

## Commentary

### Plants circling in outer space

#### Oscillatory movements are ubiquitous in plants

Plants do not grow in a strict linear manner, rather they circumnutate. That is, they exhibit an oscillatory or helical growth pattern around an axis. Circumnutation is readily apparent in vines such as morning glory or grape (Fig. 1), but in fact it is nearly ubiquitous in plants. Circumnutation occurs in almost all plant organs throughout all stages of development (Johnsson, 1997; Larson, 2000). In the late 19th century, plant scientists noted that plant organs, including roots, shoots, stems, hypocotyls, branches, leaves and flower stalks, did not grow exactly in a linear direction. The mean growth direction may be maintained for long periods of time, but the organ's instantaneous growth direction usually rotates or oscillates slowly around a mean. Circumnutation is best visualized using time-lapse photography (Fig. 2), and numerous examples, including sunflower seedlings, *Arabidopsis* stems and morning glory stems, are illustrated in movies found at the Plants-in-Motion website (<http://plantsinmotion.bio.indiana.edu/>). The paper by Johnsson *et al.* (pp. 621–629) in this issue of *New Phytologist* uses microgravity as a tool to study this interesting phenomenon in plants.

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*'... this paper suggests not only that endogenous nutations occur in stems as Darwin predicted, but also that gravitational accelerations amplify these circumnutations.'*

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Plant movement was the subject of two of Charles Darwin's books, *The movements and habits of climbing plants* (Darwin, 1875) and *The power of movement in plants* (Darwin & Darwin, 1880). Darwin hypothesized that circumnutation is the major developmental phenomenon in plants and that understanding this oscillatory movement was the key to understanding all aspects of plant movements, including gravitropism, phototropism, thigmotropism and nastic movements. He viewed all of these phenomena as an outgrowth of circumnutation.

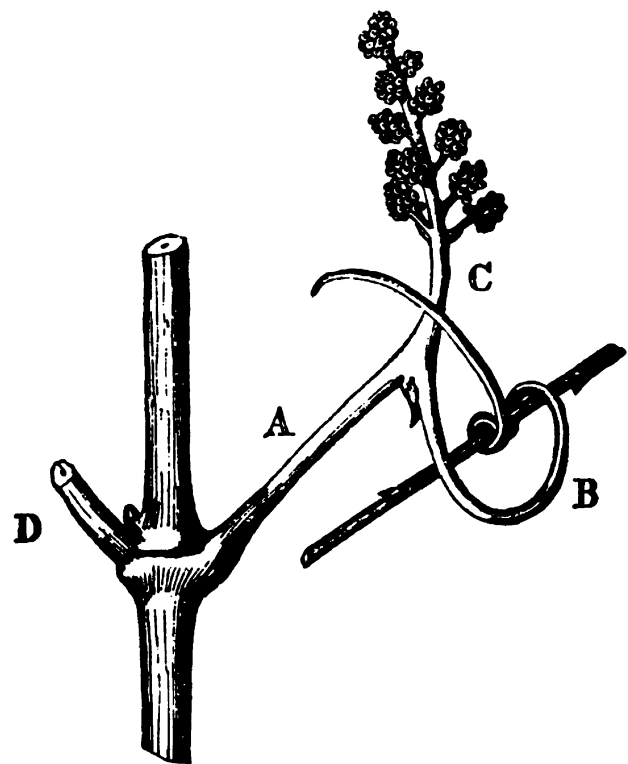
In order to verify his observations, Charles Darwin performed his studies on many types of plants. In his book, he lists the 320 plant species he studied and concludes 'every growing part of every plant is continually circumnutating,

though often on a small scale' (Darwin & Darwin, 1880). Darwin hypothesized that circumnutation was an endogenous mechanism found in all plants that they employ in order to explore their immediate environment.

#### Is circumnutation dependent upon gravity?

Supporting Darwin's endogenous hypothesis, Shabala (2006) summarized other possible functions of circumnutation, including synchronizing events between cells at different sites, functioning as a filter that helps separate signal from environmental noise and decreasing the response time when reacting to external stimuli. However, an alternate hypothesis is that circumnutation is dependent upon gravity and therefore is not a strictly endogenous feature of plants (Brown, 1993).

Several modern experiments support Darwin's 'endogenous hypothesis'. For example, in a spaceflight experiment, 93% of sunflower (*Helianthus annuus*) seedlings exhibited circumnutation in microgravity compared with 100% of control seedlings on the ground (Brown *et al.*, 1990). The circumnutation of the seedlings in microgravity had a reduced period and amplitude



**Fig. 1** Circumnutation is most obvious in climbing vines, as shown in this illustration from Darwin (1875). In this flower stalk, circumnutation leads the flower tendril (B) to wrap around an object. A, peduncle; C, subpeduncle with flower buds; D, petiole.



**Fig. 2** Circumnutation demonstrated in a time-lapse image of a single shoot of the vine *Lonicera japonica* (Larson, 2000). Sixteen photographs taken over a 2-h period were superimposed to illustrate the clockwise rotation in this vine.

relative to the ground control plants. By contrast, a series of papers by the Takahashi group in Japan suggested that gravity was required for circumnutation to occur. In their first report, they suggested a link between circumnutation and gravity based on their finding that an agravitropic mutant of morning glory (*Pharbitis nil*) also was defective in circumnutation (Hatakeda *et al.*, 2003). In a follow-up study, this group also showed that mutants of *P. nil* and *Arabidopsis thaliana* lacking the endodermal layer, which is involved in gravity sensing in shoots (Kiss, 2000), exhibited severely reduced circumnutations – thus linking this phenomenon directly to mechanisms of gravity perception (Kitazawa *et al.*, 2005).

### Space experiments provide a research opportunity for fundamental biology

How can the results between the Brown group and the Takahashi group be reconciled? Kitazawa *et al.* (2005) suggested that the sunflower seedlings in the experiments by Brown *et al.* (1990) sensed gravity before the space experiment started because some seedlings germinated prior to the launch of the spacecraft. Thus, an experiment in which all seeds were germinated in space (and seedlings developed completely in microgravity) would help to resolve these controversies. The paper by Johnsson *et al.* uses this very approach with *Arabidopsis* plants.

