Most Water in the Tomato Truss Is Imported through the Xylem, Not the Phloem: A Nuclear Magnetic Resonance Flow Imaging Study [W][OA]

Carel W. Windt*, Edo Gerkema, and Henk Van As
Laboratory of Biophysics, Wageningen University, 6703 HA Wageningen, The Netherlands

In this study, we demonstrate nuclear magnetic resonance flow imaging of xylem and phloem transport toward a developing tomato (Solanum lycopersicum) truss. During an 8-week period of growth, we measured phloem and xylem fluxes in the truss stalk, aiming to distinguish the contributions of the two transport tissues and draw up a balance between influx and efflux. It is commonly estimated that about 90% of the water reaches the fruit by the phloem and the remaining 10% by the xylem. The xylem is thought to become dysfunctional at an early stage of fruit development. However, our results do not corroborate these findings. On the contrary, we found that xylem transport into the truss remained functional throughout the 8 weeks of growth.

During that time, at least 75% of the net influx into the fruit occurred through the external xylem and about 25% via the perimedullary region, which contains both phloem and xylem. About one-half of the net influx was lost due to evaporation. Halfway through truss development, a xylem backflow appeared. As the truss matured, the percentage of xylem water that circulated into the truss and out again increased in comparison with the net uptake, but no net loss of water from the truss was observed. The circulation of xylem water continued even after the fruits and pedicels were removed. This indicates that neither of them was involved in generating or conducting the circulation of sap. Only when the main axis of the peduncle was cut back did the circulation stop.

Fruits are terminal organs that depend completely on long-distance transport to supply them with sugars and water for growth. Water is imported by means of both the xylem and the phloem, whereas sugars are only imported by means of the phloem. Fruits have to compete for water with the rest of the plant, and for that reason, xylem influx is expected to be sensitive to changes in plant water potential. Xylem influx into fruits may thus be lower during the day and higher during the night. When in the apoplast the water potential is especially low, for instance, when the plant is transpiring a lot of water during a hot day, fruits may even experience a xylem efflux and lose water to the vegetative parts of the plant (Johnson et al., 1992; Guichard et al., 2005). It has been suggested that in several species, in order to reduce the sensitivity of fruits to changes in plant water status, during fruit development the xylem connection between fruit and plant is reduced or even severed (Findlay et al., 1987; Lang, 1990; Creasy et al., 1993; Lang and Ryan, 1994; van Ieperen et al., 2003; Drazeta et al., 2004). In contrast to the xylem, the phloem is expected to be relatively insensitive to diurnal changes in water potential (Ehret and Ho, 1986; Ho et al., 1987). For instance, in the main stem of a number of plants, the phloem was found not to respond to diurnal differences in plant water status, whereas the xylem did (Peuke et al., 2001; Windt et al., 2006).

The tomato (Solanum lycopersicum) plant has been the subject of many studies dealing with long-distance transport to fruits and has been chosen as a model system in this study as well. It has been estimated that in tomato fruits, about 80% to 90% of the influx of sap takes place by means of the phloem (Ho et al., 1987; Plaut et al., 2004; Guichard et al., 2005). It has been proposed that the low xylem contribution is due to the presence of some form of restriction in the xylem connection between plant and fruit, possibly in the knuckle (Lee, 1989; van Ieperen et al., 2003). Despite the expected low xylem contribution and limited conductivity of the xylem connection between plant and fruit, fruits have been shown to exhibit a diurnal pattern of growth. In most cases, fruits have been observed to grow fastest at night (Lee, 1989; Grange, 1995; van de Sanden and Uittien, 1995; Guichard et al., 2005). The opposite has been found to occur as well (Ehret and Ho, 1986; Pearce et al., 1993), but in these cases, the faster daytime growth was probably caused by a low diurnal stress environment. In a number of studies, even an efflux of xylem sap and fruit shrinkage during the day was reported (Johnson et al., 1992; Leonardi et al., 1999, 2000). It has been proposed that,
if the phloem and xylem operate under different diurnal cycles or if their relative contributions can be modified in any way by adjusting the environmental conditions in a greenhouse, it might become possible to control and regulate fruit yield as well as fruit quality and taste.

Considering the importance of fruit for the world’s food production, surprisingly little is known about the dynamics of sap flow to fruits. Since the conception of the cohesion tension theory (Dixon and Joly, 1894) and the Munch pressure flow hypothesis (Münch, 1930), there has been a decent theoretical understanding of the basic forces that govern phloem and xylem flow. It has already been attempted to apply this understanding to model fruit growth for a variety of fruits and applications (e.g. Daudet et al., 2002). However, many of the parameters that are needed to model long-distance transport to fruits are currently outside of experimental reach. First, little is known about the pressure and water potential gradients that drive flow to fruits. The xylem and the phloem are extremely sensitive to invasive experimentation and are easily disturbed, and the water potentials in the fruits’ symplast and apoplast are difficult to assess. Second, it is not clear whether xylem and phloem sap only enters the fruit (unidirectional flow), or if return flow is possible as well, and if it is, under which conditions it may occur. As the results of this study show, NMR flow imaging can provide answers to these important questions.

