Are xylem radial development and hydraulic conductivity in downward growing grapevine shoots influenced by perturbed auxin metabolism?

Concise paper title: Does auxin control xylem development of inverted shoot?

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Summary

Downward-growing grapevine shoots have smaller and more frequent vessels and as a consequence a lower hydraulic conductivity. In a first experiment, grapevine shoot growth orientation was manipulated in order to test whether downward shoot orientation negatively affects vessel growth in the apex via a shortage of water and nutrients. The orientation of the central shoot portion in vines was inverted by two consecutive 135° bends, the first one downwards and the second one upwards, resulting in double-bent N-shaped vines; the plant central downward shoot portion

was of different length in the experimental treatments, in order to induce increasing reductions of shoot conductivity. These treatments effectively reduced shoot conductivity and water flow, but had no effects on vessel development and frequency in the apex. In a second experiment, auxin concentration was assessed in shoots of upward and downward growing plants. IAA concentration at the apical internodes was higher in downward oriented shoots than in shoots growing upwards. In addition, a higher density and a lower vessel diameter were observed in the lower shoot side of the shoot than in the upper, suggesting an increased accumulation of auxin in the lower shoot side. These results suggest that the downward orientation induces accumulation of auxin in the apex which in turn affects the density and the size of the xylem vessels, causing reduction of hydraulic conductivity.

Key-words: apex orientation, auxin, IAA, gravitropism, hydraulic conductivity, *Vitis vinifera* L, vessels, xylem.

Introduction

The plant xylem is the main transport pathway of water, nutrients and hormonal signals from the roots to the transpiring organs (Zimmermann and Milburn 1982). Xylem sap flow is affected by a series of conductances, which can be regulated by environmental and physiological factors. Traditionally, root (Steudle 2001) and stomatal (Jones 1983, Comstock 2002) conductances have been considered the main controlling factors of water flow. In addition, the hydraulic conductivity of the shoot

can also affect water flow, and it is regulated by physiological and environmental factors (Tyree *et al.* 1999). Shoot hydraulic conductivity is affected by changes in average vessel diameter (Nijsse *et al.* 2001): in grapevine, water stress (Lovisolo and Schubert 1998), leaf shading (Schultz and Matthews 1993), low temperature (Flexas *et al.* 1999) and the downward orientation of the shoot (Schubert *et al.* 1999) reduce hydraulic conductivity by decreasing vessel diameter. Modifications of vessel size have an important role in the adaptation to unfavourable environmental conditions (Comstock and Sperry 2000), as smaller vessels are less likely to cavitate and therefore to embolize (Salleo *et al.* 1985). On the other hand, smaller vessels have lower conductivity, and this can reduce xylem transport to the leaves from the roots (Mapfumo *et al.* 1993).

It is well known that in higher plants a downward orientation of the apex leads to negative gravitropic curvature of the shoot (Firn *et al.* 2000), response often associated with perturbations of auxin metabolism and transport (Mertens and Weiler 1983, Kiss *et al.* 1998). Xylem development is, among other factors, under auxin control (Berleth *et al.* 2000, Aloni 2001), and experiments based on transgene technology (Klee *et al.* 1987, Sitbon *et al.* 1992, Tuominen *et al.* 1995, 2000) and on hormone analysis (Tuominen *et al.* 1994, Edlund *et al.* 1995) have confirmed that auxin affects the process of vessel growth. Results are, however, at contrast. On one hand, exogenous auxin was found to stimulate expansion of xylem vessels (Zakrzewsky 1991); on the other hand, a negative correlation between endogenous auxin concentration and vessel size was proposed by Aloni and Zimmermann (six-point hypothesis 1983) and confirmed by Klee *et al.* (1987) on transgenic *Petunia*, and by Tuominen *et al.* (1995) on transgenic hybrid aspen.

Our previous studies on *V. vinifera* showed that the downward shoot orientation induces a decrease in average xylem vessel diameter and shoot hydraulic conductivity (Schubert *et al*. 1999) associated with a reduction in water transport and shoot growth (Lovisolo and Schubert 2000). By using plants with upward oriented apexes but with a central, downward oriented shoot portion, we showed that these effects are not due to bending of the shoots but to their orientation. Two hypotheses can be made to explain these results. One possibility is that the lower conductivity of the downward oriented shoot decreases transport of water, nutrient and hormones to the growing apex, thus depressing its growth with a feed-forward mechanism. As an alternative, as the downward orientation of the shoot apex is likely to affect auxin distribution (Muday and Murphy 2002), the auxin metabolism in the apex can be affected by apex orientation. The objective of this work was to test these two hypotheses.

Materials and methods

Growing conditions

Two experiments were carried out in two subsequent years: 1998 and 1999.