In these elegant spaceflight studies (Johnsson *et al.*), a laboratory incubator facility with a centrifuge, termed the European Modular Cultivation System (EMCS), was used on the International Space Station (Kiss *et al.*, 2007). Centrifuges provide important controls for spaceflight studies but have not been available for most biological experiments performed in space to date (Perbal & Driss-Ecole, 2002). *Arabidopsis* plants developed from seeds in microgravity, and once inflorescence stems were formed, the centrifuge provided 0.8 *g* of acceleration, which is similar to the earth nominal control. After acceleration, the centrifuge was turned off so that the plants would again experience microgravity.

Johnsson *et al.* detected small nutational movements (with minute amplitude) of the side stems in microgravity before centrifugation. However, when the gravitational acceleration was provided to the level of 0.8 *g*, the amplitude of the circumnutations increased five to ten times. Light also had an effect on circumnutations in that the period was decreased from 85 min (dark) to 60 min (light). Thus, the results presented in this paper suggest not only that endogenous nutations occur in stems, as Darwin predicted, but also that gravitational accelerations amplify these circumnutations.

What is the overall significance of these results? Johnsson *et al.* seem to favor a model that incorporates both hypotheses – the endogenous model and the idea that circumnutation is related to, and dependent upon, gravity. This space study was able to show that small, endogenous circumnutations do occur in microgravity, but that the gravitational accelerations provided by the centrifuge clearly increased their magnitude. Johnsson *et al.* and others in the field have referred to this idea as the ‘combined model’, which has been outlined in Brown (1991) and in Johnsson (1997).

In summary, the unique microgravity environment was used to test the hypothesis that circumnutations are an internal, endogenous feature of plant organs. This is important because, in previous studies, researchers could not study circumnutations in plants without the ‘complicating’ effects of gravity. In a similar manner, the microgravity environment obtained in orbiting spacecraft has been used effectively to study phototropism without the interference of gravity or gravitropism (Heathcote *et al.*, 1995; Kiss *et al.*, 2007). Thus, the experiments of Johnsson *et al.* provide a fine example of using the microgravity environment aboard orbiting spacecraft as a unique research tool to study important problems in fundamental biology (Perbal & Driss-Ecole, 2002). We look forward to further contributions from the science programs of the European Space Agency and the National Aeronautics and Space Administration from the laboratories aboard the International Space Station.

**John Z. Kiss**

Department of Botany, Miami University,  
Oxford, OH 45056, USA  
(tel + 1 513 529 5428;  
email kissjz@muohio.edu)

## References

- Brown AH. 1991. Gravity perception and circumnutation in plants. *Advances in Space Biology and Medicine* 1: 129–153.
- Brown AH. 1993. Circumnutations: from Darwin to space flights. *Plant Physiology* 101: 345–348.
- Brown AH, Chapman DK, Lewis RF, Venditti AL. 1990. Circumnutations of sunflower hypocotyls in satellite orbit. *Plant Physiology* 94: 233–238.
- Darwin C. 1875. *The movements and habits of climbing plants*. London, UK: John Murray.
- Darwin C, Darwin F. 1880. *The power of movement in plants*. London, UK: John Murray.
- Hatakeeda Y, Kamada M, Goto N, Fukaki H, Tasaka M, Suge H, Takahashi H. 2003. Gravitropic response plays an important role in the nutational movements of the shoots of *Pharbitis nil* and *Arabidopsis thaliana*. *Physiologia Plantarum* 118: 464–473.
- Heathcote DG, Brown AH, Chapman DK. 1995. The phototropic response of *Triticum aestivum* coleoptiles under conditions of low gravity. *Plant, Cell & Environment* 18: 53–60.
- Johnsson A. 1997. Circumnutations: results from recent experiments on earth and in space. *Planta* 203: S147–S158.
- Johnsson A, Solheim BGB, Iversen T-H. 2009. Gravity amplifies and microgravity decreases circumnutations in *Arabidopsis* stems: results from a space experiment. *New Phytologist* 182: 621–629.
- Kiss JZ. 2000. Mechanisms of the early phases of plant gravitropism. *Critical Reviews in Plant Sciences* 19: 551–573.
- Kiss JZ, Kumar P, Bowman RN, Steele MK, Eodice MT, Correll MJ, Edelmann RE. 2007. Biocompatibility studies in preparation for a spaceflight experiment on plant tropisms (TROP). *Advances in Space Research* 39: 1154–1160.
- Kitazawa D, Hatakeeda Y, Kamada M, Fujii N, Miyazawa Y, Hoshino A, Iida S, Fukaki H, Morita MT, Tasaka M *et al.* 2005. Shoot circumnutation and winding movements require gravisensing cells. *Proceedings of the National Academy of Sciences, USA* 102: 18742–18747.
- Larson KC. 2000. Circumnutation behavior of an exotic honeysuckle vine and its native congener: influence on clonal mobility. *American Journal of Botany* 87: 533–538.
- Perbal G, Driss-Ecole D. 2002. Contributions of space experiments to the study of gravitropism. *Journal of Plant Growth Regulation* 21: 156–165.
- Shabala S. 2006. Oscillations in plants. In: Baluška F, Manasco S, Volkmann D eds. *Communications in plants*. Berlin, Germany: Springer-Verlag, 261–275.

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## New insights into bordered pit structure and cavitation resistance in angiosperms and conifers

