

RESEARCH PAPER

Distribution of xylem hydraulic resistance in fruiting truss of tomato influenced by water stress

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Abstract

In this study xylem hydraulic resistances of peduncles (truss stalk), pedicels (fruit stalk) and the future abscission zone (AZ) halfway along the pedicel of tomato (*Lycopersicon esculentum* L.) plants were directly measured at different stages of fruit development, in plants grown under two levels of water availability in the root environment. The xylem hydraulic connection between shoot and fruits has previously been investigated, but contradictory conclusions were drawn about the presence of a flow resistance barrier in the pedicel. These conclusions were all based on indirect functional measurements and anatomical observations of water-conducting tissue in the pedicel. In the present study, by far the largest resistances were measured in the AZ where most individual vessels ended. Plants grown at low water availability in the root environment had xylem with higher hydraulic resistances in the peduncle and pedicel segments on both sides of the AZ, while the largest increase in hydraulic resistance was measured in the AZ. During fruit development hydraulic resistances in peduncle and pedicel segments decreased on both sides of the AZ, but tended to increase in the AZ. The overall xylem hydraulic resistance between the shoot and fruit tended to increase with fruit development because of the dominating role of the hydraulic resistance in the AZ. It is discussed whether the xylem hydraulic resistance in the AZ of tomato pedicels in response to water stress and during fruit development contributes to the hydraulic isolation of fruits from diurnal cycles of water stress in the shoot.

Key words: Abscission zone, hydraulic conductance, hydraulic resistance, *Lycopersicon esculentum* L., pedicel, tomato, water stress.

Introduction

As in most fruits, fruits of tomato (*Lycopersicon esculentum* L.) possess strong sink capacities and also specific metabolite requirements. Fruit growth of tomato is largely determined by the import of water, nutrients and assimilates from other parts of the plant. It has been shown that, during fruit development, 90% of all water enters the fruit via the phloem and that water import via the xylem almost completely ceases approximately 25 d after anthesis (DAA) (Ehret and Ho, 1986a, b; Ho *et al.*, 1987). This is remarkable by itself because the hydraulic resistance of the xylem is usually much smaller than that of the phloem (Nobel, 1983). It therefore seems logical to assume that somewhere along the xylem transport path between shoot and fruits a large hydraulic resistance exists. This resistance may function as a safety valve to minimize the effects of diurnal water stress in the plant on fruit development (Lee, 1989). However, by restricting water import via the xylem it also limits the import of calcium, which causes local calcium deficiency in fruits. This negatively influences fruit development and causes sporadic necrosis at the distal part of tomatoes ('blossom end rot'), a problem often occurring during the production of tomato fruits (Adams and Ho, 1993).

In tomato, the water transport path between shoot and fruit consists of the truss stalk (peduncle) and fruit stalk (pedicel). In most varieties a knuckle is found midway along the pedicels that contains a future abscission zone

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(AZ) (André *et al.*, 1999; Lee, 1989). Although AZs in leaves and fruits were identified as possible safety devices or flow resistance barriers along the xylem water transport path (Zimmermann, 1983; Lee, 1989; Van der Schoot and Van Bel, 1989), study of the hydraulic properties of water-conducting tissues in these zones is limited (André *et al.*, 1999). Contradictory views exist about the presence of a large hydraulic resistance in the abscission zone in the pedicel (Lee, 1989; Lee *et al.*, 1989; cf. Malone and Andrews, 2001). Lee and coworkers (Lee *et al.*, 1989) reported a high hydraulic resistance in the knuckle in the pedicel, while Malone and Andrews (2001) recently reported the opposite. However, directly measured hydraulic resistances along the water transport path between shoot and fruits have never been reported up to now. Lee and co-workers based their opinion upon functional measurements (differential water potential measurements between plant and fruit) and anatomical observations. The anatomical observations demonstrated a reduced xylem cross-section in the AZ in the tomato pedicel (Lee, 1989; André *et al.*, 1999), which was interpreted as the cause of a high hydraulic resistance. On the other hand, Malone and Andrews (2001) used linear displacement transducers to check the response time of calyx leaflet water status in response to changes in apoplastic water availability in either leaves or fruits. They observed a fast response of calyx water status upon sudden water availability in leaves induced by scorching, and concluded that the resistance in the knuckle in the pedicel was unimportant for water transport to a tomato fruit. They supported this view by a long-term staining of the xylem pathway (from stem via peduncle, pedicel into fruits), which showed no significant hydraulic constrictions in the xylem proximal to the calyx. Instead, they reported a rapidly increasing transverse xylem area in pedicel cross-sections during fruit development.