Estimating Long-Distance Transport to Fruits

So far, the most important methods to estimate xylem and phloem influx in fruits have been the subtractive method (Lang and Thorpe, 1989) and the mineral accumulation method (Ho et al., 1987). In the subtractive method, the contribution of xylem and phloem are estimated by heat girdling the pedicel (fruit stalk) of a fruit. Heat girdling destroys the sieve tubes, stopping phloem influx, while the xylem is assumed to remain intact and functional. By comparing the growth of nongirdled fruits to that of girdled fruits, the phloem contribution can be estimated. The most critical assumption in this method is that the xylem sap flow is not affected by heat girdling. However, the validity of this assumption is not evident. First, because xylem and phloem flow to fruits are coupled. Xylem influx is driven by a water potential difference between the xylem and the fruit symplast, which is maintained by osmotically active compounds (sugars), which in turn are imported by means of the phloem. Fishman et al. (2001) showed that the coupling between phloem and xylem influx could give rise to significant errors when using the pedicel girdling technique. A second reason is that heat girdling may profoundly affect xylem function. The xylem tissue may apparently escape heat girdling unscathed, as demonstrated by Guichard et al. (2005), but if the surrounding cells are damaged, it is not unlikely that functional damage will occur. For instance, it has been proposed that the cells that surround the xylem protect it against embolisms by preventing the entry of air (Hacke and Sperry, 2001). Van Ieperen et al. (2003) found that in the tomato pedicel, the abscission zone is the site of highest xylem resistance and that only a few xylem conduits traverse it. If an obstruction would occur in these conduits, either by embolisms or by particles of debris, it could significantly affect xylem resistance and have large implications for xylem transport to the fruit.

In the second method, the mineral composition of the fruit is used to estimate the relative xylem and phloem contribution. Ho et al. (1987) measured calcium accumulation, net water import, and fruit respiration in tomato fruits. The xylem contribution to fruit growth was then estimated based on a number of assumptions: (1) the calcium content of phloem sap can be neglected compared to that of xylem sap; (2) the calcium content of xylem sap is similar to that measured in root stump exudate; and (3) xylem backflow from fruits does not occur. However, in view of current knowledge, the first and third assumptions are questionable. Calcium is used in signal transduction and as such is known to be present in the phloem. The question is, in what concentration. In phloem sap exudate of castor bean (Ricinus communis) and eucalyptus (Eucalyptus globulus), calcium concentrations have been found that were about 66% and 25% of the concentration in the root stump exudate, respectively (Pate et al., 1998; Peuke et al., 2006). In Banksia prionotes, the calcium concentration in phloem exudate was even found to be 10 times higher than that of the xylem sap (Pate and Jeschke, 1995). It should be noted that in these studies phloem sap was harvested by cutting. This may have elicited a wounding response, causing elevated calcium levels in the phloem (Knoblauch et al., 2001). Still, we argue that these findings illustrate that the calcium concentration in the phloem cannot be assumed to be negligible, especially when the majority of influx of sap is thought to take place via the phloem. The assumption that backflow does not take place also may not hold. In a number of studies, backflow from tomato fruits has already been observed, especially under summer conditions or high vapor deficit (Johnson et al., 1992; Leonardi et al., 1999, 2000; Guichard et al., 2005). The subtractive and the mineral accumulation method thus are likely to be subject to large systematic errors. Better methods to estimate or measure long-distance transport to fruits are needed.

NMR Flow Imaging

Over the last 10 years, it has been demonstrated that NMR flow imaging can provide an excellent tool to measure xylem and phloem transport (Van As, 2007). NMR flow imaging does not only give information about the average flow velocity, such as heat pulse based methods do, but gives access to all properties of the flowing water, such as the flow conducting area, the distribution of flow velocities, and the
volume flow, all on a per pixel basis (Scheenen et al., 2000b). So far, studies have been conducted measuring flow in the stem of a variety of plants, ranging from castor bean seedlings (Köckenberger et al., 1997) to fully developed tomato, castor bean, and tobacco (Nicotiana tabacum) plants, and a small poplar tree (Populus spp.; Windt et al., 2006). The technique has been used to study the diurnal variation in long-distance transport (Peuke et al., 2001; Windt et al., 2006), the effects of cold girdling (Peuke et al., 2006), and xylem embolism repair (Scheenen et al., 2007) and has been used as a reference technique to provide detailed velocity maps for comparison with different heat pulse methods (e.g. Helfter et al., 2007; D. Chavarro, C.W. Windt, M.W. Lubczynski, J. Roy, and H. Van As, unpublished data). These studies have in common that flow was only measured in the main stem of the plant. This is a convenient place to do flow imaging for a variety of reasons. In comparison with other flow-conducting structures in the plant, the stem is large, sturdy, and stable. It conducts the largest fluxes, and the xylem and phloem can be easily distinguished on the basis of their direction of flow. These properties make imaging xylem and phloem transport relatively easy.