The question of what structural features underlie differences in resistance to xylem cavitation is a long-standing issue fundamental to our understanding of water transport in plants. Plants routinely face xylem tensions great enough to cause cavitation and embolism, which may result in

significantly increased hydraulic resistance, limitations on leaf gas exchange and ultimately carbon starvation and plant death (Tyree & Zimmermann, 2002; McDowell *et al.*, 2008). The relative resistance of a plant to embolism is a major determinant of species distribution and the ability of plants to survive in the face of environmental stresses such as drought and freezing (Stuart *et al.*, 2007). The xylem consists of a highly compartmentalized network of conduits in which emboli can be isolated while water transport continues in adjacent conduits. The continued function of this network depends to a large degree on the nano-porous primary cell walls (pit membranes) that separate conduits from one another. Pit membranes function as safety valves in the xylem, allowing the free passage of water between cells as it moves from the roots to the leaves, but limiting the spread of gas or pathogens. However, the fine porosity of pit membranes also results in significant hydraulic resistance, with pit hydraulic resistance accounting for a large proportion of total xylem hydraulic resistance (Zwieniecki *et al.*, 2001; Choat *et al.*, 2006). The structure and function of pit membranes is therefore of great importance in both the hydraulic efficiency of the xylem and cavitation resistance (Choat *et al.*, 2008). Although there is a great breadth of diversity in bordered pit structure across higher plants, pit membranes can generally be divided into two major forms: homogeneous pit membranes, typical of angiosperm species; and margo–torus pit membranes of tracheid-bearing conifers (Fig. 1). In this issue of *New Phytologist*, two exciting studies extend our understanding of the relationship between xylem structure and resistance to cavitation: Christman *et al.* (2009, pp. 664–674) examine the anatomical underpinning of cavitation resistance in angiosperm species, while Hacke & Jansen (2009, pp. 675–686) report a detailed investigation of margo–torus pit structure and its influence on cavitation resistance in three conifer species.

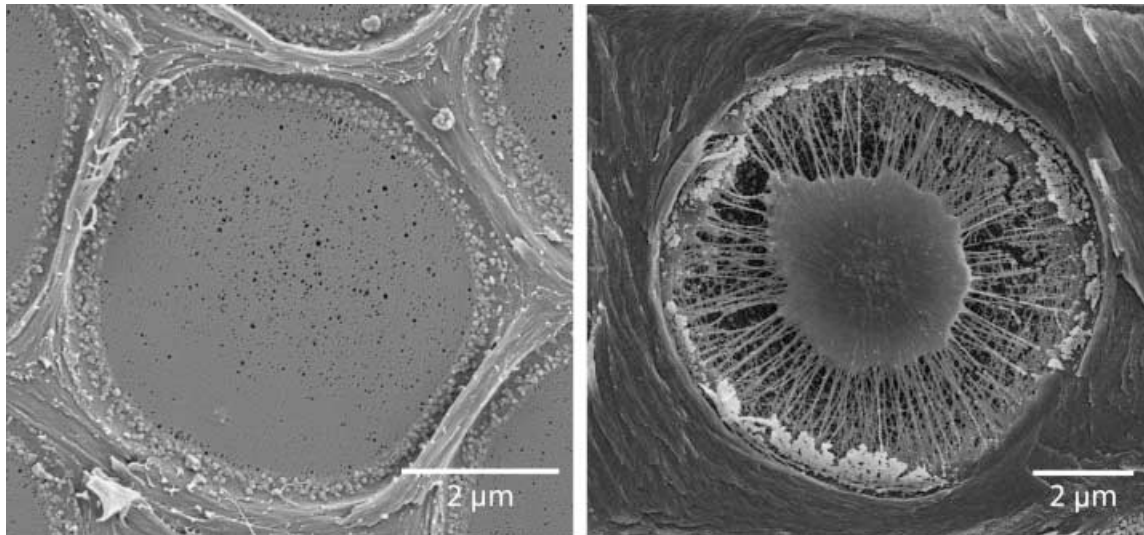
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*‘It is obvious from this work that rare, leaky pits have dramatic consequences for the ability of plants to sustain water transport as water stress and xylem tension increases.’*

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### Cavitation resistance in angiosperms

Pores of homogenous pit membranes are of sufficiently small dimensions that they will prevent gas being drawn into an adjacent water-filled vessel until a critical threshold is reached (e.g. a 100-nm-diameter pore will prevent gas penetration up to



**Fig. 1** Variation in pit structure. (a) A homogeneous pit membrane of an angiosperm species, *Acer negundo*, and (b) a margo–torus type pit membrane of a conifer, *Calocedrus decurrens*. Homogeneous pit membranes, typical of angiosperm species, have a relatively uniform array of microfibrils, whereas in margo–torus pit membranes of tracheid-bearing conifers, the conductive and protective functions of the membrane are spatially distinct as a porous outer region (margo) that allows for movement of water between conduits and a central thickened plug (torus).

a pressure difference of 2.88 MPa across the pit membrane). The potential for the spread of embolism between vessels and throughout the xylem is therefore dictated by the porosity of pit membranes and the minimum value of xylem water potential (negative hydrostatic pressure in the xylem fluid). Species with smaller pit-membrane pores are predicted to have greater cavitation resistance, and thus to tolerate greater degrees of water stress, than those with larger pit-membrane pores. However, although the relationship between pit-membrane pore size and cavitation resistance has sound theoretical underpinnings, it has been difficult to confirm this empirically by matching observed pore sizes to measured cavitation resistance across a range of species, with many studies failing to find pores large enough to be responsible for air seeding at realistic pressures (Wheeler, 1983; Shane *et al.*, 2000; Choat *et al.*, 2003). One explanation for this discrepancy is that the pores responsible for air seeding are actually extremely rare. Because air seeding will always occur first at the largest pore, it is only required that there be one large pore present in all of the many thousands of pit connections between two vessels. A rare, large pore may therefore escape detection by electron microscopy or particle-exclusion experiments. Support for this idea is provided by the work of Wheeler *et al.* (2005), which shows a strong correlation between cavitation resistance and the average area of pit overlap between vessels. This suggests that cavitation resistance might be determined stochastically, with the probability of having a rare, large pore increasing with the area of contact between vessels.

Christman *et al.* provide further support for this hypothesis, using an elegant pairing of theory and empirical data. Probability theory was used to model the cavitation threshold

of pit membranes in three *Acer* species that have differing resistances to cavitation. The model incorporates the theory that if there is a normal distribution of pore diameters in any connection between vessels, only the extreme tail of the distribution will be responsible for air seeding. In fact, the model suggests that only one in 10 000 pits would be 'leaky' enough to cause air seeding at measured air-seeding thresholds. To test this model, Christman *et al.* measured air-seeding thresholds on different stem lengths of the three *Acer* species. This is analogous to a membrane-filter bubble test, where the pore diameter of a filter can be predicted from the pressure required for gas penetration through the filter. The model predicts that short stem segments with fewer vessel end walls should air seed at lower pressures than longer stem sections in which air must penetrate an increasing number of intervessel end walls to move through the entire segment. The empirical data matched the modeled predictions of air-seeding pressures closely. As the stem length increased, air-seeding pressures also increased, indicating that the effects of rare, large pit-membrane pores was masked by the majority of end walls, which lack very leaky pits. In the shortest stem segments, air-seeding pressures were consistently lower than the average cavitation pressures of each species. This evidence confirms that there is wide variation in the porosity of pit connections within each stem, and strongly suggests that a very small variation in the frequency of the rare, large pores can have a significant effect on cavitation resistance, which is independent of the number of pits or the total pit area.

