

## **Stomata and starch in grape berries**

by

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### **Stomata und Stärke in Traubenbeeren**

**Zusammenfassung.** — Dünnschnitte durch sich entwickelnde Traubenbeeren der Sorten Sultana und Cabernet Sauvignon wurden nach Einbettung in Kunstharz lichtmikroskopisch untersucht. An Fruchtknoten und jungen Beeren wurden die Stomata aufgrund der Stärkekörner in den Schließzellen aufgefunden. An älteren Beeren bildeten sich unter den Stomata Lenticellen. Je nach Sorte variierte die Anzahl der Lenticellen zwischen 2 und 16 pro Beere, wovon bis zur Hälfte mit Stomata vergesellschaftet sein konnten.

Bei der Anthese enthielten die meisten Zellen des Perikarps Stärkekörner. Diese verschwanden mit fortschreitendem Beerenwachstum; bei der Reife kamen sie nur noch in der Epidermis und in einigen subepidermalen Zellschichten vor.

### **Introduction**

Grape berries have a slight but measureable photosynthetic activity which falls as the berry grows (GEISLER and RADLER 1963, KRIEDEMANN 1968). The purpose of this investigation was to establish whether stomata, to permit gas exchange, occur on the surfaces of ovaries and berries. Further, the cells of ovaries and berries were examined to see whether they contained starch granules.

### **Materials and Methods**

Small fruiting plants of grapevine (*Vitis vinifera* L.) were established in a glasshouse according to the method of MULLINS (1966). Cultivars grown are listed in Table 1, except that berries of Bruce's Sport (a sport of Sultana) and one group of Sultana berries were selected from field grown plants.

For light microscopy, berries of Cabernet Sauvignon were selected at anthesis, at colour change and at maturity, and berries of Sultana and Bruce's Sport at maturity only. Small slices of these berries were fixed in 6% glutaraldehyde (0.025M phosphate buffer, pH 7.0) for 24 hours at 4° C. The specimens were then dehydrated and embedded in glycol methacrylate (FEDER and O'BRIEN 1968). Transverse 2  $\mu$ m sections were cut and stained with a) toluidine blue 0, b) the periodic acid — SCHIFF's reaction (PAS) using toluidine blue 0 or fast green FCF as a counterstain, or c) Sudan black B.

Counts of the numbers of stomata on ovaries of Cabernet Sauvignon and lenticels on berries of all cultivars (Table 1) were made using fresh material. Ovaries were cut in half longitudinally, placed in a solution of 0.2N iodine in 0.18N potassium iodide for 5 minutes, then mounted on a slide and examined under a microscope. Stomata were detected by the presence of starch grains, which stained blue-black, in their guard cells. With berries, the "skin" was removed carefully and cleared in 80% methanol for 2 hours before staining with the iodine/potassium iodide solution. The dark orange suberized material of the lenticels stood out clearly from the surrounding epidermal cells.

## Results

### Developing berries

The mean diameter for twenty Cabernet Sauvignon berries was recorded at regular intervals from anthesis to full maturity, and a plot of berry size against time resulted in the usual double sigmoid growth curve.

At anthesis the pericarp was about 6 cell rows wide external to the vascular bundles and about 5 cells internal to them (Figs. 1, 2). The contents of many cells throughout the pericarp stained green or blue-green with toluidine blue, indicating the presence of phenolic compounds. In addition, these compounds appeared orange in sections treated with the PAS reaction, but this is a non-specific effect and should not be confused with the intense red staining of PAS-positive components such as starch grains and cell walls (The PAS reaction, as performed in this study, is a specific histochemical test for polysaccharides with vicinal hydroxy groups, and starch and hemicelluloses of the cell wall are strongly stained). Starch granules, recognized on the basis of their shape and intense staining after PAS treatment, were distributed throughout the pericarp (Fig. 2). Very occasionally stomata were seen in sections, but they were not a common feature (Fig. 1). Their guard cells contained large starch granules (Fig. 2). Cell walls in the pericarp were thin, except for those at either surface of the pericarp. Small wall-protuberances were apparent on the outer epidermal cell walls (Fig. 2).