In 1998, thirty two-year-old plants of *Vitis vinifera* cv. Pinot noir, grafted on *Vitis riparia* x *berlandieri* 'Kober 5BB', were grown in 18 L containers filled with a substrate composed of a sandy-loam soil / expanded clay / peat mixture (4:2:1 in volume), with a final pH of 7.3. Containers were placed in a greenhouse with no supplementary light or heating. Plants were watered twice a week to soil field capacity and were fertilised once a month with 30 g of a complex (*20-10-10*) fertiliser. At

budbreak, only the basal bud of each plant was allowed to grow. Budbreak took place on 6 April. When the single shoot of each plant reached 0.65 m length (12.5 ± 0.9 internodes, 37 ± 2.5 days after budbreak, DAB), it was bent 135° to the downward position for 24 vines, while it was allowed to grow upwards for 6 control vines. In order to affect vessel development and hydraulic conductivity, shoots were bent gradually to minimise mechanical strains and the downward shoots were periodically tied to a trellis, in order to hinder spontaneous gravitropic upward bending. In three groups of six downward bent plants the shoot was again bent 135° upwards when the downward portion reached respectively 0.55, 1.10 and 1.65 m length. With this operation, N-shaped vines (Schubert *et al.* 1999) with a central downward portion of different length in a proportion of 1:2:3 were obtained (Tab. 1). The 6 remaining plants were forced to continue growing downwards. The experiment thus consisted of five treatments of six replicate plants, which were labelled C (**control** plants growing vertically), S (N-shaped plants with **short** central downward portion, 0.55 m long), M (N-shaped plants with **medium** central downward portion, 1.10 m long), L (N-shaped plants with **long** central downward portion, 1.65 m long), and D (**downward** plants) (Fig.1).

Containers with modified shoot orientation were placed 1.0 m higher than containers of control plants, so that shoots of all plants were at similar height above the ground. All lateral shoots and clusters were removed immediately after beginning growth. Containers were placed in the greenhouse following a completely randomised design.

In 1999, six replicate three-year-old C and D plants were used, and the same growth conditions of 1998 were applied. Budbreak took place on 15 April.

Measurements of plant growth, shoot hydraulic conductivity and water flow.

In both experiments, length and node number of the shoot were recorded three times per week from budbreak to the beginning of August, for a total of 128 days.

Shoot hydraulic conductivity (k_h) was assessed at the end of the 1998 experiment (128 DAB) by direct measurements on excised shoot portions, forcing pressurised distilled water in the xylem. Excision was made under water to avoid air entry into the vessels cut open at both ends. Conductivity was measured on the basal, central and distal shoot portions of four plants per treatment. The basal portion consisted of the basal 0.65 m long, upward oriented shoot. The central portion consisted of the adjacent distal shoot portion, which was 1.10 m long and upward oriented in C plants, downward oriented and of different length in N-shaped plants (treatments S, M, and L), downward oriented and 1.10 m long in D plants. The distal portion consisted of the further distal adjacent shoot portions, upward oriented in C, S, M, and L plants, and downward oriented in D plants. The shoot portions used for the conductivity assay were thus never shorter than 0.55 m, which is about the average length of vessels in grapevine (Sperry *et al.* 1987). In order to be sure that different length of the samples could misrepresent results (Van Ieperen *et al.* 2000), all measurements were taken on 0.55 m long shoot samples of each tested portion. Conductivity measurements were taken immediately after cutting. A controlled pressure system was used, according to Schubert *et al.* (1995). After 10 min at 0.3 MPa m^{-1} to eliminate shoot embolisms (Sperry *et al.* 1988, Van Ieperen *et al.* 2001) a measurement, representative of conductivity as affected only by vessel size, was taken at 0.1 MPa m^{-1} for two minutes. After a one-minute interval without pressurisation the measurement was

replicated. Shoot hydraulic conductivity k_h was calculated from measured pressure gradient and flow. Shoot specific conductivity k_s (Tyree and Ewers 1991) was calculated dividing k_h by the xylem cross-sectional area (measured as described in the following section) at the middle of the central internode of the shoot portion.

Water flow was assessed, on three replicate plants, on N-shaped and control plants 126 DAB by the Stem Heat Balance (SHB) technique (Baker and Van Bavel 1987). Gauges were adapted to the grapevine stems (Lovisolo and Schubert 1998) and clamped the base of the shoot.

Xylem anatomy

In 1998, the xylem cross-sectional area, the number of vessels and the diameter of vessel lumina were measured at the end of the experiment (128 DAB) on two replicate plants of the four plants per treatment used for conductivity analysis. Internode sections about 200 μm thick were cut with a hand-held scalpel midway between the nodes. Sections were cut at every 4th internode along upward shoot portions and at every 2nd in downward shoot portions, beginning with the most basal one. One section was observed with a stereomicroscope for each sampled internode. Xylem area and vessel diameter were calculated from the average of two orthogonal measurements of xylem width (120x) and of vessel diameter (500x) per section. Vessel number and diameter were measured on all the vessels observed within four xylem wedges (xylem sectors delimited by rays), 90° one to the other, per section.

In 1999, the same measurements were taken at the end of the experiment 100 DAB on six replicate C and D plants. In addition, in the downward-growing portion of D plants, the lower (soil-facing) shoot side (LSS), and the upper, opposite side (USS),

were discriminated by marking the LSS of whole shoots before sectioning. We labelled as LSS and USS the shoot sides on the basal upward-growing portion contiguous to the LSS and USS of the downward portion. Two of the wedges where measurements were made belonged to the LSS and two to the USS (Fig. 2).

