CUTICULAR TRANSPIRATION AND WAX STRUCTURE AND COMPOSITION OF LEAVES AND FRUIT OF *VITIS VINIFERA*

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Summary

The fine structure of the surface wax of leaves of sultana vines (*Vitis vinifera* var. *sultana*) has been examined using the carbon replica technique. Leaf wax was found to be morphologically similar to the wax on the surface of grapes and to consist of a series of overlapping platelets. A brief period (30 sec) of exposure to light petroleum vapour disorganized the platelet structure of both leaf and fruit wax. This treatment markedly increased the cuticular transpiration of both fruits and leaves. The results are discussed in relation to the known chemical composition of these waxes. It is suggested that the surface wax, which consists of overlapping platelets that are hydrophobic in nature, may be important in controlling cuticular transpiration in both the fruit and leaves of grape-vines.

I. Introduction

Investigations of the factors controlling water loss from grape berries have suggested that the important layer controlling water loss in these fruits, which lack stomata, is the waxy outer layer of the cuticle (Martin and Stott 1957; Dudman 1962; Grncarevic 1963). The physical appearance and structure of the surface wax of grapes have been examined by Chambers and Possingham (1963) who suggested that the physical arrangement of overlapping wax platelets and their hydrophobic nature was important in controlling water loss.

The chemical nature of the wax layer on the surface of grapes has been investigated by Radler and Horn (1965). They have shown the wax to consist of a "hard" wax component, oleanolic acid, together with a "soft" wax component, soluble in light petroleum vapour. The soft wax is a mixture of long-chain (chain lengths 14–33) acids, alcohols, aldehydes, esters, and hydrocarbons. In further work the chemical nature of the wax of leaves and fruit was compared (Radler 1965*a*) where it was shown that the wax of leaves contained only the soft wax fraction. The available data on wax composition of fruit and leaf surfaces is summarized in Figure 1. Radler (1965*b*) showed that the water loss from grape berries is greatly increased following brief periods of exposure of the berry to light petroleum vapour. Treatments as short as 10 sec in light petroleum vapour exerted a demonstrable increase in the rate of water loss.

The present study compares the fine structure of wax of leaves and fruit of *Vinis vinifera* var. *sultana*, and the effects of light petroleum vapour on the morphological appearance of both waxes and on leaf cuticular transpiration.

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II. Methods

Carbon replicas of the surface of both leaf and fruit waxes were prepared as described by Chambers and Possingham (1963). Water loss from grapes was measured by frequent weighings of samples placed in a forced draught oven and the drying rates were obtained over an initial 5-hr period. The light petroleum vapour treatments were applied by holding mature grape berries approximately 10 cm above a beaker of boiling petroleum (b.p. 40–60°C) for various times. A Soxhlet assembly was used for the chloroform treatment. Leaf transpiration was measured by cementing with Vaseline a 10-cm^2 section of a leaf to a microscope slide, the lower side of the leaf towards the glass. The leaves were held at 20°C and were weighed at frequent intervals. All water loss in this arrangement occurred through the upper leaf surface, which in the case of grape leaves is stomate free. This method gave an estimate of the rate of cuticular water loss.