It is obvious from this work that rare, leaky pits have dramatic consequences for the ability of plants to sustain water transport as water stress and xylem tension increases. The

**Box 1** Cavitation resistance in conifers

Under functional, water-filled conditions where the xylem pressure ( $P_x$ ) is below zero, the conifer pit membrane is centrally located in the pit chamber allowing water to move through the margo unimpeded. Should one tracheid become air-filled ( $P_x = 0$ ), the pressure difference across the menisci in the margo pores will be sufficient to deflect the membrane towards the adjoining functional tracheid where  $P_x < 0$ . This way, the torus is appressed against the pit aperture, thereby isolating the water-filled tracheid from its dysfunctional neighbor. Cavitation presumably occurs when  $P_x$  becomes negative enough to cause the torus to slip from its sealing position, allowing air to enter the water-filled tracheid. Indeed, the sealing action of the torus over the pit aperture may be one reason why only a weak correlation between pit area and cavitation resistance has been observed in conifer stems (Pittermann *et al.*, 2006).

important question now becomes how plants would control the frequency of such pits in the xylem. Christman *et al.* suggest that such pits might be the result of 'mistakes' occurring during the development and hydrolysis of the primary walls that make up the pit membranes in secondary xylem. Weak spots in the pit membranes, where the density of cellulose microfibrils is lower, would be particularly susceptible to air seeding if large pressure differences are present across the membrane, as is the case when embolized vessels border water-filled vessels under tension. In this study there was no correlation between the average pit area and the cavitation resistance. Therefore, the frequency of large pores cannot be explained by a uniform stochastic model, as presented by Wheeler *et al.* (2005). The most likely alternative explanation is that changes in the intrinsic properties of pit membranes (average porosity and thickness) are responsible for differences in cavitation resistance. If pit membranes are, in general, more porous and flimsy, then it is easy to imagine that there would be a greater chance of a large pore developing somewhere in the many pit membranes that connect each vessel to others. This is supported by the large between-species variation in pit membrane properties and evidence that air-seeding pressures of individual vessels are correlated with the average pore diameter of pit membranes, as observed by electron microscopy (Jansen *et al.*, 2009).

Perhaps the most important point to acknowledge is that cavitation resistance will not be controlled exclusively by either tissue-level properties (vessel length, diameter, pit overlap area) or pit-level properties (such as average porosity and thickness). Characteristics at both pit and tissue levels will influence cavitation resistance in plants. It is simply a question as to what extent the selective pressures that act to match cavitation resistance of angiosperm species to their environment have been satisfied by shifts in tissue-level traits rather than variation in microscopic pit structure. It will be most intriguing to see how the relationship between these traits evolves as more data become available.

**Control of cavitation resistance in conifers**

In a structural departure from the homogenous pit membrane of angiosperms, the conifer torus–margo pit membrane combines a high degree of cavitation protection with efficient water transport that allows conifer xylem to achieve hydraulic

efficiencies similar to those of angiosperms. In conifer wood, water transport occurs through single-celled conduits, known as tracheids, rather than through the long multicellular vessels characteristic of angiosperms; consequently, as water moves from one tracheid to another at a given segment length, it encounters a much higher frequency of the pitted end-wall regions than it would in vessels. Thus, in the absence of low-resistance end-walls, conifer xylem can potentially represent a hydraulically inefficient, high-resistance network requiring large pressure gradients to drive water transport. Conifers have avoided this problem by developing the torus–margo pit membrane in their tracheid end-walls. This structure reduces the end-wall hydraulic bottleneck because water travels through the net-like margo region of the membrane, a substantially more porous structure than the homogenous pit membrane. Consequently, pit resistance in conifers is almost 60 times lower than in angiosperms, effectively compensating for the frequent end-wall crossings presented by short tracheids (Pittermann *et al.*, 2005; Sperry *et al.*, 2006). Importantly, the hydraulic efficiency of conifer xylem is equivalent to that of angiosperm xylem for a given conduit diameter.

Despite the structural differences, conifers achieve the same degree of cavitation resistance as angiosperms, albeit using a different mechanism (Box 1). This raises the question of which xylem features control cavitation resistance in conifers. Hacke & Jansen use a combination of scanning electron microscopy and transmission electron microscopy to examine the relationship between fine pit structure and cavitation resistance in the root and shoot tissue of three conifer species. Comparisons between root and shoot tissue are ideal because root tissue is consistently more susceptible to cavitation than stem tissue within the same plant.

Hacke & Jansen's study reveals tight correlations between cavitation resistance and discrete features of pit anatomy. Specifically, cavitation resistance was positively related to the ratio of torus to aperture diameters whereby a larger torus for a given aperture diameter resulted in greater resistance to cavitation. This is intuitive because the greater the torus overlap against the pit chamber, the lesser the chance of the torus being pulled through the aperture and allowing air seeding to occur (Pittermann *et al.*, 2006; Domec *et al.*, 2008). In addition, the thickness of the torus and the depth of the pit chamber were inversely related to cavitation resistance, suggesting that

a combination of a thinner torus and a shallow pit chamber may form a tighter seal over the pit aperture. The authors suggest that deeper pit chambers may require the margo to stretch further to seal the aperture, thereby predisposing the fibrils to irreparable damage by tearing.

