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Nucleation/Nucléation

Cavitation in trees

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Abstract

Sap is transported under tension (i.e. negative pressure) in trees, according to the tension-cohesion theory. Since water is physically instable under negative pressure, a risk of cavitation is possible. Techniques have been developed during the past two decennia to study cavitation in trees. Trees appear remarkably immune to cavitation events. Cavities form only when extreme water stresses occur or when sap freezes. Nucleation is heterogeneous in trees, presumably caused by the aspiration of air bubbles through conduit walls. Threshold xylem pressures for cavitation vary greatly between species, in concordance with the great functional and ecological diversity of trees. ***To cite this article: H. Cochard, C. R. Physique 7 (2006).***

Résumé

Cavitation chez les arbres. Le transport de l'eau dans les arbres s'effectue sous tension (pression négative) selon la théorie de la tension–cohésion. L'eau étant physiquement instable dans ces conditions, les colonnes de sève sont sujettes à un risque de cavitation. Au cours des deux dernières décennies, des techniques ont été développées pour étudier ce phénomène de cavitation chez les arbres. Bien qu'opérant constamment sous pressions très négatives, les arbres sont remarquablement prémunis du risque de cavitation. Ce n'est que lors de contraintes hydriques exceptionnelles (grandes sécheresses) ou face à des cycles de gel–dégel, que la cavitation se produit. La cavitation à pour origine une nucléation hétérogène, très probablement par l'aspiration d'une bulle d'air à travers les parois des conduits. Les pressions seuils de cavitation sont extrêmement variables entre espèces, caractère que l'on peut mettre en relation avec la grande diversité fonctionnelle et écologique des arbres. ***Pour citer cet article : H. Cochard, C. R. Physique 7 (2006).***

Keywords: Cavitation; Embolism; Tree; Tension; Drought; Xylem

Mots-clés : Cavitation ; Embolie ; Arbre ; Tension ; Sécheresse ; Xylème

1. Introduction

Each day, hundreds of litres of water circulate in the trunk of a mature tree and are lifted up to its canopy tens of meters above ground. Leaves have to allow the evaporation of such a large amount of water to uptake carbon dioxide and regulate their surface temperature. The machinery behind the process of water transport has to be remarkably efficient and reliable, as any dysfunction may impair tree hydration and, hence, its survival. Trees have evolved a very efficient and cost-effective pumping system. However, as the system is operating under large negative pressures, trees live under the constant threat of water cavitation.

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In this article I will briefly present our current understanding of long distance water transport in trees and review recent investigations on sap cavitation. If this short review can promote further interactions between physicists and physiologists on this field, its main objective will be fulfilled.

2. Long-distance water transport in trees

To understand water transport in trees, it is essential to visualize how trees are constructed and where water can flow (Fig. 1).

Trees, like any plants, are made of cells with rigid and hygrophilous walls formed of cellulose and hemi-cellulose microfribrils. Most cells are surrounded by a lipid bilayer *plasmalemma membrane* in contact with the wall. The plasmalemma membrane delimits the *cytoplasm*, a solution with a high solute concentration (the osmotic potential Π is typically in the range of -1 to -2 MPa). This membrane is hemi-permeable, i.e., it is permeable to water molecules but selectively permeable to solutes. This selectivity is quantified by the reflection coefficient σ of the membrane for a given solute [1]. From the point of view of water relation, a hemi-permeable membrane enables uptake of water by osmosis, but the permeability of the bi-lipidic layers is relatively low. However, this permeability is greatly enhanced by specific water-channel proteins, *aquaporins* [2]. Cytoplasm can communicate between cells in contact through *plasmodesmata* without crossing plasmalemma membranes. Therefore, all the interconnected cytoplasm determines a specific water compartment, the *symplasm*. Water located outside of the symplasm, e.g. in the wall matrix, forms a second compartment: the *apoplasm*.