Xylem hydraulic resistance strongly depends on the number and radii of xylem vessels along the transport path (Nobel, 1983). According to Hagen–Poiseuille's law, the hydraulic resistance of an individual vessel is inversely proportional to the 4th power of its radius (Zwieniecki *et al.*, 2001). Besides vessel diameter, finite vessel length and concomitant transport from one vessel to the other may also strongly influence hydraulic resistance (Nijssen *et al.*, 2001). The locations for water transport between vessels, the pit membranes (modified regions in the primary cell walls between neighbouring vessels), greatly influence xylem hydraulic resistance because of their high hydraulic resistance (Schulte *et al.*, 1987). Therefore, regions with shorter vessels significantly increase xylem hydraulic resistance. Combinations of an increased tendency of vessels to end and increased hydraulic resistance were observed in the AZ between stem and peduncle in *Rose hybrida* (Darlington and Dixon, 1991) and in the nodes of several species with diffuse porous wood (Salleo *et al.*,

1984). Anatomical observations on tomato pedicels (Lee, 1989; André *et al.*, 1999) also indicate an increased tendency of vessels to end near the AZ in the knuckle. Water stress during growth may influence xylem hydraulic resistance by inducing the development of xylem vessels with a smaller diameter. Indeed, in the stems of *Zea mays* L. (Abd el-Rahim *et al.*, 1998) and *Vitis vinifera* L. (Lovisol and Schubert, 1998) low water availability in the root environment resulted in xylem elements of smaller diameter. The effect of water stress during growth on xylem hydraulic resistance between the shoot and fruits of tomato is unknown.

The aim of this study was to determine by direct hydraulic resistance measurements whether a large flow resistance barrier exists in the AZ in the pedicel of tomato fruits. The aim was also to investigate whether the distribution and magnitude of hydraulic resistances along the water transport path between shoot and fruits changed during fruit development and whether they are influenced by water stress during growth.

Materials and methods

Plant material

Tomato plants (*Lycopersicon esculentum* L. Mill. cv. Counter, the same cultivar as previously used by Malone and Andrews (2001)), were grown during the autumn and winter of 2001/2002 in a greenhouse at Wageningen University, The Netherlands. Plants were grown in 10 l pots filled with perlite (Agra-perlite; No 1; grading 0.6–1.5 mm) at 70% RH and 18/22 °C night/day temperature under natural light conditions. During growth, two constant levels of volumetric water content were maintained in the pots: 35% v/v% (substrate at field capacity, further referred to as high water availability treatment) and 2% v/v% (further referred to as low water availability treatment). Levels of volumetric water content were continuously controlled using measurements with a TDR (Time Domain Reflectometer) water content meter (Grodan, Roermond, The Netherlands) in combination with a computer-controlled drip irrigation system. After the formation of six fruits on the first truss the truss was pruned. Plants were de-topped just below the second truss and allowed to grow further until they were used for measurements. Axial shoots were regularly removed. Before measuring, plants were transferred from the greenhouse to a climate room (60% RH, 20 °C) close to the laboratory. Dates of flowering of all fruits of the first truss were measured. In order to characterize the effect of the water availability treatments on the plant material, the weights of fruits, calyx and pedicels as well as the total area of the sepals and lengths of peduncles and pedicels were measured. Osmotic potentials were measured by freezing-point depression in sap expressed from fruit pericarp tissue after one freeze and thaw cycle using an osmometer (Vogel, Type 3B, Gießen, Germany).

Hydraulic resistance measurements

Hydraulic resistance measurements were done on peduncles, whole pedicels and pedicel stem segments on both sides of the AZ, at two stages of fruit development (11 and 31 DAA) and replicated 4 or 6 times per treatment and development stage. The apparatus to measure hydraulic resistance was described in full earlier (Van Ieperen *et al.*, 2000). In short, measurements are based on accurate recordings of the rate of weight loss of containers filled with solution (10 mM CaCl₂) on high precision balances. Weight loss was caused