Determination of free IAA

In order to measure auxin concentration, we used 1 cm long shoot fragments, taken from the central part of the internodes used for xylem anatomy measurements in C and D plants in 1999. Shoot samples were dipped in cold 70% (v/v) aqueous acetone (1:5, w/v). They were then minced and homogenised with an Ultra-Turrax T25 (Janke & Kunkel GmbH & Co KG, Staufen, Germany). Each homogenate was supplemented with a suitable amount of internal standard, namely $^{13}C_6$-IAA (synthesised as described in Di Gregorio *et al.* 1995), then was stirred for 12 h at 4°C with a magnetic stirrer. After centrifugation at 2000 g for 15 min, the pellet was re-extracted twice with the same solvent. The supernatants were pooled and reduced to the aqueous phase under vacuum at 35°C, the pH was adjusted to 7.0 with concentrated KOH, and the aqueous extracts were stored at -20°C for 12 h. After thawing they were centrifuged at 13000 g at 4°C for 30 min. Supernatants were adjusted to pH 2.8 with concentrated HCl and partitioned five times against equal volumes of peroxide-free diethyl ether. The diethyl ether extracts were stored at -20°C overnight, the remaining frozen water was discarded and the ether was removed by evaporation. The dried extracts were re-suspended in 2 ml of 20% (v/v) aqueous acetonitrile containing 0.5 % (v/v) acetic acid and purified by HPLC, derivatised and analysed with GC-MS following the method described by Sorce *et al.* (2000).

Statistical analysis

Data were submitted to analysis of variance, and averages were separated with the Duncan test.

Results

Growth

At the end of the 1998 experiment (128 DAB), the number of internodes, shoot length and leaf area of the manipulated vines did not differ among N-shaped and C plants, while in D plants, shoots were significantly shorter and leaf area was lower than in all other treatments (Tab. 2).

The shoot node emission rate of downward and N-shaped plants became progressively lower than in control plants , starting nine days after the date of downward bending, and for further 12 days; afterwards, it remained more or less stable at about 75% of the shoot node emission rate of control plants. In downward plants shoot growth rate remained constant up to the end of the experiment. On the contrary in N-shaped plants, when the shoot was again bent upwards, shoot node emission rate recovered in comparison to controls. The recover started 17 ± 3.5 days after upward bending without significant differences among the three N-shaped treatments. The increase of shoot node emission rate continued from this time on, so that the N-shaped plants showed in the apical upward shoot portion higher shoot node emission rates than control plants. (Fig. 3).

In the 1999 experiment, shoot growth was similar as in 1998 (data not shown).

Shoot hydraulic conductivity(k_h)

In the basal, upward growing shoot portion, k_h showed no significant differences among treatments. As expected, in the central portion, k_h was significantly higher in C plants (upward oriented shoot portion) than in N-shaped plants, and it was lowest in D plants. In distal shoot portions, which were upward oriented in all treatments with the exception of D plants, k_h was slightly, even if not significantly, higher in N-shaped plants than in controls, while it was again significantly lower in D plants than in all other treatments (Fig. 4a).

Shoot specific conductivity showed a similar pattern, although in this case the differences observed in distal shoot portions were more evident and significant; in N-shaped plants, the length of the central inverted downward portion affected k_s negatively in central portions and positively in distal portions (Fig. 4b).

Xylem anatomy and water transport

The vessel density per xylem area unit in the apical shoot portions was always significantly higher in shoot portions where the apex grew downwards (downward shoots) than in those where it grew upwards (control and N-shaped shoots). In N-shaped shoots vessel density was not significantly different than in control plants (Tab. 3).

The mean diameter of the xylem vessels of the apical shoot portions of D plants was significantly lower than in all other treatments, while N-shaped plants had greater vessel diameter than the control plants (Tab. 3).

In 1999, measurements were repeated on D plants and controls by discriminating the lower shoot side (LSS) from the upper shoot side (USS) of shoots. In LSS of distal portions of D plants the vessel density per xylem area unit was higher and average vessel diameter was smaller than in the USS. These differences were observed in the central portion, while they were absent in the basal upward-growing portion (Fig.5). On the contrary, in sections of control plants no differences were found in vessel diameter and density among the four xylem wedges where measurements were made (data not shown).

As expected, water flow was negatively affected by downward bending of the shoot. In N-shaped plants, water flow was lower than in control plants, and the decrease in water flow was larger when the length of the downward shoot portion was longer, and conductivity was lower (Tab. 3).

Auxin concentration

As expected, auxin concentration in internodes decreased from the apex towards the base of grapevine C plants. In D plants a similar pattern was observed, but auxin concentration was significantly higher than in C plants in the distal portion; in the other portions auxin concentration decreased to levels similar as in controls (Fig. 6). Within the distal portion, significant differences in auxin concentration were observed at last six internodes.

Discussion

Although shoots of higher plants naturally grow upward oriented due to negative gravitropism, a horizontal or downward orientation is very common, due either to the attempt of the plant to evenly distribute its foliage in relation to light cues (Givnish 1995) or, in crop plants, to manipulations intended to increase plant production or yield quality (Tassie and Freeman 1992). A common response to a downward growth orientation is reduced shoot growth (Prasad and Cline 1985, Kliewer *et al.* 1989). Furthermore, the downward shoot orientation negatively affects the radial development of xylem vessels and thus the shoot hydraulic conductivity (Schubert *et al*. 1995, 1999).

When forced in the downward orientation, the growing shoot is subjected to different perturbations of its metabolism: in particular, the downward position of the apex may affect auxin metabolism and distribution (Bandurski *et al.* 1984). However, the downward oriented shoot portion below the apex has lower hydraulic conductivity, and this can potentially limit the transport of water, nutrient and hormones to the growing internodes (Lovisolo and Schubert 2000). In this work we attempted to uncouple these two factors, in order to assess their relative role on vessel development in downward oriented shoots. Given that downward shoots have lower conductivity (Schubert *et al.* 1999), experimental treatments with the same (upward) growth direction of the apex, but with decreasing shoot water conductivity were obtained in N-shaped vines by increasing the length of the central inverted portion. As expected, reductions in conductivities per unit shoot length were observed, which were about proportional to the length of the inverted shoot portion. The effect becomes even more evident when whole-portion conductivities (conductivity per unit length multiplied by

the length of the inverted portion) are computed, and this shows that inverting shoots was successful at partly blocking the water pathway.