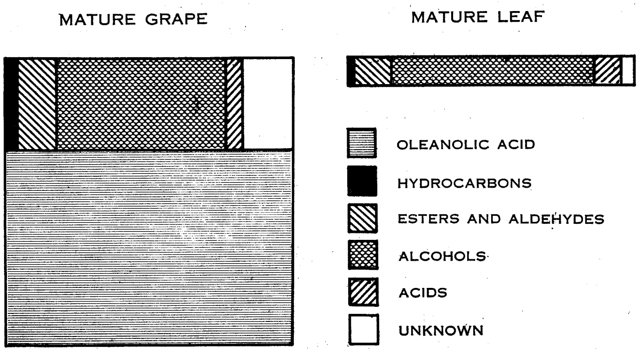
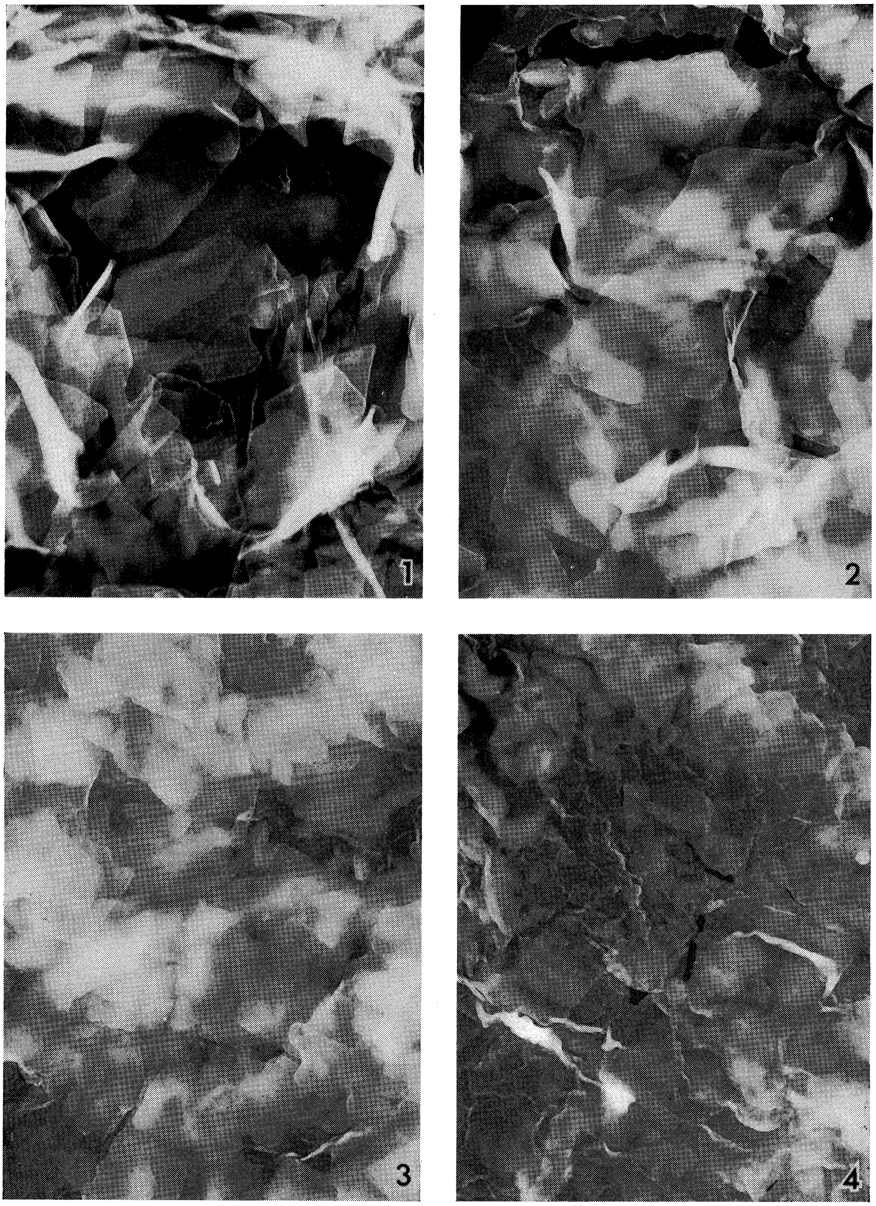


Fig. 1.—Summary of the relative quantities by weight and the individual components present in the wax of mature grapes and mature leaves of *Vitis vinifera* var. *sultana*.