At colour change the number of cells across the pericarp had increased, with approximately 6 cells internal to and 13 cells external to the vascular bundles (Fig. 3). Of the 13 external cells, the outer 10 layers were relatively small with thicker walls, and most contained phenols, whereas the inner 3 layers were large, thin-walled and lacked phenols. Similarly, phenols were absent from the large, thin-walled cells internal to the vascular bundles. Starch granules were present only in the outermost cells of the pericarp. Stomata were no longer recognized, but occasionally sections showed lenticels (Figs. 3, 4). The thick cuticle over the epidermis and the lipid-rich cork material of the lenticels stained strongly with Sudan black B (Fig. 4). (Sudan black B stains triglycerides and some steroids). The cuticle also stained lightly after the PAS reaction, suggesting a polysaccharide content.

The number of cells across the pericarp at full maturity was the same as at colour change. Phenols in the outer pericarp cells appeared less diffuse than at colour change and frequently formed dense aggregates, rather than being evenly dispersed throughout the cells (Fig. 5 cf. Fig. 3). Other features were unchanged, so that, except for cell size, berries at both stages appeared identical. Again, starch was present, but confined to the outermost few layers of cells.

Sultana and Bruce's Sport at maturity had phenol and starch distribution similar to that of Cabernet Sauvignon (Fig. 6). However, the number of cells across transects of the pericarp interior to vascular bundles was approximately 17 for Sultana and 14 for Bruce's Sport.

### Stomata and lenticels

There was an average of 4 stomata per ovary for Cabernet Sauvignon (Table 1). Fig. 7 illustrates the appearance of stomata with iodine-stained starch grains in their guard cells; under the microscope, stained pollen grains were readily distinguished from stomata. There was invariably a large number of stomata on the receptacle. In the mature Cabernet Sauvignon berry there were 6 lenticels per berry, and in the other cultivars investigated there was a range from 2 to 16 lenticels per berry (Table 1). Fig. 8 illustrates a lenticel on a Cabernet Sauvignon berry at

three focus levels. In Fig. 8c focus was on the apex of this lenticel, which carried two stomatal guard cells at this point, although only one cell contained starch

