# Revealing catastrophic failure of leaf networks under stress

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**The intricate patterns of veins that adorn the leaves of land plants are among the most important networks in biology. Water flows in these leaf irrigation networks under tension and is vulnerable to embolism-forming cavitations, which cut off water supply, ultimately causing leaf death. Understanding the ways in which plants structure their vein supply network to protect against embolism-induced failure has enormous ecological and evolutionary implications, but until now there has been no way of observing dynamic failure in natural leaf networks. Here we use a new optical method that allows the initiation and spread of embolism bubbles in the leaf network to be visualized. Examining embolism-induced failure of architecturally diverse leaf networks, we found that conservative rules described the progression of hydraulic failure within veins. The most fundamental rule was that within an individual venation network, susceptibility to embolism always increased proportionally with the size of veins, and initial nucleation always occurred in the largest vein. Beyond this general framework, considerable diversity in the pattern of network failure was found between species, related to differences in vein network topology. The highest-risk network was found in a fern species, where single events caused massive disruption to leaf water supply, whereas safer networks in angiosperm leaves contained veins with composite properties, allowing a staged failure of water supply. These results reveal how the size structure of leaf venation is a critical determinant of the spread of embolism damage to leaves during drought.**

embolism | drought | xylem | vein | leaf

One of the most striking features of leaves is the network of veins that function as microfluidic circuits responsible for importing water and nutrients and exporting sugars. These microfluidic circuits supply water to tissues engaged in photosynthesis (1), thus governing the rate of water and $CO_2$ exchange between plants and the atmosphere (2, 3). Despite the essential function of the leaf vasculature, its integrity is constantly under threat of failure because of the uncontrolled air embolization of the network when water stress exceeds a critical threshold value (4). Hence, leaf networks have evolved under competing selective pressures to simultaneously maximize efficiency (in terms of flow and construction cost) and safety (from embolism disruption) of water transport within the leaf (5). Major evolutionary transitions have affected the transport efficiency of the leaf vein network (6, 7), but little is known about adaptation of leaf networks to avoid catastrophic failure by air embolism.

High water tension in the water-transporting xylem cells of plants originates in drying soils and, according to the "air-seeding" hypothesis, is responsible for pulling air into the continuous xylem water column through submicron "pit" pores in the thin, porous membranes that separate neighboring xylem conduits (8). Typically, the closure of stomatal valves on the leaf surface dramatically slows the pace of plant dehydration before damage to the xylem begins (9), but without rain, drying soil will ultimately cause water tension to exceed the air-seeding threshold (4). At this critical tension, the capillary forces that prevent air from bursting into the xylem through microscopic pores in the pit membranes are exceeded, causing a bubble to invade the water-filled lumen and expand rapidly to form an air embolism that blocks the xylem conduit. These embolisms are theoretically able to propagate between interconnected conduits (which range in length from microns to meters) by traversing pit membranes between xylem conduits, although this process has only been visualized in wood (10). Subsequent blockage of xylem cells by air embolisms progressively reduces water transport, further increasing xylem tension in a positive feedback loop that can cause catastrophic failure of the xylem vasculature unless plants are rewatered (11). Progress in understanding the dynamics of embolism spread using traditional hydraulic methods to quantify the effect of drought-induced embolism formation on plant function have been hampered by the presence of high xylem hydraulic tension, which makes intrusive measurements prone to exogenous embolism formation during manipulation (12). Recent techniques such as X-ray and magnetic resonance imaging have provided new insights into when and where embolisms form in stems (10, 13, 14), but a lack of resolution, and the fact that leaves are damaged by ionizing radiation, currently prevents this technique from being used to investigate the spread of embolism through the leaf water transport network. The resultant knowledge gap is significant not only because of the critical role of water transport failure in determining plant mortality during drought (15) but also because the dominant leaf vascular architectures found in modern land plants are the product of >400 million years of evolutionary selection and should therefore represent diverse solutions for building failsafe supply networks (16). The properties of these leaf vascular supply networks may have applications to a wide range of large-scale man-made networks for fluid delivery (water, gas, oil, and other fluids), energy distribution (17) (electrical current), data communication (internet, landline, and cellular telephone) (18), or urban architecture. Here we develop a simple technique that provides the first opportunity, to our knowledge, to visualize where embolism damage initiates in leaf networks and how it propagates as leaves are exposed to water stress. Our aim was to use this new optical technique to discover the

