

# In Vivo Observation of Cavitation and Embolism Repair Using Magnetic Resonance Imaging<sup>1[w]</sup>

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Magnetic resonance imaging (MRI) was used to noninvasively monitor the status of individual xylem vessels in the stem of an intact, transpiring grape (*Vitis vinifera*) plant over a period of approximately 40 h. Proton density-weighted MRI was used to visualize the distribution of mobile water in the stem and individual xylem vessels were scored as either water or gas filled (i.e. embolized). The number of water-filled vessels decreased during the first 24 h of the experiment, indicating that approximately 10 vessels had cavitated during this time. Leaf water potentials decreased from  $-1.25$  to  $-2.1$  MPa during the same period. Watering increased leaf water potentials to  $-0.25$  MPa and prevented any further cavitation. Refilling of xylem vessels occurred as soon as the lights were switched off, with the majority of vessels becoming refilled with water during the first 2 to 3 h in darkness. These measurements demonstrate that MRI can be used to monitor the functional status of individual xylem vessels, providing the first method to study the process of cavitation and embolism repair in intact plants.

Transport of water through xylem vessels may become disrupted by breakage of water columns under high levels of tension or freezing temperatures (Tyree and Sperry, 1989). Because gas-filled vessels cannot transmit tensions, embolized vessels are permanently lost from the water transport system unless a mechanism exists to reconnect the water column. The idea that embolized vessels might be restored to their functional state is not new, but has generally been thought to be limited to situations in which the entire vascular system could be pressurized due to active solute transport by the roots (Cochard et al., 1994; Fisher et al., 1997). Recent studies, however, suggest that cavitated vessels may be repaired even when the water in neighboring conduits is under tension (Salleo et al., 1996; McCully et al., 1998; Zwieniecki and Holbrook, 1998; Pate and Canny, 1999; Tyree et al., 1999; Melcher et al., 2001). Embolism removal is thought to require positive pressures to force the gas into solution, making it difficult to understand how

this process could take place against a background of negative water potentials (Holbrook and Zwieniecki, 1999). Although there has been some progress on how this local compartmentalization might occur (Zwieniecki and Holbrook, 2000), a mechanism that reconciles xylem tension and embolism repair has not, in our opinion, been fully articulated.

A major factor limiting our understanding of embolism repair is the lack of an *in vivo* method for examining changes in the functional status of individual vessels. All of the methods currently used to study refilling, such as temporal changes in hydraulic conductivity (Zwieniecki and Holbrook, 1998), percent loss conductivity (Salleo et al., 1996), or proportion of gas-filled conduits (McCully et al., 1998; Pate and Canny, 1999), require destructive sampling. In this paper, we use high-resolution magnetic resonance imaging (MRI) to follow the status of individual xylem vessels in the stem of an intact grape (*Vitis vinifera*) plant. MRI is well suited for studies of embolism repair because it can image inside optically opaque subjects, it is noninvasive, it is compatible with longitudinal investigations, and it does not require the use of any exogenous chemical tracers (MacFall and Van As, 1996; Chudek and Hunter, 1997). However, previous studies using MRI to study water transport in plant stems do not have the spatial resolution to distinguish individual xylem vessels (Johnson et al., 1987; Köckenberger et al., 1997). In addition, MRI studies of water transport have tended to use plants small enough to fit entirely within the magnet (Kuchenbrod et al., 1996; Köckenberger et al., 1997). Because vines have large diameter xylem vessels and relatively flexible stems, they are well suited

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for MRI studies of water transport. Here we present the first direct observations of xylem cavitation and embolism repair in an intact plant.

## RESULTS

As expected, regions of high water density in the  $M_r$  images corresponded with xylem vessels (Fig. 1). At the start of the experiment, leaf water potential was  $-1.25$  MPa and the  $M_r$  image contained several regions with a low density of vessels, indicating that some cavitation had already occurred (Fig. 2). Over the next 24 h, approximately 10 additional vessels cavitated (Fig. 2). During this period, leaf water potentials fell to  $-2.1$  MPa. There was no obvious spatial pattern to which vessels cavitated; on one side of the stem the cavitated vessels were somewhat clumped, whereas on the other side they were evenly dispersed.