Trees, like other vascular plants, have developed a very specific tissue devoted to long-distance water transport: the *xylem*. The xylem contains cells that have undergone profound modifications during their maturation: the lateral walls have been reinforced by a deposit of lignin, a hydrophobic polymer, and the cytoplasm has been dissolved. This type of cells is called *tracheids*. In broadleaves trees, cell end walls are partly or completely dissolved, thus forming very long conduits: the *vessels*. Xylem conduits (tracheids and vessels) and hence capillaries of various dimensions (1 mm

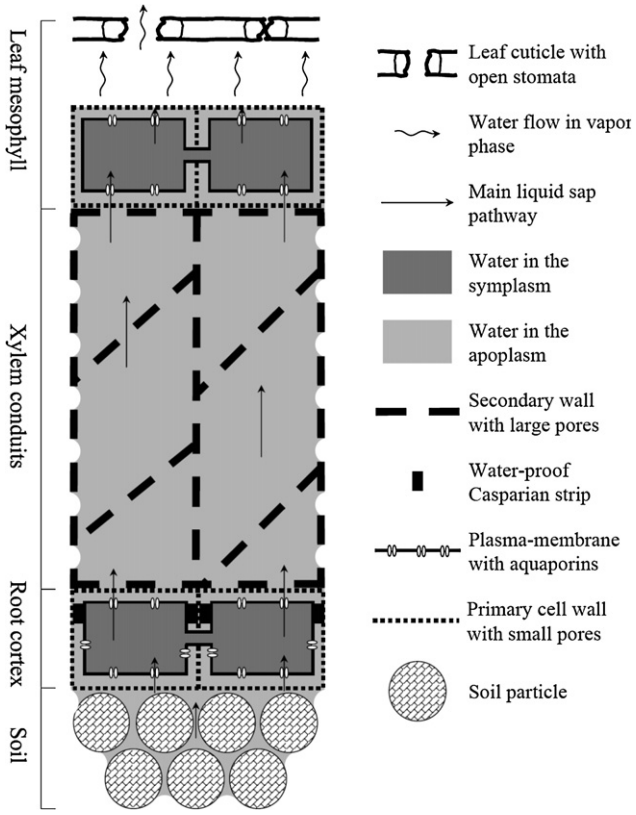

Fig. 1. A schematic representation of the structure of water pathways in trees. See text for details.

to several meters in length and 10 to 400 μm in diameter). These pipes are made of dead cells and water moves from one conduit to another across cell walls without crossing any membrane. Hence, water in the xylem conduits belongs to the *apoplasm*.

Water movements within the symplasm and within the apoplasm are caused by differences in *hydrostatic pressures* ΔP:

$$F = K.\Delta P \tag{1}$$

with F the bulk water flow (kg s^{-1}) and K a diffusive coefficient.

To account for the water movements across a plasmalemma membrane (i.e. from the symplasm to the apoplasm between two cytoplasts not connected by plasmodesmata) we must further consider the difference of *osmotic potential* $\Delta\Pi$ between the two compartments and the reflection coefficient of the membrane:

$$F = K.(\Delta P + \sigma\Delta\Pi) \tag{2}$$

If the membrane is perfectly hemi-permeable ($\sigma = 1$) the $\Delta P + \Delta\Pi$ represents the difference in *water potential* Ψ, namely, the chemical potential of water μ divided by its partial molar volume. Hence:

$$F = K.\Delta\Psi \tag{3}$$

All the water crossing the tree comes from the soil. Soil is a porous media made of small aggregates and particles retaining water between them. Water in the soil is very dilute, containing only a few millimols of different ions. When the soil is very wet, its water potential Ψ is hence close to the water potential of pure water at atmospheric pressure. By convention this potential is set to 0 MPa. When the soil dehydrates, the large pores empty first, water then retracts into pores of smaller dimension. This shape change of the interface between air and water lowers the negative hydrostatic pressure P_{soil} of water according to the Laplace law [3]:

$$P_{\text{soil}} = -2\gamma/r \tag{4}$$

with γ the water–air surface tension and r the radius of curves meniscus. Note that Eq. (4) assumes that the contact angle between the meniscus and the particles is nil.

As an example, for a soil that lost 80% of its water available for trees, $r = 0.3$ μm and $P_{\text{soil}} = -1$ MPa [4]. Differences in hydrostatic pressure ΔP_{soil} explain water movements in soils:

$$F = K_{\text{soil}}.\Delta P_{\text{soil}} \tag{5}$$

with K_{soil} a diffusive coefficient defining the soil *hydraulic conductivity*. K_{soil} is greatly influenced by the porosity of the soil fraction containing water, and, hence, P_{soil}. When soil dries out, K_{soil} decreases exponentially and can limit water transport to tree roots [5].