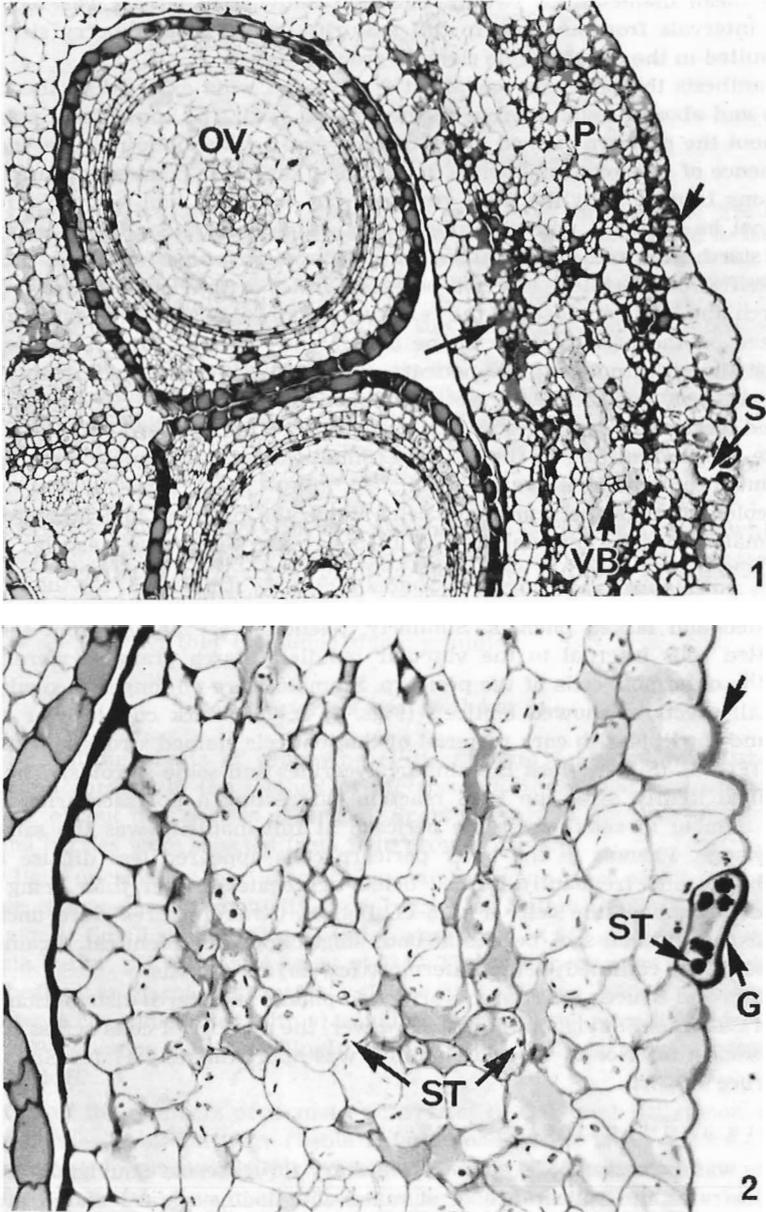


Fig. 1: Transverse section showing part of a Cabernet Sauvignon ovary at anthesis. Pericarp cells with dark contents (arrows) contain phenols. Toluidine blue 0.  $\times 260$ .  
 Fig. 2: As for Fig. 1, but stained with PAS/fast green. A guard cell of a stoma has been sectioned longitudinally. Arrow indicates protuberances of outer epidermal cell wall.  $\times 660$ .

P = pericarp; OV = ovule; S = stoma; G = guard cell; ST = starch.

Table 1

Numbers of lenticels on whole berries of different cultivars of grapevine  
Anzahl der Lentizellen bei ganzen Beeren verschiedener Rebsorten

Variety	Stage of development	Lenticels	
		Total number/ whole berry	Proportion having guard cells with starch, %
Gordo Blanco	Colour change	9.2 ± 0.8	35.1 ± 4.2
Rhine Riesling	Mature	6.6 ± 1.1	23.3 ± 5.6
Sultana (glasshouse)	Mature	6.3 ± 0.4	55.5 ± 5.4
Sultana (field)	Mature	16.3 ± 0.3	not assessed
Bruce's Sport (field)	Mature	15.0 ± 1.0	43.5 ± 5.7
Shiraz	Mature	2.3 ± 0.4	22.5 ± 10.8
Cabernet Sauvignon	Mature	6.0 ± 0.5	13.8 ± 5.1

Statistical comparison of numbers of stomata at anthesis and lenticels at maturity on half berries of Cabernet Sauvignon<sup>1)</sup>:

Cabernet Sauvignon	Anthesis	2.0 ± 0.2 Stomata/half berry
(half berries)	Mature	3.1 ± 0.4 Lenticels/half berry
(L.S.D. (5%) — 0.93)		

<sup>1)</sup> These figures represent a different sample to those given for whole Cabernet Sauvignon berries in the table above.

grains. Fig. 9 illustrates starch-containing guard cells from the apex of another lenticel. Not all lenticels on mature berries had guard cells with starch, and Table 1 lists the proportion. With this technique for identification of guard cells (i.e., presence of starch) it was not possible to determine whether lenticels without starch were also without guard cells.

### Discussion

PRATT (1971) concluded that ripe berries have no stomata, but that lenticels may occur. This study has established that stomata are present on the ovary at anthesis and that stomatal guard cells may occur at the apex of lenticels on mature berries. It is recognized that lenticels often originate beneath stomata (ESAU 1960), and this appears to occur in grape berries. It is of interest here that BESSIS (1972) has recently revealed the presence of stomata on Pinot grapes using the technique of stereoscanning microscopy.

The actual frequencies (Table 1) of stomata at anthesis (and of guard cells on mature berries) may be underestimated as our observations relied on the existence of starch which may not necessarily have been present. Considering the data for

Abb. 1: Querschnitt durch Teil eines Fruchtknotens von Cabernet Sauvignon während der Anthese. Die Perikarp-Zellen mit dunkel gefärbtem Inhalt (Pfeile) enthalten Phenole. Toluidinblau 0. — Vergrößerung 260X.