Hacke & Jansen's study arrives on the heels of recent work that underscores the functional significance of conifer pit membranes on tree height. The pit aperture may represent a significant proportion of transport resistance in their xylem, so if apertures shrink to improve cavitation resistance, the resulting decrease in aperture conductance thus represents a clear trade-off in hydraulic efficiency at the pit level. Indeed, a linear relationship between the torus : aperture ratio and cavitation resistance has been observed with increasing height in very tall Douglas-fir trees: at greater branch heights, cavitation resistance increases to compensate for increasing xylem tensions but at the cost of reduced transport efficiency through the pit aperture (Domec *et al.*, 2008). Given the linear relationship between the torus : aperture diameter and height, this compromise in pit structure places an important constraint on the maximum height that these trees can reach. Whether any clear relationship exists between pit architecture and tree height in tall angiosperms remains to be seen.

### Future directions for research

While great strides have recently been made in our understanding of structure–function relationships in the xylem, important gaps still remain. For example, the spatially complex structure of the angiosperm vessel network has not often been incorporated into measurements of xylem function. The three-dimensional arrangement and connectivity of vessels has enormous potential to influence the efficiency and the propagation of embolism through the xylem. As imaging technology, such as X-ray computed tomography and magnetic resonance imaging, is refined, our ability to resolve flow and propagation of embolism, in three dimensions and in real time, will be greatly improved. We can now measure hydraulic function directly at the pit level, so given the great variation in the structure of interconduit pits and their importance to hydraulic function, further direct measurements are warranted. In conifers, additional evaluation of the margo structure, and its implications for hydraulic trade-offs, should be considered. Because of its delicate nature the margo is often difficult to visualize using scanning electron microscopy. A combination of careful observation of margo structure and improved capability to simulate flow through complex structures should allow an improved resolution of the role that variation in margo structure plays in trade-offs at the tissue and whole-plant levels.

**Brendan Choat<sup>1\*</sup> and Jarmila Pittermann<sup>2</sup>**

<sup>1</sup>Functional Ecology Group, Research School of Biological Sciences, The Australian National University, Canberra,

ACT, 2601, Australia; <sup>2</sup>Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, California, 95064, USA (\*Author for correspondence: tel +61 2 6125 4558; email brendan.choat@anu.edu.au)

### References

- Choat B, Ball M, Lully J, Holtum J. 2003. Pit membrane porosity and water stress-induced cavitation in four co-existing dry rainforest tree species. *Plant Physiology* 131: 41–48.
- Choat B, Brodie TW, Cobb AR, Zwieniecki MA, Holbrook NM. 2006. Direct measurements of intervessel pit membrane hydraulic resistance in two angiosperm tree species. *American Journal of Botany* 93: 993–1000.
- Choat B, Cobb AR, Jansen S. 2008. Structure and function of bordered pits: new discoveries and impacts on whole-plant hydraulic function. *New Phytologist* 177: 608–625.
- Christman MA, Sperry JS, Alder FR. 2009. Testing the 'rare pit' hypothesis for xylem cavitation resistance in three species of acer. *New Phytologist* 182: 664–674.
- Domec JC, Lachenbruch B, Meinzer FC, Woodruff DR, Warren JM, McCulloh KA. 2008. Maximum height in a conifer is associated with conflicting requirements for xylem design. *Proceedings of the National Academy of Sciences, USA* 105: 12069–12074.
- Hacke UG, Jansen S. 2009. Embolism resistance of three boreal conifer species varies with pit structure. *New Phytologist* 182: 675–686.
- Jansen S, Choat B, Pletsers A. 2009. Morphological variation of intervessel pit membranes and implications to xylem function in angiosperms. *American Journal of Botany* 96: 409–419.
- McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG *et al.* 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* 178: 719–739.
- Pittermann J, Sperry JS, Hacke UG, Wheeler JK, Sikkema EH. 2005. Torus–margo pits help conifers compete with angiosperms. *Science* 310: 1924–1924.
- Pittermann J, Sperry JS, Hacke UG, Wheeler JK, Sikkema EH. 2006. Inter-tracheid pitting and the hydraulic efficiency of conifer wood: the role of tracheid allometry and cavitation protection. *American Journal of Botany* 93: 1265–1273.
- Shane MW, McCully ME, Canny MJ. 2000. Architecture of branch–root junctions in maize: Structure of the connecting xylem and the porosity of pit membranes. *Annals of Botany* 85: 613–624.
- Sperry JS, Hacke UG, Pittermann J. 2006. Size and function in conifer tracheids and angiosperm vessels. *American Journal of Botany* 93(10): 1490–1500.
- Stuart SA, Choat B, Martin KC, Holbrook NM, Ball MC. 2007. The role of freezing in setting the latitudinal limits of mangrove forests. *New Phytologist* 173: 576–583.
- Tyree MT, Zimmermann MH. 2002. *Xylem structure and the ascent of sap*. New York, USA: Springer-Verlag.
- Wheeler EA. 1983. Intervascular pit membranes in *Ulmus* and *Celtis* native to the United States. *International Association of Wood Anatomists Bulletin* 4: 79–88.
- Wheeler JK, Sperry JS, Hacke UG, Hoang N. 2005. Inter-vessel pitting and cavitation in woody rosaceae and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant, Cell and Environment* 28: 800–812.
- Zwieniecki MA, Melcher PJ, Holbrook NM. 2001. Hydrogel control of xylem hydraulic resistance in plants. *Science* 291: 1059–1062.

**Key words:** air seeding, bordered pit, cavitation, embolism, torus–margo pit membranes, xylem.

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## Letters

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### Mycorrhizas in Upper Carboniferous *Radiculites*-type cordaitalean rootlets

Mycorrhizas are mutualistic associations between plants and fungi; > 90% of embryophytes are capable of forming symbioses of this type. The fungus uses the host as a source of carbon, while the host is supplied with mineral elements by the fungus. Endomycorrhizal fungi associated with prostate axes of *Aglaophyton major* (paramycorrhizas *sensu* Strullu-Derrien & Strullu, 2007) from the Lower Devonian Rhynie chert represent the oldest occurrence of mycorrhizas (Remy *et al.*, 1994; Taylor *et al.*, 2005). The fungi involved in this and other mycorrhizal associations from the Rhynie chert belong to the *Glomeromycota*, a fungal phylum established by Schüßler *et al.* (2001) using molecular data. Evidence from these plant-bearing deposits indicates that all main spore types in the *Glomeromycota* were in existence before the evolution of true roots (Dotzler *et al.*, 2008).