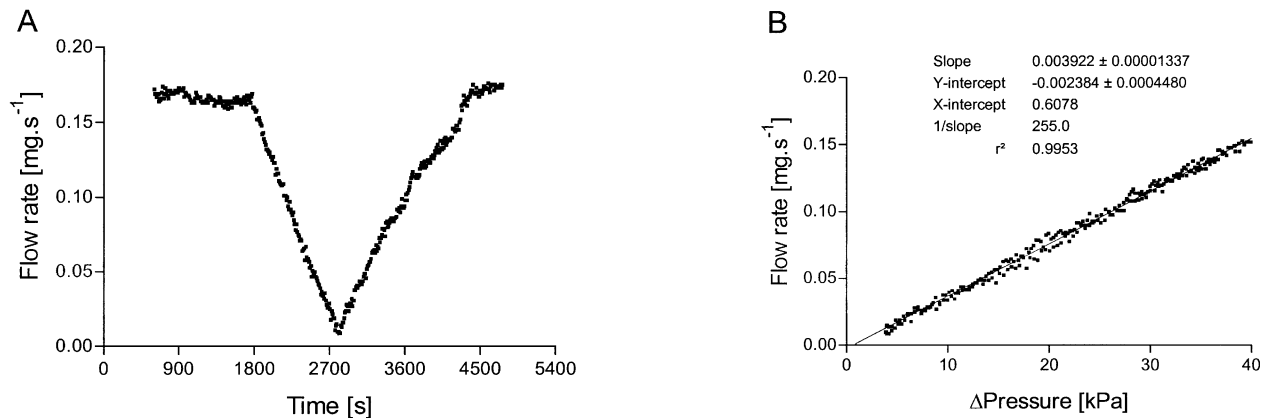


Fig. 1. (A) Measured water flow rates in a tomato pedicel induced by slowly changing suction pressures applied at the outflow cut end of the pedicel. After several minutes at a constant level (-40 kPa), the suction pressure was slowly released to 0 kPa and subsequently decreased to -40 kPa again. (B) The relationship between suction pressures and corresponding flow rates obtained from (A). The reciprocal of the slope of the linear regression represents the hydraulic resistance of the pedicel. All data points correspond with individual flow rate measurements, measured over 10 s intervals after noise reduction (running averages over six subsequent flow rate measurements).

by water uptake at one cut end of a sample, which was induced by a known suction pressure at the other cut end of the same sample. Measurements of flow rate and suction pressure were made each 10 s. Suction was used instead of pressure to prevent flooding of intracellular air spaces and to avoid artificial flow paths (Canny, 1990). Measurements were done in a climate chamber at constant temperature of ambient air and solution (20 ± 0.5 °C) to avoid viscosity differences of water solutions. The only modification of the previously described method was in the measuring protocol: after recording the flow rate in a sample for several minutes at -40 kPa pressure difference, suction pressure was slowly released up to a pressure difference of almost 0 kPa and then slowly decreased back to -40 kPa. Meanwhile flow rate and pressure measurements were measured continuously. The whole procedure took around 90 min. This procedure resulted in an approximately V-shaped flow curve, where each flow rate corresponded to a known suction pressure (Fig. 1A). Plotting measured flow rate (after noise reduction by calculating the running average over six subsequent measurements) against pressure (Fig. 1B) produced a linear relationship of which the slope represented the conductance (reciprocal of resistance) of the sample. This protocol allowed checking whether resistance was constant over the measurement period (initial and final flow rates had to be approximately equal) and whether resistance was independent of flow rate and pressure. After measuring its hydraulic resistance each sample was photographed together with a scale (Sony digital still camera DSC-S70) for later length determination using the free UTHSCSA ImageTool program (developed at the University of Texas Health Science Center at San Antonio, Texas). Resistance per unit length was calculated.

Measurements of the distribution of hydraulic resistances between stem and fruit

To determine the distribution of resistances in the flow path towards a tomato fruit, the first truss was cut from the plant under water to prevent air entrance in the xylem conduits. Length of the peduncle (between stem and pedicel of the first fruit) was measured. Then, peduncle and pedicel of the first fruit were carefully cut using a sharp razor blade. Resistances of the resulting pieces of the peduncle and pedicel were determined using the procedure described above. Resistances of whole peduncles and pedicels (R_{peduncle} and R_{pedicel}) were calculated from the corresponding resistances per unit length and their actual lengths in the truss before cutting. Afterwards, the

pedicel was cut (under water) into three pieces: (i) the pedicel segment between peduncle and knuckle, (ii) the knuckle including AZ, and (iii) the pedicel segment between knuckle and fruit. The two stem segments of the pedicel were carefully re-cut with a sharp razor blade and resistances and lengths were measured. Resistances per unit length and actual resistances in the whole pedicel were calculated

