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Patterns of water flow in Riesling berries in relation to developmental changes in their xylem morphology¹⁾

by

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Wassertransport in Rieslingbeeren und entwicklungsbedingte morphologische Veränderungen ihres Xylems

Zusammenfassung: Kurz nach Beginn der Reifung („Veraison“) ließen sich im Xylem von Rieslingbeeren mit Hilfe eines wasserlöslichen Farbstoffes Veränderungen im Wassertransport feststellen. Der Wassertransport im Xylem erscheint verlangsamt und umgeleitet. Vor dem Einsetzen der Reifung fließt das Wasser vorzugsweise im peripheren Xylemsystem der Beeren. Während der Beerenreifung ist der Wassertransport hier stark reduziert, und die verbleibende, in ihrer Leitfähigkeit abnehmende Xylemkomponente ist ausschließlich das axiale (zentrale) System, das der Versorgung der Samen dient. Diese Veränderungen, die einen entsprechend verstärkten Wassertransport im Phloem notwendig machen, scheinen durch eine morphologische Veränderung hervorgerufen zu werden: das Auftreten von Brüchen in den peripheren Gefäßen. Diese Brüche treten in der Phase des erneuten Beerenwachstums unmittelbar nach Beginn der Reifung auf.

Eine Umleitung der Wasserversorgung in dieser Phase der Beerenentwicklung vom peripheren zum axialen Xylemsystem und vom Xylemsystem zum Phloemsystem hat letztlich wichtige Konsequenzen für die Mineralstoffzusammensetzung der Beeren.

Key words: berry, water, transpiration, translocation, growth, maturation, anatomy.

Introduction

During their second stage of rapid growth, i. e. after the start of ripening, grape berries accumulate mainly hexoses and water (WINKLER *et al.* 1974; COOMBE 1976, 1984; WILLENBRINK 1982). While much attention has been paid to the routes and mechanisms of sugar transport to the pericarp cells of the berry (SWANSON and EL-SHISHINY 1958, HALE and WEAVER 1962; KOBLET 1969; KRIEDEMANN 1969; DÜRING and ALLEWELDT 1980, 1984) the transport of water into the berry has not been explored.

In recent experiments with cv. Silvaner it had been shown that over the period 30—85 d after full bloom the transpirational water loss of the grape berry starts to decline and that simultaneously — in contrast to potassium — the rate of calcium uptake by the berry decreases (FRIEDEN 1984; DÜRING and OGGIONI 1986). The reduction in transpirational water loss is most likely attributable to changes in the skin since this is the region of lowest hydraulic conductance between soil and atmosphere (SLATYER 1967) and also is known to develop waxy layers and to suffer the degeneration of stomata to lenticels over this period. However, the opposite going changes in the rates of accumulation of calcium and potassium imply in addition a shift in the balance of the

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xylem and phloem flows with an increase in the phloem component and a decrease in the xylem component.

While structural changes at the epidermis have already been studied in some detail (CHAMBERS and POSSINGHAM 1963; RADLER 1965; HARRIS *et al.* 1968; BELIN 1972; ALLEWELDT *et al.* 1981; BLAICH *et al.* 1984), to our knowledge, little research has been done on changes in xylem structure within the berry. Here we present evidence for a correlated change in xylem structure and function in grape berries during their ripening which most likely is the major factor responsible for the shift in the balance of phloem and xylem flows, and thus in the mineral composition of the mature fruit so important to winemaking (HALE 1977).

Materials and methods

Clusters at different stages from around the onset of ripening to maturity were collected from field-grown Riesling (*Vitis vinifera* L.) vines in the early morning to minimise air embolism of the xylem. The shoulder (15–20 berries) of each bunch was then cut off under water in the laboratory and a 100 mm length of silicon rubber tube containing dye (0.15 % aqueous black amide (ESCHRICH 1976)) slipped over the cut end. Shoulders treated in this way placed under reasonably standard conditions (23 ± 2 °C, RH 30–40 %) in the draught of a small fan to enhance transpiration and thus dye infiltration. After allowing dye infiltration for varying periods of time, berries were removed using a sharp blade, cutting through the berry 1–2 mm from the stalk end to expose a surface some 8 mm in diameter. The axial and peripheral vascular bundles were clearly visible and were scored for the presence of dye (0 = nil, 1 = some, 2 = much).