## Significance

**Water sustains photosynthesis and growth of land plants, but it must be transported from the soil to leaves under high tension. Drying soil leads to an increase in water tension, exposing plants to the problem of breakage of the water column, causing embolisms that cut off water supply, leading to tissue death during drought. The ability of leaves to resist embolism formation is a key adaptive axis in plant evolution, and yet the process itself has never been visualized in the leaf venation. We describe a new optical method that allows the evolution and spread of embolism in the entire leaf network to be mapped, thus revealing general rules in the sequence of leaf vein transport failure.**

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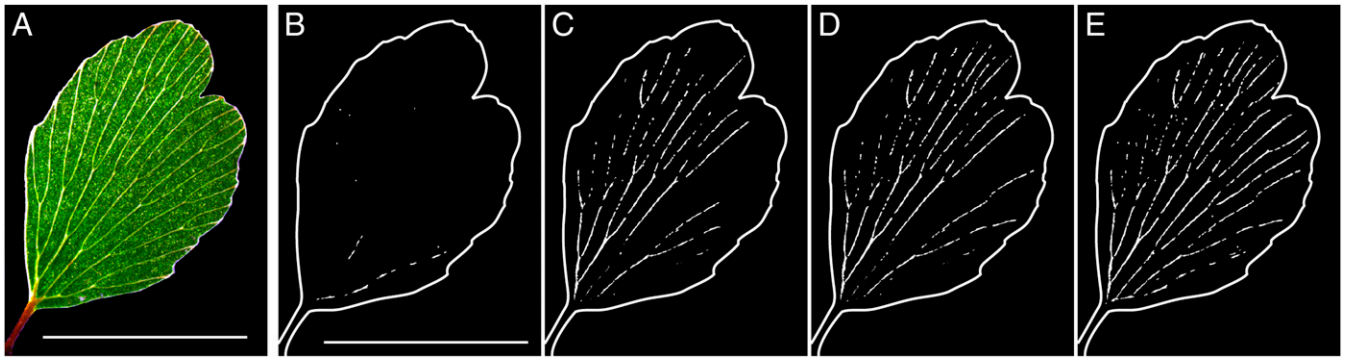


**Fig. 1.** Digital representation of embolism recorded optically during leaf drying in the fern *Adiantum*. (*A*) Leaf illuminated by transmitted light. (Scale bar, 5 mm.) (*B–E*) The result of image analysis, showing the progression of embolism after (*B*) 2 h 5 min, (*C*) 4 h 10 min, (*D*), 6 h 15 min, and (*E*) 8 h 20 min. The darkening of pixels between successive images (30 s apart) by embolism is detected by image difference. If the darkening exceeds a threshold, these pixels are assigned as white (see Movies S1 and S2 for further explanation).

basic rules of network failure in leaves and to determine whether species with markedly different network architecture perform differently in terms of preserving network connection under imposed stress.

To capture embolism events, we recorded rapid changes in light transmission through the venation as leaves were allowed to desiccate while attached to branches. Leaf veins are composed of tight bundles of water-filled xylem tubes, relatively transparent to light (Fig. 1 and Fig. S1). These tubes are segmented by membranes that temporarily stop gas interface propagation. When embolism occurs in these xylem cells, they immediately fill with air, changing the refractive properties of the venation as the result of an increase in the number of air–water interfaces (19). By recording images at a relatively high frequency (one image every 30 s) on a low-magnification microscope or slide scanner (see *SI Methods* for details), we were able to distinguish rapid, embolism-induced changes in light transmission, from slower changes in light transmission resulting from processes such as changing leaf density as specimens desiccated. This technique allowed us to produce what are, to our knowledge, the first ever temporally resolved maps of xylem embolism in leaves exposed to water stress over many hours or days (Fig. 1 and Movies S1–S2).

