Xylem conduit deformation across vascular plants: an evolutionary spandrel or protective valve?

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Summary

- The hydraulic system of vascular plants and its integrity is essential for plant survival. To transport water under tension, the walls of xylem conduits must approximate rigid pipes. Against this expectation, conduit deformation has been reported in the leaves of a few species and hypothesized to function as a 'circuit breaker' against embolism. Experimental evidence is lacking, and its generality is unknown.
- We demonstrated the role of conduit deformation in protecting the upstream xylem from embolism through experiments on three species and surveyed a diverse selection of vascular plants for conduit deformation in leaves.
- Conduit deformation in minor veins occurred before embolism during slow dehydration. When leaves were exposed to transient increases in transpiration, conduit deformation was accompanied by large water potential differences from leaf to stem and minimal embolism in the upstream xylem. In the three species tested, collapsible vein endings provided clear protection of upstream xylem from embolism during transient increases in transpiration.
- We found conduit deformation in diverse vascular plants, including 11 eudicots, ginkgo, a cycad, a fern, a bamboo, and a grass species, but not in two bamboo and a palm species, demonstrating that the potential for 'circuit breaker' functionality may be widespread across vascular plants.

Introduction

Xylem transport is essential for sustaining the hydration of photosynthetic tissues, and the loss of hydraulic capacity is a crucial determinant of tree mortality under drought (Rowland *et al.*, 2015; Anderegg *et al.*, 2016; Adams *et al.*, 2017; Choat *et al.*, 2018; Bittencourt *et al.*, 2020; Arend *et al.*, 2021; Chen *et al.*, 2021a). According to the cohesion–tension theory (Dixon & Joly, 1895), water is pulled from the roots to the canopy by transpiration-generated negative pressures (tensions) in xylem conduits. In woody stems, the water transport system is generally conceived of as a rigid tubing system, in which embolism preempts the implosion of conduit walls (Zimmermann, 1983; Choat *et al.*, 2018). Embolism is preferred, in an adaptive sense, over implosion, as in the former the structural integrity of the stem is not destroyed (Zimmermann, 1983; Hacke *et al.*, 2001). Alternatively, some studies have shown reversible conduit collapse, a form of deformation that reverses rapidly upon xylem tension relaxation with minimal to no hysteresis (Cochard *et al.*, 2004; Brodribb & Holbrook, 2005; Zhang *et al.*, 2014, 2016; Chin *et al.*, 2022). If conduits can deform without damaging the surrounding tissues, collapse might be expected to be preferred over embolism by natural selection. In assessing the fitness impact of a hydraulic failure mode, a key consideration is the time scale over which the loss of hydraulic capacity is reversible. In the absence of positive xylem pressures, complete dissolution of xylem embolism is unlikely to occur in a timely manner (Hochberg *et al.*, 2016; Knipfer *et al.*, 2016), and so recovery from embolism generally requires new growth. The functional role and generality of nondestructive conduit deformation across vascular plants deserve further investigation.

While nondestructive collapse would not seem possible for the heavily lignified matrix of secondary stem xylem, it has been shown to occur in the terminal, less lignified, vascular bundles or transfusion tissue of leaves. In conifer needles (Cochard *et al.*, 2004; Brodribb & Holbrook, 2005; Zhang *et al.*, 2014; Chin *et al.*, 2022) and the smallest veins of red oak leaves (Zhang *et al.*, 2016), once a critical threshold in xylem tension is crossed, the vascular conduits develop regions of negative (i.e. inward) curvature, easily distinguished from their normally circular to slightly elliptical shapes in cross section. In red oak, negative curvature increases with further increases in xylem tension until the walls are appressed against themselves, and the lumen space in which flow occurs is almost entirely occluded, reducing the theoretical Hagen–Poiseuille conductance effectively to near zero (Zhang *et al*., 2016). Minor conduit deformation was also recently observed in wheat stems, but complete collapse (walls pressed against each other) was not detected within the range of xylem tension as low as −3.3 MPa (Corso *et al*., 2020).

Leaves are in a dynamic environment with changes in wind speed driving high-frequency variations in incident solar radiation (due to leaf angle) and boundary-layer thickness, both of which impact leaf temperatures. While the question of how far plants operate from the brink of hydraulic dysfunction remains unresolved (Tyree & Sperry, 1988; Brodribb & Holbrook, 2004; Meinzer *et al*., 2009; Choat *et al*., 2012), transient environmental changes such as thermally driven increases in transpirational demand could push water potentials to levels causing xylem embolism. For example, cloud-to-sun transitions can heat leaves by as much as 10°C (Singsaas & Sharkey, 1998), which may be expected to about double transpiration. In a steady state, stomatal regulation protects the xylem from excessive tensions that cause embolism (Brodribb & Holbrook, 2003; Hochberg *et al*., 2017). However, stomata close slowly, on the order of minutes in seed plants (Lawson & Blatt, 2014; Merilo *et al*., 2014; Elliott-Kingston *et al*., 2016), during which time damaging water potentials may develop. Moreover, in angiosperms, increases in evaporative demand initially cause stomatal pores to open, rather than close (Darwin, 1898; Iwanoff, 1928; Mott & Parkhurst, 1991). Effective stomatal control requires 'active' (abscisic acid (ABA)-mediated) control to rectify the so-called 'wrong-way response' that arises as a result of the mechanical advantage of surrounding epidermal cells over guard cells (Franks & Farquhar, 2007; McAdam & Brodribb, 2016; Brodribb & McAdam, 2017). Passive (i.e. tension-induced) conduit deformation may play a role in leaf response to transient water stress.

Previously, we used a modeling approach to show that reversible conduit collapse in minor veins could function as a 'circuit breaker' in leaves, protecting the upstream xylem from embolism when plants are exposed to a transient sudden increase in transpiration (Zhang *et al*., 2016). To date, no experimental evidence has been reported to support this hypothesis, and it remains possible that conduit collapse is a spandrel, that is, a byproduct feature of the evolvement of another function (Gould & Lewontin, 1979). Reversible deformability could simply result from selection for high conduit wall permeability to facilitate the movement of water from the xylem to the mesophyll. Here, we undertook experiments to test the circuit breaker hypothesis.

We first imposed a step increase in transpiration to test whether we could induce three states whose co-occurrence is predicted by our hypothesis: (1) conduit deformation of minor veins; (2) a large water potential gradient from the stem to the leaf, consistent with the presence of a large resistance; and (3) a lower degree of embolism than expected based on slow drying on the bench top. The latter would arise during the transient if an increase in resistance blocked the transmission of mesophyll water potential to the upstream xylem, and capacitive mesophyll discharge supplied the increase in transpired water instead. One challenge is that the water potentials at which deformation and embolism occur are determined under slow equilibrium drying, while transpiration creates a nonequilibrium gradient in water potential. For example, we previously found during slow bench-top drying of red oak branches that minor vein deformation coincided with hydraulically inferred petiole embolism in the water potential domain (Zhang *et al*., 2016), yet the relevant *local* water potentials driving these two processes might diverge during transpiration. Such nonequilibrium water potentials are inaccessible to current techniques for measuring plant water potential, though this may be changing (Jain *et al*., 2021). To address this type of challenge, Hochberg *et al*. (2017) used slow drying and the optical method (Brodribb *et al*., 2016a) to turn small differences in the critical potentials for stomatal closure and embolism into large differences in the timing of those events. Here, we used a similar approach to test whether xylem deformation occurs before embolism in whole potted plants of two crop species. Finally, we investigated whether conduit collapse is widespread across vascular plants by surveying diverse lineages, including woody and herbaceous, bamboo, ginkgo, a grass, a cycad, and a fern species. For this survey, we simply sought to determine whether conduit deformation could occur, rather than obtain the entire response of conduit deformation to water potential, due to constraints imposed by time and material. Our goals were to (1) test experimentally the 'circuit breaker' hypothesis, (2) monitor the temporal progress of deformation and embolism in the water potential domain under slow ('equilibrium') drying in species whose physiologies are otherwise well understood, and (3) explore the generality of conduit collapse across a phylogenetically broad sample of vascular plants.