Because it was suspected that developmental changes in xylem conductivity might occur, it was clearly desirable to know the physiological age of the berries assayed for dye infiltration. Each berry was therefore analysed subsequently, young (smaller and harder) berries for titratable acidity (weighed, homogenised, and titrated with 0.133 M NaOH) and older (larger and softer) berries for sugar (crushed and a drop of juice placed in an Abbé refractometer). From these results the physiological age of each of the more than 1000 berries involved in the dye flow experiment was determined by reference to standard time curves of titratable acidity or sugar level as appropriate, generated from the analysis of separate collections of fruit from the same vineyard and season. The physiological age of a berry is expressed as so many days after flowering (mid anthesis) with veraison occurring at day 70 and our data covering the period day 60 to day 110.

For anatomical study some 200 berries were chosen in the age range 60–105 d after flowering. This range was a little different from that in the dye infiltration experiment for reasons which will become apparent. These berries were halved axially, one half being reserved for anatomical study and the other used to assess physiological age as above. For anatomical study the skin was carefully removed using fine forceps and the exposed surface flooded with phloroglucin solution (2.5 g in 50 ml of 70 % aqueous ethyl alcohol) for 1 min and then with concentrated HCl. This rendered the (lignified) xylem in the peripheral bundles clearly visible. The xylem of these bundles was found to be broken at regular intervals in the older berries (Fig. 1). A number of tests and controls established that these breaks were not artifactual due, say, to our skin removal or staining procedures. The sum of the lengths of the gaps was then measured and expressed as a percentage of the sum of the lengths of the broken segments for each berry.

Berry size was calculated from the weights of the berries involved in the dye infiltration experiment. Allowance was made for the change in density accompanying a rising sugar concentration and the result expressed as a percentage increase in berry size after veraison (day 70). The berries were assumed spherical (a close approximation to reality for Riesling) and the linear rather than the cubic measure of size was adopted since this represents the change in berry perimeter to which the peripheral vascular bundles would be subject.

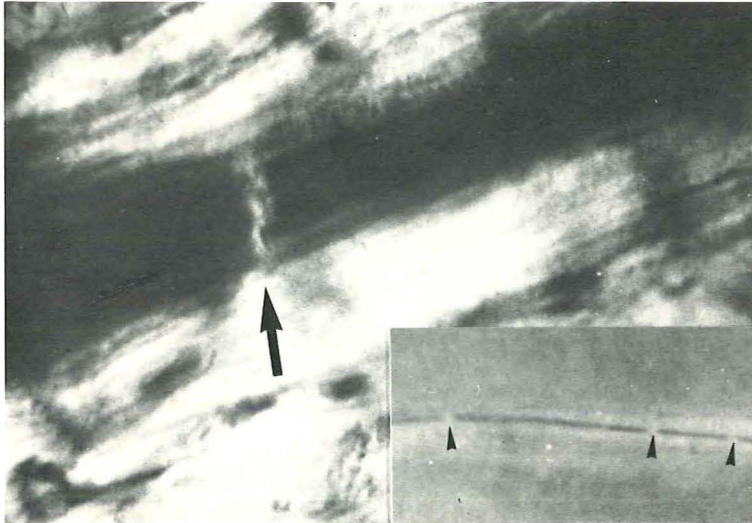


Fig. 1: Peripheral xylem system of a berry in the post veraison period, the arrows indicating gaps.
Peripheres Xylem einer Beere in der Phase der Nachveraison; die Pfeile weisen auf Brüche hin.