The modifications of hydraulic conductivity induced by inverting the central portion of N-shaped plants caused the expected reduction in water flow, however they did not affect neither shoot growth rate nor xylem development in the apical part of the shoot. Growth rate in the apical, upward portion of N-shaped plants was even higher than in control plants. Also vessel radial development of N-shaped plants was not reduced by decreasing shoot conductivity, on the contrary it increased in comparison to controls. These results are exactly the opposite than expected if the downward position of the shoot were to affect its growth and vessel development only because of lower hydraulic conductivity. Thus, on the basis of these data, we reject the hypothesis that vessel development was limited by insufficient supply of water (and nutrients) by the xylem.

Maximum reductions in conductivity which were recorded in longer central portions of N-shaped plants (L plants) were about 32 % of the controls. In our experiment, this reduction did not affect vessel development downstream the reduction, neither did it affect shoot growth and leaf area, which were similar in N-shaped plants as in controls. In previous experiments on grapevine, downward orientation of field-grown shoots reduced conductivity more than 80 % (Lovisolo and Schubert 2000), water stress about 73 % (Lovisolo and Schubert 1998), and a more severe water stress about 96% (Schultz and Matthews 1988). In D plants of our experiment reduction in k_h was meanly 80 % of the controls. In those conditions, water (and nutrient) availability to distal portions were markedly reduced, and effects on growth of shoot distal portions were evident. Thus, it is possible that a more marked reduction of k_h may have an

effect on vessel development and shoot growth that in the conditions of N-shaped treatments was not observed.

Results on N-shaped plants of the present study leave thus the downward apex position itself as a candidate for negatively affecting vessel radial development. On the other hand, parameters as growth rate expressed as nodes emitted per day and vessel size and number in the apical portion are strongly influenced by the morphogenetic activity of the shoot apex (Berleth and Sachs 2001). Downward positioning of shoot apexes can perturb auxin metabolism and transport (Mertens and Weiler 1983), in particular increasing the concentration of auxin in the lower part of the downward oriented shoot (Wright 1982, Bandurski *et al.* 1984). Auxin is transported through plant tissues, moving from cell to cell in a unique polar manner, requiring light and gravity vectors (Muday 2001). Polar auxin transport controls important growth and developmental processes in higher plants (Muday and De Long 2001, Weyers and Paterson 2001). In roots and in shoots, auxin is implicated in controlling growth (Dietz *et al.* 1990, Obermeyer and Bentrup 1996, Vissenberg *et al.* 2001) and in promoting vascular differentiation. According to the 'six-point hypothesis' (Sachs 1981; Aloni and Zimmermann 1983), higher concentrations of auxin are thought to increase the density of vessels in the developing xylem and to decrease the average vessel diameter (Aloni 1987, Tuominen *et al.* 1995, Klee *et al.* 1987, Sachs 2000). We thus established an experiment to gain evidence of changes in auxin concentration in downward shoots as opposed to upward shoots.

In this (1999) experiment we obtained a direct evidence of auxin metabolism perturbation, when we observed that auxin concentration increased at the apical internodes of downward oriented shoots compared with upward-growing controls.

This observation confirms previous measurements on upward and downward shoots (Schubert *et al.* 1999) and, following the six-point hypothesis, agrees with the higher frequency and lower diameter of vessels in downward shoots. Moreover, while downward-growing grapevine shoots had more and thinner vessels (as observed in 1998), they also showed differences in xylem structure between shoot sides. We observed a greater density of vessels and a lower vessel diameter in the LSS than in the USS of downward growing shoots, while in upward growing shoots vessel density and diameters were homogenous throughout the xylem section. Although we made no direct measurement of auxin on the two shoot halves, based on the six-point hypothesis we hypothesise that these effects were due to a higher concentration of auxin in the lower side of downward-growing shoots. A preferential transport of auxin to the downward side of tilted shoots has been observed in several studies of shoot gravitropism, and it would have the scope of promoting cell elongation in order to curve upwards the shoot (Hejnowicz 1997, Chen *et al.* 1999, Tasaka *et al.* 1999). In our experiment we did not only tilt the apex, but we forced it to the downward position and as a consequence the difference in vessel density and diameter was constantly observed along the whole of the downward portion. The situation in our experiment was similar to the vegetative reproduction of woody plants by layers, where root initiation is stimulated by endogenous auxin that accumulates in the apex bent downward and covered with soil.

Our results have some applicative aspects, helping to understand hydraulic effects on growth of downward-growing shoots, when either the weight of fruits wins the mechanical resistance of wood fibers of the shoot, or downward growth is artificially imposed by growers. In addition, they add further information on some biological

systems, i.e. variously oriented forest lianas and other flexible-wood plants, whose complex hydraulic architecture (Tyree and Ewers 1991) should keep in account the shoot orientation and growth direction of the apex. Further work will be needed to clarify growth mechanisms on variously oriented flexible-wood plants, as a continuous supply of polarly transported auxin is required to stimulate xylem production in cambial tissues (Sundberg *et al.* 2000, Mellerowicz *et al.* 2001).