Tree roots are in direct contact with soil water. Root hairs, mycorrhisal hyphes and mucilages greatly amplify this contact. When water enters the roots it can theoretically travel through the cell walls or enter the symplasm. However, the flow is mostly directed to the symplasm for two reasons. First, in the root cortex the wall apoplasmic pathway is usually interrupted by the Casparian strip, a deposit of hydrophobic suberin. Hence the water flow is here forced to enter the symplasm. Second, aquaporins in the plasmalemma membrane greatly increase its permeability and water will flow in this least resistive pathway. There are two main advantages for interrupting the apoplasmic pathway between the root and the soil. First, this structure enables a selective and active uptake of ions from the soil solution. Specifics proteins in the plasmalemma membrane are implicated in this process. Second, the tree will be able to build up a positive hydrostatic pressure in the xylem compartment under certain circumstances (i.e. spring root pressures) that may refill cavitated conduits.

Water enters the root xylem by reverse-osmosis (Eq. (2)) and flows to the leaves in the xylem conduits. Water in the xylem is virtually as dilute as water in the soil and moves according to difference in hydrostatic pressures (Eq. (1)). This xylem pathway represents about 99% of the total water pathlength in the tree. This explains why trees have evolved a complex structure, maximising transport efficiency while minimizing risk of failure. The most striking feature of xylem conduits is their lack of plasmalemma membrane and, hence, cytoplasma. Xylem conduits are made of dead cell walls. This greatly increases their hydraulic efficiency. Wall permeability is also increased by the presence of many pores (*pits*).

The water flow from the xylem conduits to the leaf mesophyll is virtually symmetrical to flow from the root cortex to the xylem, with the difference that leaves lack casparian trip. Recent evidence [6] suggests that aquaporin

inhibition strongly reduces this flow, suggesting that most water flows across the leaf symplasm and a limited amount in the apoplasm. Water eventually evaporates between the microfibrils of the mesophyll cell walls, in the stomatal chambers. As in the soil, water forms curved meniscus in this wall and the same Laplace law applies here (Eq. (4)). Wall porosity at the evaporation sites is very small (tens of nanometers) and the meniscus can, in theory, compensate a difference in hydrostatic pressure of tens of MPa. In other words, these meniscuses can resist the load of a water column of several Km high! However, we will see that trees never achieve such low pressures because of their xylem's vulnerability to cavitation.

From this description of water pathways in trees, it is important to realise that liquid water forms *continuous* columns from the soil to the sites of evaporation in the leaves where the columns are 'suspended' to tiny meniscuses. Hydrostatic pressures in the soil being at most nil and usually under negative pressures and the force of gravitation lowering this pressure by −0.1 MPa every 10 m, the pressure in the xylem conduits is under negative pressures (i.e., under *tension*). Hence, at the top of a 100 m tall *Sequoia*, the xylem pressure is lower than −1 MPa. When water evaporates in the leaves, the radius of air/water meniscuses tend to decrease, thus increasing the capillary forces due to the surface tension of water. These forces pull upwards the water molecules at the meniscuses, but because the cohesive forces between water molecules are much larger, it is the entire water column which is pulled upwards. Water then ascents the tree just like when one pulls up a rope. Because water has to find a path through pores and membranes, there is a resistance to the water flow. This resistance further lowers the negative xylem pressure proportionally to the water flow. As a consequence, xylem pressures of most temperate trees typically reach values as low as −2 MPa in the middle of the day. Much more negative values are measured in Mediterranean trees. The mechanism described above is known as the Cohesion–Tension Theory and was first proposed in the late 19th century [7,8].

3. Cavitation in trees

Since the sap pressure is most of the time under large negative pressures, trees live under the threat of cavitation. Cavitation would break the continuity of water columns and hence the water supply to transpiring leaves. Although the risk of cavitation was considered by earlier proponents of the Cohesion–Tension Theory, it is only recently that techniques have been developed to assess its significance for sap transport in trees.