After the plant was rewatered, leaf water potentials increased rapidly to  $-0.25$  MPa. During the period in which the lights remained on there was no change in the number of visible vessels in the MRI image. Once the lights were turned off, the number of water-filled vessels increased markedly (Fig. 2). After the first hour in the dark, the number of water-filled vessels was approximately equal to the start of the experiment. The number of water-filled vessels continued to increase over the next 12 h, although the rate of increase slowed with time. During this period there was no evidence of root exudation from a short side branch at the base that had been freshly cut. At the end of the experiment the plant was severed at the base and there were no visible signs of root exudation.

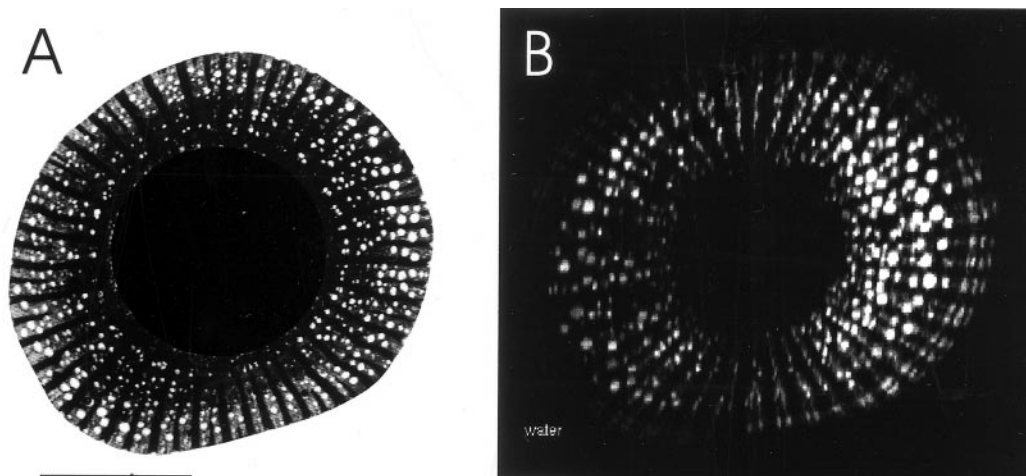
## DISCUSSION

The images presented demonstrate that MRI can be used noninvasively to monitor the functional state of