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Table 1. Length, number of internodes and growing period of the inverted (downward) portion of grapevine shoots subjected to the three orientation (N-shaped) treatments; S=short, M=medium, L=long, (means ± standard error).

Orientation Treatment	**length (m)**	**internodes**	**Growing period (days)**
S	0.55	8.6 ± 0.5	22.2 ± 1.5
M	1.10	14.4 ± 1.5	29.2 ± 1.6
L	1.65	20.6 ± 1.9	40.1 ± 2.0

Table 2. Number of internodes, total shoot length and total leaf area measured at the end of the experiment (128 DAB) in grapevine shoots subjected to different orientation treatments (C, S, M, L and D: see Fig. 1), (means± standard error; averages followed by a common letter do not differ significantly among orientation treatments at P=0.05, according to variance analysis and Duncan test).

Orientation treatment	**number of internodes**	**shoot length m**	**Total leaf area m^2**
C	51.3 ± 0.61 a	4.03 ± 0.35 a	1.02 ± 0.10 a
S	51.2 ± 0.89 a	3.97 ± 0.40 a	0.98 ± 0.12 a
M	50.2 ± 0.62 a	4.03 ± 0.34 a	0.97 ± 0.13 a
L	48.8 ± 2.42 a	4.12 ± 0.49 a	0.99 ± 0.15 a
D	39.2 ± 2.07 b	2.58 ± 0.34 b	0.76 ± 0.11 b

Table 3. Vessel density per xylem area unit and diameter of vessel lumina in the apical shoot portion, and water flow per unit leaf area in grapevine shoots subjected to different orientation treatments (C, S, M, L and D: see Fig. 1), (means ± standard error; for each shoot portion, averages followed by a common letter do not differ significantly among orientation treatments at P=0.05, according to variance analysis and Duncan test).

		orientation treatment	
vessel density per xylem area unit	**mm^{-2}**	C	73.8 b
		S	66.7 b
		M	70.5 b
		L	82.0 b
		D	132.5 a
vessel diameter	**μm**	C	35.2 b
		S	41.7 a
		M	39.4 a
		L	38.4 a
		D	22.5 c
water flow	**g m^{-2} s^{-1}**	C	76.7 a
		S	68.2 ab
		M	64.8 ab
		L	57.8 b
		D	-

Figure 1. Experimental manipulation of single-shooted grapevines. C = upward - growing **control** plants; S = N-shaped plants with **short** central downward portion, 0.55 m long; M = N-shaped plants with **medium** central downward portion, 1.10 m long; L = N-shaped plants with **long** central downward portion, 1.65 m long; D = **downward** – growing plants. Lines representing shoots are drawn in scale according to the 1998 growth 128 days after budbreak. Numbers onto shoot portions are the average number of internodes of each portion.

Figure 2. Cross section of grapevine shoot. In four xylem wedges, 90° one to the other (arrows), anatomical measurements were made. On downward-growing D shoots, two of the 4 wedges belonged to the upper shoot side (USS) and two to the lower shoot side (LSS).

Figure 3. Shoot node emission rate of grapevine shoots subjected to different orientation treatments (C, S, M, L and D: see Fig.1) calculated in relation to shoot node emission rate of C shoots. Downward- and upward-oriented arrows mark the dates in which shoots were downwardly or upwardly bent according to the orientation treatment. Bar (lower right) represents the standard error of the means.

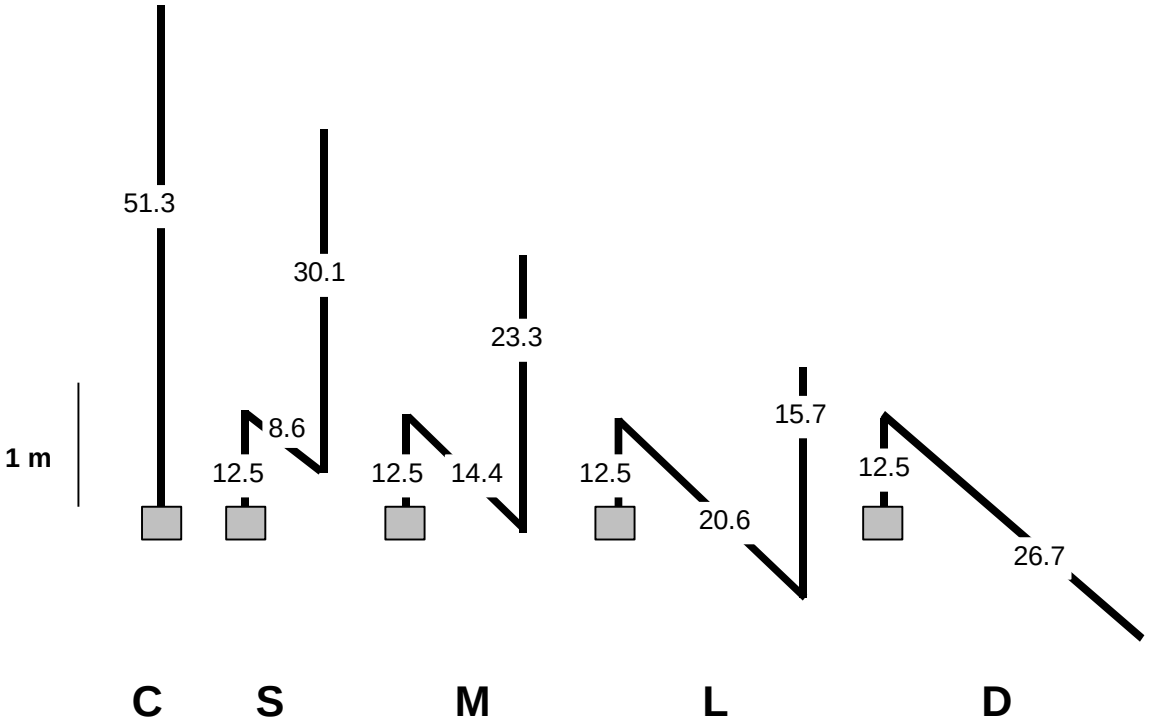
Figure 4. Shoot hydraulic conductivity k_h (a) and shoot specific conductivity k_s (b) measured on basal, central and distal portions of grapevine shoots subjected to different orientation treatments (C, S, M, L and D: see Fig.1). Measurements were taken after a high-pressure flow designed to eliminate vessel embolisms (within each