Abb. 2: Wie Abb. 1, jedoch mit PAS/Fastgreen behandelt. Eine Schließzelle einer Spaltöffnung ist längs getroffen. Der Pfeil rechts oben weist auf Vorsprünge der äußeren Epidermiszellwand hin. — Vergrößerung 660X.

P = Perikarp; OV = Samenanlage; S = Stoma; G = Schließzelle; ST = Stärke.

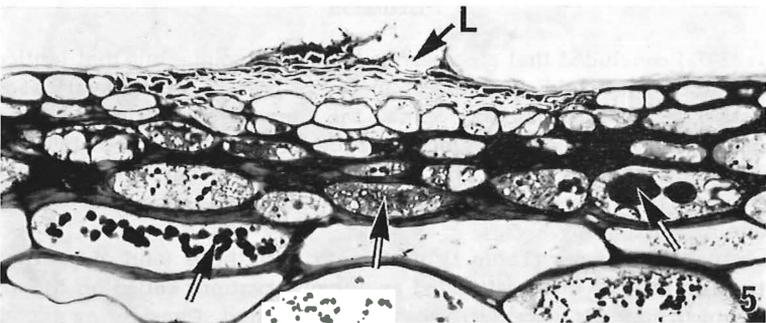
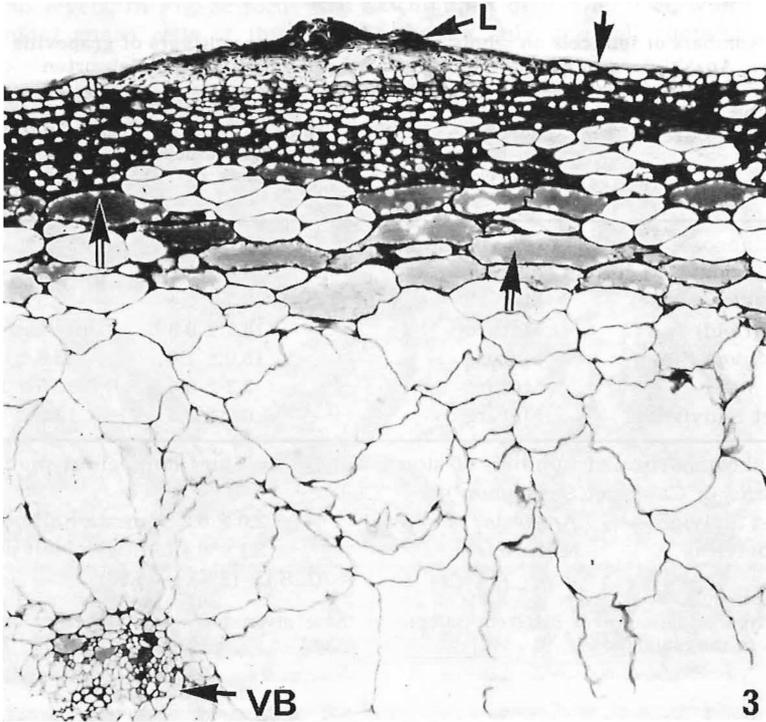


Fig. 3: Transverse section of part of the outer pericarp of a Cabernet Sauvignon berry at colour change. Single arrow indicates pale, PAS-stained layer over epidermis and double arrows indicate cells containing phenols. PAS/toluidine blue.  $\times 160$ .

Fig. 4: View of a lenticel at colour change after staining with Sudan black B. Thick cuticle over epidermis is shown at arrowhead.  $\times 240$ .

Fig. 5: View of a lenticel and portion of the outer pericarp of a mature Cabernet Sauvignon berry. Compare the appearance of the phenol-containing cells (double arrows) in this section with those in Fig. 3. PAS/toluidine blue.  $\times 370$ .

VB = vascular bundle; L = lenticel.