Aims and Research Questions

In this study, we used NMR flow imaging to measure long-distance transport to fruits. As a model plant, tomato was chosen. The anatomy of the tomato truss, as well as the dimensions of the magnetic resonance imaging (MRI) device and its components, made it impossible to image the pedicel of a single fruit. The pedicels were too short and too close together to fit them with the radio frequency (RF) coil that is needed for MRI. For this reason, we chose to perform flow imaging on the peduncle of tomato, measuring the transport toward the entire developing truss. After fitting the plant in the imager, it was impossible to remove the plant without damaging it. The plant was therefore left in the imager and allowed to grow there for 8 weeks. In this period, we continuously monitored long-distance transport into the truss, aiming to answer the following questions: (1) can xylem and phloem flow into the truss be visualized and distinguished; (2) what transport tissues conduct sap into the truss during truss development; (3) is phloem and xylem transport into the truss unidirectional, or does backflow occur; and (4) can NMR flow imaging be used to draw up a quantitative balance of xylem and phloem influx into the truss?

RESULTS

NMR Flow Imaging in the Peduncle of a Tomato Truss

Throughout the growth of the truss, the weight and size of the growing fruits caused the peduncle to shift and move to a certain degree, requiring constant attention to keep the peduncle within the field of view (Fig. 1A). During the first 2 weeks, the pattern of flow in the peduncle consisted of a central ring, with small rays extending outwards (Fig. 1B). From week 3 onwards, a second, outer ring appeared. The central
inner ring remained functional, but the ray-like structures in between the two rings became less intense. From week 4 onwards, the intensity of the inner ring decreased as well, but it remained active and visible. After 3.5 weeks, the first backflow (i.e. water transport out of the truss) became apparent in the daytime measurements (image not shown). During the night, backflow could be observed from week 5 onwards. Backflow was only observed in the outer ring, and more and more bundles were observed to conduct water back from the truss as the truss matured.

After the experiment, microscopy slides were prepared of the section of the peduncle where flow imaging was done, in order to compare the microscopic anatomy of the peduncle to the flow images made at week 8 (Fig. 2). In the outer ring, both influx and efflux were observed, shown in blue and red, respectively. In the inner ring, only influx was found, here shown in green. The flow images of Figure 2D were scaled to match the size of the microscopy image and plotted over it, giving Figure 2B. The outer ring matches perfectly with the ring of the widest xylem vessels in the peduncle, indicating that it contains xylem only. Further evidence that the outer ring corresponds with xylem sap flow is given in Figure 2E. At the end of the experiment, the truss was cut back in a number of steps. As the cut approached the site of imaging, xylem conduits started to become embolized (Fig. 2E, first image). As the cut moved even closer, the number of embolies increased strongly, showing up as dark spots in the image (Fig. 2E, second image). In the last image, in addition to cutting the peduncle back to 5 cm from the imaging site, the base of the peduncle was also cut loose from the rest of the plant. This presumably led to the embolization of all xylem vessels in the peduncle.

The ray-like structures that are located between the inner and outer ring (which were especially visible in the first weeks of truss growth) can also be assigned to the xylem. They matched nicely with

![Figure 2](image-url) Assignment of flow conducting tissues. A, Microscopy image (light microscopy) of the peduncle after flow imaging. B, Volume flow map of influx and efflux in the peduncle before truss pruning, plotted over image in A. Influx in the outer ring is shown in blue and efflux in red. Influx in the inner ring is shown in green. The influx in the inner ring corresponds with the position of the perimedullary tissue, shown in detail in C. In the perimedullary tissue, phloem (ph) and xylem (x) are present. D, Quantitative volume flow map. E, Water content maps based on multiecho T2 measurements (amplitude maps) acquired during truss pruning. Left image: embolisms appeared in the outer ring after cutting back the main axis of the truss (Fig. 6, position II). Multiple embolisms are visible, and one is pointed out (asterisk). Middle image: after cutting at position III. Right image: after cutting the truss stalk loose from the stem of the plant. a.u., Amplitude in arbitrary units.
the rays of wider xylem vessels that start at the perimedullary tissue in the center and extend outwards to the widest xylem vessels in the outer ring. For convenience, the ray-like structures are counted as outer ring.

For the inner ring, the tissue assignment is less straightforward. The inner ring corresponds nicely with the perimedullary tissue, which is visible as a ring of darker blotches in the parenchyma (Fig. 2A). This tissue consists of bundles of phloem conduits that surround a single, relatively wide xylem vessel (Fig. 2C). The inner ring is therefore expected to contain a large contribution of phloem sap flow, but may also contain a significant amount of xylem flow. Because the two tissues are very close together and are flowing in the same direction, into the truss and toward the fruits, it was not possible to separately measure the xylem and phloem flows that are likely to contribute to the sap flow in the inner ring.