3.1. Techniques to measure cavitation and embolism in trees

3.1.1. Direct observations

Direct evidence of cavitation in trees is readily obtained when a cut branch of a tree is allowed to absorb a coloured liquid (such as safranine of phloxine). On a cross-section we can observe some stained conduits, while others remained uncoloured. The former transported the liquid while the latter were air-filled and thus non-functional. There is, of course, a lot of drawbacks with this simple procedure (for instance, water filled conduits may be hydraulically isolated and remain uncoloured), and therefore more sophisticated direct techniques have been used to detect cavitation in trees. For instances, samples have been frozen with liquid nitrogen and the content of xylem conduits observed, still frozen, with cryogenic scanning electronic microscopes [9]. However, it has been demonstrated that freezing-induced cavitation could occur during sample preparation which of course biases the observation [10].

3.1.2. Acoustic emissions

Acoustic emissions are produced during the sudden breakdown of a water column by cavitation. The range of theses emissions is extremely broad, from audible to ultrasonic frequencies. Earlier investigators [11] have used detectors in the audible range. Although the technique was very laborious and hence rapidly abandoned, it must be emphasized that these pioneering investigations have called the attention of plant physiologists to the problem of cavitation in plants and have been decisive in promoting further research. Tyree and Dixon [12] have detected acoustic emissions in the ultrasonic frequencies (typically 100–300 kHz). The technique was really appealing at first sight because cavitation could be monitored under field conditions without injuring the trees. However, it has been demonstrated that acoustic events do not necessary correspond to cavitation events in xylem conduits [13,14] so the techniques will remain imprecise until more cavitation-specific acoustic signals have been identified [15].

3.1.3. Hydraulic techniques

Cavitation causes an embolism, i.e., a thrombosis that blocks the water flow in the xylem conduits. As a consequence, xylem hydraulic conductance (K) is decreased. Techniques have been developed to detect the degree of loss

of hydraulic conductance due to embolism [16]. The percent loss of hydraulic conductance (PLC) can be determined as:

$$\mathrm{PLC} = 100(1 - K/K_{\mathrm{sat}}) \tag{6}$$

where K_{sat} is the saturated xylem hydraulic conductance. K is computed according to Eq. (1), usually with a positive pressure head (typically 4 kPa).

The technique is destructive because K is determined on small wood samples (typically 3–10 cm long) but is preferred to other techniques because it measures the quantitative effect of cavitation on water transport capacity. A specific apparatus based on this technique has been recently released (XYL'EM, Bronkhorst-France).

3.2. Occurrence of cavitation in trees

Typical time courses of xylem embolism are shown in Fig. 2 for two contrasted species. The behaviour of the first species is representative of species with long and large conduits (i.e. ring porous species like Oak or Ash). The second is representative of species with more narrow conduits (i.e. diffuse porous species like Birch or Beech). The first year was characterized by a very dry summer which induced a significant level of embolism for both species. When negative air temperatures were recorded, a drastic increase in embolism was noticed for the ring-porous species, whereas embolism developed more progressively all over the winter in the species with smaller conduits. In spring time, the ring porous species produced new functional conduits but the older conduits remained permanently embolised. On the contrary, xylem conduits recovered from embolism in spring.

From the analysis of the above data, we can conclude that: (i) summer drought can induced embolism; (ii) cold temperature provokes embolism during winter time but xylem anatomy greatly influences the process; and (iii) recovery mechanisms exist for some species.