Extensive collections of thin sections of petrified plant material were manufactured during the early twentieth century. These collections are an invaluable source of information about associations between plants and microorganisms (Krings *et al.*, 2007). Our study focuses on fungal associations in permineralized *Radiculites* rootlets of the *Radiculites*-type (assigned to cordaitalean *reticulatus*) from the flora from Grand'Croix (France) based on the original slides contained in the Lignier, Florin and Carpentier collections. The thin sections were prepared in the early twentieth century from material collected from the Late Pennsylvanian (Upper Carboniferous) 'Poudingue Mosaïque' of Grand'Croix, which belongs to the Saint Etienne coal basin (Massif Central, central France). This basin is situated *c.* 50 km southwest of the town of Lyon. Information about the geological setting of Grand'Croix can be found in Doubinger *et al.* (1995). Thin sections were prepared according to standard techniques. A piece of silicified rock was cemented to a glass slide and then ground to a thickness sufficiently thin to allow for examination in transmitted light. Slides were studied using dry or oil immersion objectives. The Lignier slide collection is today housed in the Herbarium (C.N.) of the University of Caen (France), the Carpentier slides are kept in the collections of Lille Catholic University (France) and the Florin collection is housed in the Natural History Museum of Stockholm (Sweden).

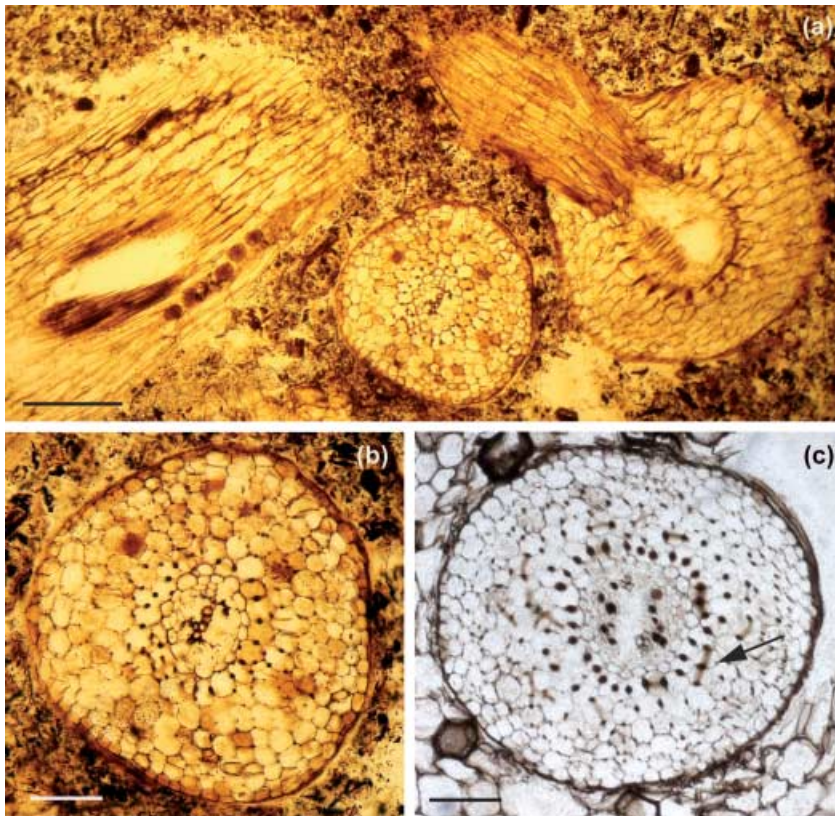
### Characterization of the rootlets

The rootlets are up to 0.65 mm in diameter. In transverse section, they appear well-delimited by an epidermis composed of dark cells (Fig. 1). An exodermis occurs beneath the epidermis; it is one or two layers in thick and consists of cells characterized by thickenings on the periclinal walls. The cells of the outermost exodermal layer are brown in colour. Root-hairs were not observed. The cortex is composed of parenchymatous cells that become more elongated toward the centre of the root. The cells of the cortex are characterized by phi thickenings (Fig. 1b,c), which are prominent in cells located close to the vascular cylinder and gradually become smaller towards the periphery. The name phi thickenings, based on the resemblance of these structures in cross-section to the Greek letter phi, was given by Russow (1875; cited in van Tieghem, 1888). The thickenings form a frame that corresponds to the frame of the adjoining cell (Fig. 1c). An endodermis with a distinct casparian strip on the anticlinal walls separates the cortex from the vascular cylinder. The rootlets generally possess a diarch primary xylem.

The cortical network described above is obvious in all specimens studied and shows a pattern similar to that seen in the rootlets of extant *Cupressaceae* (s.l.) (Gerrath *et al.*, 2002). There are distinct differences with regard to the shape of the thickenings, which may be linked to habitat because the specimens come from three different localities (Grand'Croix, Cuzieu and Assailly) within the 'Poudingue mosaïque' (Fig. 1b,c).

### Evidence for endomycorrhizal colonization in the rootlets of *Cordaites*

Lignier (1906) was the first to report endophytic fungi in *Radiculites reticulatus* cordaitalean rootlets and referred to them as mycorrhizas. Zimmermann (1933), who also studied material from Grand'Croix, suggested that mycorrhizal infection was restricted to a few large cells that lack a casparian strip. In fact, the occurrence of mycelial hyphae was only documented in the outer cortex. This meant that the mycorrhizal status of *R. reticulatus*, as well as that of other supposedly mycorrhizal cordaitalean rootlets (e.g. *Amyelon radicans*; see Osborn, 1909; Halket, 1930), was contested (Cridland, 1962). Cridland suggested that the fungus in Lignier's material represents a parasite or saprotroph, rather than a mutualist. He also restudied Carpentier's material, but from the Lower Permian, while we focused on specimens from the Upper Pennsylvanian of Grand'Croix (Carpentier,



**Fig. 1** Overview of the Cordaites rootlets and transverse sections of the rootlets showing the cortical network of phi thickenings (Lignier's and Florin's materials). (a) Overview of the rootlets in different sections: on the left, longitudinal section of a rootlet; in the centre, transverse section of another rootlet; on the right, young root rising out from another rootlet. (Lignier's slide no. 194). Bar, 0.25 mm. (b) Cordaites rootlet from Grand' Croix. The cortex comprises a reticulum of phi thickenings that are prominent in cells located close to the vascular cylinder (Lignier's slide no. 194). Bar, 0.1 mm. (c) Cordaites rootlet from Cuzieu. Note the frame (arrow) that form the phi thickenings from cell to cell. (Florin's slide no. S 4626 0.14). Bar, 0.1 mm.

