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Xylem development and function in the grape peduncle: Relations to bunch stem necrosis*)

by

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Summary: Using a simple flow test evidence is provided that in some grape varieties xylem development is suppressed just distal to each node of the peduncle system. A little further along, development is again normal giving a bottleneck appearance. The bottleneck would appear to offer a high resistance to xylem sap flow both because of its reduced area of cross-section and because of its having only small, primary vessels. The varieties exhibiting a distinct bottleneck tend to be those susceptible to bunch stem necrosis whereas the ones without it tend not to be susceptible.

Key words: xylem, peduncle, water flow, bunch stem necrosis, disorder.

Introduction

Bunch stem necrosis occurs from time to time in grapevines in almost all vine-growing countries and is known to be a non-pathogenic, physiological disorder. The symptoms appear after the onset of ripening as dark, sunken, necrotic spots on the surface of the peduncle usually towards the distal region of clusters. As the condition develops the necrosis penetrates to the vascular tissue damaging them and slowing or preventing water and solute transport to the berries. This causes shrinking and a decrease in quality of the berries (CLAUS 1965). In advanced stages berries or clusters fall and serious losses of yield occur (STELLWAAG-KITTLER 1975).

Since the early observations of OSTERWALDER (1937) many aspects of bunch stem necrosis have been studied including its symptomatology, environmental and endogenous causes, and preventive measures have been suggested. In a series of publications it has been shown that bunch stem necrosis can be associated with a local, relative Ca deficiency (ALLEWELDT and HIFNY 1972, FREGONI *et al.* 1973, STELLWAAG-KITTLER 1975, BRECHBUHLER 1975, FEUCHT *et al.* 1975, COCUCCI *et al.* 1988). Thus it is probable that factors which reduce xylem sap flow in the peduncle, and thereby Ca transport through the cluster, will induce bunch stem necrosis.

It has been shown, for example, that altering the transpiration of berries affects Ca transport to them (DÜRING and OGGIONNI 1986) and that a lowering of berry transpiration is associated with an increased occurrence of bunch stem necrosis (STELLWAAG-KITTLER and HAUB 1964, CLAUS 1965, KOBLET and THEILER 1970). Water flow through plants is also known to be affected by the hydraulic resistance of the xylem (HUBER 1956, MILBURN 1979, ZIMMERMANN 1983) so we may predict that the hydraulic resistance of the peduncle xylem in grape will affect the water and hence the move-

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ment of Ca through it. In this way the seasonal variation in the incidence of bunch stem necrosis can be traced to the effects of weather around the time of flowering and its effects on xylem development (THEILER and MÜLLER 1986, LANG and DÜRING 1990). While engaged in a preliminary study of xylem structure and function in the grape berry-peduncle system (DÜRING *et al.* 1987) we noticed that xylem development in some varieties is rather reduced in a short region just distal to each branchpoint (node). The phenomenon is quite marked and is much more than the expected reduction in vascular area as one moves downstream (viz. xylem cross-sectional area at any point in the peduncle is linearly related to the number of berries downstream (LANG and DÜRING 1990)). Instead, the appearance is of a bottleneck where, over a short length, xylem development has been arrested at an early stage. A little further along towards the berry, vascular development is again normal, usually with a full secondary xylem ring.

In a preliminary study to see how generally these xylem bottlenecks were distributed amongst grape varieties, we noticed them to be more obvious in some varieties (e.g. Riesling) than in others (e.g. Silvaner). Moreover there seemed to be a relation between the presence of bottlenecks and the varietal susceptibility to bunch stem necrosis. Because of the possible causal relation between xylem water flow, Ca transport in the clusters and the incidence of bunch stem necrosis, this was particularly interesting.

We therefore initiated a long term (5 years) investigation of xylem development in the peduncles of 17 grape varieties of known susceptibility to bunch stem necrosis. We also carried out simple hydraulic tests to check that what appeared under the light microscope as a bottleneck did indeed represent a region of high hydraulic resistance able to slow down xylem sap flow significantly. This work we report here.