By severing the contact between branches and soil, we were able to simulate the onset of severe drought conditions during a relatively short period. On the initiation of drying, leaves attached to branches were observed to resist any embolism for variable periods ranging

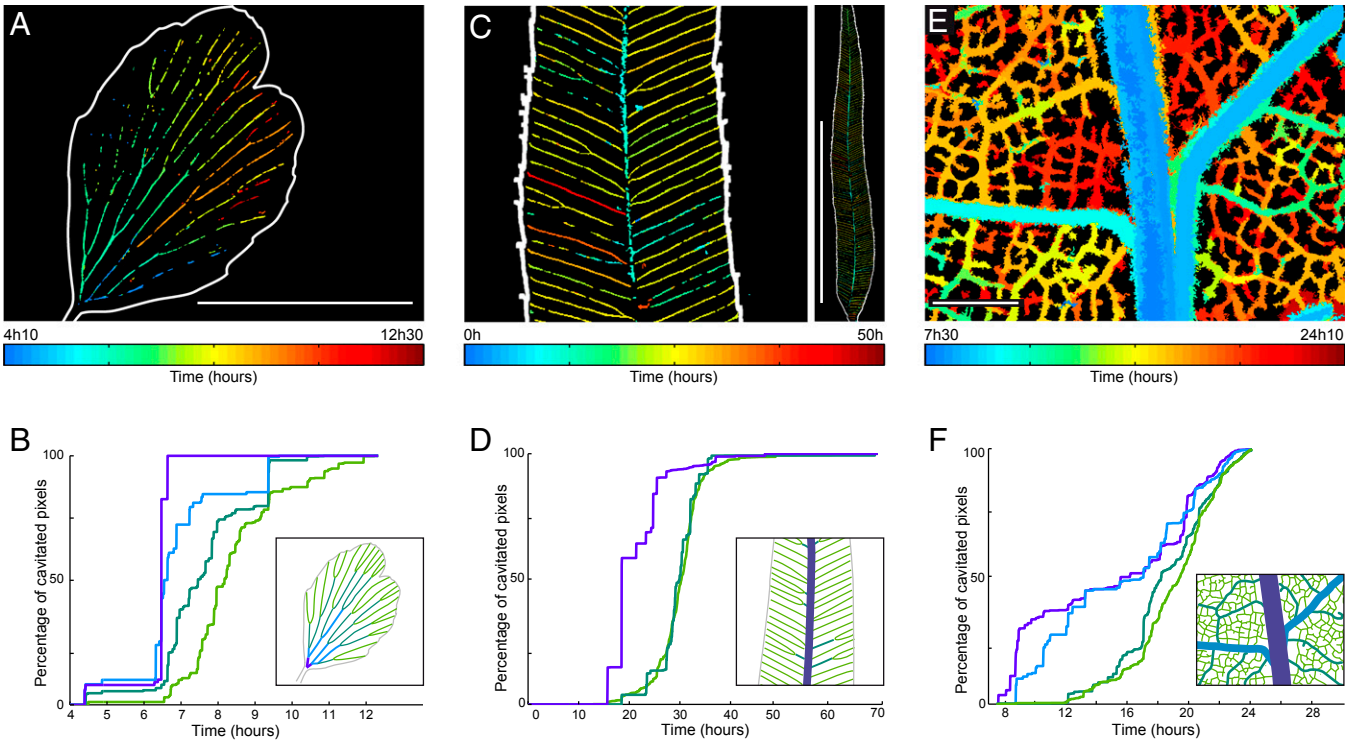


**Fig. 2.** Spatiotemporal evolution of embolism formation in leaves exposed to acute water stress. (*A*) *Adiantum*: progression map of embolism in a single pinna. (Scale bar, 5 mm.) (*B*) Progression of embolism for different vein orders. (*Inset*) Definition of the colors of each order. (*C* and *D*) Embolism map for the fern *Pteris* and progression of emboli for the whole leaf; the orders are here defined in the usual way from the petiole. (*Left*) Zoom. (*Right*) Whole leaf. (Scale bar, 50 mm.) (*E* and *F*) The same for *Quercus*. Colors represent the first embolism. See Fig. S4*B* for the progression of embolism in a whole *Quercus robur* leaf. (Scale bar, 1 mm.)

from 30 min (in the fern *Adiantum capillus-veneris*) to 70 h (in the woody angiosperm *Eucalyptus globulus*). After this quiescent period, large embolism events were observed to spread through the vascular network, continuing until leaf water potentials reached levels causing cellular damage, whereupon embolism was no longer observed. We have recently shown that these embolism events in leaf veins are responsible for the decline in leaf vein water transport function that occurs during severe water deficit after the closure of stomata and immediately before leaf death (9). Here we used the technique of image subtraction (*SI Methods*) to map the dynamics of embolism spread within the leaf xylem network (Fig. 2). These spatial observations of leaf embolism in situ enabled us to determine whether there were "weak links" in the leaf vein network that always embolized early, or whether the process of embolism spread was entirely random. We were also able to assess the performance of different vein network architectures under exposure to high water tension generated by acute leaf water stress.