Calculation of hydraulic resistance of the abscission zone

The hydraulic resistance of the AZ in the knuckle (R_{AZ} ; kPa s mg^{-1}) was calculated as the difference between the measured hydraulic resistance of the whole pedicel (R_{pedicel}) and the resistances of the pedicel stem segments on the shoot side (R_{ped_s}) and fruit side of the AZ (R_{ped_f}) after correction for length due to recutting. $R_{\text{AZ}} = R_{\text{pedicel}} - R_{\text{ped}_s} - R_{\text{ped}_f}$. To accomplish this calculation correctly R_{ped_s} and R_{ped_f} were calculated from their respective measured resistances per unit length and their actual lengths within the whole pedicel of origin. These lengths were obtained from the digital images of whole pedicels. Outer characteristics of the knuckle, which indicate the location of the AZ, were used as an indication for the separation between the pedicel stem segments. The thickness (length) of the abscission zone, which is usually only a few cell layers, was assumed to be negligible.

Visualization of the water flow path

To test the flow path for water in samples, toluidine blue O (0.1%) was sucked through peduncles and pedicels during a few seconds by sub-atmospheric pressure (-40 kPa) using the measurement system for hydraulic resistance and the preparation protocol. Toluidine colours cell walls of all water-conducting vessels blue, since it easily passes via vessels and through pit membranes. Immediately after perfusion free-hand cross-sections of pedicels were quickly cut, rinsed with ethanol (80%) and examined under a binocular microscope (Leica MZ 12).

Determination of vessel endings

To determine whether vessels have a tendency to end near the AZ, a red latex particle suspension was sucked through the pedicels by sub-atmospheric pressure. Latex particles can freely move through the lumen of xylem vessels but cannot pass pit-membranes at vessel-to-vessel connections (Zimmermann and Jeje, 1981). Consequently, only vessels with an open connection with the cut end in the latex

Table 1. Characteristics of tomato fruits^a and associated calyx and pedicels from plants subjected to two levels of water availability in the root medium during growth (averages \pm standard errors of means)

Treatment ^b (v/v%)	Fresh weight fruits (g)	Fresh weight calyx (mg)	Fresh weight pedicel (mg)	Osmotic potential fruits (MPa)	Calyx surface area (mm ²)	Diameter pedicel (mm)	
						Shoot side	Fruit side
35%	57.7 \pm 9.1	541 \pm 40	347 \pm 52	-0.81 \pm 0.02	714 \pm 48	3.8 \pm 0.2	4.1 \pm 0.3
2%	35.1 \pm 1.4	366 \pm 20	249 \pm 16	-1.02 \pm 0.01	558 \pm 26	3.2 \pm 0.1	3.4 \pm 0.1

^a Green tomato fruits (harvested 42 d after anthesis).

^b 35% v/v%: High water availability in substrate (field capacity), 2% v/v%: low water availability in substrate. Statistical significant differences between the two water availability treatments were found in all parameters shown ($P=0.05$, Student *t*-test, $n=6$).

suspension became filled with latex. An aqueous 1% (w/w) suspension of red latex particles (Nijse *et al.*, 2001) was sucked through during approximately 10–15 h after preparation of the pedicels under water. Pedicels were used from fruits from the first and the second positions on the first truss (harvested 20 DAA). Pedicels were cut (A) at the peduncle–pedicel junction, leaving the complete pedicel stem segment before the AZ on the pedicel sample, and (B) at the knuckle, approximately 2 mm before the AZ to maximize the number of latex-filled vessels at the entrance of the AZ. This enabled the distinction of a normal tendency of vessels to end from an increased tendency at the AZ. After perfusion with latex suspension, pedicels were cut at several distances from the entrance point and images of the sections were made using a digital colour camera (Nikon DXM-1200) attached to a binocular microscope (Leica MZ 12). Numbers of latex-filled vessels at different distances from the entrance point were counted from the images using the image analysis software, as mentioned above.