**Dynamics of Long-Distance Transport**

Because of the position of the truss in the center of the magnet, it was difficult to determine the stage of development that the fruits were in. However, we were able to observe whether or not fruits were beginning to turn red (advanced mature green [AMG] stage or slightly older than that if the red coloration would have started at a position that was out of sight). In Figure 3, the gray bands indicate at what time the tomato fruits passed the AMG stage. Band II indicates the point in time when the last and most distal tomato passed the AMG stage; band I indicates when all other (the more proximal) fruits passed it.

The inner ring holds the perimedullary tissue and therewith all of the phloem flow that is transported to the tomato fruits, plus an unknown contribution of xylem flow. It conducted the highest volume of flow in week 1, with a volume flow rate of 0.13 μL/s during the day and 0.11 μL/s during the night (Fig. 3A). After the first week, these flows dropped rapidly to about half those values. At time 3.5 weeks, the volume flows dropped once again by about 50%, to values of around 0.03 μL/s. From week 4.5 onwards, i.e. 1 week before all except one fruit passed the AMG stage, the volume flows dropped to a very low base level, staying at that level up to the end of the experiment without disappearing completely. Clear diurnal differences or trends were not observed; the day and night time volume flows were close together throughout the experiment.

The volume flow that was transported through the outer ring was at all times equal to or higher than the volume flow in the inner ring (Figs. 3 and 4). Note that the ray-like structures in between the inner and the outer ring are assumed to be xylem and in this context are counted as belonging to the outer ring. The nighttime influx in the outer ring was relatively constant up to week 3.5, with values around 0.12 μL/s. The daytime influx appeared to be slightly higher than nighttime influx at week 1 and lower afterward until week 4, but the differences were small and in all cases smaller than the SD. From week 5.5 to 8, after all tomato fruits except one reached the AMG stage, the daytime fluxes appeared to be higher than the nighttime fluxes. The highest volume flow was observed at night at week 4 (0.27 μL/s). After week 4, the volume flows slowly decreased with each passing week.

From week 3.5 onwards, backflow was observed in part of the outer ring, while in the rest of the ring,
influx remained visible. At first, backflow could only be observed during the day but not during the night, indicating that day-night pressure differences are felt inside the truss and influence the xylem circulation therein. After week 5, bundles of xylem conduits conducting backflow could also be observed during the night (Fig. 1). During the day, significantly more backflow occurred than during the night, partially counteracting the increase in daytime influx. Backflow was observed in only one-half of the truss stalk, roughly in the top section in between the 8 and 2 o’clock positions. In all cases, the backflow conducting conduits were interspersed with inflow conducting conduits.

When the values for influx and efflux in the two rings are combined, a net influx can be calculated (Fig. 3C). The net influx equals the total amount of water that is taken up by the truss for growth and evaporation of all its components, including the fruits. The net influx appeared to be highest from week 1 to week 4, as might be expected because of the presence of the developing fruits. Peaks in influx were observed at weeks 1 and 4. After week 4, the net influx gradually decreased until the end of the experiment at week 8, but a net loss of water from the truss was never observed. At week 8, a daytime influx remained of approximately 0.02 μL/s. After 8 weeks, six fruits had developed with a volume of 318 mL in total (average volume per fruit 53 mL). During these 8 weeks, an estimated cumulative net influx of 660 mL had been transported through the truss, indicating that about half the net influx in the truss was lost due to evaporation. Approximately 25% of the cumulative net influx was transported by the inner ring, whereas about 75% was transported by the outer (xylem) ring (Fig. 4).

The NMR flow imaging method that was used not only gives information about the volume flows but also gives access to flow conducting area and average linear velocity (Windt et al., 2006). The average linear velocities in the inner ring are highest in the first 2 weeks, with values of around 0.6 mm/s and higher (Supplemental Fig. S1A). After week 2, the velocity gradually drops to a value of about 0.3 mm/s around weeks 3 and 4 and to 0.2 mm/s toward the end of the experiment. The velocities of the influx in the outer ring start with values of 0.6 mm/s for nighttime flow at week 1 and slowly decline to values of about 0.25 mm/s at the end of the experiment (Supplemental Fig. S1B). The daytime influx velocities did not decrease as much, staying almost constant from week 4 onward. The outer ring efflux velocities were comparable with the average linear influx velocities but showed more variability. The changes in the flow conducting areas of the different flow conducting regions largely match those that were observed for the volume flows (Supplemental Fig. S2, A and B).

**DISCUSSION**

**Which Tissues in the Peduncle Conduct Flow?**

In the peduncle, there are three pathways that may support a flow of sap: the external xylem, the external phloem, and the perimedullary region, which contains both internal xylem and internal phloem (Fig. 2, A and C; Bonnemain, 1968, 1970; Guichard et al., 2005). The literature suggests that, certainly in the first weeks of fruit growth, the xylem and phloem flow is unidirectional and directed toward the fruits (Lee, 1990). In a number of studies, diurnal shrinkage of fruits was observed, suggesting that backflow took place (Leonardi et al., 1999, 2000; Guichard et al., 2005). However, even if backflow is observed, it is not a priori evident which
tissue would be conducting it, even though the xylem would be the most likely candidate. Thus, flow direction alone is not an appropriate basis to distinguish phloem from xylem flow in the peduncle.