Materials and Methods

Plant materials

This study was carried out in summer seasons of 2016, 2017, and 2021. We used irrigated red oak (*Quercus rubra* L.), eastern cottonwood (*Populus deltoides* W. Bartram ex Marshall), red maple (*Acer rubrum* L.), ginkgo (*Ginkgo biloba* L.) trees growing on the Harvard campus and grapevine (*Vitis vinifera* L.), cotton (*Gossypium hirsutum* L.), tomato (*Solanum lycopersicum* L.), tobacco (*Nicotiana tabacum* L.), soybean (*Glycine max* (L.) Merr.), sunflower (*Helianthus annuus* L.), Boston fern (*Nephrolepis exaltata* (L.) Schott), areca palm (*Dypsis lutescens* (H. Wendl.) Beentje & J. Dransf.), clumping bamboo (*Fargesia robusta* Yi (OHRN)), and cycad (*Zamia floridana* A. DC.) plants growing in glasshouses at Harvard University, Cambridge MA, USA (most species). Wild blueberry (*Vaccinium angustifolium* Aiton) leaves were sampled from a glasshouse at the University of Maine, Orono, ME, USA. Additionally, leaves of two tree species *Parashorea chinensis* Wang Hsie, *Castanopsis indica* (Roxburgh ex Lindl.) A. DC., two bamboo species *Bambusa vulgaris* Schrad. ex J. C. Wendl., *Bambusa multiplex* (Lour.) Raeusch. ex Schult.f., and one grass species *Microstegium ciliatum* (Trinius) A. Camus were collected from the Xishuangbanna Tropical Botanical Garden, Yunnan, China. An *in situ* transient experiment with red oak canopy leaves was carried out in Harvard Forest, Petersham, MA, USA, using an aerial lift.

Leaf water potentials

Leaf water potentials were measured using a Scholander-type pressure chamber (Soil Moisture Equipment, Goleta, CA, USA) fitted with a digital gauge with one psi (6.9 kPa) resolution (DPG500; Omega Engineering Inc., Stamford, CT, USA, or PMS1505D-EXP; PMS Instrument Co., Corvallis, OR, USA).

Detecting conduit deformation with cryo-scanning electron microscopy

Cryo-scanning electron microscopy (cryo-SEM) was used to detect the deformation of water-conducting conduits (minor veins for eudicot species, parallel and cross veins for dicots, second-order veins for the fern, and transfusion tracheids for gymnosperms). Leaf samples were frozen with a copper clamp precooled by liquid nitrogen, stored in liquid nitrogen, and transported to the Center for Nanoscale Systems (CNS) at Harvard University or the Central Laboratory at the Xishuangbanna Tropical Botanical Garden for imaging with cryo-SEM. For each sample, an *c.* 5 × 5 mm leaf section was fixed in a custom sample holder while remaining submerged in liquid nitrogen. The leaf section was then transferred to a cooled vacuum chamber (*c.* −150°C) using a cryo-transfer Shuttle VCT 100 (Bal-Tec, Balzers, Liechtenstein) or VCT 500 (Leica, Wetzlar, Germany) for freeze-fracturing and coating. The sample was fractured using a knife cooled to −150°C to expose the leaf veins in cross section, etched for 5–10 min depending on water content at −100°C, and sputter-coated with platinum at −130°C with a Bal-Tec MED020 (Bal-Tec) or ACE 900 (Leica) freeze-fracture–etching–coating system. The samples were then imaged at an accelerating voltage of either 2 or 3.5 kV in a Zeiss NVision 40 Dual-Beam Focused Ion Beam and SEM (Zeiss, Oberkochen, Germany) or Zeiss EVO LS10 (Zeiss) with a cryogenic stage maintained at −150°C.

Responses of leaves to a transient increase in transpiration

To detect the dynamics of potential embolism spread and conduit collapse when leaves are exposed to a transient increase in transpiration, we carried out three experiments using red oak trees and potted soybean plants.

Experiment 1 Oak leaves *in situ.* To study leaf response to transient increases in transpiration in intact mature oak trees, we used an aerial lift (bucket truck) to reach the canopy (*c.* 20 m aboveground) of red oak trees in Harvard Forest. In the morning between 10:00 h and 11:30 h when the leaves achieve their highest transpiration rates, two adjacent sun-exposed leaves were exposed to hot air flow generated by the hairdryer (Conair 1875 watt, low setting, East Windsor, NJ, USA) to achieve a transient increase in transpiration (see Fig. 1). Before the treatment, a leaf was excised and kept in a prehumidified plastic bag for water potential measurement with the pressure chamber. After exposing the leaf to hot air for 3 min (peak transpiration, Fig. 1), one leaf was frozen with a copper clamp precooled by liquid nitrogen, and the other leaf was cut and stored in a prehumidified plastic bag for water potential measurement. The frozen leaf was stored in liquid nitrogen until imaging.

Experiment 2 Potted soybean plants. To study the leaf response of soybean plants to a transient increase in transpiration, we moved potted soybean plants from one growth chamber with relatively low vapor pressure deficit (VPD) (0.96 kPa; RH 73%; T 26.9°C) to another with higher VPD (4.3 kPa; RH 24%; T 35°C). The soils of the pots were covered with plastic bags to avoid evaporation, and the transpiration of the whole plants (water loss rate over time) was measured with a balance connected to a computer, working as a lysimeter. After the plants were in the high VPD chamber for 3 min, a mature leaf was frozen with a copper clamp precooled with liquid nitrogen. The frozen leaf sample was kept in liquid nitrogen until imaging.

Experiment 3 Excised red oak branches. In the morning between 10:00 and 11:30 h when the leaves typically achieve their highest transpiration rate during the day, *c.* 2-m-long sun-exposed branches were cut from trees on campus and transported to the laboratory immediately (within 2 min). Once in the laboratory, one leaf close to the top of the branch was fixed to the microscope stage (adaxial side down) using strong double-sided tape for embolism detection with the optical technique (see details of the method described later). An adjacent leaf was bagged to equilibrate it with the stem water potential for *c.* 5 min before sampling. Then, the leaf on the microscope and a second adjacent leaf were exposed to hot air for 3 min. The leaf on the microscope was imaged every 3 s within these 3 min to detect potential embolism events. After these 3 min, the leaf exposed to hot air was frozen with a copper clamp precooled with liquid nitrogen, and the leaf on the microscope was excised and kept in a moist plastic bag for transpiring leaf water potential measurement with the pressure chamber. The prebagged leaf was also excised for stem water potential measurement. The images were then analyzed to detect embolism events, and the total cumulative number of embolized pixels was counted. Percent embolism was calculated using this total of embolized pixels divided by the average total number of embolized pixels for four completely embolized leaves. The leaves frozen with the copper clamp were stored in liquid nitrogen until imaging.

Temporal dynamics of embolism spread and conduit collapse

Potted soybean and cotton plants were used to detect the temporal dynamics of embolism spread and conduit collapse. After one mature leaf was fixed to a stereomicroscope (M80; Leica Microsystems, Heerbrugg, Switzerland) stage for detecting embolism using the optical technique (Brodribb *et al.*, 2016a,b;

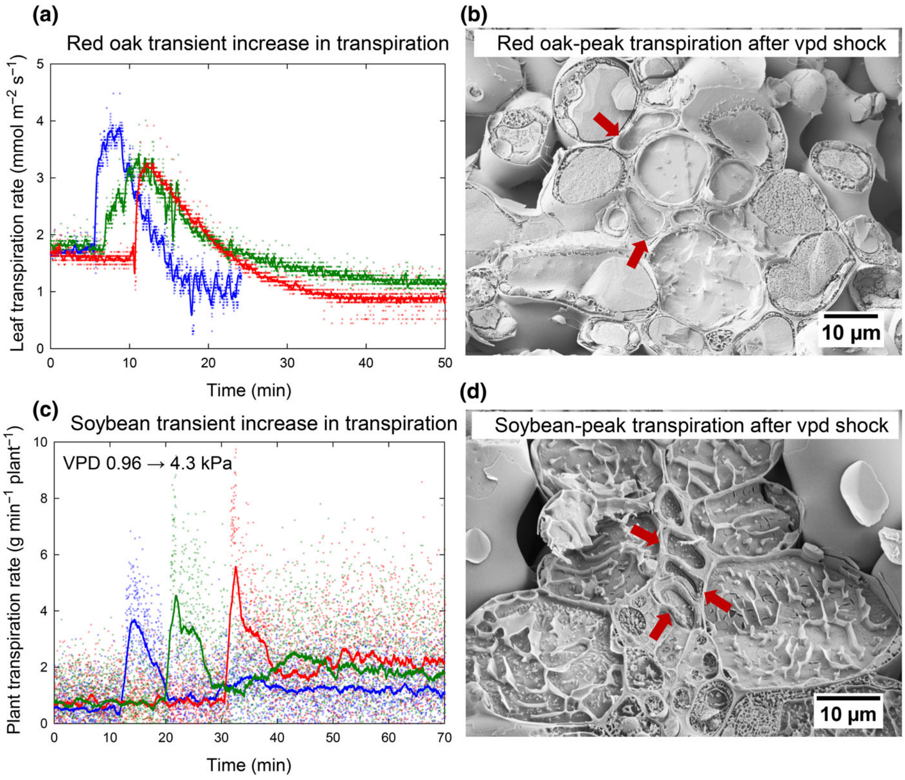

Fig. 1 Transient increase in transpiration (exposed to higher vapor pressure deficits) resulted in minor vein collapse in red oak and soybean leaves. (a, c) The dynamics of leaf or plant transpiration rate when the leaves or plants (different colors indicate different samples) were exposed to a transient increase in evaporative demand. Red oak leaves were exposed to hot air generated by a hairdryer, while potted soybean plants were moved from a humid chamber (VPD = 0.96 kPa) to a hot and dry chamber (VPD = 4.3 kPa). The lines are the moving averages. (b, d) Representative images showing the conduit collapse in the vascular bundle of red oak and soybean leaves 3 min after transient dehydration. Red arrows in (b, d) indicate conduits showing collapse.