Results and analysis

The data in the first part of the experiment contained information on the relative flows of xylem fluid in the axial and peripheral vascular systems of Riesling berries. These data were in the form — berry age (x) vs fan exposure time (y) vs dye level (z). For the axial bundles there were more than 1000 evaluations of dye level and for the peripheral bundles more than 2000. It was inevitable that for some combinations of berry age : fan exposure time there was multiple replication whereas for others, values were missing altogether. To render the data suitable for graphing a single value of dye level for each cell in a regular grid of berry age versus fan exposure time was computed using all of the data, weighting the dye level data (z) according to the inverse square of their x , y distances from that cell. The axial and peripheral dye level data being treated separately, this procedure yielded two complete tables of dye level : berry age : fan exposure time one for each part of the vasculature. Note that because of a sharp fall off to 0 in dye infiltration to the peripheral bundles over the period 70—85 d after flowering it was necessary to split the data (for the peripheral bundles only) at

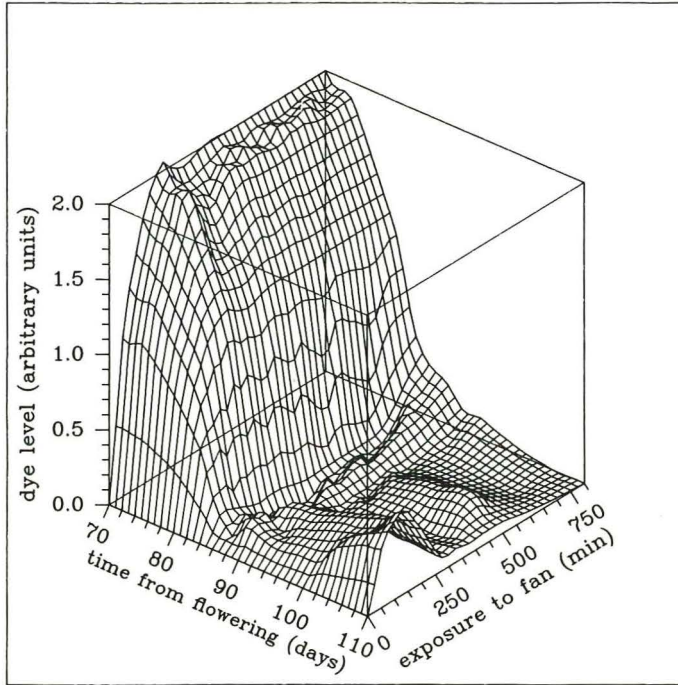


Fig. 2: Dye accumulation (z) in the peripheral vascular system of Riesling berries is plotted as a function of the period of exposure (y) to the draught of the fan and of berry age (x). (Dye level was scored as nil = 0, some = 1, much = 2.)

Die Farbstoffanreicherung (z) im peripheren Gefäßsystem von Rieslingbeeren in Abhängigkeit von der Ventilationsdauer (y) und vom Beerenalter (x). (Klassifizierung des Anfärbungsgrades: 0 = nicht, 1 = schwach, 2 = stark angefärbt.)

this discontinuity before applying the interpolation and smoothing computations in order to achieve a meaningful representation of dye distribution. The two parts were spliced together again prior to graphing which was performed using a package (P79) available on the VAX system.

Results are presented in Figs. 2 and 3 for peripheral and axial dye distribution respectively. Notice that as might be anticipated, dye level rises with increasing exposure time to the draught of the fan. The tendency to plateau after around 250 min of exposure arises from our method of assessing dye level which was unable to distinguish between 'much = 2' and higher accumulations of dye in the bundles. From these figures it is evident that in the peripheral bundles dye flow decreases steadily following veraison and ceases entirely around day 85 after flowering whereas that in the axial bundles decreases only slightly and more or less smoothly over the entire experimental period (day 70 to day 110). These trends have the result that for the younger berries dye appears first in the peripheral bundles and only later in the axial ones, while for older berries it appears only in the axial ones.