Results

Size, fresh weight and osmotic potential of fruits, as well as fresh weight and area of the calyx were clearly lower in plants grown at low water content in the root environment (i.e. with water stress; Table 1). Lengths of peduncles and pedicels were not influenced by the water availability treatments, although the length of peduncles tended to be lower in plants grown at low water availability ($P=0.052$, Student *t*-test; data not shown). Indeed, later experiments in summer confirmed clear differences in peduncle length due to similar water availability treatments (data not shown). Diameters and weights of peduncles and pedicel stem segments were lower in plants grown at low water availability.

Generally, hydraulic resistance of the pedicel ($R_{pedicel}$) was much higher than that of the peduncle ($R_{peduncle}$) (Table 2). Reduced water availability during growth resulted in an increased $R_{pedicel}$ as well as $R_{peduncle}$. During fruit development $R_{peduncle}$ decreased, whereas $R_{pedicel}$ did not decrease (the increases in means were not statistically significant ($P=0.05$, Student *t*-test)). As a result the $R_{peduncle}$: $R_{pedicel}$ ratio changed from 1:40 to 1:200 between 11 and 31 DAA. Measurements on the distribution of hydraulic resistance along the pedicel revealed that $R_{pedicel}$ was almost completely due to the high resistance located in the relatively thin AZ in the knuckle (R_{AZ}) (Table 3). Hydraulic resistances of the pedicel stem

Table 2. Hydraulic resistances of peduncle ($R_{peduncle}$) and pedicel of the first fruit ($R_{pedicel}$) in fruiting truss of tomato at two fruit development stages subjected to two levels of water availability in the root medium during growth (averages \pm standard errors of means)

Treatment ^a (v/v%)	Fruit development stage (days after anthesis)	$R_{peduncle}$ (kPa s mg ⁻¹)	$R_{pedicel}$ (kPa s mg ⁻¹)
35%	11	4.2 \pm 0.8 a	152.2 \pm 13.7 a
2%	11	8.3 \pm 1.1 b	286.3 \pm 13.2 b
35%	31	1.0 \pm 0.2 c	180.4 \pm 41.2 a
2%	31	1.5 \pm 0.1 d	374.0 \pm 80.0 b

^a See Table 1. Statistical significant differences in hydraulic resistance (within a column) are indicated by different letters ($P=0.05$, Student *t*-test, $n=4-6$).

segments (R_{ped_s} and R_{ped_f}) and of the AZ (R_{AZ}) were larger after growth at reduced water availability in the root environment. Remarkably, R_{AZ} also tended to increase with fruit development while R_{ped_s} and R_{ped_f} clearly decreased. The decrease of peduncle and pedicel stem segments' resistances during fruit development can be explained by the formation of new vessels during secondary growth, which was also reflected in the increase in peduncle and pedicel diameters. Microscopic observations of the knuckle area showed a clear restriction of cross-sectional xylem area close to the abscission zone (data not shown), indicating severely reduced or the absence of secondary xylem in the abscission zone. Outer knuckle diameter, however, clearly increased with development, probably due to the increase in cross-sectional phloem area and pith parenchyma (see also André *et al.*, 1999; Lee, 1989).

Latex perfusion experiments on pedicels showed that vessels tend strongly to end in and near the AZ in the pedicel (Fig. 2): of all xylem vessels entering the knuckle at the shoot side more than 90% ended in the 2 mm thick AZ (Table 4). This is in contrast to the relative small percentage of vessels that ended in the 1 cm long pedicel segment before the knuckle (about 25%) after entering the pedicel at the peduncle–pedicel junction. Near and in the AZ, latex-filled vessels were clearly reduced in diameter compared to latex-filled vessels in the pedicel stem segment before the AZ (Fig. 2). Microscopic observations

Table 3. Distribution of hydraulic resistances within the pedicels of Table 2

Resistances of the pedicel stem segments on the stem- (R_{ped_s}) and fruit-ends (R_{ped_f}) of the pedicel and within the knuckle with future abscission zone (R_{AZ}) (averages \pm standard errors of means).