In this study, we observed flow in three regions: an outer ring, an inner ring, and some ray-like structures that were visible in between the two rings and were especially prominent during the first weeks of truss.
growth (Fig. 1). When the flow images are compared with the microscopy images of the pedicel (Fig. 2), the inner ring matches perfectly with the perimedullary tissue. The ray-like structures could be traced back unambiguously to the ray-like strings of larger xylem vessels. These must have been the largest vessels in the first weeks of truss growth, thus causing the clear ray-like structures in that period of development.

The tissue assignment of the outer ring of flow is less straightforward. Normally in a plant stem, the external xylem and phloem would become visible as two separate rings of flow, provided that both tissues conduct sap (Windt et al., 2006). The same pattern was observed in the main stem of the tomato cultivar that was used in this experiment (data not shown). In the truss stalk, however, only one outer ring was observed. Microscopy showed that the outer ring corresponded perfectly with the outermost layer of the largest vessels in the xylem, suggesting that it contained xylem only (Fig. 2, A and B). Further proof for the involvement of xylem rather than phloem came from the observation that embolisms appeared when the pedicel was cut back (Fig. 2E). We therefore conclude that in this experiment, the external phloem did not conduct a discernible amount of flow and that the outer ring contained xylem flow only.

Dynamics of Long-Distance Transport to the Tomato Truss

During 8 weeks of truss development, at least 75% of the total net influx was imported by means of the xylem. This is a conservative estimate as only the contribution of the outer ring was taken into account (Fig. 4). Because the perimedullary tissue in the inner ring contains xylem as well as phloem conduits (Fig. 2C), the xylem contribution might in reality have been larger still. A net xylem influx remained visible throughout the 8 weeks of truss development, and at all times the contribution of the xylem in the outer ring was larger than the combined contribution of xylem and phloem in the inner ring. A comparison of the cumulative net influx over 8 weeks of truss growth, with the total fruit volume after 8 weeks, indicates that about half of the total net influx was lost to evaporation by the fruits and the vegetative parts of the truss. This value cannot be compared with literature because, as far as we know, no data are available with regard to evaporation by the truss as a whole. However, a 50% evaporative loss from the truss does not seem improbable when compared with the estimates of fruit evaporative loss by Guichard et al. (2005).

In this study, some diurnal trends in the fluxes toward the truss were observed, but no significant differences. In the first 5 weeks, the nighttime influx tended to be larger than the daytime influx (with the exception of week 1), and in the last weeks of the experiment, the situation was reversed (Fig. 3). This trend suggests that in the first weeks, during rapid fruit expansion, the fruits grew most quickly at night. In the following period, after the first fruits passed the AMG stage, the uptake of water for fruit growth may have declined, while the evaporative loss from the vegetative parts of the truss stayed constant, thus causing the daytime influx to become larger than that during the night. In the literature, tomato fruits have been observed to grow more quickly during the night than during the day (Lee et al., 1989; Grange and Andrews, 1994; van de Sanden and Uittien, 1995; Guichard et al., 2005), but the opposite has been observed as well (Ehret and Ho, 1986; Pearce et al., 1993).

The observation that >75% of the influx took place by means of the xylem is surprising and, as far as we know, opposite to all estimates in the literature. In the literature, the consensus appears to be that most water by far enters the fruit through the phloem and that this rule holds in a wide range of species. For tomato, it has been estimated by various authors that 80% to 90% of the water enters the fruit through the phloem, versus 10% to 20% by means of the xylem (Ho et al., 1987; Wolterbeek et al., 1987; Plaut et al., 2004; Guichard et al., 2005). Similar estimates exist for other fruits as well, such as apple (Malus domestica; Lang, 1990), grape (Vitis vinifera; Lang and Thorpe, 1989), cereals...
(Cook and Oparka, 1983), and legumes (Pate et al., 1977). As discussed earlier, the subtractive and the mineral accumulation methods that have been used to estimate these ratios are based on assumptions that are likely to be invalid and may give rise to large errors. We argue that this explains the large difference between our findings and earlier estimates. However, it also has to be remembered that to fit the plant into the imager, it was necessary to remove the two leaves underneath the truss. The topmost of these leaves would normally serve as a source leaf for the truss, together with the leaf above it (Ho and Hewitt, 1986). To compensate and maintain a constant source-to-sink ratio, we also removed trusses upstream and downstream of the truss under observation. The truss in the imager grew rapidly and produced fruits that in size and appearance were comparable to other fruits on the plant, as well as to fruits on similar plants in the greenhouse. This indicates that the fruits grew normally and xylem and phloem influx were not inhibited.