In Fig. 4 are presented the data for xylem breakage vs age (gaps are expressed as the percentage of the length of the broken segments). Breakage is clearly evident in all

except the youngest berries appearing suddenly after veraison (day 70), with the displacement of the broken segments increasing steadily as time proceeds. Comparison with Fig. 2 suggests that it is this increase which gives rise to the progressive reduction in the conductivity of the xylem in the peripheral bundles, with flow through them finally ceasing around day 85. At this stage the gaps have opened to around 8 %. Notice that the gaps continue to open as the berry grows peaking around day 95, at which time they represent some 12 % of the broken segment lengths. Notice too that this time (day 95) corresponds to the time at which berry size peaks (Fig. 5) have expanded by some 16 % since veraison (day 70). Both gap size and berry size reduce slightly after day 95 due to a net water loss from the fruit.

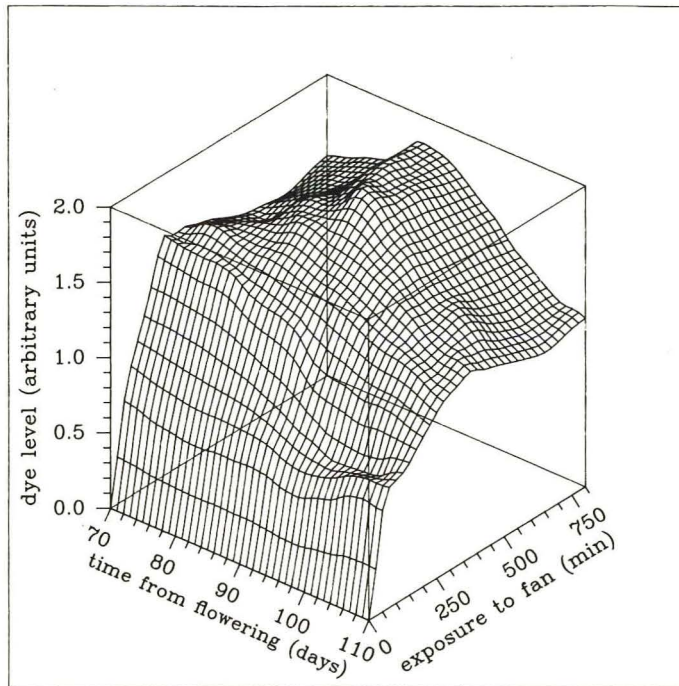


Fig. 3: Dye accumulation (z) in the axial vascular system of Riesling berries is plotted as a function of the period of exposure (y) to the draught of the fan and of berry age (x). (Dye level was scored as nil = 0, some = 1, much = 2.)

Die Farbstoffanreicherung (z) im axialen Gefäßsystem von Rieslingbeeren in Abhängigkeit von der Ventilationsdauer (y) und vom Beerenalter (x). (Klassifizierung des Anfärbungsgrades: 0 = nicht, 1 = schwach, 2 = stark angefärbt.)

Discussion

From the dye infiltration results it seems clear that within the Riesling berry a decrease in xylem conductance occurs soon after veraison (day 70). This decrease follows the collapse of the, initially more conductive, peripheral xylem system to leave

only the axial xylem system still functional. The collapse in peripheral xylem conductivity is probably due to the distinctive multiple breakages which occur in these bundles about this time and which open up progressively as berry growth continues. The gap size seems to reach a maximum of around 12 % on day 95 and then declines a little in synchrony with corresponding changes in berry size (compare Figs. 4 and 5).

At first sight there may seem to be a difficulty with this explanation since it is to be noted that flow continues for some 12 d after breakages suddenly appear (day 70). Moreover, linear extrapolation of the fitted line in Fig. 4 seems to suggest that breakage occurs several days earlier than veraison, at around day 63, with gap size at the time of veraison (day 70) already advanced to around 3 %. These discrepancies appear to deny a simple causal relation between the sudden resumption of berry growth at veraison and xylem breakage on the one hand, and between xylem breakage and hydraulic conductance decrease on the other. They may however be accommodated as follows. Persistence of flow between days 70 and 82 clearly indicates that breakage does not in itself cause an immediate and drastic decrease in xylem conductance. Rather, the results suggest that conductance declines steadily following the appearance of the breaks, flow becoming negligible only after some days and after the gaps have opened up to around 6 %. It is possible, for example, that a wound response initiated at breakage, renders the initially conductive gaps between the broken xylem strands progressively less conductive — this idea we did not explore experimentally. As for the extrapolative prediction that breakage occurred several days before veraison and

