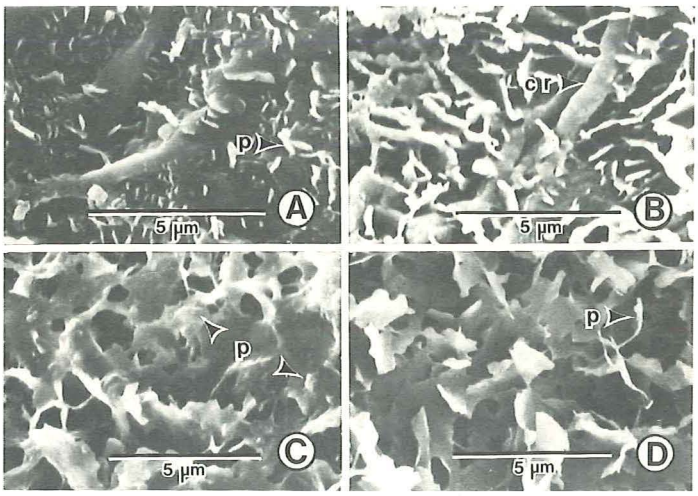


Fig. 3: The development of the epicuticular wax platelets. — A) Small, simple wax platelets on the berry surface a few days after bloom. B) The surface of the berry 3.5 weeks after anthesis; wax platelets almost obscure the cuticular ridges. C) The surface wax of the berry 8.5 weeks after anthesis. D) The surface of the berry at maturity, 13 weeks after anthesis. — All samples were freeze-dried without fixation. p = wax platelet.

Développement des plaques de cire épicuticulaire. —A) Plaques de cire simples et petites sur la surface de la baie de raisin quelques jours après floraison. B) Surface de la baie de raisin 3,5 semaines après floraison; les plaques de cire recouvrent presque les stries cuticulaires. C) La cire de la surface du raisin 8,5 semaines après floraison. D) Surface de la baie de raisin à maturité, 13 semaines après floraison. — Tous les échantillons ont été lyophilisés sans fixation. p = plaque de cire.

when the cuticle has become a smooth, uniform layer underlying the epicuticular wax, supports this suggestion.

Epicuticular wax was ordinarily not present on the ovary surface prior to anthesis, although an amorphous substance, possibly wax, was occasionally seen on the cuticle surface of berries sampled just prior to bloom. Within a few days after anthesis, the wax layer began rapid development in the form of small, individual, upright wax platelets that appeared both between and covering the cuticular ridges (Fig. 3 A). The wax platelets increased in both size and number during the post-anthesis period of berry growth. By 21 d after bloom, the wax platelets almost completely obscured the cuticular ridges which covered the surface of the developing berry (Fig. 3 B). The platelets were most densely distributed during the lag phase of growth, then spread apart somewhat as the berry resumed rapid growth after veraison (Fig. 3 C). YAMAMURA and NAITO (1983) similarly found that the amount of berry wax per unit surface area increased rapidly during the first 30 d after bloom in the cultivar Delaware, then remained relatively constant thereafter. RADLER (1965) and RADLER and HORN (1965) reported that the amount of extractable wax per unit surface area did not change from very young fruit through maturity in Thompson Seedless. CHAMBERS and POSSINGHAM (1963) found, however, that brief exposures to chloroform such as those used in that study do not completely remove surface waxes. The anatomical evidence reported here follows the pattern described for Delaware; platelets increased in both size and density through veraison, then decreased slightly in density of distribution in the final period of berry expansion. Although SEM cannot accurately quantify the total mass of the epicuticular wax, it seems unlikely that the changes in platelet size and density observed here could occur without increasing the amount of wax per unit surface area.

In addition to an increase in size, the wax platelets also increased in complexity during berry development. When first secreted, they had the form of small, simple plates with blunt edges. The edges of the platelets became progressively sharper and more serrated during berry development. At maturity, 90 d after anthesis, the wax platelets were overlapping and lace-like, terminating in sharply lobed edges (Fig. 3 D). Structural variation has been correlated with chemical differences in epicuticular waxes of other species (BAKER and HUNT 1981), and changes have been reported in the chemical composition of the wax from Thompson Seedless berries during berry growth (RADLER and HORN 1965). Thus, the changes in the appearance of the wax platelets seen here may be related to chemical changes in the wax.

## Summary

The cuticle and epicuticular wax of Thompson Seedless grape berries developed as morphologically and developmentally distinct layers covering the epidermal cells of the pistil. The ovary surface had no cuticle or wax covering until approximately 4 weeks before anthesis. The cuticle began to form about 3 weeks before anthesis as highly organized, tightly appressed cuticular ridges. These ridges began to spread apart late in the period of pre-anthesis pistil expansion. The ridges continued to spread apart and flatten, and became progressively more disorganized during post-anthesis berry growth. Epicuticular wax formation began with the appearance of small, simple wax platelets within a few days after anthesis. The wax platelets increased in size, number and complexity as the berries matured.

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