Hochberg *et al.*, 2019) as detailed later, the soils of the potted plants were removed to accelerate dehydration in the dark. Leaf water potentials were measured before dehydration using the pressure chamber and then measured repeatedly during dry-down using leaves from the same plants. The plants were not exposed to light and rates of water loss were low, allowing us to assume water potential equilibrium among leaves of the same plant. When a leaf was sampled for water potential, an adjacent leaf was frozen with a copper clamp precooled with liquid nitrogen. The frozen samples were kept in liquid nitrogen and later transported to the Center for Nanoscale Systems for detecting conduit shape change using the cryo-SEM.

Optical detection of embolism spread was recorded by imaging changes in light transmission through the xylem conduits (Brodribb *et al.*, 2016a,b; Hochberg *et al.*, 2019). Leaves were imaged every 2 min with a stereomicroscope (M80; Leica Microsystems) until the leaf was completely desiccated. The adaxial side of the leaf was fixed to the stereomicroscope stage using double-sided tape, leaving the stomata uncovered. The leaf was illuminated from below to create an image of transmitted light, which was recorded by a digital camera (DFC290; Leica Microsystems). The imaged area of 17 × 10 mm encompassed all vein orders including the midrib; each image consisted of 1920 × 1080 pixels, resulting in an 8.8 µm pixel^{-1} resolution.

The images were then analyzed using IMAGEJ (National Institutes of Health, Bethesda, MD, USA) to quantify embolism spread and dynamics. Consecutive images were subtracted to produce a set of difference images. In this series of subtracted images, white pixels represent optical events. These optical events derive from embolism (signal), as well as a variety of artifacts (noise) due to the instability of light conditions, movements of the leaf, a trichome, or a bug. To separate the embolism signal from noise, the difference images were processed according to the following sequence: a brightness increase (25%), a median filtering using 3 × 3 neighboring pixels, a threshold adjustment (8%), and a second median filtering. In some cases, the resulting images still contained recognizable artifacts, normally originating from movements of trichomes or insects, which therefore bear their shape: Such artifacts were removed manually. The degree of embolism was calculated as the cumulative number of embolized pixels normalized to the total number of embolized pixels throughout dehydration. Finally, to visualize the dynamics of embolism spread through the leaf, events were colored on a continuous scale identifying the time at which they appeared.

Survey of diverse species for conduit deformation/collapse

To test whether conduit deformation or collapse is widespread across vascular plants, we sampled plants from diverse lineages (see Plant materials in the Materials and Methods section) for cryo-SEM. Sun-exposed branches 1.5–2 m long of trees growing on campus or in the garden or potted plants were bench-dried for various times and then placed in black plastic bags for at least 2 h for water potential equilibration. Fully developed leaves were frozen with a precooled (with liquid nitrogen) copper clamp while still attached to the branch or potted plants and then stored in liquid nitrogen for cryo-SEM. An adjacent leaf was excised simultaneously for water potential determination using the pressure chamber.

Data and image analyses

Cryo-SEM images were analyzed using ImageJ. The change in conduit shape of xylem conduits of red oak in Experiment 3 (mentioned in the previous section) was quantified with the hydraulic index (HI), which describes a hydraulically weighted measure of roundness based on the measured area and perimeter of each conduit (Zhang *et al.*, 2016). The HI for an individual conduit was calculated by dividing the area of a conduit by the area of a circle with the equivalent perimeter of that conduit and then squaring this ratio to make it proportional to the fourth power of the radii, as in the Hagen–Poiseuille equation for pipe flow. The HI, therefore, has a theoretical maximum of 1 that decays toward 0. To calculate the HI for all the conduits imaged from a particular cryo-SEM sample, the ratio of the sum of squared measured areas to the sum of squared equivalent circle areas was calculated. Slow dehydration vulnerability curve data for four red oak leaf samples constructed using the optical method were collected from a previously published paper for the same individuals (Hochberg *et al.*, 2019) and pooled together. Then, a four-parameter sigmoid function was fitted to the relationship between percent leaf xylem embolism detected by the optical method and water potential.

Results

Conduit deformation can be triggered by transpiration transients

Experiment 1 We tested whether exposing intact leaves to a step (transient) increase in transpiration results in conduit deformation in red oak trees. We reached the canopy leaves of red oak trees with an aerial lift in the morning (10:00–11:00 h) when their transpiration rates were close to diurnal peaks (stomatal conductance ranged from 0.23 to 0.31 mol m^{-2} s^{-1}). While conduit size and shape varied among conduits and samples, no conduit deformation, as indicated by negative curvatures or walls pressed against themselves, in minor veins was detected in four freely transpiring, unheated leaves (Supporting Information Fig. S1). The average HI of the free-transpiring samples was 0.82 (based on four leaf samples from different trees with 12 veins and 46 conduits per sample; Fig. S1). After a transient increase in transpiration treatment achieved by forcing with hot air (equilibrium water potential minima -1.6 ± 0.05 MPa), conduit deformation in minor veins was detected with cryo-SEM (Fig. 1b) in four out of seven samples imaged with an average HI of 0.68 (an average of 18 veins and 78 conduits per sample). Although the difference in HI was not enormous, the occurrence of negative curvature (Fig. 1), which was not found in hydrated samples, indicated deformation. Since only a 5 × 5 mm sample (*c.* 0.1% of the leaf) was imaged under cryo-SEM, it is possible that we might have missed collapse in certain leaves.

Experiment 2 For this experiment, we used potted soybean plants. When intact plants were moved from a humid chamber to a chamber with higher VPD (see Materials and Methods section for details), peak transpiration and water potential minima (-0.91 ± 0.14 MPa; equilibrium leaf water potential) were reached after 3 min (Fig. 1c), and conduit deformation in minor veins (negative curvature) was detected in three out of five tested samples (Fig. 1d). As in red oak, the occurrence of negative curvature (Fig. 1d), which was not found in hydrated samples, indicated deformation.

Experiment 3 Here, we monitored the dynamics of both embolism events (optical method) and leaf xylem deformation (cryo-SEM) when actively transpiring leaves on cut branches of red oak were exposed to enhanced transpiration load. Two-meter-long actively transpiring red oak branches were excised from trees exposed to full sun, and two leaves were then exposed to a hot airflow. Tests showed that the transpiration rates of detached leaves were doubled in 3 min by this treatment, after which transpiration started to decline (Fig. 1a). At peak transpiration (3 min after exposure to the hot air; Fig. 1a), leaf and stem water potentials were measured, embolism level was quantified optically, and minor veins of a treated leaf were frozen and imaged with the cryo-SEM (see Materials and Methods section for details).

As opposed to slow dehydration, during which water potentials of all leaf tissues remain in near-equilibrium, transpiration induces a water potential gradient from the stem–petiole junction to the leaf mesophyll (see a conceptual diagram in Fig. 2a). During transpiration, the water potentials in the upstream leaf vascular system will be 'wetter' (less negative) than the average potential in the mesophyll (as measured by the pressure chamber) to the degree that the hydraulic resistance of the path from the upstream leaf vasculature (major veins) into the mesophyll is large (Fig. 2a). This resistance is influenced by conduit shape and increases rapidly with conduit deformation. During a transient increase in transpiration, this increase in resistance results in transpiration being supplied by water lost from mesophyll cells, rather than the xylem, with the result that the upstream xylem is protected from increasingly negative water potentials in the mesophyll. We, therefore, expected less embolism in the vascular system during transpiration transients than predicted by the near-equilibrium vulnerability curve as a function of the average leaf water potential measured by the pressure chamber (Fig. 2a).