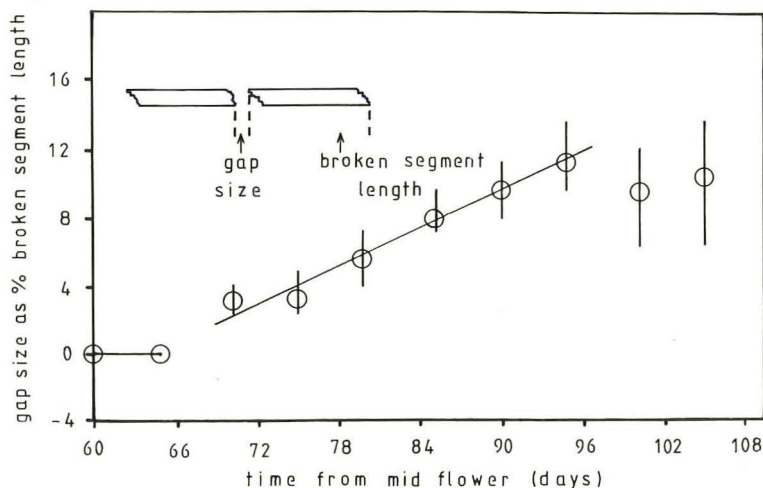


Fig. 4: Mean gap size (with 95 % confidence bars) expressed as a percentage of the broken segment length for the peripheral vascular system, is plotted as a function of berry age. Note that the regression line is fitted to data only for the period days 70 to 95 inclusive, over which gap size increased more or less linearly. Note too that while the x-intercept of this line suggests breakage to have occurred around day 63 no breakage was evident in the data before veraison (day 70).

Die durchschnittliche Ausdehnung der Lücken des peripheren Gefäßsystems in Abhängigkeit vom Beerenalter. Die Gesamtlänge der Lücken ist in Prozent, bezogen auf die Gesamtlänge der unterbrochenen Gefäßabschnitte, ausgedrückt (Vertrauensgrenzen für $P = 0,05\%$). Es ist zu beachten, daß die Regressionsgerade nur aus den Daten der Tage 70 bis 95 erstellt wurde. In dieser Phase nahm die Ausdehnung der Lücken mehr oder weniger linear zu. Obwohl der x-Achsenabschnitt dieser Geraden vermuten läßt, daß die Brüche etwa am 63 Tag auftraten, war aus den Daten kein Bruch vor der Veraison (70. Tag) erkennbar.

therefore before the second phase of berry enlargement was initiated, it should be recalled that the breaking strain of xylem tissues is in the range of 2—5 % (KOLLMANN and CÔTÉ 1968; NOBEL 1974). This means that, on breaking, there will be an almost instantaneous release of this strain as the stretched tissues relax and thus the abrupt appearance of a gap size of about this order. Our observation of the sudden appearance of already well developed gaps at veraison (day 70), the time the second phase of berry growth is initiated, is entirely consistent therefore with the likely physical properties of the xylem tissues and of post veraison growth initiated breakage.

There is a slight discrepancy between the percentage increase in gap size and that of berry size. Comparison of Figs 4 and 5 shows gaps size to increase a little more slowly than berry size would suggest it should. This is probably the result of an underestimate of gap size and overestimate of broken segment length due to the slightly jagged and oblique nature of the broken surfaces (see inset Fig. 4).