Treatment ^a (v/v%)	Fruit development stage (days after anthesis)	R_{ped_s} (kPa s mg ⁻¹)	R_{AZ} (kPa s mg ⁻¹)	R_{ped_f} (kPa s mg ⁻¹)
35%	11	9.0 \pm 0.8 a	118.7 \pm 14.1 a	24.5 \pm 7.1 a
2%	11	22.6 \pm 2.9 b	234.1 \pm 11.4 b	29.5 \pm 3.7 a
35%	31	3.6 \pm 0.5 c	168.5 \pm 39.5 a	8.3 \pm 2.0 b
2%	31	5.7 \pm 0.5 d	346.8 \pm 78.4 b	21.5 \pm 2.3 a

^a See Table 1. Statistical significant differences in hydraulic resistance (within a column) are indicated by different letters ($P=0.05$, Student *t*-test, $n=4-6$).

after toluidine perfusion also indicated that at both sides of the AZ vessels were much larger in diameter than in the AZ (results not shown). The number of latex-containing xylem elements at 1 mm from the entrance cut surface was much higher when latex was directly sucked into the knuckle than when it entered the pedicel at the peduncle–pedicel junction (Table 4). This could have been due to the presence of more xylem elements per cross-section at the entrance of the knuckle, but also to the method used. It is possible that much more of the short vessels and/or tracheids near the cut surface at AZ were involved in latex transport than near the cut surface at the peduncle–pedicel junction. Also short vessels and tracheids could have been continuously open from cut end to AZ in the case of cutting at the knuckle. Nevertheless, the percentages of vessel endings in the AZ were comparable for different cutting procedures (Table 4).

Toluidine staining was attributed to the vessels, which were assumed conductive under the conditions of the dye application. Staining was intensive near the place where the dye was sucked into the pedicel (stem part of pedicel), remained relatively the same until the knuckle in the middle of the pedicel. At the point of knuckle, staining by toluidine concentrated mainly at the sides of the knuckle, but never at its middle. After the knuckle in the fruit part of the pedicel, blue toluidine rings surrounded fewer but still many vessels.

Discussion

Temporary shrinkage of plant organs is a common phenomenon during periods of water stress. It reflects the loss of water by direct transpiration or back-flow of water from the organ towards the rest of the plant via the xylem. However, in tomato no or only little variation in fruit size due to transient water shortage in the shoot has been observed *in vivo* (Ehret and Ho, 1986a, b; Johnson *et al.*, 1992; Kitano *et al.*, 1996; Pearce *et al.*, 1993). Fruit shrinkage was little even when apoplastic water potential gradients between fruits and shoot clearly favoured water transport in the direction of the shoot during periods of water stress (Johnson *et al.*, 1992). However, the absence of fruit shrinkage during periods of water stress does not

necessarily imply the absence of back-flow via the xylem. Water import via the phloem may occur against a water potential gradient (e.g. phloem flow from leaves to roots) and may have simply overruled back-flow via the xylem in magnitude resulting in net import of water. Experimentally, back-flow from fruits to leaves has been shown in bench-drying tomato explants, containing 1–3 leaves and one fruiting truss (Malone and Andrews, 2001). This indicated at least the presence of a functional xylem connection between shoot and fruit, which was clearly confirmed by long-term experiments with dyes in whole plants. However, the extent of xylem back-flow was small (Malone and Andrews, 2001) despite the probably large driving force (assuming a low leaf water potential in bench-drying explants). The evidence above indicates a large hydraulic resistance between plant and tomato fruit tissue.

Previous detailed anatomical observations on the xylem transport system clearly showed xylem restrictions in tomato pedicels, especially at the AZ in the knuckle (André *et al.*, 1999; Lee 1989; Lee *et al.*, 1989). They revealed a sharp decrease in xylem cross-sectional area at the AZ in the knuckle of the pedicel, which was interpreted as a localized large hydraulic resistance for xylem water transport (Johnson *et al.*, 1992; Lee, 1989). Regular microscopic observations in this study (results not shown) also confirmed these results. On the other hand, Malone and Andrews (2001) argued that visual reduction in total xylem area at the AZ does not necessarily mean that hydraulic resistance actually increases; xylem area proximal to the AZ could have represented non-conducting fibres and parenchyma cells. Present experiments with toluidine do not support this view: for most of the xylem vessels proximal and distal to the AZ were involved in xylem water transport, while only a few functional vessels penetrated the AZ. Malone and Andrews (2001) mentioned that, with fruit development, transverse xylem area rapidly increases in pedicels. This increase clearly correlates with the presently measured decreasing hydraulic resistances of peduncle and pedicel stem segments during development (Tables 2, 3). However, the present measurements also demonstrate that a relatively large hydraulic resistance exists in the AZ located in the knuckle of a tomato fruit