Material and methods

Determination of hydraulic resistance. Riesling grapes were collected in the early morning at a maturity stage midway between veraison and harvest. Clusters were selected which had a single first sidebranch the first internode of which was several cm long. The entire cluster was removed by a cut close to the subtending cane and placed immediately in a polyethylene bag which was promptly sealed. In the laboratory the proximal end of the peduncle was recut with a sharp blade to expose a fresh, uncrushed surface and connected, via a short length of thick walled silicone rubber tube, to a source of recently distilled, filtered and deaerated water. The supply pressure was raised to 0.1 MPa and held at this pressure for the duration of the experiment. Berries were then removed from the sidebranch by cutting just proximal to its first node. From this cut surface xylem borne water immediately exuded. Side branches thus prepared were up to 100 mm long.

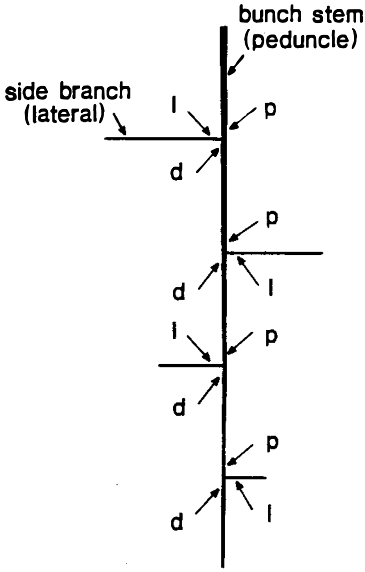
The flow rate from the cut surface was determined by noting the time taken to fill a 10 μl glass microcapillary. Time intervals were of the order of 15—30 s representing flows of 40—20 $\mu l \cdot min^{-1}$. After some minutes, when a mean value from 15—20 flow records had been obtained, the branch was recut about 10 mm further back from the previous cut and a new mean flow rate was determined for the, now shorter, branch. This procedure was repeated until the length of the branch was reduced to zero and the flow rates had increased considerably as successively shorter lengths of xylem lay between the pressurised water source and the exuding surface.

In this simple system any variation in the hydraulic resistivity of the xylem along the side branch is expected to be reflected in a change in the rate at which flow increased with successive cuts. A subsidiary experiment confirmed that water flow in the experimental system described was stable for 4 h or more and that the rates of flow were linearly related to the applied pressure. Measured flows were remarkably steady, lying within about ± 5 % of the mean values.

Determination of xylem development. During the second half of August each year from 1987 to 1991, several clusters of each of 17 varieties were collected from the vine collection of the Institute for Grapevine Breeding Geilweilerhof (Germany). In the laboratory, berries were cut off near the brush and the peduncles fixed in 3.7 % aqueous formaldehyde and stored pending sectioning.

Starting at the first node (branch point) of each peduncle, 100—200 µm thick hand sections were cut as close as possible to the node. The sections from each node were marked proximal (p), distal (d) and lateral (l), depending on their position (see Fig. 1). The three sorts of section were cut from 3—6 nodes per peduncle, from 8—10 replicate clusters to yield some 100 sections per variety per year.

After cutting, the sections were placed for about 1 min in phloroglucin solution (2.5 g in 50 ml of 70 % aqueous ethyl alcohol) and then flooded with concentrated HCl. This staining procedure is specific for lignin, colouring the lignified cell walls of the xylem tissue a distinct red. The extent of xylem development (D) was then estimated by comparison with a set of standards (Figs. 2 and 3). In 1991 the subjectivity of the method was checked by a blind test in which the varieties were given numbers instead of names. A sub-experiment was carried out to confirm that no xylem development occurred over the period in which samples were taken. Ongoing xylem development was not anticipated (THEILER and COOMBE 1985) but might have confused the results.