group of histograms, values labelled by the same letter do not differ significantly at P=0.05, according to variance analysis and Duncan test).

Figure 5. Vessel density per xylem area unit (a) and diameter of vessel lumina (b) of basal, central and distal portions of downward-growing (D) and vertical control (C) grapevine shoots. In D shoots the upper shoot side (USS) of the section was discriminated from the lower shoot side (LSS) (means ± standard error; within each group of histograms, values labelled by the same letter do not differ significantly at P=0.05, according to variance analysis and Duncan test).

Figure 6. Auxin concentration of basal, central and distal portions of downward-growing (D) and vertical control (C) grapevine shoots (means ± standard error; within each group of histograms, values labelled by the same letter do not differ significantly at P=0.05, according to variance analysis and Duncan test).

Figure 1



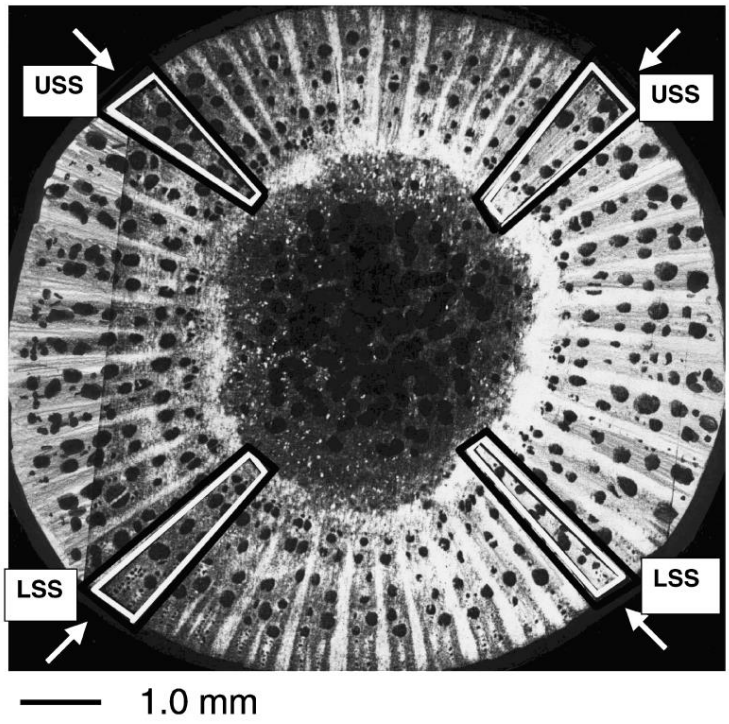
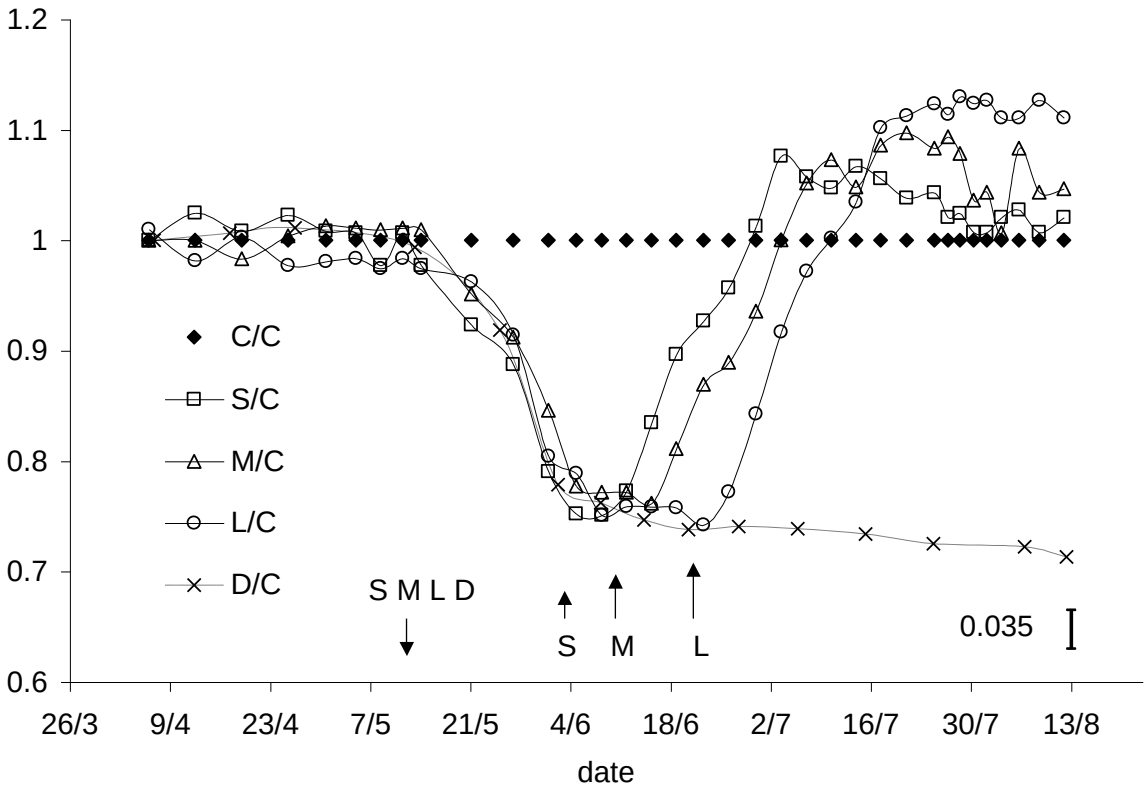