III. Results

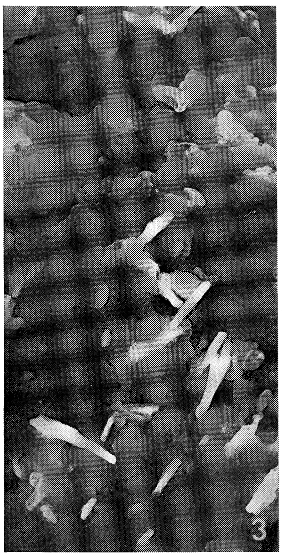
A replica of untreated (control) sultana fruit (Plate 1, Fig. 1) shows the overlapping arrangement of wax platelets. There are, however, some differences in appearance of the replicas obtained in the present experiment with those previously published (Chambers and Possingham 1963). The wax platelets in the present study are more angular in outline and their margins are less undulate. These differences may be due to variation in growing conditions which occur between years, or they may be due to the fruit having been sprayed at earlier stages in its development with fungicides containing detergents. Detergents are known to affect wax structure (Wortmann 1965).

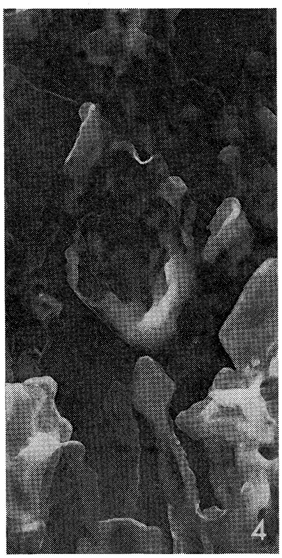
Fruits treated with light petroleum vapour (Plate 1, Figs. 2, 3, and 4; Plate 2, Fig. 1) showed a progressive, although variable, reduction in the surface platelets, and these became disorganized with longer periods of treatment. They were completely removed by chloroform extraction (Plate 2, Fig. 2). However, the significant result is that a treatment as brief as 10 sec has an observable effect on the arrangement of the wax.

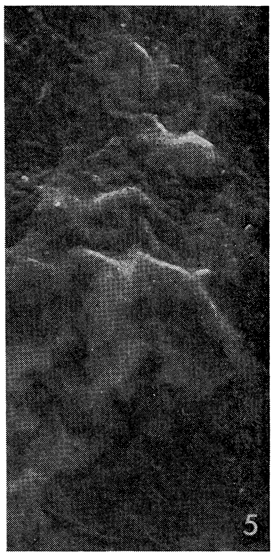


Figs. 1–4.—Untreated (control) sultana berry [Fig. 1 (No. 11017)] and sultana berries treated with light petroleum vapour for 10 sec [Fig. 2 (No. 11023)], 20 sec [Fig. 3 (No. 11026)], and 60 sec [Fig. 4 (No. 11035)]. All figures × 18,500. Numbers in parentheses in Plates 1 and 2 refer to plates stored in the Electron Microscope Laboratory, Botany School, University of Melbourne.

CUTICULAR TRANSPIRATION OF SULTANA LEAVES AND FRUIT







Figs. 1 and 2.—Replicas of sultana berry treated with light petroleum vapour for 3 min [Fig. 1 (No. 11044)] and after complete Soxhlet extraction in chloroform for 2 hr [Fig. 2 (No. 11047)]. Both figures × 18,500.

Figs. 3–5.—Replicas of lower [Fig. 3 (No. 8193)] and upper [Fig. 4 (No. 8182)] surface of sultana leaf (control), and of upper surface of leaf treated for 10 sec with light petroleum vapour [Fig. 5 (No. 8183)]. All figures × 18,500.

When mature sultana berries (1·5–2·0 g) were exposed to boiling light petroleum vapour, considerable amounts of soft wax were removed. Approximately one-quarter of the total soft wax is removed in 10 sec (see Table 1). By contrast only very small amounts of hard wax are removed, even after 300 sec. The cuticular transpiration rate of the berries exposed to light petroleum vapour increased with the time of treatment; this can be correlated with the amount of soft wax removed.

Replicas of leaf surfaces indicate that the wax on the upper surface of untreated leaves is somewhat similar in morphology to that of fruit. There are a series of regular overlapping platelets (Plate 2, Fig. 4). These platelets are a little smaller than those on the fruit, and generally they become damaged as the leaf matures.