Indeed, following the transient increase in transpiration treatment, we detected conduit deformation when the leaf water potentials were driven lower than -2.2 MPa (Fig. 2b, cryo-images in Fig. 2c–f). The HI (see Materials and Methods section) of the minor vein conduits, based on measurement of 12–23 vascular bundles and 50–104 conduits per sample, decreased from the hydrated value of 0.82 (four transpiring leaves, as mentioned in the previous section and Fig. S1) to 0.51 (Fig. 2b,d), corresponding to a 38% decline in hydraulic conductance. While we also detected xylem embolism at these same stress levels (< -2.2 M), the amount of embolism was distinctly less than that predicted by the near-equilibrium vulnerability curve (Fig. 2b; Dataset S1). This result agrees with our hypothesis that the deformation of minor vein conduits can reduce the propagation of highly negative water potentials in the upstream xylem during transient increases in transpiration. Notably, changes in leaf temperature can change the

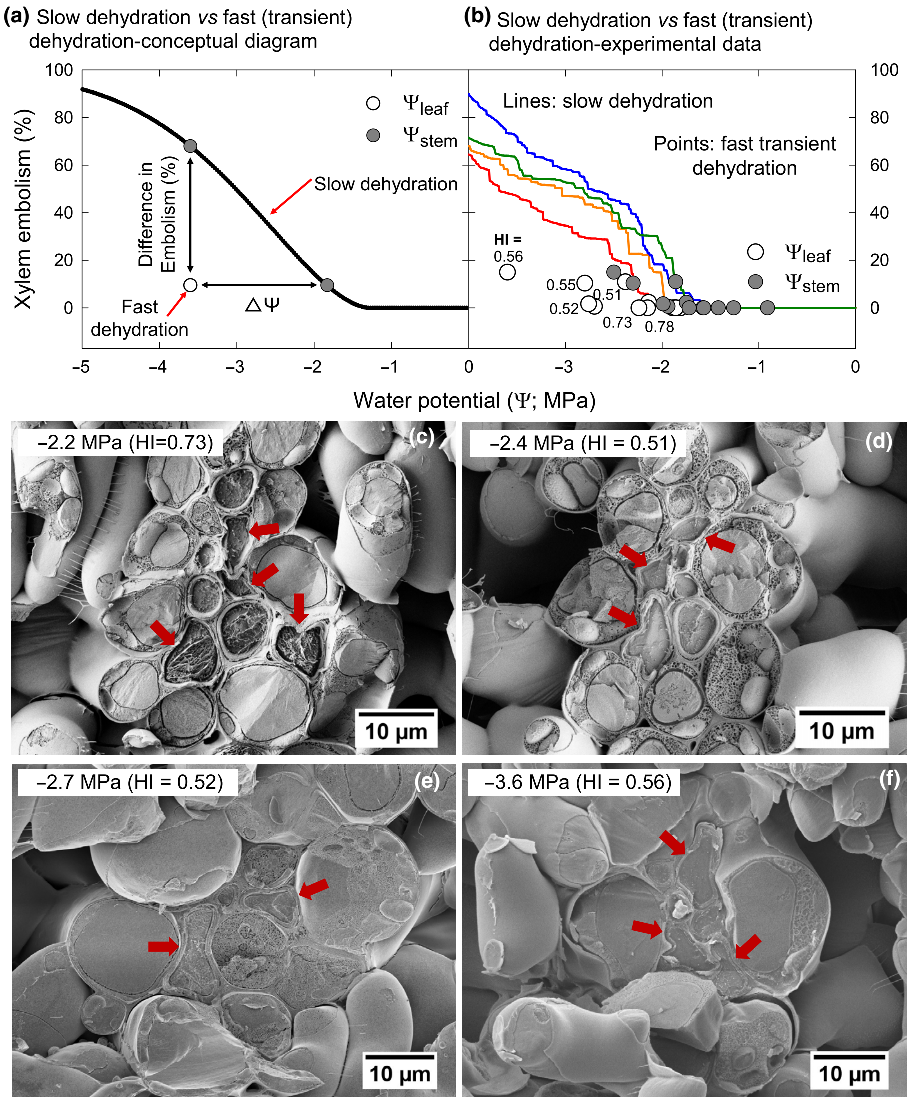

Fig. 2 Transient increase in transpiration (i.e. exposure to higher vapor pressure deficit) resulted in minor vein collapse and less embolism compared with slow dehydration of red oak leaves. (a) A graph showing the theoretical interpretation of the experiment. The thick line in (a) is a Weibull function fitted to the relationship between percent leaf xylem embolism and water potential for the four samples in (b). The lines in (b) show the increase in the percentage of leaf xylem embolism as the water potentials decreased for red oak branches dehydrated slowly on the lab bench (slow dehydration vulnerability curve data for red oak from Hochberg *et al*., 2019). Different colors indicate different branch samples. The points in (b) are the percentage of xylem embolism of leaves exposed to a transient increase in transpiration plotted against both the transpiring leaf (open points) and the 'bagged leaf' or stem water potential estimate (closed points) of adjacent leaves. (c–f) Representative images of conduit collapse in minor veins in the vascular bundle of leaves exposed to transient dehydration as labeled in (b). Red arrows indicate conduits showing deformation. Grand hydraulic index (HI; a hydraulically weighted measure of roundness) of the samples is labeled in the figure.

viscosity of water, leaf hydraulic conductance, and the water potential gradient, which was not tested in the present study.

Conduit deformation precedes xylem embolism in soybean and cotton

To investigate whether conduit deformation precedes embolism in potted soybean and cotton plants, we used slow drying and nondestructive embolism detection to transform small, difficult-to-measure differences in the response to water potential into large, easier-to-measure differences in temporal responses (Hochberg *et al.*, 2017). While monitoring the occurrence of xylem embolism using an optical method (Brodribb *et al.*, 2016b), we sampled adjacent leaves to detect xylem conduit deformation using cryo-SEM. We found that conduit deformation in minor veins (indicated by the arrows in Fig. 3(a,b)) preceded embolism (indicated by the increase in % of xylem embolism in Fig 3(a,b)) in both cotton and soybean ($n = 4$ per species). Both negative curvature and conduit inner walls pressed against themselves were detected (Figs 3, S2), which we have not observed in well-hydrated samples (Figs 4, 5). In soybean leaves, there were large temporal separations between these two processes; embolism occurred 4–19 h after conduit deformation was first detected (Figs 3a,e, S2; Dataset S1). In cotton plants, the interval was more variable: Minor vein conduit deformation always occurred before embolism, but the first embolism events were within 1 h of conduit deformation being detected in two of the four samples (Figs 3b,f, S2; Dataset S1). The water potentials at which conduit deformation was detected ranged from −1.4 to −1.8 MPa for soybeans and −1.8 to −2.4 MPa for cotton (Fig. 3a,b). The high temporal resolution of embolism spreading afforded by the optical method allowed us to show that conduit deformation precedes embolism during slow dehydration in both soybean and cotton. The optical method is likely better at capturing cavitation, as the imaged area was large compared with the sampled area for cryo-SEM. This makes our estimate of how much earlier deformation is than cavitation likely to be very conservative.