From these first observations of drying leaves, we discovered that embolism invades the network by abrupt steps (Movie S3), contrary to the smooth physiological flow of sap observed in veins (20). This is expected because according to the "air-seeding" hypothesis, air should only break into a water-filled vessel when the water tension exceeds a threshold that cannot be contained by capillary forces in the walls or membranes of individual xylem vessels (21). The progressive invasion of air into xylem conduits indicates that a range of thresholds for air entry into xylem conduits exists in the xylem network of a single leaf. The size of these embolism steps was highly variable, with the first embolism events generally propagating further than those occurring later in the drying cycle (Fig. 1). A strong, size-dependent pattern was observed in vein diameter, such that a typical embolism sequence begins in the largest veins and progresses into smaller veins (Fig. 2). These observations were common to all vein networks sampled across phylogenetically and architecturally diverse group of fern and angiosperm species (Fig. 3), suggesting that a gradient in xylem vulnerability to embolism is present within leaves. We found that widespread embolism of minor veins often occurred after the bulk of the larger vein orders were embolized, and thus deactivated (Fig. 2 *B*, *D*, and *F*). In contrast to previous studies (22), our observations show that major veins are the first conduits to become damaged by water stress. This pattern of drying from the leaf interior is contrary to that observed in inert porous materials, which initiate embolism from the periphery (23).

In all species, we observed a consistent relationship between relative embolism vulnerability (as represented by time to reach 25% of total embolism in each vein order) and vein size in different vein orders (Fig. 3). The form of this relationship was close to $T = k/D$ (where $T$ = time to 25% embolism and $D$ = vein order diameter). Relating this timing of embolism to the properties of the xylem conduits, which are bundled together to form the leaf veins (24), is simplified by the observation of a consistent scaling relationship between mean conduit diameter and vein diameter in leaves (25). Thus, if the sizes ($d$) of air-seeding pores in xylem membranes [through which air enters a conduit during embolism (21)] are proportional to conduit size (26), and the water tension ($P$) in the xylem is a linear function of drying time, then an inverse proportionality between $T$ and $D$ would be predicted by the Young-Laplace equation that states an inverse proportionality between $P$ and $d$. Given that a gas–liquid interface presents a surface tension σ, this interface can seed an embolism through an opening of size $d$ by curving its shape with a curvature radius $d/2$, which requires a tension $-P = 2\sigma/(d/2)$, according to the Young-Laplace equation. The observed relationship between vein size and resistance to embolism within leaves supports the idea of an efficiency–safety trade-off within the leaf network, whereby larger conduits that transport water more efficiently are more exposed to loss of function by embolism. Thus, the scaling between conduit size and vulnerability within each leaf appears to mirror the larger-scale pattern observed among species from diverse plant lineages (27).