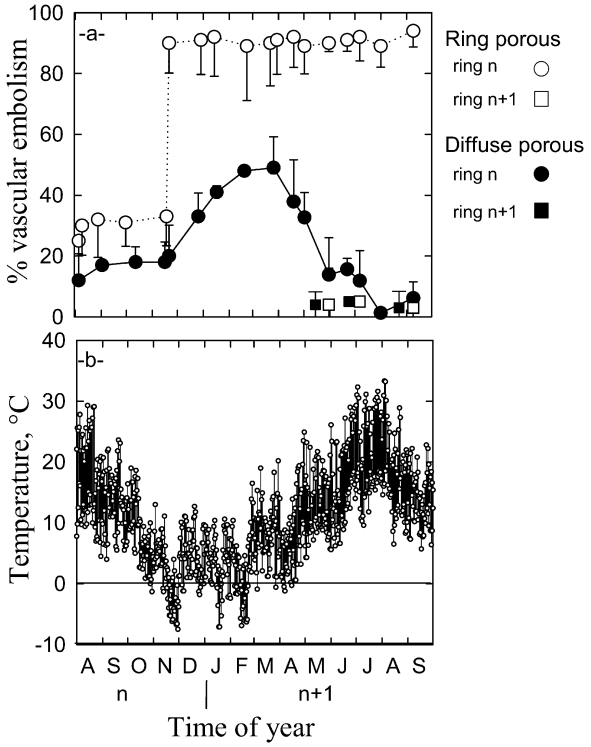

Fig. 2. (a) Typical time courses of xylem embolism for diffuse and ring porous species in temperate areas. Embolism is measured in terminal branches for two consecutive years (year n and year $n + 1$). (b) Minimum and maximum daily temperatures.

3.3. Xylem vulnerability to freezing stress induced-cavitation

The process of cavitation induced by a freezing stress in trees has been well documented. Because gas is not soluble in ice, and because sap is saturated with gas, air bubbles form when sap freezes in xylem conduits. The fate of these bubbles upon thawing will determine conduits vulnerability. The stability of a bubble in a solution is determined by the Laplace law, i.e. by the radius of the bubble and the negative pressure in the liquid (Eq. (4)). The larger the bubble, or the lower the pressure, the higher the chance for the bubble to spread and cavitate the conduit. Large bubbles are more likely to form in larger conduits, and therefore large conduits are more sensible for freezing-induced cavitation. Davis et al. [17] have shown that for conduit diameters below 30 μm, the risk of freezing-induced cavitation is very low, but increases very rapidly above this diameter. This explains why conifers, which have small conduits, are usually very resistant to freeze-thaw cycles. However, Mayr et al. [18] have shown that a combination of low xylem pressure and repeated freeze-thaw cycles could provoke cavitation by a process more related to drought stress-induced cavitation (see below).

3.4. Xylem vulnerability to drought stress-induced cavitation

The xylem vulnerability of a tree species to drought stress-induced cavitation is assessed by determining how cavitation relates to xylem pressure P_x. This response curve is called a *vulnerability curve* and is typically obtained by plotting percent loss conductance (PLC) versus xylem pressure. Various techniques have been used to dress vulnerability curves (bench drying, air pressurisation) but the newly introduced 'Cavitron' technique has overcome most of the previous limitations [19]. The technique uses the centrifugal force to lower the xylem pressure in wood segments centred on a dedicated rotor. By varying the angular velocity, the xylem pressure can be adjusted with great accuracy (±0.01 MPa). Sample hydraulic conductance is determined during centrifugation by measuring the water flow rate through the sample. Thus by measuring the impact of decreasing xylem pressures on sample conductance, a vulnerability curve can be obtained in typically 30 minutes. The technique is fast and reliable, except when most conduits are longer than the sample length.

Vulnerability curves have now been dressed for hundreds of tree species. A typical curve is show on Fig. 3. It can be seen that embolism forms only when pressure decreases below a threshold value and then increases rapidly to 100 PLC. Vulnerability curves have hence typically a sigmoid shape and are satisfactorily described by the following two parameters equation [20]:

$$\mathrm{PLC} = \frac{100}{1 + \mathrm{e}^{\frac{\sigma}{25}(P_x - P_{50})}} \tag{7}$$

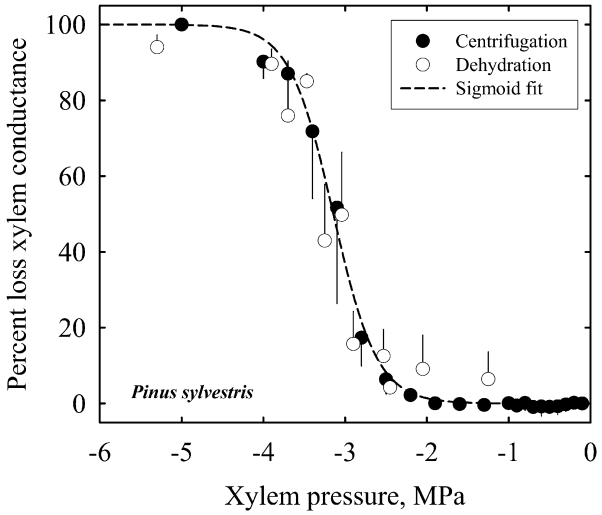

Fig. 3. Xylem vulnerability curves for apical branches of *Pinus sylvestris*. Curves were obtained by dehydrating shoots on a bench or by centrifugation of cut stems. The two techniques give typical sigmoid curves.