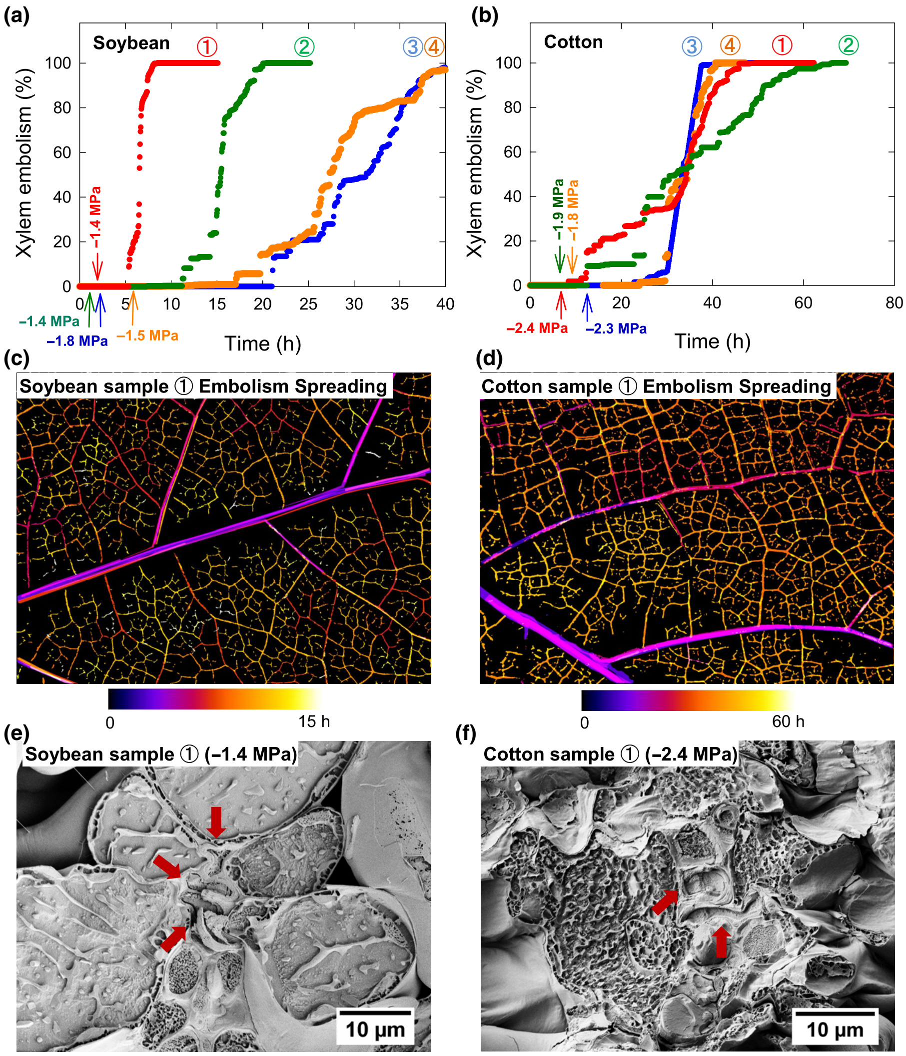

Fig. 3 Xylem conduit collapse in minor veins detected before leaf xylem conduit embolism in soybean and cotton leaves during slow dehydration. (a, b) The accumulation of xylem embolism over time in soybean and cotton leaves detected by the optical method. (c, d) The spatial pattern of xylem embolism accumulated over time in one soybean and one cotton leaf as detected by the optical method. (e, f) Freeze-fracture cryo-SEM images showing conduit collapse (red arrows) in sample 1 of both soybean and cotton at the time indicated by the arrows in (a, b). The arrows in (a, b) indicate times and water potentials at which conduit deformation was detected (different color arrows indicate different samples). Images showing the conduit collapse of samples 2–4 are in Fig. S2.

Conduit deformation occurs across vascular plants and plant growth forms

We also surveyed the generality of conduit deformation and collapse under nonlethal water stress (as may be induced by peak transpiration and/or mild soil water deficits) in leaves across plant lineages. Here, we aimed to explore the generality of the occurrence of conduit deformation across multiple taxa, rather than quantifying the water potential initiating the deformation for individual taxa, which would require an independent study for each species. Conduit deformation, indicated by negative wall curvatures, or complete collapse, indicated by walls pressed against themselves, was not found in hydrated leaves across all species examined here (Figs 4, 5) nor in previous studies (Zhang *et al*., 2014, 2016). Cryo-SEM imaging showed conduits in various stages of collapse in the minor veins in all six woody and five herbaceous eudicot species studied (Figs 6, S3). The water potentials of samples ranged from −0.9 to −3.2 MPa depending on species (Figs 6, S3). The woody plants examined covered a range of growth forms, including a vine (Chardonnay grape; *V. vinifera*), two temperate trees (*P. deltoids* and *A. rubrum*), two tropical trees (*P. chinensis* and *C. indica*), and a shrub (wild lowbush blueberry; *V. angustifolium*). The herbaceous species included soybean (*G. max*), cotton (*G. hirsutum*), tobacco (*N. tabacum*), tomato (*S. lycopersicum*), and sunflower (*H. annuus*) (Figs 6, S3). Conduit deformation or complete collapse was also found in parallel veins of a bamboo (*B. multiplex*; −4.1 MPa) and a grass species (*M. ciliatum*, −5.0 MPa), but not in their cross veins (Fig. 7). By contrast, no conduit deformation was observed in three monocotyledons (two bamboos *F. robusta* and *B. vulgaris*; and a palm *D. lutescens*), despite large vascular tensions (water potentials of −4.2 and −4.6 MPa, respectively; Fig. 7). The HI of samples showing conduit deformation ranged from 0.13 to 0.55 (Figs 6, 7), compared to 0.78 to 0.93 in hydrated samples. The reversibility of conduit deformation was tested in one species, *C. indica*. When a sample dried to −3.2 MPa was rehydrated to 0.2 MPa and imaged, no conduit deformation was detected (Fig. S4), compared with obvious conduit deformation and complete collapse in a sample imaged at −3.1 MPa (Fig. 6d).

All of the nonangiosperms tested (the fern *N. exaltata*, the cycad *Z. floridana*, and *G. biloba*) also showed instances of vascular deformation (Fig. 8b–d). In *N. exaltata* (Boston fern), conduit deformation was found in the xylem tracheids of the second-order (also the highest vein order) veins at −2.3 MPa. In the

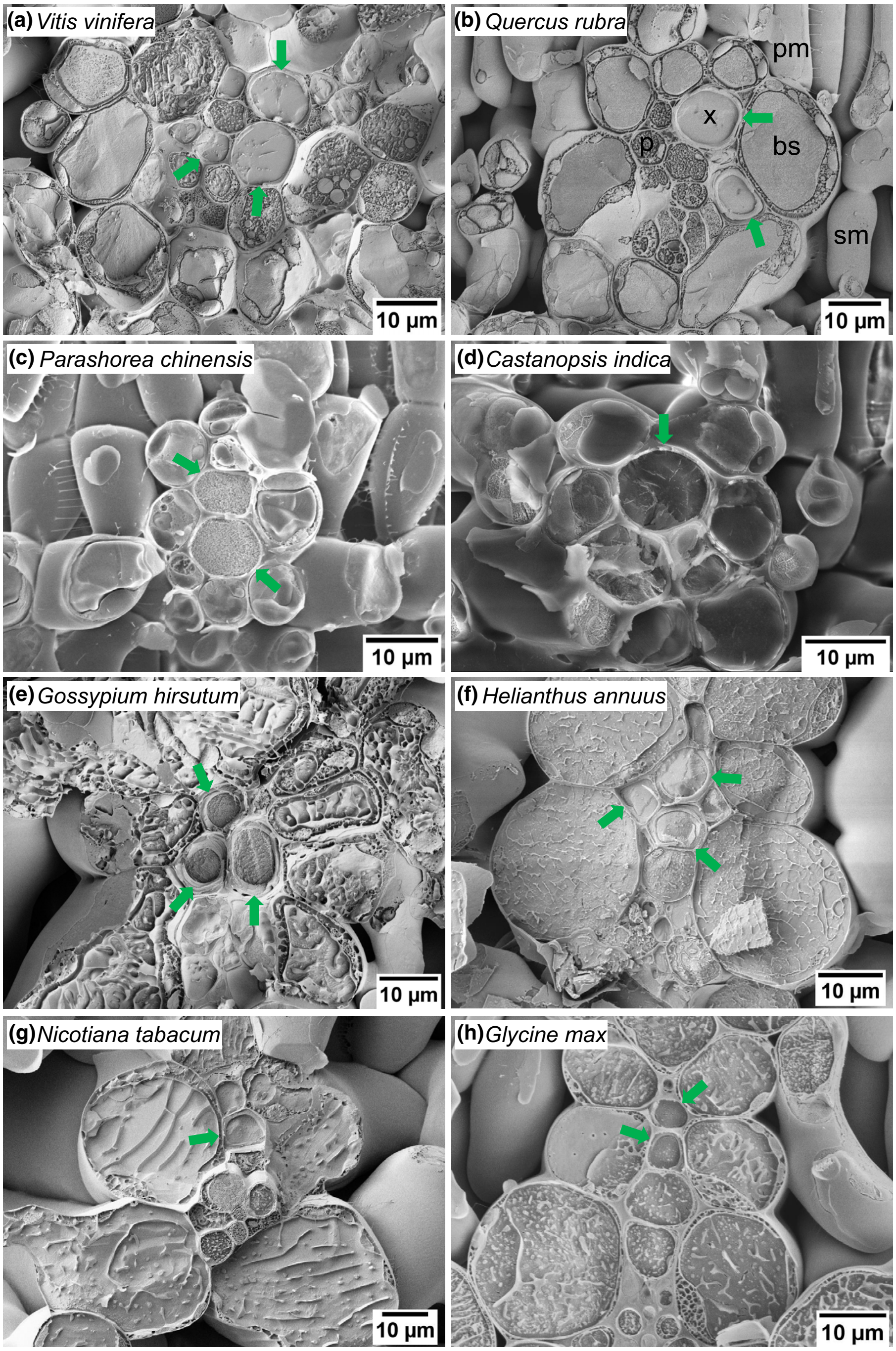

Fig. 4 Freeze-fracture cryo-SEM images showing the xylem conduit shape of minor veins of hydrated leaves (water potentials close to zero) in four woody (a–d) and four herbaceous (e–h) species. No xylem conduit deformation was found in any of these species. Green arrows in the figure indicate xylem conduits without deformation. Example cell types are shown in (b): bs, bundle sheath; sm, spongy mesophyll; p, phloem sieve tube; pm, palisade mesophyll; x, xylem conduit.

gymnosperms *Z. floridana* and *G. biloba*, conduit deformation was detected in the transfusion tracheids (radially oriented tracheids occurring outside of the axially oriented vasculature) at −2 and −1.7 MPa, respectively, but not in the axial xylem. These results are consistent with previously observed deformation and collapse in the transfusion tissue of another gymnosperm, *Taxus baccata* (Zhang *et al*., 2014).