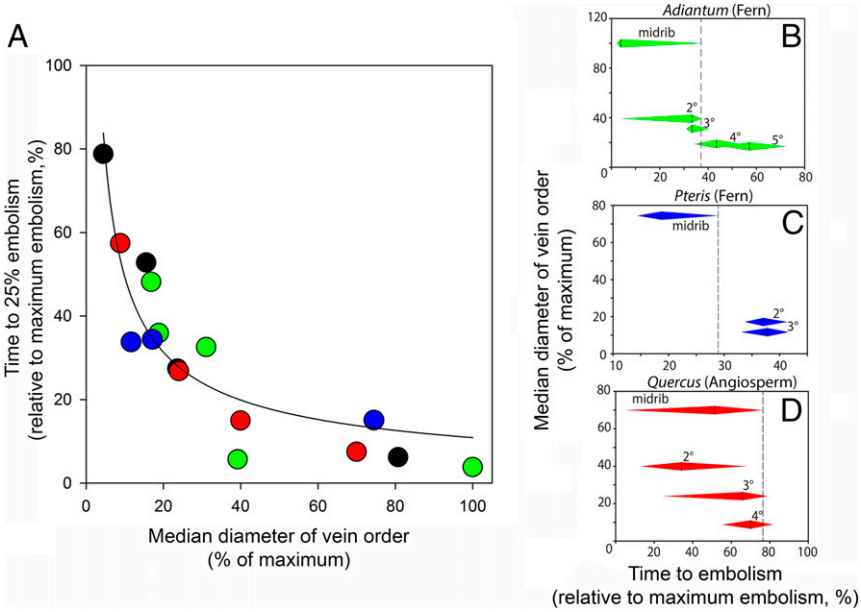


**Fig. 3.** Normalized plot showing that within each leaf, the larger vein diameters are the first to embolize. (*A*) Relative vein sizes (in each species, the sizes of vein orders are expressed relative to the largest vein size) and time to embolism (in each species, the time for 25% embolism of each vein order is expressed relative to the time to 99% embolism of all veins) are plotted for two ferns (*Adiantum*, green; *Pteris*, blue) and two angiosperms (*Quercus*, red; *Eucalyptus*, black). The median vein diameter $D$ of the considered order is normalized by $D_{max}$, the maximum vein diameter in the corresponding leaf. The time $T_{25}$ to reach 25% of vein order embolism is normalized using $(T_{25} - T_{min})/(T_{max} - T_{min})$, where $T_{min}$ and $T_{max}$ are times for 1% and 99% embolism in the whole leaf, respectively). A best-fit regression with the equation $T_{25} = k/D$ is fitted to all points. (*B–D*) Overlap between vein orders in terms of timing of embolism is shown for species with contrasting vein architectures (note that axes are inverted compared with *A* to emphasize functional overlap between veins). The dotted line shows the point in time where the midrib is completely cavitated, and leaves are no longer able to function. Diamonds for each vein order extend between times for 25th–75th percentile embolism for the particular vein order.

In terms of function, it was surprising to observe a progression of damage from larger upstream (supply) nodes to smaller downstream (delivery) nodes during exposure to water stress, because this is counterintuitive in terms of maintaining a functional vein network poststress. Early cavitation of upstream venation means downstream venation becomes redundant once disconnected from the water source (in this case, the stem of the plant), leading to catastrophic desiccation unless the connection can be restored. Nevertheless, we found that the same size–vulnerability scaling rules operated in all species measured, despite the fact that other xylem anatomical features such as conduit length are very different among leaves (28). It should be noted that the absolute relationships between embolism vulnerability and leaf vein size are only likely to be conserved within species (9), but the within-leaf scaling pattern among diverse lineages observed here appears to be a general rule.

Network architecture was observed to play a major role in the propagation of embolism. Within our small sample of fern and angiosperm species, we found three types of network architecture that produced contrasting patterns of embolism, leading to contrasting characteristics of network safety. We have classified these architectures according to the presence or absence of hierarchy in veins (i.e., the presence of several classes, or vein orders, with a clear distinction in size with respect to each other) and their reticulation (i.e., reconnections of veins after branching points).

### Nonhierarchical, Nonreticulate Venation (as in *Adiantum*: Fern; Fig. 2 *A* and *B*)

This represents the earliest type of leaf vasculature observed in the first vascular land plants (29), and today only in gymnosperms, ferns, and lycophyte relatives of early-diverging tracheophyte clades. This category describes topologies in which veins are typically linear, tapering and bifurcating without reconnections. Because of the tapering in venation from leaf base to tip, we observed that embolism always initiated in the source node of the network and progressed to the tip. In *Adiantum*, the largest vein contained few xylem conduits with a narrow size range (Fig. S2), leading to rapid loss of function during embolism because distal veins were immediately severed from the supply node (Figs. 2 *A* and *B* and 3*B*). This network type was highly prone to the formation of very large embolism events that deactivated entire pinnae in single embolism events (Figs. S3*B* and S4).

### Hierarchical, Nonreticulate Venation (as in *Pteris*: Fern)

Common among ferns, but never occurring in flowering plants, this topology also describes networks with linear tapering veins, but vein sizes are disjunct, leading to "major" and "minor" vein size classes. In the fern *Pteris*, we found that embolism in the large midrib occurred before in the minor veins, and that there was no overlap in the size or the embolism sequence of these different orders (Fig. 2 *C* and *D*). As a result, embolism in minor veins occurred randomly along the length of the leaf, but most minor veins were cut off from water supply (by midrib embolism) before they reached their embolism threshold (Fig. 3*B*).