A second striking observation is that the xylem influx into the truss continued uninterrupted throughout the 8 weeks of truss growth. This, too, is contradictory to earlier reports. It has previously been proposed that at an early stage during fruit development, the xylem connection with the rest of the plant is significantly reduced or lost completely (Ehret and Ho, 1986; Ho et al., 1987). Our results do not support this view but match well with more recent studies in grape that show that the xylem connection between fruit and plant is maintained throughout the development of the fruit (Keller et al., 2006; Chatelet et al., 2008; Tilbrook and Tyerman, 2009), even though the resistance of the pathway may be relatively high. The latter may serve to dampen the transmission of sudden differences in water potential from the plant to the fruits (van Ieperen et al., 2003).

**Backflow**

The literature suggests that tomato fruits can lose water to the rest of the plant. It has been observed that under summer conditions, when large diurnal stresses were present, fruit shrinkage occurred that could not be explained by the evaporative loss of water from the fruit alone. It was concluded that in these cases, the fruit shrinkage was caused by a backflow to the plant (Johnson et al., 1992; Guichard et al., 2005). In other studies, where the diurnal

![Figure 7. MRI setup and the positioning of plant and truss. A, Schematic depiction of a typical plant (without fruits) in the MRI setup. B, Side view of the truss-bearing tomato plant as it was positioned inside the imager. With regard to the drawing, the plant was rotated one-quarter of a turn to insert it. The dotted square (truss stalk) was positioned in the center of the magnet, in between the gradient plates.](image-url)
differences were less severe, diurnal differences in fruit growth were seen, but no fruit shrinkage was recorded. The latter matches well with our results. Throughout the experiment, a net influx into the truss was observed, for the inner as well as the outer ring, and during the night as well as during the day (Fig. 3). Backflow was observed, but the overall balance remained positive: at no time did the truss effectively lose water to the rest of the plant.

From week 3.5 onwards, an efflux of xylem sap was observed in parts of the outer ring of flow, while the influx in other regions of the same ring continued like before (Figs. 3 and 5). At first, backflow only took place during the day, but after week 5, it also was measured at night. As the truss grew older, the proportion of xylem water that flowed back to the plant progressively grew bigger, up to the point that the amount of water that was circulating became larger than the amount of water that was deposited in the truss (Fig. 3).

The fact that water circulated into the truss and out again is puzzling. The truss pruning experiment (Fig. 6) demonstrated that the fruits, calyxes, pedicels, or knuckles were not involved in maintaining the circulation of sap. The circulation of sap continued even after the fruits were mature, and the circulation was not markedly affected when the fruits, calyxes, and pedicels were successively removed from the truss (Fig. 5). Only when at the end of the experiment the main axis of the truss was cut back in a number of steps did the pattern of flow start to change (Fig. 5). As the position of the cut came closer to the site of imaging, xylem embolisms became apparent (Fig. 2E), showing that the gradual disappearance of circulation was caused by xylem vessels that had become cut during the pruning procedure. During the process of cutting back, the average linear velocity of the remaining xylem flow was hardly affected, indicating that the pressure gradient in the xylem vessels that remained uncut was not altered by the procedure (Supplemental Fig. S1). We therefore conclude that the circulation in the peduncle was not driven by the truss itself but must have been caused by pressure differences in the main stem of the plant.

It is difficult to imagine why xylem sap would take a detour into a truss stalk from which the fruits have been removed. A number of explanations might account for the phenomenon. First, the pressure gradient along the xylem in the stem might cause a small pressure difference to exist between the xylem vessels at the lower side of the base of the pedicel and the ones at the top side of it, causing a circulation of xylem sap in the peduncle. A second explanation could be that there is something special about the way that the peduncle is plumbed into the vasculature of the plant. There might be a site of high resistance in the stem at the position of the peduncle, promoting the circulation of sap through the truss, or the peduncle might have especially wide vessels, providing a pathway of low resistance. On the basis of our results, it cannot be concluded which explanation is the most likely one.

Some top-down sectoriality could be observed in the truss stalk, but even in the backflow conducting section, the backflow conducting vessels were interspersed with influx conducting ones (Fig. 1). Although in a terminal organ such as the truss stalk, this type of xylem circulation might be beneficial in that it would, for instance, prevent water to become stagnant, the prevalence, function, and significance of this type of what might be called futile xylem circulation remains obscure.

CONCLUSION

We demonstrated that xylem and phloem transport toward a tomato truss can be measured by means of NMR flow imaging, making it possible to draw up a balance between influx and efflux. Surprisingly, xylem transport into the truss remained functional throughout truss development, and in that time, at least 75% of the net influx into the fruit took place through the external xylem. About 25% was transported by means of the perimedullary region, which contains both phloem and xylem. We therefore conclude that most water in the tomato truss is imported by means of the xylem. Roughly 50% of the net influx was lost due to evaporation. Halfway during truss development, a xylem backflow to the plant appeared, the magnitude of which increased as the truss matured. Despite the backflow, the truss did not experience a net loss of water. Because the circulation of xylem water continued even after the fruits and pedicels were removed, we conclude that neither of them was involved in the generation or conductance of this circulation.