Cabernet Sauvignon in Table 1, it could be proposed that 2 stomata per ovary had guard cells lacking starch to give a total of 6 stomata per ovary, which would correspond to the 6 lenticels per berry observed at maturity. Alternatively, some lenticels may develop in stomata-free areas as found in other fruits (ESAU 1960). Differences between field and glasshouse values suggest that lenticels may develop as a response to damage. At all events there is evidence that stomata on ovaries are sites of lenticel-formation on mature berries. It is unlikely that the guard cells on the apices of lenticels are functional, since these cells appear to be isolated from other living cells by dead, corky material. It is noted that the density of stomata on ovaries of Cabernet Sauvignon (approx.  $1/\text{mm}^2$ ) was of the order of 100 times less than that normally found on mature leaf tissues (e. g., CURTIS and CLARK 1950). This difference corresponds to the hundredfold difference between immature grape berries and disks of grape leaves in their rates of oxygen evolution under standard conditions of illumination (KRIEDEMANN 1968). Reduction in photosynthetic activity of berries as they grow (KRIEDEMANN 1968) may be related to the isolation of stomata by corky material as lenticels develop beneath them, as well as to changes in volume to area ratios.

It is now clear that most cells in the pericarp of grape berries were capable of storing starch at anthesis, although at maturity only epidermal and a few layers of sub-epidermal cells still contained small granules. Starch may have been formed only as a direct result of photosynthesis in the chloroplasts. This could be one reason for the continued presence of granules in only the outermost cell layers, despite a rich supply of sugars within the berry. In common with many other plants with fleshy fruits, the grapevine contains large amounts of starch but negligible amounts of hexose in stems, roots and leaves. The converse applies in the ripe pericarp where carbohydrate storage is in the form of hexose. It is therefore an interesting biochemical problem to elucidate the mechanism controlling the fate of sugar entering the grape berry.

### Summary

Developing grape berries of the varieties Sultana and Cabernet Sauvignon were examined by light microscopy following plastic embedding. In ovaries and young berries stomata were detected by the presence of starch grains in their guard cells. In older berries lenticels formed beneath the stomata. Dependant on variety, lenticel numbers ranged between 2 and 16 per berry with up to half of these being associated with stomata.

At anthesis most cells of the pericarp of grape berries contained starch granules. These disappeared as the berries grew, and at maturity they were present only in the epidermis and a few layers of sub-epidermal cells.

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Abb. 3: Querschnitt durch Teil des äußeren Perikarps der Beere, Sorte Cabernet Sauvignon, während der Verfärbung. Helle, PAS-gefärbte Schicht auf der Epidermis durch einfachen Pfeil, phenolhaltige Zellen durch Doppelpfeile gekennzeichnet. PAS/Toluidinblau. — Vergrößerung 160×.

Abb. 4: Lentizelle während der Verfärbung, Sudanschwarz B. Die Pfeilspitze zeigt auf die dicke Kutikula der Epidermis. — Vergrößerung 240×.

Abb. 5: Lentizelle und Teil des äußeren Perikarps einer reifen Beere von Cabernet Sauvignon. Das Erscheinungsbild der phenolhaltigen Zellen (Doppelpfeile) des vorliegenden Schnittes hat sich gegenüber den entsprechenden Zellen der Abb. 3 verändert. PAS/Toluidinblau. — Vergrößerung 370×.

VB = Leitbündel; L = Lentizelle.