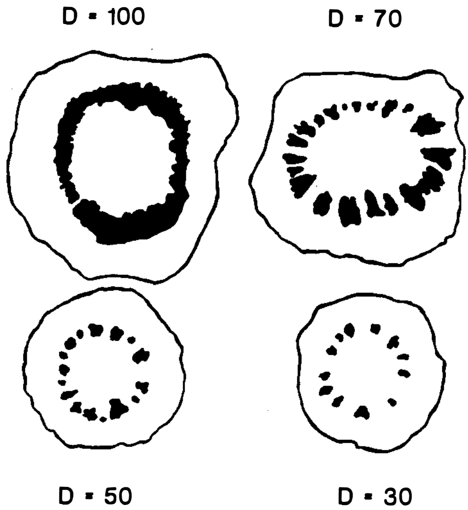


Fig. 1 (left): The grape peduncle system to show the regions from which proximal (p), distal (d) and lateral (l) transverse sections were taken.

Fig. 2 (right): Four generalised diagrams of the xylem tissues in transverse sections of the grape peduncle show different amounts of xylem development. These were arbitrarily assigned the semiquantitative values 100, 70, 50 and 30 to indicate the extent to which the xylem tissue had developed. These diagrams were used to rate the extent of xylem development.

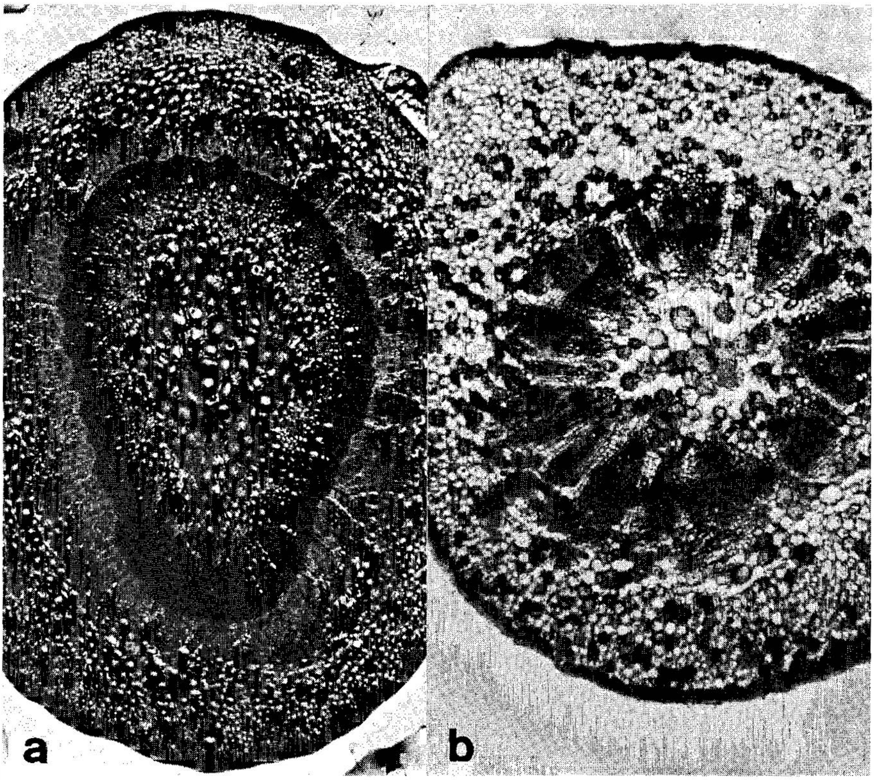


Fig. 3: Cross-sectional area of grape peduncles differing in the relative xylem development. a: D = 100 %; b: D = 80 %. Under the microscope the differences are most distinct due to the red colour of the stained xylem tissue.

Results and analysis

Hydraulic resistance along a sidebranch. Several tests were carried out of the hydraulic properties of sidebranches of the Riesling peduncle system; Fig. 4 represents a typical result. It shows exudation flow from 9 progressively exposed surfaces. The figure includes a schematic to show the experimental setup and a bar graph assessment of xylem development (D) at the mid point of each length removed. Values of D towards the ride hand side are about 80, those close to the node fall to 30.