Figure 2

Figure 3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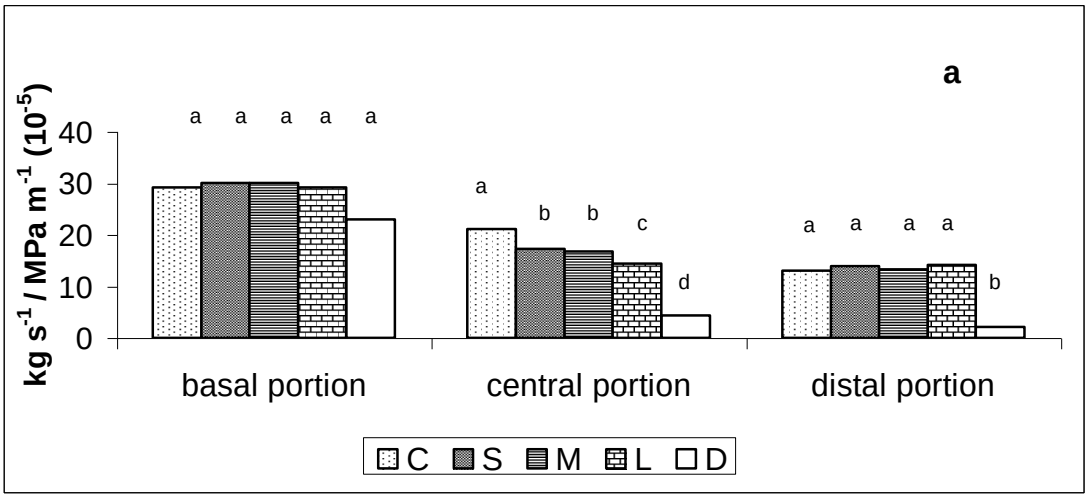
1.1
1
0.9
0.8
0.7
0.6
C/C
S/C
M/C
L/C
D/C
S M L D
S
M
L
0.035
26/3
9/4
23/4
7/5
21/5
4/6
18/6
2/7
16/7
30/7
13/8
date

Figure 4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30
20
10
0
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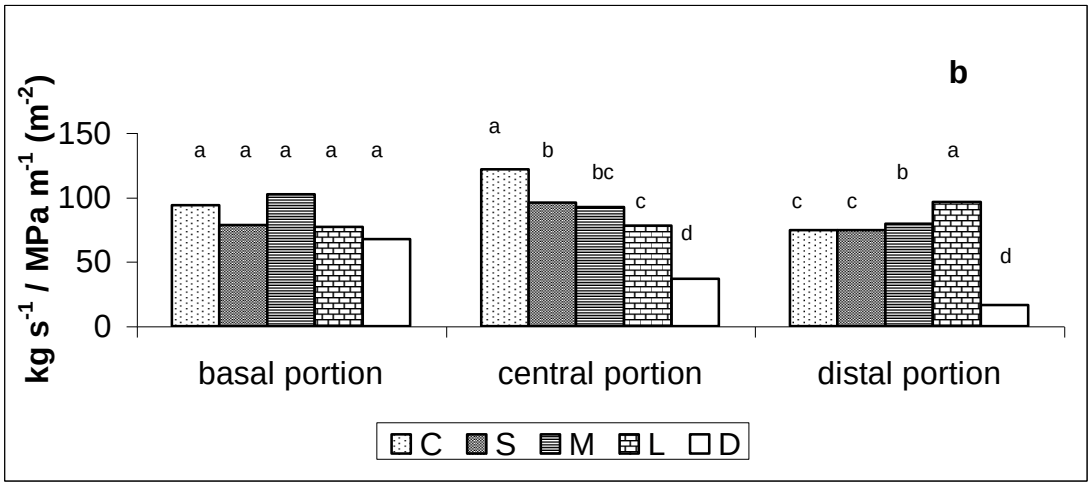
a b b c d
a a a a b
basal portion
central portion
distal portion
C S M L D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100
50
0
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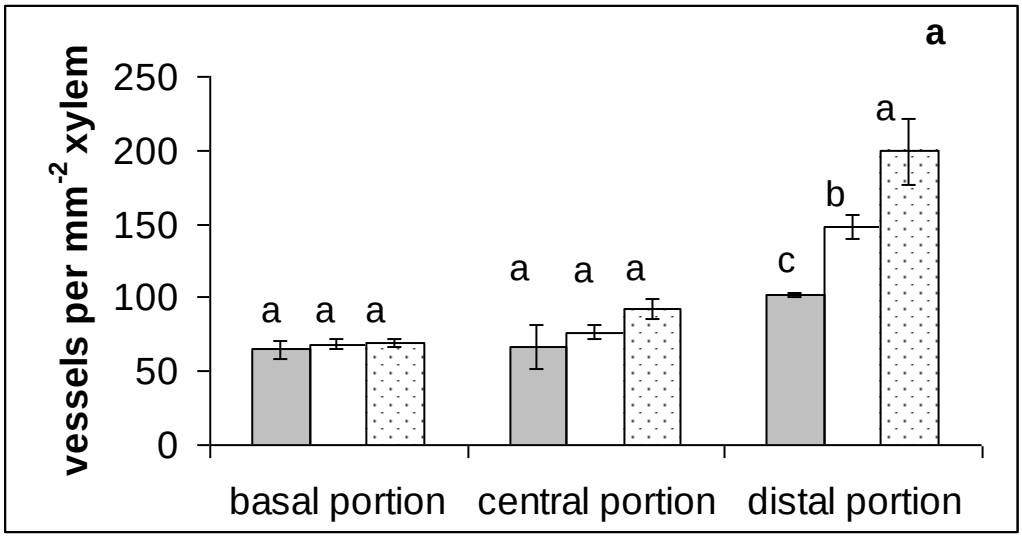
a b bc c d
c c b a d
basal portion
central portion
distal portion
C S M L D