Discussion

The three transient experiments provide evidence supporting the hypothesis that xylem deformation in minor veins can protect upstream xylem from embolism when leaves are challenged with a rapid increase in transpiration, as previously predicted by modeling (Zhang *et al*., 2016). Thus, elastic conduits may not simply be an evolutionary spandrel (Gould & Lewontin, 1979). The elastic reversibility of conduit deformation in leaves has been confirmed in pines (Cochard *et al*., 2004), *Podocarpus* (Brodribb & Holbrook, 2005), English yew (Zhang *et al*., 2014), redwood (Chin *et al*., 2022), red oak (Zhang *et al*., 2016), and *C. indica* here (Fig. S4). While protoxylem is often destroyed by growth stresses, we have not observed any deformed leaf metaxylem in unstressed tissues, which would be expected if conduit buckling was not reversible. It, therefore, seems likely that leaf conduit

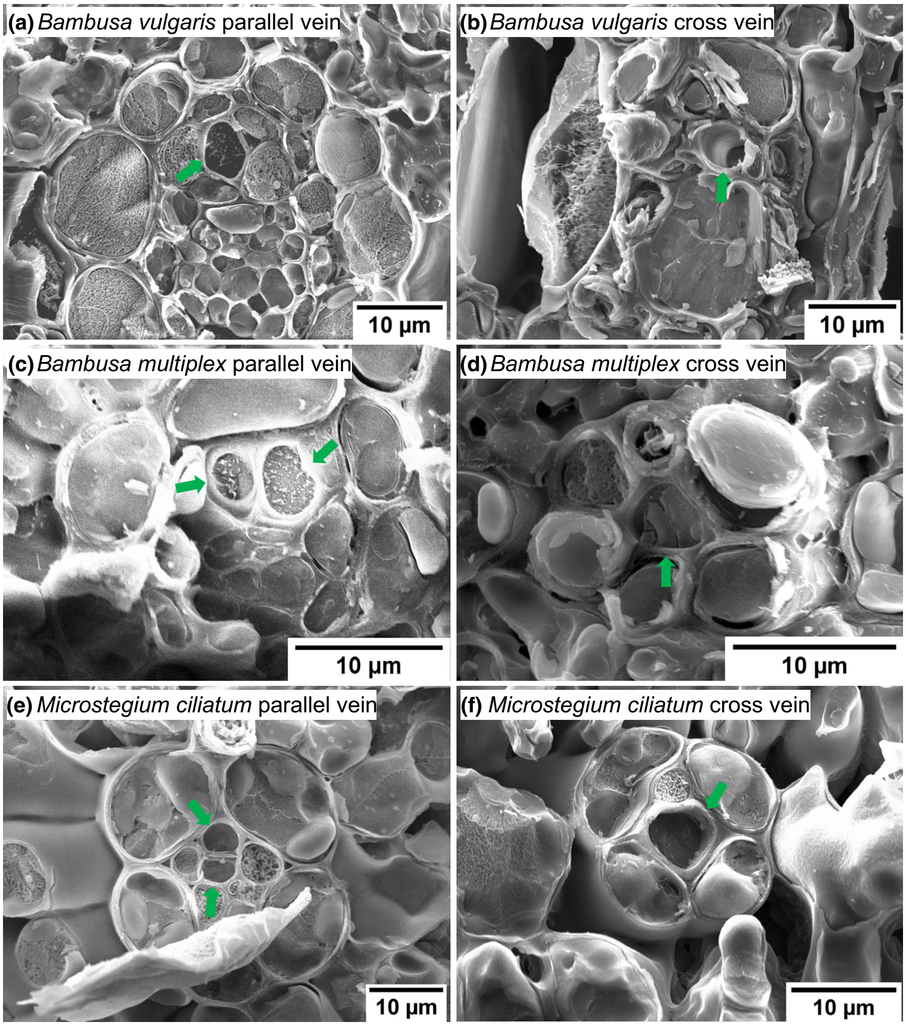

Fig. 5 Freeze-fracture cryo-SEM images showing the xylem conduit shape of parallel and cross veins of hydrated leaves (water potentials close to zero) in two bamboo and one grass species. No xylem conduit deformation was found in the parallel veins (a, c, e) and cross veins (b, d, f) of hydrated leaves of two bamboo (*Bambusa vulgaris and Bambusa multiplex*) and a grass species (*Microstegium ciliatum*). Green arrows indicate xylem conduits without deformation.

deformation is reversible in most vascular plants, although further research is needed to confirm the reversibility and the water potential range for recovery to occur.

The potential functional roles of conduit deformation

In our transpiration perturbation experiments, without the deformation in the vein endings (highest order veins), there would have been a smaller water potential gradient from the mesophyll to the upstream xylem, less discharge from the mesophyll 'capacitor', and consequently more negative upstream xylem water potentials and a greater risk of embolism. Other sources of variable conductance that might act alongside the deformation of minor vein endings have been identified. For example, reductions in mesophyll (outside of the xylem pathway) hydraulic conductance (Scoffoni *et al.*, 2013) could help isolate the upstream xylem by further promoting mesophyll capacitive discharge, yet mesophyll resistance may be bypassed ('shunted') by internal vapor transport and so possibly less effective in this role (Rockwell *et al.*, 2014; Buckley, 2017). With respect to stem xylem, conduit deformations have been detected in wheat stems, but were not physiologically significant compared with embolism over the same range of water potential (Corso *et al.*, 2020). In some trees and lianas, vessel lumen areas tend to increase despite an overall shrinkage of the xylem tissue during dehydration probably due to the release of xylem tension after embolism (Chen *et al.*, 2021b).

Water release from minor vein conduits as they deform could also be physiologically significant under both fast and slow dehydration. The absolute volumes of minor vein conduits appear to be relatively large. For red oak, our calculation based on the highest order (terminal, free-ending) vein length, vessel size, and leaf hydraulic capacitance indicated that water released from collapsed terminal (i.e. the highest order) free-ending veins was equal to the volume that can increase mesophyll water potential by 0.4 MPa before turgor loss, though only 0.05 MPa after turgor loss. Further, the collapse of all minor veins releases water that can increase water potential by 0.8 or 0.1 MPa (before or after turgor loss; see Notes S1). In a *Podocarpus* species, the release of water from collapsed conduits of the accessory transfusion tissue was estimated as equivalent to a volume of water that would increase the water potential of the mesophyll by 2 MPa (Brodribb & Holbrook, 2005). In redwood (*Sequoia sempervirens*), deformation of transfusion tracheid can sustain transpiration at maximum g_s and local mean VPD for *c.* 400 s (Chin *et al.*, 2022). Therefore, elastic