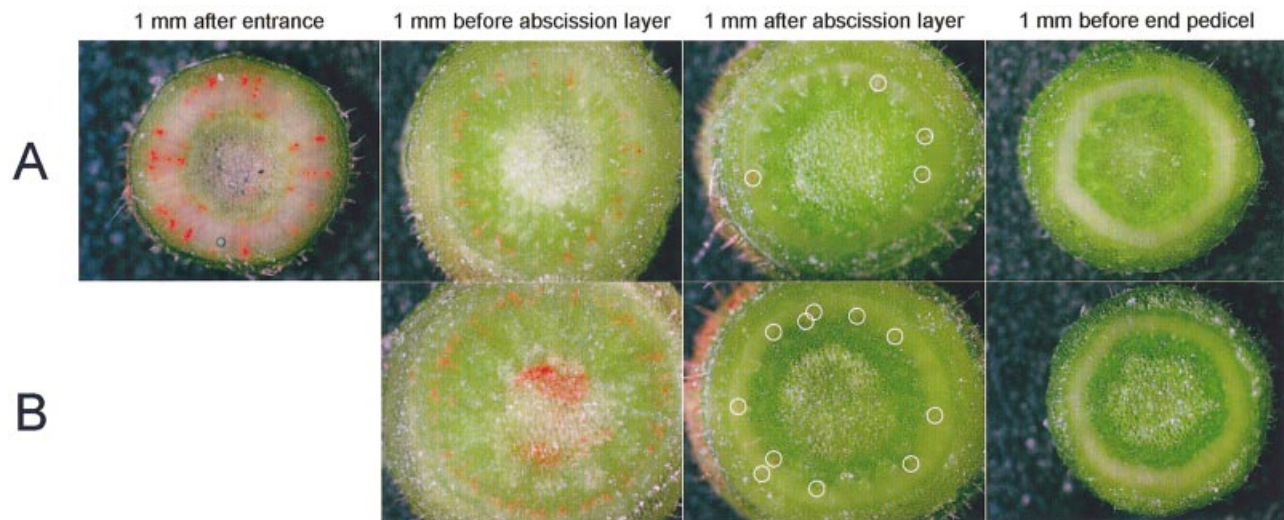


Fig. 2. Images of transverse sections of tomato fruit pedicels after 12 h of latex uptake. The red dots in each image are latex-filled xylem elements that are continuously open from the latex entrance point (cut surface) towards the indicated position in the pedicel. Latex entrance point (cut surface) was varied between (A) peduncle–pedicel junction or (B) 2 mm before the knuckle half-way along the pedicel.

Table 4. Number of continuous xylem vessels (without pits in the flow path) starting from (A) the peduncle–pedicel junction or (B) 2 mm before the knuckle towards specific downstream locations in the pedicel

Measured on pedicels of the first or the second fruit in a fruiting truss of tomato at 20 d after anthesis grown at two levels of volumetric water content of the root medium during growth (averages \pm standard errors of means).

Treatment ^a (v/v%)	Inflow	1 mm after peduncle–pedicel junction (No. vessels)	1 mm before abscission zone (No. vessels)	1 mm after abscission zone (No. vessels)
35%	A	55 \pm 5	42 \pm 5	3 \pm 1 (8%) ^b
2%	A	58 \pm 3	43 \pm 4	5 \pm 1 (12%)
35%	B	–	183 \pm 18	12 \pm 1 (7%)
2%	B	–	137 \pm 13	12 \pm 5 (9%)

^a See Table 1.

^b Between brackets: percentage of the coloured vessels at 1 mm before the abscission zone that did not end in the abscission zone. No statistical significant differences were found in the number of latex coloured vessels between the water availability treatments nor between % continuous vessels through the abscission zone ($P=0.05$, Student *t*-test, $n=4$ (last column tested after log transformation)).

pedicel (R_{AZ} ; Table 3). This R_{AZ} dominates the hydraulic resistance of the xylem water transport path between shoot and fruit–pedicel junction by far (Tables 2, 3). It should be noted, however, that in this study the hydraulic resistance inside the fruit was not included. This hydraulic resistance, located distal to the fruit–pedicel junction, may be substantial and might overrule the R_{AZ} as the major resistance in the xylem pathway between the stem and fruit tissue.