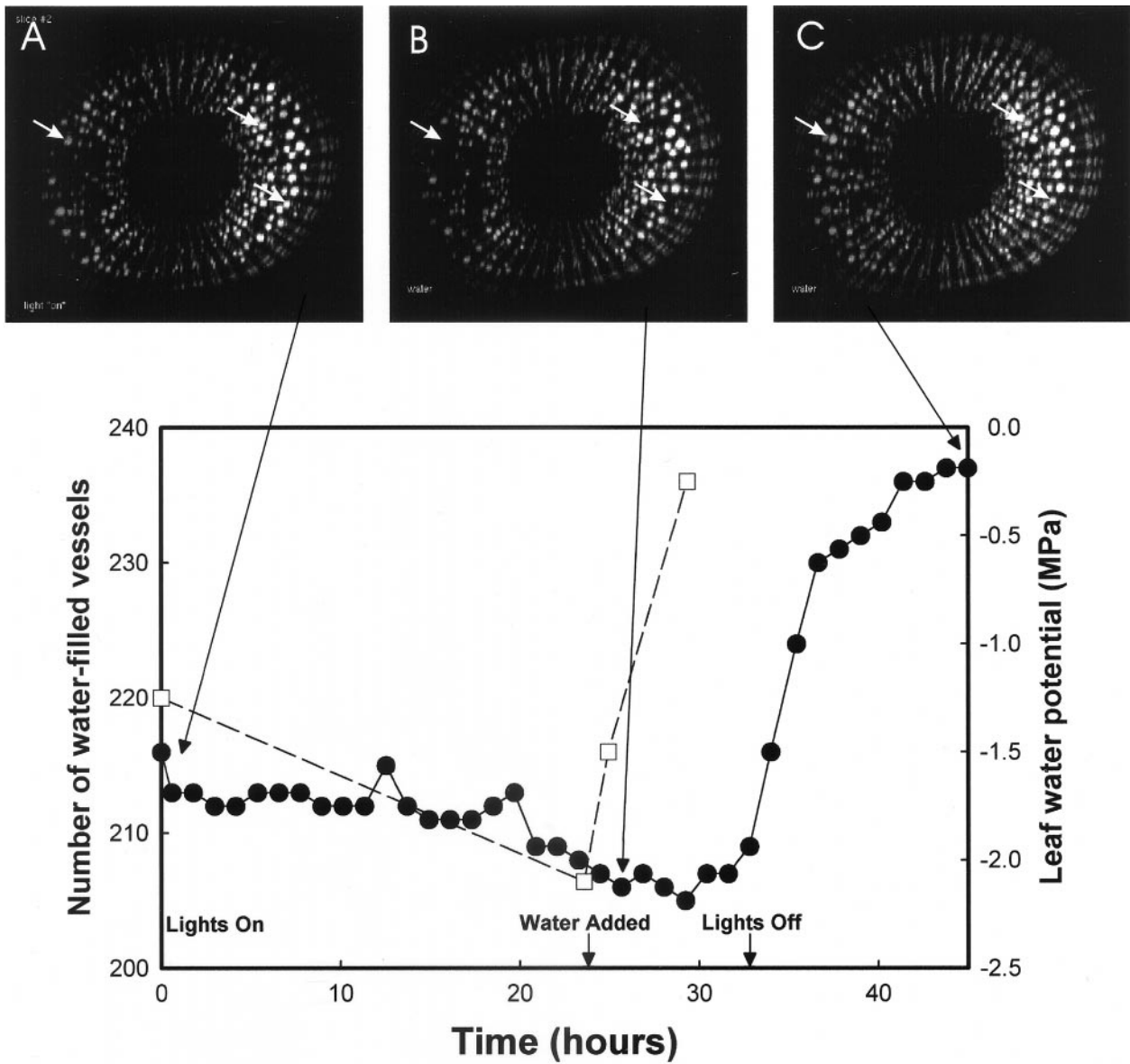
individual xylem vessels, thus opening new possibilities for studying embolism repair in intact plants. By combining MRI with more detailed physiological measurements such as sapflow and in situ thermocouple psychrometry, we will be able to delineate the conditions under which repair occurs. Because MRI can be used to image flow velocities (Bourgeois and Decorps, 1991; Köckenberger et al., 1997; Kuchenbrod et al., 1998) as well as water density, further studies could determine whether refilled vessels are able to subsequently transport water during periods of active transpiration. In addition, monitoring through more than one drying cycle could be used to determine if previously cavitated vessels are more prone to cavitation in the future.

In the grapevine examined in this study, cavitation occurred while the plant was actively transpiring and the leaves were turgid. Cavitation appeared to occur at random within the stem, although some areas lost more vessels than did others. There was no evidence of embolism repair, as determined by the reappearance of water in xylem vessels, although the leaves were illuminated. This was true even after the plant was watered and leaf water potentials substantially increased. Repair was first observed in the measurement immediately after the lights were turned off, and continued to occur, although at a decreased rate, over the next 12 h. This suggests that in grapevines embolism repair may require both an increase in water potential and a cessation of flow through the xylem. Other species, however, are reported to repair cavitated vessels during periods of active transpiration (McCully et al., 1998). MRI studies of these species will allow us to pinpoint the conditions under which such repair occurs.

Grapes are well known for their capacity to generate root pressure (Hale, 1727; Sperry et al., 1987). Prior to leaf expansion, grapes use root pressure to refill xylem vessels that had become air filled during the winter (Sperry et al., 1987). We did not observe



**Figure 1.** Cross section of grape stem using light microscope (A) or MRI (B). Scale bar in A = 2 mm. Note that the bark can be seen in the MRI image, but only the wood is present in the anatomical cross section (A).



**Figure 2.** Time course of total number of vessels visible in the MRI images and leaf water potential as a function of time. A through C show representative MRI images, with white arrows marking vessels that were initially water filled (A), then gas-filled and hence not visible (B), and finally refilled with water (C). A QuickTime movie of all 39 images can be viewed at [www.plantphysiol.org](http://www.plantphysiol.org).

any signs of root exudation during this study despite careful visual examination. However, in the absence of additional measurements, we recognize that the possibility of root pressure being responsible for the observed repair cannot be eliminated.