Figure 5


a
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200
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c
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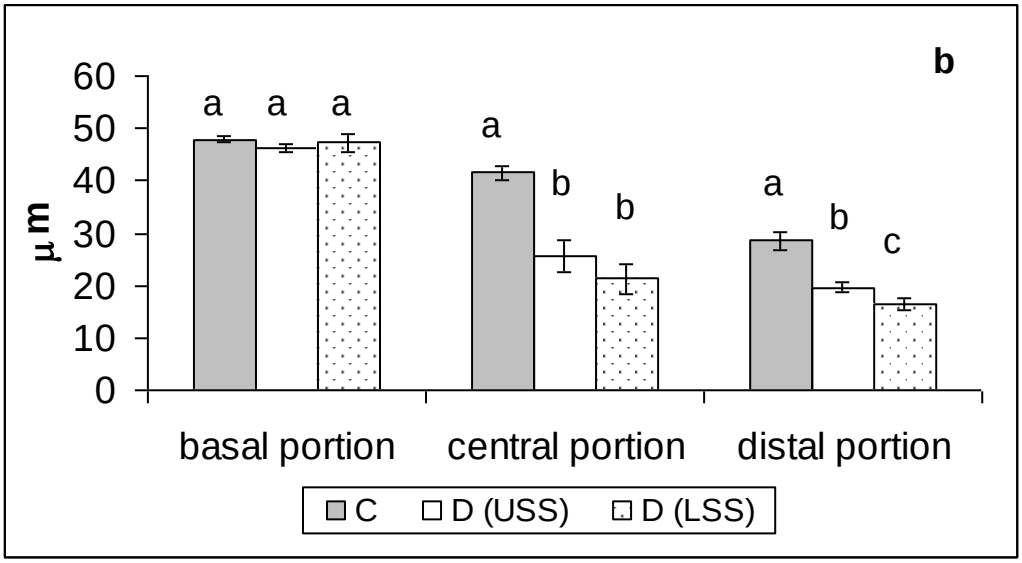
central portion
distal portion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a
b
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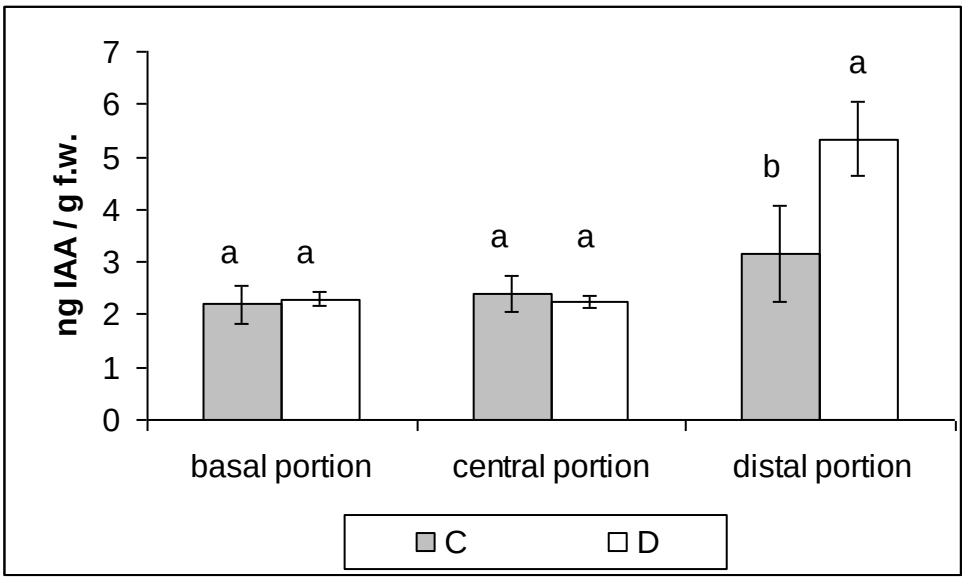
basal portion
central portion
distal portion
C
D (USS)
D (LSS)

Figure 6


ng IAA / g f.w.
7
6
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4
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2
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a
b
a
basal portion
central portion
distal portion
C
D