While other hydraulic resistances within peduncle and pedicel stem segments before and after the AZ are rather low and decrease with fruit development this is not the case for R_{AZ} . The relatively large R_{AZ} clearly resulted from the discontinuity of more than 90% of the vessels entering the AZ (Table 4), combined with a reduced vessel diameter of the vessels in the AZ. These results are in agreement with the anatomical observations of André et al. (1999), who reported approximately similar percentages of dis-

continuous vessels through the AZ and reduced vessel diameters, measured by a completely different technique (microcasting). Continuous vessels in the AZ are mostly primary and sometimes heterogeneous (helical wall thickenings at the AZ level and pitted on both sides; (André et al., 1999)). The absence of significant secondary growth at the AZ probably explains why R_{AZ} does not decrease with fruit development as was measured in all other parts of the xylem water transport path. Primary vessels are formed during the early phases of pedicel development. Due to the helical structure of the wall thickenings, primary as well as the heterogeneous vessels allow some stretching, which is probably necessary to avoid excessive conductivity loss when transverse surface area of the knuckle increases during fruit development (André et al., 1999). The primary character of the vessels in the AZ also suggests that, already, at an early fruit development stage (when primary vessels are formed in

the AZ), R_{AZ} is importantly determined. Effects of water stress during the early stages of fruit development might therefore determine the overall hydraulic xylem resistance during later fruit development, since there is no possibility to compensate by secondary vessel formation in the AZ. This should be verified by further research. The small number of continuous vessels through the AZ makes the xylem system in the pedicel vulnerable for conductivity loss. Cavitation due to water stress, clogging or mechanical rupture of only a very few vessels in the AZ may already lead to significant (temporary or permanent) loss of xylem conductivity. The present measurements did not show a statistically significant increase in R_{AZ} between 11 and 31 DAA, although the mean values suggest an increase in R_{AZ} in time. Preliminary experiments with almost harvest-ripe tomatoes indicate a further increase in hydraulic resistance in the AZ with fruit development (W Van Ieperen, VS Volkov, U Van Meeteren, unpublished results).

A restricted xylem connection between shoot and fruit and the consequential hydraulic isolation of the fruit from the rest of the plant may have clear advantages as well as disadvantages for tomato fruit development. It may protect fruits and seeds from diurnal cycles of severe water stress, which are thought to be harmful for fruit and seed development, since stable water relations are required for normal embryo development (Bradford, 1994). On the other hand, a restricted xylem connection may negatively influence calcium import into low-transpiring fruits, which may also lead to negative effects on fruit development (e.g. blossom end rot) (Adams and Ho, 1993). The absence of alternating changes in fruit volume seem to impede the use of diurnal cycles in water stress to transport additional calcium-rich xylem water into tomato fruits by periodic flow reversal (Malone and Andrews, 2001; Wiebe *et al.*, 1977). However, another driving force for xylem water (and calcium) transport through the peduncle and pedicel might be the transpiration of the calyx. Ehret and Ho (1986a) showed significant transpiration of the calyx in detached fruits that were able to take up water via the pedicel. They argued that the calyx may be involved in the calcium supply of the fruits because removal of the calyx increased the incidence of blossom-end rot in the berry (Ehret and Ho, 1986b).

Although it was clearly shown that R_{AZ} increasingly dominates the hydraulic resistance of the xylem water transport between the stem and the pedicel–fruit junction with fruit development, the question remains how important this resistance is for hydraulic isolation of the fruit. The highest measured hydraulic resistance (approximately 400 kPa s mg⁻¹; Table 2) of the transport path between the stem and the pedicel–fruit junction of the first fruit of a truss is equivalent to a hydraulic conductance of approximately 9 mg h⁻¹ kPa⁻¹ (at 32 DAA). A rough estimation of water use of the fruit and calyx of comparable fruits reveals rates of maximal 100 mg h⁻¹ for water loss and growth of the berry

and calyx (calculated from Ehret and Ho, 1986a, b). This demand can already be fulfilled by a relatively small pressure gradient (≈ 11 kPa) between stem and fruit. This calculated gradient is much smaller than the apoplastic water potential differences (up to 1 MPa) between stem and fruit tissue reported by Johnson *et al.* (1992). These calculations suggest the existence of another major resistance for water flow located distal to the pedicel–fruit junction as was previously suggested by others (Ehret and Ho, 1986a; Malone and Andrews, 2001). Increasing conductivity loss at the AZ in the pedicel with further fruit development might finally lead to hydraulic isolation located at the knuckle. This seems to be more related to the process of abscission of the ripe fruit than to a role in reducing water fluxes between fruit and stem.

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