### Hierarchical, Reticulate Network (as in *Quercus, Eucalyptus*: Angiosperms)

This type of venation network is present in all modern angiosperms, but also in some fern species. Veins typically branch unequally, leading to loopiness and reconnection between neighboring veins and among vein orders. In our sample leaves, we found considerable overlap in the embolism profiles of all vein orders (Figs. 2 *E* and *F*, 3*D*, and Fig. S3 *C* and *D*), probably because of the large number and size range of xylem vessels that made up major veins. Of particular note was the fact that the midrib maintained a degree of functionality throughout much of the embolism of the network (Table S1 and Fig. S5). The extended functionality of the midrib, in combination with the reticulation of vein orders, explains the observation that embolism of minor veins occurred homogeneously across the leaf surface despite being preceded by large embolism events in the major veins.

The three patterns described here span the range of evolved possibilities among leaf vascular supply networks, yet the general principal of size-dependent scaling of network nodes with respect to embolism vulnerability remained consistent throughout. Thus, we propose that this rule is conserved among vascular plants and that it provides a reliable means to predict the behavior of leaf networks during water stress, based on their size structure. These vein size structures are extremely diverse among leaves, yet they appear to be conserved within species (30), thus providing critical information about leaf adaptation to water stress in both living and fossil plant species (31).

Ultimately, the progression of embolism within the leaf network must be a combination of the vein network topology and the structure of the xylem conduits that make up the leaf veins (Fig. S2). Although our data suggest significant relationships between conduit size and embolism vulnerability within leaves, it is not expected that a general relationship exists between species. Furthermore, it is unknown whether differences in embolism propagation observed in the sampled fern and angiosperm leaves occurred as a result of peculiarities of the fern xylem (an absence of vessels in leaves) or because of size distributions of conduits between leaf network nodes. Despite these uncertainties, the general size-dependent pattern of embolism spread within leaf veins remained consistent among species, thus providing a strong foundation for future study.

In terms of maintaining supply under water stress, the reticulate networks represented by *Quercus* and *Eucalyptus* performed better than the fern species (even compared with ferns with reticulate venation; Fig. S3 *A* and *B* and Movie S4), in the sense that minor veins failed randomly whereas the rest of the network retained some connection to the source node (the petiole). Minor veins tended to embolize only once, presumably terminating hydraulic connection to the downstream leaf tissue (Movie S4). The fact that these minor veins are the last to lose function means that as long as some nonembolized conduits remain in the larger veins, the supply of water to the mesophyll can continue until the minor veins fail. The result is a gradual decline, rather than a catastrophic decline in water transport efficiency, as larger (major) veins begin to embolize during acute water stress (9). Thus, the dense reticulation of the minor venation in angiosperms (which appears at first glance to be an inefficient redundancy) represents an efficient means of bypassing local disruption in major veins and avoiding rapid and catastrophic hydraulic failure when leaves are drought stressed (32). In addition, if it is assumed that the production of embolism resistance comes at a cost in terms of construction (33) and transport efficiency (26, 34), then the optimal network architecture occurs when there is overlap in the vulnerability of different nodes (thus avoiding both catastrophic failure and excessive redundancy). Overlapping vulnerability of network nodes in *Quercus* and *Eucalyptus* was no doubt aided by the composite structure of the larger veins (Fig. S2) in these species, which contained hundreds of connected xylem conduits spanning a large range of sizes and, presumably, vulnerabilities (Table S1 and Fig. S2). A degree of hydraulic isolation between conduits seems to prevent embolisms from spreading immediately between neighboring conduits, particularly in the radial direction (35, 36). In artificial networks, the failure of major nodes is the source of a major disruption; the lesson we learn from these natural networks it that a gradual decay of the main nodes, instead of an on–off transition, is the key to extended functionality.

The simplicity of the imaging approach we describe here brings questions about hydraulic failure within the reach of any research group with access to basic imaging tools. Thus, our study provides the departure point for a new generation of research into the evolution and function of biological networks in leaves and in roots, where high transparency will also allow quantification of xylem embolism.

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