Breakdown of peripheral xylem water flow in the berry after veraison is not as serious a problem to maintenance of adequate water content as might at first be imagined since the fruit has at this stage no functional stomata and a very thick cuticle, so minimising transpirational water loss. Moreover, the vigorous increase in import of assimilate in the phloem probably supplies a more than adequate amount of water to the fruit. The degeneration of existing structures and failure to maintain an active xylem cambium able to replace any vessels that might be broken as the berry expands, is entirely consistent with what we know of the grape's physiology in the post veraison

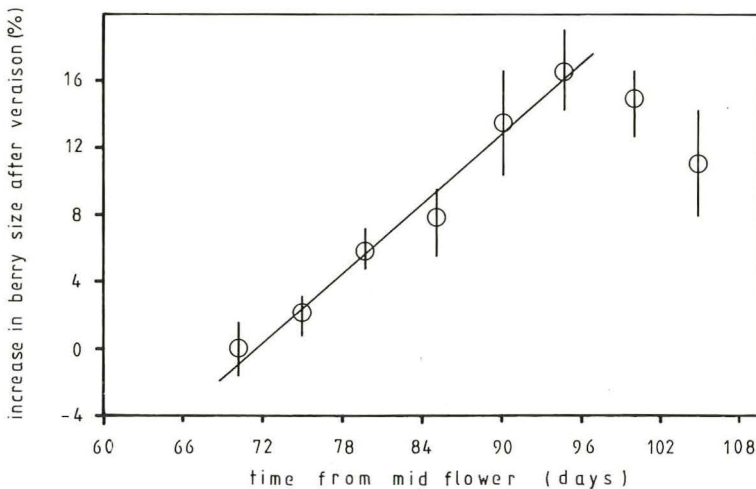


Fig. 5: The increase in berry size (with 95 % confidence bars) expressed as a percentage of berry size at veraison (day 70) is plotted as a function of berry age. Note that the regression line is fitted only to data over the period, days 70 to 95 inclusive, over which berry size increased more or less linearly. Note too the similar form of this relationship to that in Fig. 3 implying a causal relationship between berry growth and gap size.

Die Zunahme der Beerengröße in Prozent ihrer Größe zur Zeit der Veraison (70. Tag) in Abhängigkeit vom Beerenalter (Vertrauensgrenzen für $P = 0,05$ %). Es ist zu beachten, daß die Regressionsgerade nur aus den Daten der Tage 70 bis 95 erstellt wurde. In dieser Phase nahm die Größe der Beeren mehr oder weniger linear zu. Zu beachten ist auch, daß die Art dieser Beziehung derjenigen in Fig. 3 gleicht, womit sich eine Kausalbeziehung zwischen Beerenwachstum und Ausdehnung der Lücken andeutet.

phase (CONSIDINE and KNOX 1979). It is to be noted that the young seeds, whose development is substantially complete by this stage, are supplied by the axial bundles, and are less affected by these events than the fleshy pericarp. A shift in the balance of supply of xylem borne minerals from flesh to seed is implied. More important commercially is the shift in the balance of the fruits' water supply from the xylem stream to phloem stream which the decreasing xylem conductance and berry transpiration imply. These provide a most plausible explanation for the observed changes in mineral composition as the fruit matures (DURING and OGGIONNI 1986), and which ultimately are major determinants of winemaking quality.

The significance of these findings to the integrated sugar and water physiology of the developing fruit will be examined elsewhere.

Summary

Using a water soluble dye as tracer, the pattern of xylem water movement within Riesling berries was found to change soon after the onset of ripening, i. e. 'veraison'. Xylem water flow appears to be both slowed and diverted. Initially it flows predominantly in the berry's peripheral xylem system but later flow is much reduced and the remaining xylem component is exclusively in the progressively less conductive axial system serving the seeds. These changes which must force proportionately more water through the phloem path appear to be brought about by morphological change — the appearance of breaks in the peripheral xylem bundles — coinciding with the period of renewed expansion growth which occurs immediately after the onset of ripening.

The diversion at this stage of development of the berries water supply from the peripheral to the axial xylem, and from the xylem system to the phloem system has important implications for the fruits ultimate mineral composition.

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³⁾ Only after closing the manuscript the authors came to know the recently published paper by FINDLAY, N.; OLIVER, K. J.; NII, N.; COOMBE, B. G.; 1987: Solute accumulation by grape pericarp cells. IV. Perfusion of pericarp apoplast via the pedicel and evidence for xylem malfunction in ripening berries. *J. Exper. Bot.* **38**, 668—679, which is in accord with some of the results presented above.

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