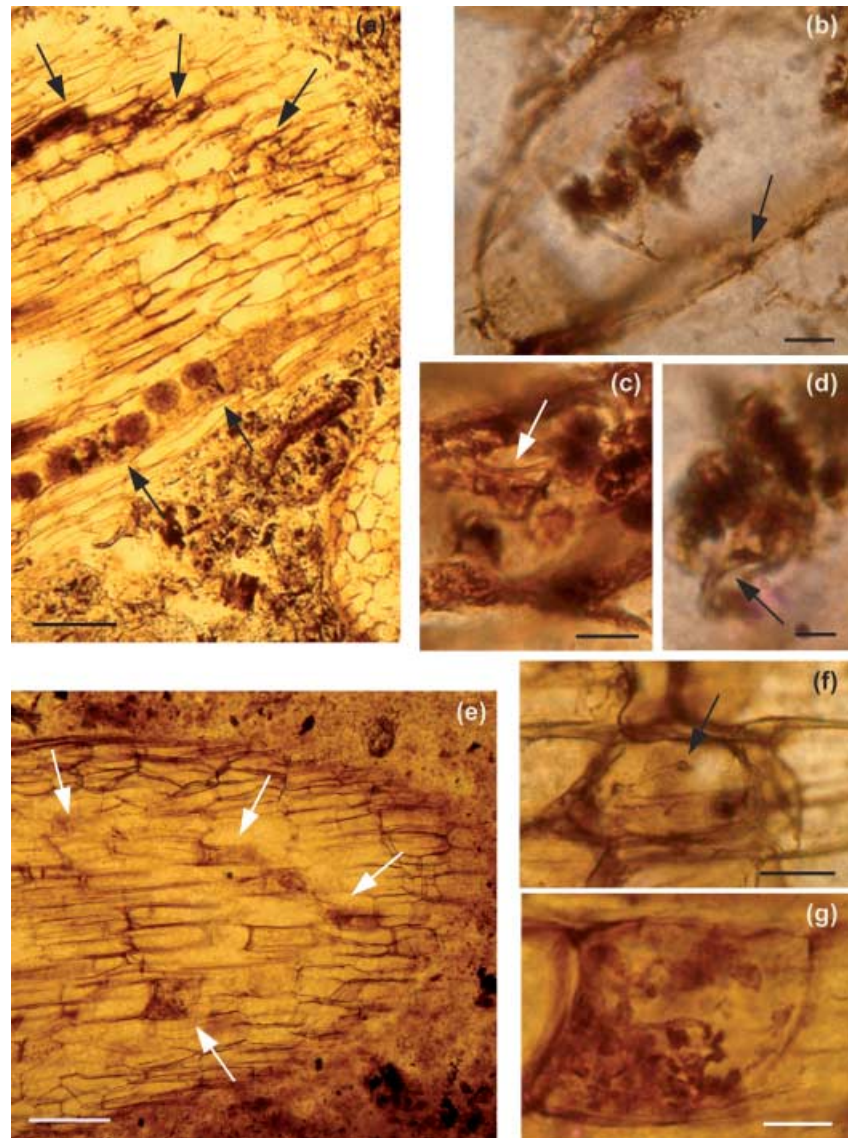
1932). However, the cortical network that characterizes the *Radiculites*-type rootlets has not been reported for *Amyelon*-type rootlets (Cridland, 1964). As a result, these two types of rootlets appear to belong to different genera of Cordaites. The colonization of *Amyelon radicans* by endophytic fungi has not yet been reinvestigated.

The relationship of *R. reticulatus* rootlets to the Cordaites was established by Lignier (1911). Attachment of the *Radiculites*-type rootlets to larger cordaitalean roots have been observed in three of the Lignier's slides. Mycorrhizal associations occur in the rootlets that correspond to the primary state of growth of cordaitalean roots (Fig. 1a). The best preservation occurs in the material from the Lignier and Carpentier collections, where many rootlets show evidence for mycorrhization. Rootlet diameters vary from 0.5 to 0.65 mm. The fungus colonizes a discontinuous fungal zone in the central layers of the cortex (Fig. 2a,e, arrows). Colonization is characterized by the absence of intercellular phase and by the development of intracellular coiled hyphae (Fig. 2f), which spread from cell to cell. While vesicles have not been observed in the cordaitalean rootlets, small arbuscules occur in some of the cortical cells. The arbuscules are most easily recognized in longitudinal sections (Fig. 2b–d). As in living plants, the cell wall shows a slight thickening, called an apposition, that forms at the point of entry of the fungal hypha (Fig. 2b). Arbuscules (Fig. 2b–d) originate from the coiled hyphae and are morphologically identical to those seen in arbuscular mycorrhizas of extant

plants. The hyphal trunk of the arbuscule is 2  $\mu\text{m}$  wide and branches repeatedly to form a bush-like tuft within the cell (Fig. 2c,d). Moreover, a few of the cortical cells appear to be filled with material similar in appearance to the amorphous masses that are the result of arbuscule degeneration in the cells of living plants (Fig. 2a). Based on the evidence assembled, we suggest that the AM association in *R. reticulatus* is of the *Paris* type (Strullu, 1985). The endophyte is only associated with ontogenetically young axes. Additional details of the association are difficult to resolve, owing primarily to the prominence of the cortical thickenings in the rootlets; a similar masking of fine details of the mycorrhiza by cortical cell thickenings has been recorded for extant plants with *Paris*-type mycorrhizas (cf. *Thuja occidentalis*, Brundrett *et al.*, 1990).