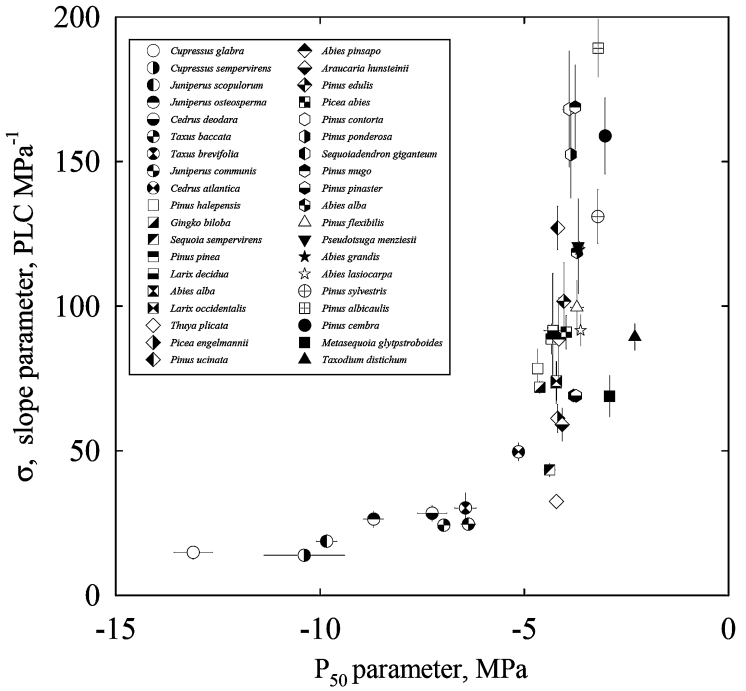

Fig. 4. Vulnerability of different coniferous species to water stress-induced embolism as assessed with the centrifugation technique. The two axes represent the two parameters of Eq. (7). P_{50} is the xylem pressure inducing 50% loss of conductance. σ is the slope of the curve at the inflection point. Unpublished data from H. Cochard and S. Delzon.

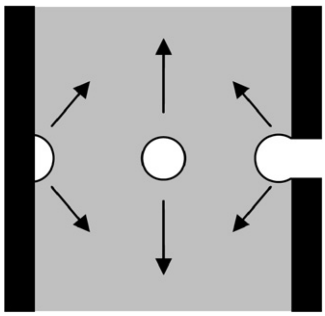

Fig. 5. Three possible sources of nucleation that may explain cavitation in trees: loss of cohesion between water molecules (centre), loss of adhesion at the inner conduit wall (left), or aspiration of a air bubble through a pore in the conduit wall (right). The later mechanism is the most probable for trees.

where P_x is the xylem pressure, P_{50} the P_x value provoking 50 PLC (inflection point), and σ, the slope of the curve at the inflection point.

Considerable variations in P_{50} and σ exist between species. Fig. 4 shows how P_{50} and σ co-vary amongst different conifer species. P_{50} ranges from -2 down to -13 MPa for the most cavitation resistant species. Interestingly P_{50} and σ vary in inverse proportion, suggesting that the most vulnerable species tend to embolise more rapidly.

3.5. Mechanisms for drought stress-induced cavitation

Theoretically, two possible mechanisms could explain the induction of cavitation in trees (Fig. 5): a loss of cohesion between water molecules in the volume of xylem conduits (homogeneous cavitation), or a loss of adhesion between water and conduit walls (heterogeneous cavitation) [21]. For the latter mechanism, a distinction can be made between a loss of adhesion at the inner wall of the conduit or through a hole in the wall. The rupture of cohesive forces between water molecules is thought to occur only at pressures below -20 MPa [22,23], i.e. much below the most negative pressures recorded in xylem sap (around -13 MPa). Therefore the hypothesis of homogeneous cavitation in trees is usually discarded. However, there are currently no reliable estimates of the tensile strength of sap. Sap being a complex solution, saturated with gas, it remains possible that its tensile strength may substantially deviate from the tensile strength of pure degassed water.