TABLE 1

CUTICULAR TRANSPIRATION RATES AND WAX REMOVAL OF SULTANA GRAPES TREATED WITH LIGHT PETROLEUM VAPOUR

Period of Exposure to Vapour (sec)	Soft Wax Removed (%)	Hard Wax* Removed (%)	Rate of Loss of Water ($mg/cm^2/hr$)
0	0	0	0·23
10	26	1	0·34
60	63	5	0·57
300	85	12	0·90

* Oleanolic acid.

In other work we have found that the more protected lower leaf surface has a denser array of wax platelets (Plate 2, Fig. 3) which are similar in morphology to those of the fruit, and these generally are less damaged as the leaf ages than those of the upper surface. The effect of light petroleum vapour on the leaf wax was essentially similar to its effect on the fruit. Disorganization of the platelets occurred in the brief exposure period of 10 sec (Plate 2, Fig. 5). After an exposure of 30 sec no further change in the fine structure of the wax was observed.

The effect of exposure to light petroleum vapour for 60 sec on cuticular transpiration in leaves is shown in the following tabulation:

	Rate of Loss of Water ($mg/cm^2/hr$)					
Control	7·3	6·8	4·0	3·5	4·3	2·5
Treated with light petroleum vapour	86·4	97·8	102·5	114·0	106·7	99·0

The mean rate of loss of water (±S.E.) for the control and vapour treatment were $4 \cdot 73 \pm 0 \cdot 78$ and $101 \cdot 1 \pm 3 \cdot 79$ respectively, the difference being significant at $P < 0 \cdot 01$.

The treatment responses of leaves are far larger than those obtained with fruit. It seems that treatment with light petroleum vapour increases the water movement rates by some 20–25 times over that of the controls. In fruit the increase is only fourfold.

IV. Discussion

The results of published data on the chemical nature of the wax of sultana fruits and leaves (Radler and Horn 1965; Radler 1965*a*) is summarized in Figure 1. The total amount of wax on the fruit is some 10 times that on the leaves on a unit area basis. As well, there is a considerable qualitative difference between the waxes of leaves and fruit. Leaf wax consists entirely of the soft wax fraction which is readily removable by light petroleum vapour, but the fruit wax contains some 70% of oleanolic acid which is not removed to any great extent.

A comparison of the replicas obtained with fruit before and after exposure to light petroleum vapour indicates that this treatment, which has increased the cuticular permeability, has also altered the physical arrangement of the wax on the surfaces. Data presented for the percentage removal of wax indicates that, in a 60-sec treatment, a significant percentage of the hydrocarbon, aldehyde, and alcohol fractions is removed, but virtually none of the hard wax oleanolic acid component comes off. Such treatment results in the almost complete disorganization of the wax platelets which were previously a conspicuous feature of the surface wax on sultana grapes. The similarity of the fine structure of the wax surface of sultana leaves and fruit and the similar behaviour in light petroleum vapour of these wax surfaces suggest that, in both cases, the wax platelets consist of soft wax material. At present it is not possible to study the relative distribution of the wax fractions on and in the fruit surface layers. However, it seems likely that the hard wax (oleanolic acid) occurs more abundantly in the layers of the fruit cuticle rather than associated with the outer platelet zone.

The experiments in which leaves were exposed briefly to light petroleum vapour suggest that wax is the important barrier in cuticular transpiration in leaves, as well as in fruit. The importance of surface wax in controlling cuticular transpiration is supported by the experiments of Grncarevic and Radler (1967) who have recently shown that the evaporation of water through plastic membranes can be markedly suppressed by coating them with soft wax components isolated from sultana grapes.

With fruit it has been suggested that it is the structural arrangement of wax platelets, together with their hydrophobic surfaces, which controls water movement. This control is exerted by restricting the pathway for evaporation from the fruit cuticle surface to the relatively long narrow and hydrophobic capillary channels between the wax platelets (Chambers and Possingham 1963). This mechanism involves the water being transferred through the waxy platelet layer in the vapour form. Evidence presented here indicates that this mechanism may also operate in the case of leaves which only bear "soft" wax platelets. It seems probable that this same mechanism controls cuticular transpiration in the case of vine and other types of leaves with waxy cuticles.

V. References

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