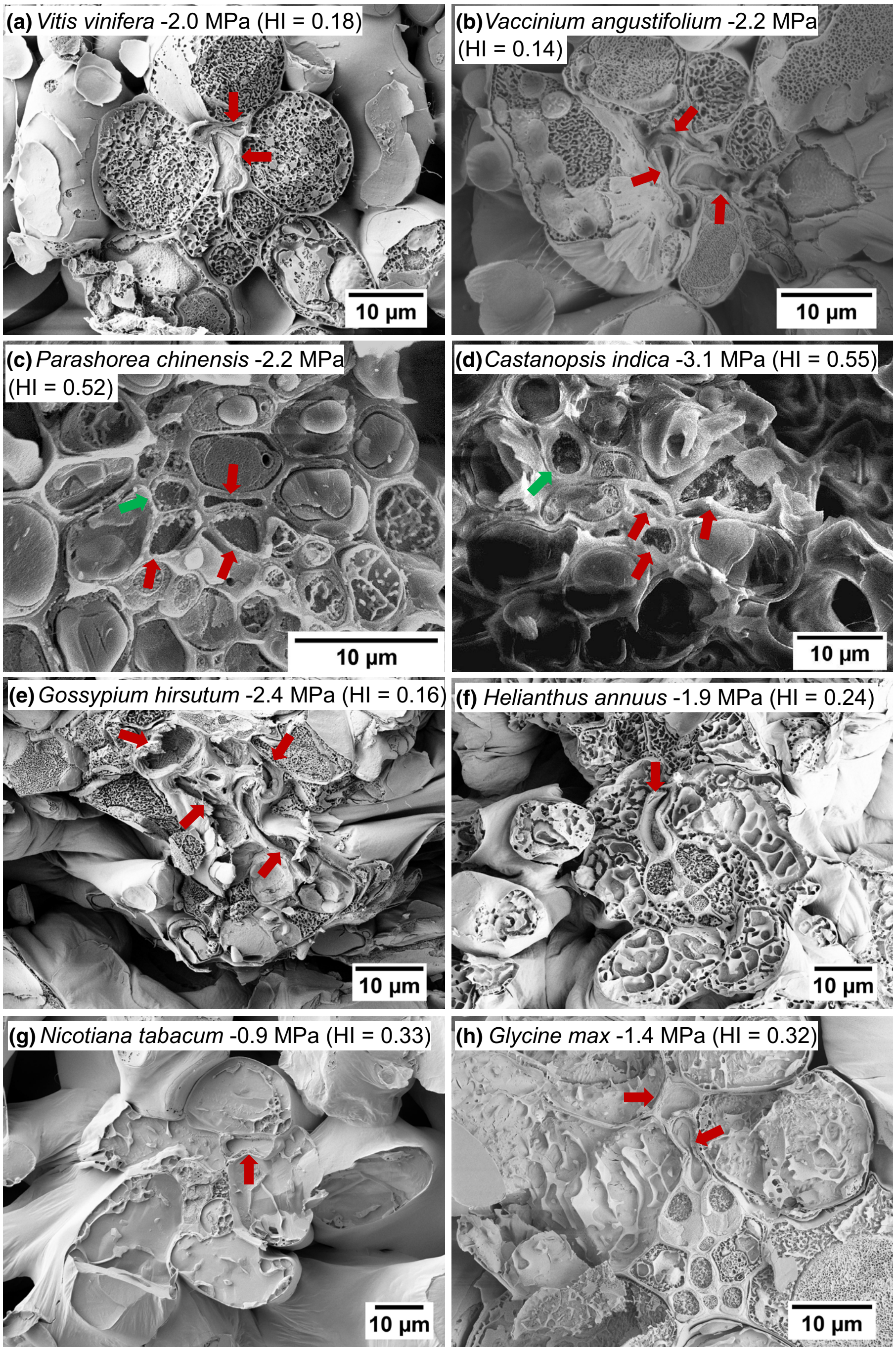

Fig. 6 Freeze-fracture cryo-SEM images showing the xylem conduit deformation in leaf minor veins of four woody and four herbaceous species. The woody species are *Vitis vinifera* (Chardonnay grape, a), *Vaccinium angustifolium* (wild lowbush blueberry, b), *Parashorea chinensis* (sky watching tree, c), and *Castanopsis indica* (Indian chestnut, d). The herbaceous species are *Gossypium hirsutum* (cotton, e), *Helianthus annuus* (sunflower, f), *Nicotiana tabacum* (tobacco, g), and *Glycine max* (soybean, h). Leaves were dehydrated to different water potentials (−0.9 to −3.1 MPa) as labeled in the figure. Red arrows in the figure indicate conduits showing deformation, while green ones without deformation. Grand hydraulic index (HI; a hydraulically weighted measure of roundness) of each sample is labeled in the figure.

conduits could also function as a significant source of capacitance to buffer water potential change when stomata are closed or to supply transpiration when stomata are open, further isolating upstream xylem from mesophyll tensions. Yet, this effect may only be physiologically significant if minor vein collapse precedes mesophyll turgor loss. As intrusive vein collapse appears to require loss of turgor in neighboring bundle sheath cells (Zhang *et al.*, 2016); the impact of capacitive release of minor vein water will be greater if bundle sheath turgor loss precedes mesophyll turgor loss.

With respect to the mesophyll, it is important to recognize that minor vein water release would only slow down, not prevent, drying during transient stomatal opening, as it is mesophyll drying that drives the increases in tension that collapse the conduits. Indeed, the feedback loop between conduit collapse and mesophyll drying could also amplify transpiration-induced declines in turgor pressure and volume of the mesophyll cells, triggering the synthesis of ABA and active stomatal closure (Zabadal, 1974; McAdam & Brodribb, 2016). In ferns, stomatal aperture passively tracks water potential (Brodribb & McAdam, 2011), and stomata could not close completely unless water potentials are extremely low (Brodribb & McAdam, 2017). In this case, conduit collapse in minor veins could be an important mechanism

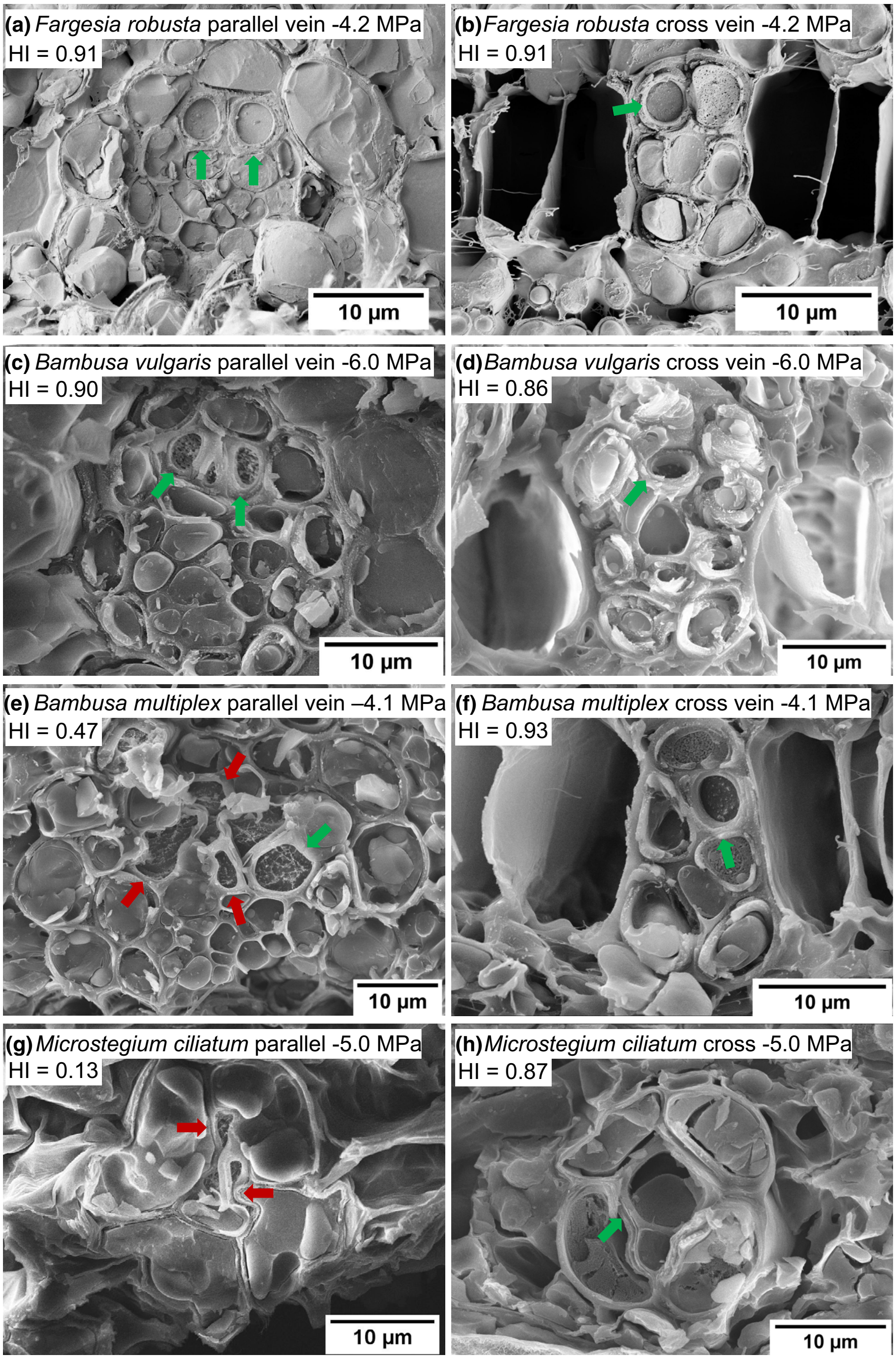

Fig. 7 Freeze-fracture cryo-SEM images showing the conduit deformation or absence of deformation in three bamboo species and a grass species. Xylem conduit deformation was not found in the parallel veins of *Fargesia robusta* (a) and *Bambusa vulgaris* (c), but found in *Bambusa multiplex* (e) and *Microstegium ciliatum* (g). No conduit deformation was found in cross veins of all the species (b, d, f, h). Red arrows in the figure indicate conduits showing deformation, while green ones absence of deformation. Grand hydraulic index (HI; a hydraulically weighted measure of roundness) of each sample is labeled in the figure.

that accelerates guard cell dehydration and stomatal closure. Physiologically, reversible conduit collapse may prove to be a broadly distributed mechanism for supplementing stomatal regulation and protecting the hydraulic system from embolism in a dynamic environment.