MATERIALS AND METHODS

Plant Material

Tomato plants (Solanum lycopersicum ‘Gourmet’) were grown in a climate chamber (day 23°C, 70% relative humidity, 16-h photoperiod 350 μmol s⁻¹ m⁻² photosynthetically active radiation; night 18°C, 70% relative humidity, 8 h) in 10-liter pots filled with commercial potting soil (Naturado potting soil; Naturado Bodenvoeding). The plants were watered daily and received nutrient solution two times a week. Flowers were pollinated artificially. For the experiment we selected a plant with a large third truss and a long peduncle (truss stalk). At the start of the experiment, the truss contained three small tomato fruits with a diameter of 1.5 cm, one with a diameter of 1 cm, three pollinated flowers, and three flowers in the bud. At the end of the experiment, six fruits had fully developed. Early in the experiment, two small developing tomato fruits fell off the truss prematurely. In the confined space inside the imager, these fruits were wedged off the truss by other rapidly growing fruits.

NMR and NMR imaging (also known as MRI) by necessity takes place in the center of a magnet. The type of electromagnet that was used in this study is especially well suited for plant studies because it allows large potted plants to be placed upright inside it. However, in imaging a growing truss, we were exploring the limits of what was possible in the confines of the imager. In order to place the truss stalk of the tomato plant in between the two poles of the electromagnet, as well as in the center of it, the plant was trimmed and put in an almost horizontal position (Fig. 7). Two leaves before the third truss were obstructing the plants’ entry and were removed. In order to maintain a constant source-to-sink ratio, the first two trusses were removed as well. An RF coil was fitted around the truss stalk as described...
below. A sturdy wooden stick was put next to the stem of the plant to support the weight of the plant and the RF tuning assembly, as well as the weight of the growing truss. The truss stalk was fixed to the wooden rod in a straight position as possible without hindering growth. The plant was then inserted horizontally into the magnet. The pot was tilted as much as needed to get the stem in a horizontal position without damaging the stem or the root system. To prevent soil or water from spilling, the lower part of the pot surface was covered with plastic. The aeration of the root system was ensured by cutting off the topmost part of the tilted pot. The top of the plant was allowed to grow upwards and out of the magnet. As the plant quickly grew, the topmost part of the plant was supported as well. Throughout the experiment, the plant grew vigorously. Two times a week developing side shoots and suckers were removed; all further leaves and tomato trusses were allowed to grow as usual. Inside the NMR setup, the plant was subjected to the following conditions: day 22 to 24°C, 40% to 60% relative humidity, 16-h photoperiod, 350 μmol s⁻¹ m⁻² PAR; night 20°C, 40% to 60% relative humidity, 8 h. The plant was allowed to remain inside the MRI system for the whole experiment. Moving the plant or the truss would have been impossible because the truss and the vegetative part of the plant quickly became too large to remove them from the confines of the MRI imager without damage.

At the end of the experiment, when all fruits were mature, it was found that the sap flow in the truss still had not ceased. In order to determine what caused this flow of sap, the fruits, pedicels (fruit stalks), and segments of the peduncle (truss stalk) were removed in a stepwise fashion (Fig. 6). First, the fruits were removed one by one, starting with the topmost fruit, leaving the calyx and pedicel intact. Then, the calyxes and distal parts of the pedicel were removed one by one by breaking at the knuckle. Subsequently, the remaining pedicel parts were then cut off, again stepwise. The main axis of the peduncle was then cut back in a number of steps; finally, the main stem of the plant was cut above and below the peduncle to ensure total stoppage of flow. The electromagnet that was at the core of the MRI setup allowed razor blades to be used inside the imager; the magnet was shut down temporarily and after cutting restarted. In between every step, flow measurements were done to follow changes in xylem or phloem flow. Further details are given in “Results.”

MRI Setup

The MRI system was homebuilt, based on a 0.72 T electromagnet with a 10-cm air gap controlled by a Bruker Avance 200 unit. In order to provide access for plants, a shielded gradient system with a plan parallel geometry was used, with a maximum gradient strength of 1 T/m in the XY and Z direction (Resonant Instruments). The 50-mm air gap between the two gradient plates provided free access to the center of the magnet, both from the front and back of the gradient set, as well as from above and below (Fig. 7). Because a resistive magnet is inherently sensitive to temperature fluctuations, the magnetic field was stabilized by means of a homebuilt external 19F lock unit.