Reading from right to left, note that the rate of flow rose slowly as successive lengths were removed from the side branch. As the main axis was approached more closely however, the rate increased much more rapidly indicating the removal of more highly resistive xylem in the vicinity of the node. The bar graph confirms that xylem development was reduced in the region of the high hydraulic resistance just distal to the main axis.

Xylem development in 17 grapevine varieties. Measurements of xylem development (D) are summarised in the Table where the values for each variety are averaged across nodes, replicates and years. For convenience the varieties are listed in an order which anticipates the results described below. In spite

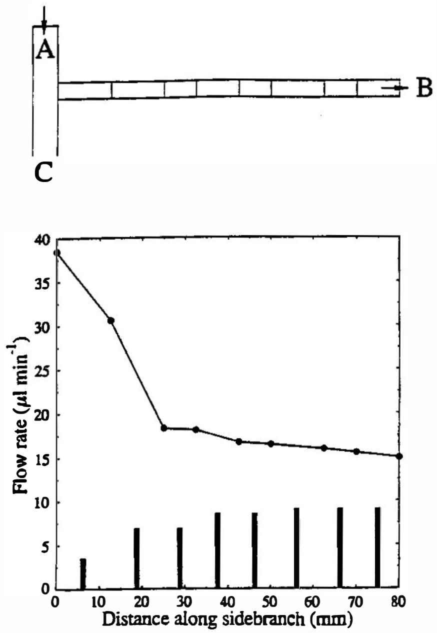


Fig. 4, above: Experimental setup with water being forced into the xylem system at A and exuding from it at the cut surface B of a side branch. The short vertical lines mark the positions of successive cuts. The remainder of the cluster attached at C is not shown.

below: Mean rates of exudation flow from the 9 exposed peduncle surfaces. Flow increased as the length of the sidebranch was reduced by successive removal of approximately 10 mm long portions. A bar graph (inset) shows the extent of xylem development D recorded for the mid point of each length of peduncle removed. — For details see text.

of simple averaging which ignores significant year effects, two trends are clearly evident upon inspection of the Table.

First, the highest values of D to be found amongst the resistant varieties at the foot of the Table (e.g. in the Pinots) whilst distinctly lower values are to be found amongst the more susceptible ones near the top (e.g. in Trollinger and Riesling). Second, the extent of xylem development close to the node is generally less in the distal (d) and the lateral (l) branches than that in the proximal branches (p).

The determination of varietal susceptibility to bunch stem necrosis by reference to the literature presented some difficulty. This was because many references were found simply to reiterate earlier statements, many of which were rather vague and often related specifically to one year and region. Frequent citation did not necessarily therefore make for a more certain assessment of susceptibility.

For similar reasons it seemed inappropriate to classify varieties more closely than into the two categories - resistant and susceptible. By this we do not mean to imply that intermediate susceptibilities do not exist, only that we have good evidence to support the resistant or susceptible classification for the 17 varieties selected. It is probable that varieties with intermediate susceptibility will have been rejected from our study as references with regard to their resistance or susceptibility are more likely to have been the equivocal ones. Note that in using the term resistant, we do not mean to imply the existence of a specific resistance mechanism but only a lack of susceptibility.

Discussion

Hydraulic resistance. The results of the water flow experiment (Fig. 4) confirm that the short bottleneck region just distal to each node of the peduncle system in Riesling grapes indeed represents a region of high hydraulic resistance. It is

Table

Varieties, variety abbreviations and classification with regard to susceptibility to bunch stem necrosis with references. On the right hand side of the Table are means over the 5 years 1987 to 1991 of measurements of xylem development *D* from grape peduncles in the three positions proximal (*p*), distal (*d*) and lateral (*l*). For further details see text.