Cavitation is most certainly heterogeneous in trees but the exact mechanism is still debated. There is experimental evidence in favour of an air-seed through pores in the wall. For instance, Cochard et al. [24] have shown that increasing air pressure on the outside wall of the conduit to $+P$ has the same effect as lowering the xylem pressure to $-P$. This suggests that cavitation is caused by the capillary rupture of an air/water meniscus located on a pore through the

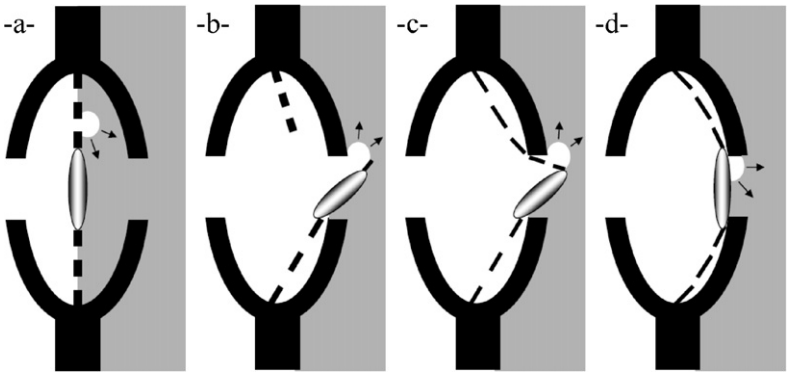

Fig. 6. Different possible processes of nucleation in conifers. See text for details.

conduit wall. The biggest pores on walls are probably found in the pit wall but it is also possible that microfractures in the wall might represent another source of nucleation that would lead to cavitation of the water column [25]. There is still a lot of incertitude about the exact process of nucleation through the pit wall. For instance, on broadleaved trees a discrepancy has been reported between the cavitation pressure, the porosity of the pit wall and the prediction of Laplace law (Eq. (4)) [26]. However, observations are been made on dehydrated material and on a relaxed pit wall and these may not represent the actual wall porosity during air-seeding.

The process or air-seeding in conifer is also not fully understood [27]. Pits in conifers look like trampolines: a thick central torus surrounded by a fibrillose margo enclosed in over-arching walls (Fig. 6). When the two conduits in contact are water filled, the pit wall lies in the middle of the pit chamber and water flows through the margo. When one conduit cavitates, air could enter the conduit in contact through pores in the margo (Fig. 6(a)). However, these pores are usually too large to account for the cavitation pressure in conifers. But these pores are small enough to displace the pit wall and aspirate it against conduit wall (as in Fig. 6(d)). This valve action is thought to prevent the propagation of air in adjacent conduits. Large xylem tensions could brake (Fig. 6(b)) or stretch (Fig. 6(c)) the fibrils in the margo and hence pull the torus through the pit aperture. This would nucleate an air seed and cavitate the conduit in contact. An important distinction between situations (b) and (c) is that in (b) the pit margo is ruptured and the conduit irreversibly damaged. In (c), the torus could still get back to its initial position. A last possibility can be imagined (Fig. 6(d)). Under scanning microscopy, the torus and the inner wall of the pit chamber never appear perfectly smooth. Therefore, the seal between the torus and the wall on aspirated pits is probably porous and air could then seed through these pores. Clearly more work is needed to understand how the structural and mechanical properties of pit walls relate to conduit vulnerability to cavitation in trees.

4. Conclusions

Water transport in trees occurs under large negative pressures and, therefore, a risk of cavitation of the water columns is possible. Plant physiologists have developed techniques and accumulated a large amount of information on this process during the past two decades. It is now clear that the risk of cavitation varies greatly between species and this has profound implications for tree water relations and their ecological preferences. However, the detail of the mechanism remains to be understood, a challenge that should stimulate cooperation between physiologists and physicists.

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