A solenoid RF coil for induction and detection of the NMR signal was custom-made to closely fit the truss stalk, providing a high filling factor and an optimal signal-to-noise ratio. To make the coil, a loosely fitting mold with a diameter of 10 mm was put around the truss stalk. The RF coil was then constructed by wrapping nine turns of 0.5-mm copper wire around the mold. The finished coil was connected to a compact, homebuilt tuning circuit, electromagnetically shielded by means of aluminum foil and copper tape, and fixed to a rod that also supported the tomato truss and part of the plant. The assembly of pot, plant, and coil was then inserted into the magnet with the RF assembly in the center of the magnet.

NMR Flow Imaging

Flow imaging was done following the methodology as described in detail by Windt et al. (2006). A pulsed field gradient-spin echo-turbo spin echo (PFG-SE-TSE) sequence (Scheenen et al., 2000a) was used to measure the average linear displacement by stepping the amplitude (G) of the PFGs from −Gmax to +Gmax to +Gmax sampling q-space completely and equalistically. After Fourier transformation of the signal as a function of G, the complete distribution of displacements of water, called propagator, in the direction of G within the labeling time Δ was obtained for every pixel in an image. For more details, see Scheenen et al. (2001). From these single pixel propagators, the following flow characteristics could be extracted for each volume element in the image: the total amount of water, the amount of stationary water, the amount of flowing water, the flow conducting area, the average linear velocity (including the direction of flow), and the volume flow (Scheenen et al., 2000b). The only assumption in this quantification method is that water in individual pixels only has one flow direction: either in or out, but not both at the same time. In the stem of plants, this has so far never been a problem. However, in the peduncle, this condition was not always met. Towards the end of the experiment, the flux of water no longer was unidirectional but also started flowing out of the truss. During this period, in a small number of pixels, simultaneous influx and efflux has been observed. This is expected to cause a minor deviation in the quantification of flow at the end of the experiment when efflux became very apparent.

The flow measurements were carried out using the following experimental parameters: image matrix 128 × 64 pixels, field of view 10 × 10 mm (pixel size 78 × 156 μm in the XY plane) or 11 × 11 mm (pixel size 86 × 171 μm in the XY plane), slice thickness 3 mm, spectral width 25 kHz, repetition time 2.5 s, four times averaging, echo time 6.8 ms, turbo factor 16; flow encoding: flow labeling time 100 ms, PFG duration 82.5 ms, 32 PFG steps, PFGstep,1/2 = 0.192 T/m; acquisition time 42 min. Data analysis was performed using IDL (Research Systems), using home-built fitting and calculation routines.

In NMR flow imaging, the process of flow labeling can cause a significant amount of signal to be lost between excitation and signal acquisition. In the PFG-SE-TSE method employed here, the signal amplitude was mainly affected by T2 relaxation. The relaxation time T2 relates to parameters such as tissue water content, cell size, and vessel diameter. It may therefore vary significantly between objects and between different types of tissue (Belton and Ratcliffe, 1986; Callaghan et al., 1994; Köckenberger, 2001; van der Weerd et al., 2002). If the relaxation rate of the reference tube (used for calibration) is not equal to that of the flowing water, quantification errors may occur. The error E will depend on the flow labeling time Δ and the difference between the T2, of the flowing water T2,flow and the T2,ref of the reference tube T2,ref:

$$E = (\exp\left(\frac{\Delta}{T2,flow} - \frac{\Delta}{T2,ref}\right) - 1) \times 100\% \quad (1)$$

Methods are available that allow T2,flow to be measured, even when (as is usually the case in plants) the spatial resolution is not good enough to distinguish the flowing water from the surrounding water (Windt et al., 2007). However, these methods are very time consuming. Non-flow-resolved T2 measurements are much faster and, for the purpose of flow quantification, still yield good results. For this reason, we used non-flow, resolved T2 measurements to estimate T2,flow and correct for differences with T2,ref. Non-flow, resolved T2 imaging was done using a multi-spin-echo imaging pulse sequence (Donker et al., 1997; Edzes et al., 1998) and the following experimental parameters: field of view 10 mm, slice thickness 3 mm, image matrix of 128 × 128 pixels, 128 echoes, repetition time 2.5 s, echo time 4.2 ms, and spectral bandwidth 50 kHz, four averages. The data sets were fitted using a monoexponential decay function, yielding quantitative maps of signal amplitude and T2.

To construct quantitative spatially resolved flow maps, the data of three or more subsequent flow measurements were averaged in order to improve the signal-to-noise ratio. The resulting data set was then analyzed on a per-pixel basis. In order to quantify flow per individual flow measurement, flow masks were constructed on the basis of such flow maps. By hand the flow masks were subdivided into an inner and an outer ring. The pixels within the selected flow masks were lumped together and the resulting total propagators analyzed to yield the total flow conducting area, total average linear velocity, and total volume flow within the respective masks. The daytime and nighttime influx and efflux were estimated on the basis of flow measurements done during a period of at least 24 h. The readings taken in the light were averaged and the SD calculated; the same was done for measurements taken during the dark period.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Average linear velocity of the flowing water during 8 weeks of truss growth.

Supplemental Figure S2. Flow conducting area (PCA) during 8 weeks of truss growth.
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