In a recent survey (Strullu-Derrien & Strullu, 2007), we reported the distribution of mycorrhizal associations in fossil and extant plants. The oldest fossil evidence for the existence of mycorrhizas occurs in *Aglaophyton major* from the Lower Devonian Rhynie chert (Remy *et al.*, 1994; Taylor *et al.*, 2005). Robust arbuscules and vesicles have been found in *Antarcticycas schopfii* from the Triassic (Stubblefield *et al.*, 1987) and mycorrhizal associations have been described in Lower Cretaceous and Middle Eocene conifer roots (Stockey *et al.*, 2001). The colonization of the Carboniferous cordaitalean *R. reticulatus* rootlets described here is the oldest unequivocal fossil evidence for eumycorrhizas (i.e. fungal–plant root associations) and the oldest evidence for mycorrhizal associations in the conifer clade.





**Fig. 2** Eumycorrhizas in *Radiculites reticulatus* rootlets in Lignier's (a–d) and Carpentier's (e–g) materials. (a) Detail of the longitudinal rootlet (see Fig. 1a) showing infected cells distributed in a distinct discontinuous zone (arrows). (Lignier's slide no. 194). Bar, 0.1 mm. (b) Detail of an arbuscule. The hyphal trunk of the arbuscule is 2 µm wide and branches repeatedly to form a bush-like tuft within the cell. A point of penetration by the hyphae can be observed (arrow). (Lignier's slide no. 194). Bar, 10 µm. (c) Detail of an arbuscule in transverse section. Note a fine-branched hypha that corresponds to the base of an arbuscule (arrow) (Lignier's slide no. 194). Bar, 8 µm. (d) Arbuscule in high magnification showing a characteristic hyphal trunk (Lignier's slide no. 194). Bar, 4 µm. (e) Longitudinal section of a rootlet showing the infected zone (arrows) (Carpentier's slide). Bar, 0.1 mm. (f) Coiled hyphae in one of the infected cell (Carpentier's slide). Bar, 20 µm. (g) One cell invaded by the fungus (Carpentier's slide). Bar, 10 µm.

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**Christine Strullu-Derrien<sup>1,2\*</sup>, Jean-Philippe Rioult<sup>3</sup> and Désiré-Georges Strullu<sup>1</sup>**

<sup>1</sup>Université d'Angers, Laboratoire Mycorhizes, Faculté des Sciences, 2 boulevard Lavoisier, F-49045 Angers Cedex, France; <sup>2</sup>Paléobotanique, Paléopalynologie, Micropaléontologie, Université de Liège, B-4000 Liège 1, Belgium; <sup>3</sup>Equipe de Recherches et d'Etudes en Mycologie, Département de Botanique, Mycologie et Biotechnologie, UFR des Sciences pharmaceutiques F-14032 Caen Cedex, France (\*Author for correspondence: tel +33 (0)2 41 73 53 56; email christine.strullu-derrien@univ-angers.fr)

## References

- Brundrett M, Murase G, Kendrick B. 1990. Comparative anatomy of roots and mycorrhizae of common Ontario trees. *Canadian Journal of Botany* 68: 551–578.
- Carpentier A. 1932. Etude de végétaux à structure conservée. Silex Stéphanien de Grand-Croix (Loire). *Mémoires et travaux des Facultés catholiques de Lille* 40: 1–30.
- Cridland AA. 1962. The fungi in cordaitan rootlets. *Mycologia* 54: 230–234.
- Cridland AA. 1964. *Amyelon* in American coal-balls. *Palaeontology* 7: 186–209.
- Dotzler N, Walker C, Krings M, Hass H, Kerp H, Taylor TN, Agerer R. 2008. Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-year-old Rhynie chert. *Mycological Progress* 112: 1107–1114.
- Doubinger J, Vetter P, Langiaux J, Galtier J, Broutin J. 1995. La flore fossile du bassin houiller de Saint Etienne. *Mémoires du Muséum d'Histoire Naturelle, Paris* 164: 1–351.
- Gerrath JM, Covington L, Doubt J, Larson DW. 2002. Occurrence of phi thickenings is correlated with gymnosperm systematics. *Canadian Journal of Botany* 80: 852–860.
- Halket AC. 1930. The rootlets of *Amyelon radicans*, Will.; their anatomy, their apices and their endophytic fungus. *Annals of Botany* 44: 865–905.
- Krings M, Dotzler N, Taylor TN, Galtier J. 2007. A microfungus assemblage in *Lepidodendron* from the Upper Visean (Carboniferous) of central France. *Comptes Rendus Palevol* 6–7: 431–437.
- Lignier O. 1906. *Radiculites reticulatus* radice fossile de Sequoïinée. *Bulletin de la Société Botanique de France* 53: 193–201.
- Lignier O. 1911. Les *Radiculites reticulatus* Lignier sont probablement des radiceles de Cordaitales. *Association Française pour l'Avancement des Sciences* 40: 509–513.
- Osborn TGB. 1909. The lateral roots of *Amyelon radicans* Will. and their mycorrhiza. *Annals of Botany* 23: 603–611.
- Remy W, Taylor TN, Hass H, Kerp H. 1994. 400 million year old vesicular arbuscular mycorrhizae (VAM). *Proceedings of the National Academy of Sciences, USA* 91: 11841–11843.
- Schüßler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research* 105: 1413–1421.
- Stockey RA, Rothwell GW, Addy HD, Currah RS. 2001. Mycorrhizal association of the extinct conifer *Metasequoia milleri*. *Mycological Research* 105: 202–205.
- Strullu DG. 1985. *Les Mycorrhizes, handbuch der pflanzenanatomie*. Berlin–Stuttgart, Germany: Gebrüder Borntraeger.
- Strullu-Derrien C, Strullu DG. 2007. Mycorrhization of fossil and living plants. *Comptes Rendus Palevol* 6–7: 483–494.
- Stubblefield SP, Taylor TN, Trappe JM. 1987. Vesicular–arbuscular mycorrhizae from the Triassic of Antarctica. *American Journal of Botany* 74: 1904–1911.
- Taylor TN, Hass H, Kerp H. 2005. Life history biology of early land plants: deciphering the gametophyte phase. *Proceedings of the National Academy of Sciences, USA* 102: 5892–5897.
- van Tieghem P. 1888. Le réseau de soutien de l'écorce de la racine. *Annales des Sciences Naturelles Botanique* 7: 375–378.
- Zimmermann W. 1933. Paläobotanische und phylogenetische beiträge 1–4. *Palaeobiologica* 5: 321–348.

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