The major technical breakthrough of this study is the application of high-resolution MRI to investigate the dynamic changes in xylem transport capacity at the level of individual vessels. Previous use of MRI to study water transport in plants has lacked the spatial resolution needed to determine the functional status of individual xylem vessels (Johnson et al., 1987; Kuchenbrod et al., 1996; Köckenberger et al., 1997). In addition, the small size of the plants used in

previous studies makes it unlikely that substantial tensions were generated within the xylem. The major limitation to MRI studies of xylem transport arises from the need to have exclusive use of the expensive microscopy instrumentation for extended periods of time and the physical constraints on suitable plant material associated with having to position the region of interest within the MRI magnet. In the case of the instrument used in this study, this meant that one-half of the plant (either all of the leaves or all of the roots and soil) had to be threaded through a 4-cm-diameter constriction in the center of the magnet bore. The ability to observe xylem processes in vivo, however, greatly outweighs these limitations

and provides a new approach for understanding factors influencing the maintenance of water transport capacity in the xylem.

## MATERIALS AND METHODS

Observations were made on a grape (*Vitis vinifera* L. var. Concord) plant growing in an 8-L pot. The plant had been previously pruned such that at the time of measurements it had an unbranched shoot approximately 4 m in length. Measurements were made using a vertical wide-bore (89-mm) 500-MHz, 11.7-tesla magnetic resonance microscopy system (Bruker Instruments Inc., Billerica, MA) located at the Biological Imaging Center (California Institute of Technology). A laboratory-built 1-cm-diameter radio frequency ( $R_F$ ) surface coil and resonant tank circuit was mounted directly onto the stem and was used for both  $R_F$  excitation and reception. A single turn surface coil was utilized because of its high quality factor and its ease in placement along the stem. The loss in reception homogeneity due to this geometry was not a serious drawback because the coil diameter exceeded that of the stem by approximately 40%, and a single, small, tip angle  $R_F$  excitation pulse (approximately  $3^\circ$ ) was utilized in the gradient echo sequence. After mounting the coil, the shoot was carefully inserted into the magnet bore. The portion of the plant protruding out the top of the magnet was coiled beneath a light assembly that provided approximately 300  $\mu\text{mol}$  photons photosynthetically active radiation  $\text{m}^{-2} \text{s}^{-1}$  to the leaves.

Images were acquired using a two-dimensional Fourier transform gradient echo protocol with a small tip angle excitation pulse (Callaghan, 1991). The repetition time and echo time were equal to 50 and 4.5 ms, respectively. Two transverse image slices were acquired simultaneously at 55-min intervals over a period of 45 h. Each slice was 1.5 mm thick and separated by 1.75 mm. The in-plane image resolution was  $20 \times 20 \mu\text{m}$ . Signal averaging was required to obtain a satisfactory signal-to-noise ratio of order of 10 within vessels, and this limited the temporal resolution per acquisition to approximately 20 min. Because the MRI method used here visualizes the distribution of mobile water (Callaghan, 1991), vessels containing embolisms are easily distinguished from filled vessels.

The plant was continuously illuminated during the first 31 h of the experiment. At 24 h into the experiment the plant was rewatered (approximately 3 L of water added to the pot). After 9 more h the lights were turned off and the imaging continued for an additional 12 h. Leaf water potentials were measured using a pressure chamber. To avoid substantial reductions in leaf area and thus changes in plant water balance during the MRI measurements, the water potentials of only six leaves were measured. After the MRI session was completed, the stem was sectioned in the plane where the MRI images were taken.

The time-dependent populations of filled and embolized vessels were quantified from time lapse data through a fixed image plane. The number of water-filled vessels in each MRI slice was counted by comparing each image to the last frame (frame 39) of that slice's sequence. The final

image was printed on a transparency and all distinct (i.e. water-filled) vessels were counted and circled. The other images (1–38) were then printed on paper and overlain, one at a time, on a back-lit reference image (frame 39). The number of missing vessels was counted for each of the frames and subtracted from the total number of vessels in frame 39.

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