Code	Variety	Bunch stem necrosis	Reference	*Dp*	*Dd*	*Dl*
Gf	Gf. GA-51-13	susceptible	4	88.4	77.9	28.8
Tr	Trollinger	susceptible	1, 2, 3	83.7	73.4	33.8
Ke	Kerner	susceptible	1, 8	91.2	86.4	59.9
Fr	Freisamer	susceptible	1, 3, 9	91.4	76.5	60.0
Ri	Riesling	susceptible	1, 2, 3, 5	81.0	62.4	64.6
Gu	Gutedel	susceptible	1, 2, 3, 5, 9	89.0	81.8	65.7
Ba	Bacchus	susceptible	1	89.2	77.0	68.8
Mü	Müller-Thurgau	susceptible	2, 3, 5, 8	86.9	71.2	70.4
Fa	Faber	susceptible	1, 8	88.7	74.0	76.7
Ge	Gewürztraminer	susceptible	2, 3, 5, 6, 9	94.7	94.3	79.6
Ca	Cabernet Sauv.	susceptible	6, 7, 11	90.3	89.2	71.8
PB	Pinot Blanc	resistant	1, 2, 3	94.9	92.3	84.0
No	Nobling	resistant	1	94.8	90.7	84.6
Si	Silvaner	resistant	1, 2, 3, 6, 10	95.8	89.2	86.2
PN	Pinot Noir	resisitant	1, 2, 3, 9	95.8	94.4	88.3
PM	Pinot Meunier	resistant	2, 3, 10	94.8	93.4	91.9
PG	Pinot Gris	resistant	1, 2, 3, 6, 9, 10	97.5	96.8	92.0

1 Hillebrand et al. (1987)
2 Stellwaag-Kittler (1975)
3 Theiler (1980)
4 Dr. Eibach, pers. inform.
5 Hartmair (1967)
6 Brechbuhler (1967, 1975)
7 Clement (1977)
8 Haub (1979)
9 Hopp (1975)
10 Roncador and Nicolli (1979)
11 Baldacchino et al. (1987)

well known that the hydraulic conductance of xylem tissue is related to the number of vessels and to their sizes. Vessel size is particularly important because flow is related to the 4th power of the radius (Poiseuille's equation for laminar flow through fine capillaries). It is also well known that the vessels of primary xylem are smaller than those of the secondary tissue. This suggests that the hydraulic resistance of the bottleneck region will be more severe than that predicted simply on the basis of tissue area. The ratio of the slopes of the two parts of the profile in Fig. 4 suggests a more than 16-fold increase in the hydraulic resistance of the bottleneck region as compared to the downstream region.

The failure of proper xylem development close to the nodes in grape peduncles is expected to restrict xylem water flow at this point. The restriction will most probably bring about a diversion of much of the xylem water flow past this region via the phloem. This will severely reduce Ca movement at this point.

Xylem development. Analysis of variance of the full data set showed strong year (1987—1991), susceptibility (1—2) and branch (p, d, l) effects (all were significant at p = 0.001 or better). The year effect was anticipated on the basis of earlier work (LANG and DÜRING 1990) but was not found to correlate significantly with a number of likely meteorological variables against which it was tested. The susceptibility effect indicates a correlative link between xylem development D and susceptibility to bunch stem necrosis. Because lower values of D are associated with greater susceptibility, the effect is in the right direction to support our hypothesis that there is a causal link also, i.e. reduced development lowers the xylem's hydraulic conductance, slowing sap flow and reducing Ca transport through the peduncle system. Low Ca levels will then predispose the tissues to necrosis.

Although values of D are reduced in the susceptible varieties in all three locations, p, d and l, the strongest effect was for measurements made at base of the lateral branches, i.e. the l positions. Because our principle interest is in the link between peduncle xylem development and varietal susceptibility to bunch stem necrosis, we shall focus the rest of our analyses on the D values in the l position. Note, however, that with only a slightly less satisfactory result, a similar analysis could have been constructed from values at either the p or the d positions.

In a forthcoming publication a probabilistic model for bunch stem necrosis will be presented to predict susceptiblility to bunch stem necrosis of newbred grapevine varieties (LANG and DÜRING, in prep.).

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