Conduit deformation as a common feature in vascular plants

From an evolutionary perspective, the fact that a fern, a cycad species, a bamboo, a grass, and ginkgo all evidenced terminal conduits that were deformed and sap-filled, rather than embolized, in response to large water potential stress, suggests that such deformability may be fairly general among vascular plants. If so, it would appear to be convergent, as the last common ancestor of ferns and seed plants is thought not to have had leaves (Boyce, 2010). Alternatively, ferns and seed plants may have converged on a common spandrel. While the upstream xylem is thought to principally transport water axially, the terminal components of the vascular system such as minor veins in angiosperms and transfusion tracheids in gymnosperms are thought to predominantly ‘leak’ water radially to the

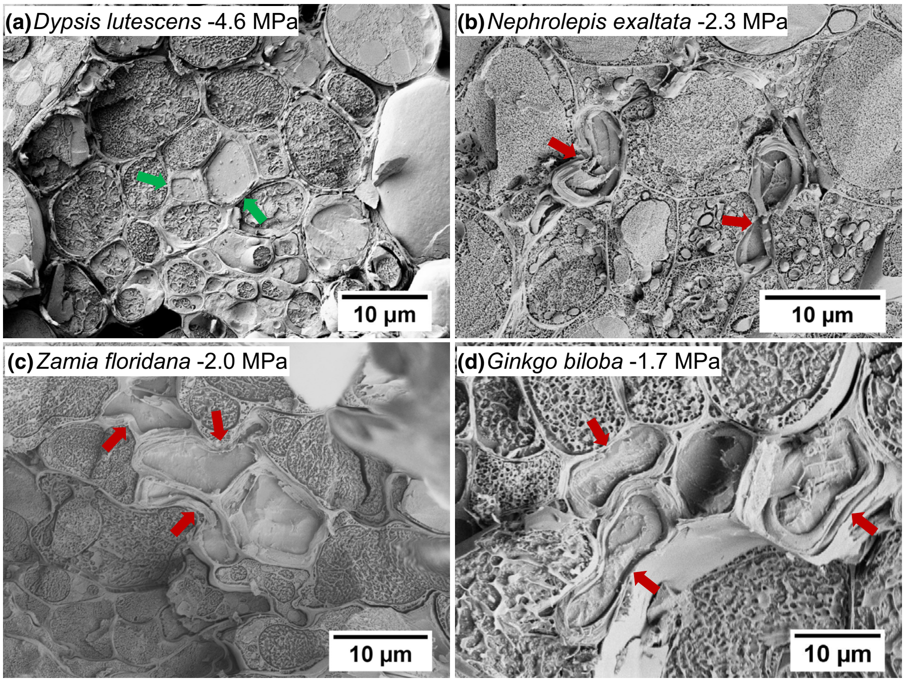

Fig. 8 Freeze-fracture cryo-SEM images showing conduit deformation/collapse or absence of deformation in a palm species, a fern species, a cycad species, and ginkgo. No xylem conduit collapse was found in the leaf of a palm (*Dypsis lutescens*) dehydrated to −4.6 MPa (a). Conduit collapse was detected in dehydrated leaves of *Nephrolepis exaltata* (b; Boston fern), *Zamia floridana* (c), and *Gingko biloba* (d). Red arrows in the figure indicate conduits showing deformation/collapse, while green ones no deformation.

mesophyll (Hu & Yao, 1981; Ohtsuka *et al*., 2018). To facilitate the radial release of water, the conduit walls need to be highly porous (Ohtsuka *et al*., 2018). Therefore, conduit deformation and eventual collapse may reflect a decrease in stiffness due to selection for high radial porosity, that is, less secondary thickening, more and larger pits increased porosity while decreased wall stiffness. However, our physiological results suggest that, at least in some taxa, the ability of such conduits to reversibly collapse can contribute to maintaining the integrity of the water transport system and so should be a product of natural selection rather than simply an evolutionary spandrel without functional significance.

Interestingly, no conduit deformation was detected in three monocotyledons (two bamboo and one palm species). Compared with plants with multiorder leaf venations, these monocotyledons have only two orders of veins: parallel veins and cross veins connecting parallel veins. It is possible that cross veins may serve a similar role as minor veins in terms of distributing water from the xylem to the mesophyll, but no deformation was detected in cross veins of all the three bamboo and one grass species, despite that conduit collapse was detected in the parallel vein of one bamboo and one grass species. Structurally, the high resistance of cross veins to deformation could be related to their thick walls as shown in Figs 5, 7. Physiologically, grasses have more rapid stomatal closure than other angiosperms (Franks & Farquhar, 2007; Lawson & Blatt, 2014; Merilo *et al*., 2014; Lawson & Vialet-Chabrand, 2019) and use other mechanisms such as the shrinkage of bulliform cells (large bubble-shaped epidermal cells) to reduce water loss. Additionally, most (if not all) bamboos have positive root pressures that can reach the top of the canopy (Saha *et al*., 2009; Cao *et al*., 2012), allowing them to refill embolized conduits during the night when transpiration is greatly reduced. The finding of conduit deformation in parallel veins in some bamboo and grass species invites further investigation to determine the functional impact.

Conclusion

Here, using a cryo-SEM survey approach, we show that the water transport pathway of vascular plants is not entirely a rigid piping system as previously thought. Rather, collapsible conduits at vein endings (terminal parts, minor veins or transfusion tracheids) are common. Further studies need to be done to quantify the relationship between conduit deformation and water potentials, relate this vulnerability to wall structure and elastic modulus, and confirm the reversibility of deformation upon relaxation of xylem tension. Such deformability can, in theory, serve as a protective mechanism for the upstream (more rigid) xylem water transport system when confronted with rapid increases in evaporative demand. However, the challenges of sampling a sufficient number of conduits in even a single leaf to quantify with any precision the impact of minor vein deformation on whole hydraulic conductance, and then replicating the study across a large number of species, mean we can only say that, to date, our physiological experiments on a restricted number of taxa continue to support the hypothesis of an adaptive circuit-breaker-like role for conduit collapse. As atmospheric evaporative demand and its perturbations are important in limiting ecosystem water and carbon fluxes (Novick *et al*., 2016), this circuit-breaker-like behavior could be important in protecting the plant hydraulic system and contributing to the maintenance of water and carbon balance of terrestrial plants.

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Competing interests

None declared.

Author contributions

Y-JZ, NMH, UH and FER designed the experiments. Y-JZ, UH, AP, AM and Y-JC carried out the experiments. Y-JZ and ACG performed the cryo-SEM imaging. Y-JZ, FER and NMH wrote the article with contribution from all co-authors.

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Data availability

The data supporting the findings of this study are available in the Supporting Information of this paper.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Supporting data for optical vulnerability curves of red oak, soybean, and cotton.

Fig. S1 Freeze-fracture cryo-SEM images showing the conduit shape of four free-transpiring leaves at the canopy of red oak trees.

Fig. S2 Freeze-fracture cryo-SEM images showing the conduit deformation in samples #2, #3, and #4 (see Fig. 3) for both soybean and cotton leaves at the times indicated by the arrows in Fig. 3.

Fig. S3 Freeze-fracture cryo-SEM images showing the xylem conduit deformation in leaf minor veins of eastern cottonwood (*Populus deltoides*), red maple (*Acer rubrum*), and sunflower (*Helianthus annuus*).

Fig. S4 Freeze-fracture cryo-SEM images showing the xylem conduit shape in leaf minor veins of *Castanopsis indica* rehydrated from −3.3 to 0.2 MPa. Green arrows in the figure indicate conduits without deformation.

Notes S1 Estimation of water release from terminal (i.e. the highest order) veins and change in leaf water potential, and of water release from all minor veins and change in leaf water potential.