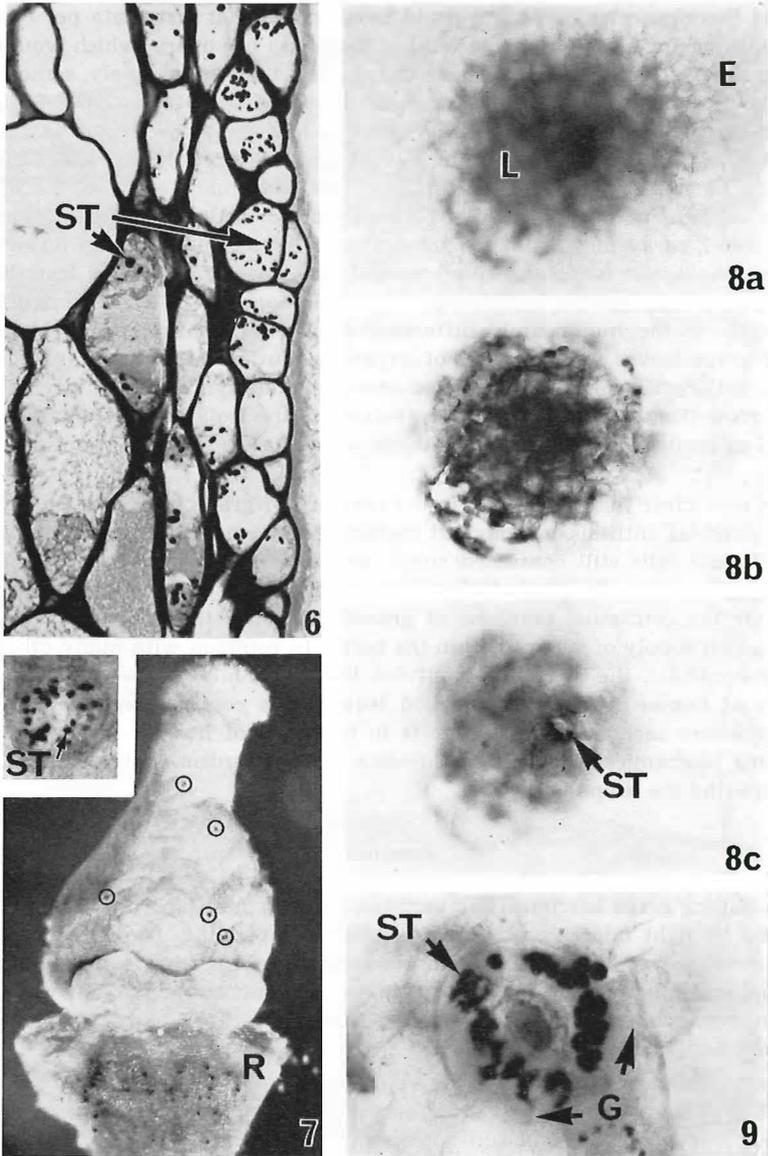


Fig. 6: Transverse section showing relatively abundant starch granules in the epidermis and a few sub-epidermal cells of a mature Sultana berry. Generally, however, starch granules were less numerous than shown here. PAS/toluidine blue.  $\times 490$ .

Fig. 7: Cabernet Sauvignon ovary after treatment with I/KI. Five Stomata (circled) were present on this half-ovary.  $\times 46$ . Inset: Higher magnification view of one of these stomata.  $\times 520$ .

Fig. 8: Lenticel on a mature Cabernet Sauvignon berry at 3 levels of focus. Focus is on the surface of the epidermis in 8a, on the mid-region of the lenticel in 8b and on the apex of the lenticel in 8c. Two guard cells, only one of which contains starch grains, are present in 8c. I/KI.  $\times 200$ .

Fig. 9: Two starch-containing guard cells at the apex of a lenticel on a mature Cabernet Sauvignon berry. I/KI.  $\times 1480$ .

G = guard cell; ST = starch; L = lenticel; R = receptacle; E = epidermis.

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Abb. 6: Querschnitt durch eine reife Sultanabeere: relativ zahlreiche Stärke-Granula in der Epidermis und in einigen subepidermalen Zellen. PAS/Toluidinblau. — Vergrößerung 490×.

Abb. 7: Fruchtknoten von Cabernet Sauvignon nach Behandlung mit Jod-Jodkalium-Lösung. Fünf Stomata (Kreise) wurden auf dieser Hälfte des Fruchtknotens gefunden. — Vergrößerung 46×. Nebenbild: Stärkere Vergrößerung eines solchen Stomas. — Vergrößerung 520×.

Abb. 8: Lentizelle an einer reifen Beere (Cabernet Sauvignon) in 3 verschiedenen Abbildungs-Ebenen. In Abb. 8a ist auf die Oberfläche der Epidermis, in 8b auf den mittleren Bereich der Lentizelle, in 8c auf die Spitze der Lentizelle scharf eingestellt. In Abb. 8c sind zwei Schließzellen vorhanden, von denen nur eine Stärkekörner enthält. Jod-Jodkalium. — Vergrößerung 200×.

Abb. 9: Zwei stärkeführende Schließzellen von der Spitze einer Lentizelle bei einer reifen Beere der Sorte Cabernet Sauvignon. Jod-Jodkalium. — Vergrößerung 1480×.  
G = Schließzelle; ST = Stärke; L = Lentizelle; R = Fruchtboden; E = Epidermis.