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Damien Lemoine · André Granier · Hervé Cochard

Mechanism of freeze-induced embolism in *Fagus sylvatica* L.

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Abstract The mechanism of freeze stress-induced embolism in *Fagus sylvatica* L. branches was analyzed under controlled conditions. Excised branches were exposed to successive freeze-thaw cycles in temperature controlled chambers. Thermocouples were placed on the bark to detect sap freezing exotherms. The degree of xylem embolism was estimated after each cycle by the loss of hydraulic conductivity. After one freeze-thaw cycle the degree of embolism was found to decrease with xylem specific hydraulic conductivity, small apical shoots being more susceptible to embolism. Exotherms revealed that distal shoots were freezing first and exuded sap as a result of water expansion. The lower water content in apical shoots upon freezing probably induced higher sap tensions which promoted air bubble expansion and vessel cavitation preferentially near the apices. When the decrease in water content was experimentally prevented, embolism developed to a lesser extent. The higher vulnerability of shoot apices may protect the rest of the branch from winter damage.

Key words Beech (*Fagus sylvatica* L.) · Cavitation · Conductivity · Xylem embolism · Freeze-thaw

Introduction

Winter embolism is probably the main cause of xylem dysfunction in temperate tree species (Sperry and Sullivan 1992; Wang et al. 1992). In species of oak, for instance, the degree of xylem embolism following prolonged water stress remains low whereas one freezing event may be sufficient to entirely impair all xylem conduits (Cochard and Tyree 1990; Cochard et al. 1992; Sperry and Sullivan 1992). Xylem vulnerability to freezing stress appears to vary considerably among species (Wang et al. 1992). This property may influence plant success in colder environments (Tyree et al. 1994; Langan et al. 1997).

The mechanism of xylem embolism production by sap freezing has been widely documented (Sucoff 1969; Sperry et al. 1988; Just and Sauter 1991; Sperry and Sullivan 1992; Lo Gullo and Salleo 1993; Améglio et al. 1995; Langan et al. 1997). Current thinking may be summarized as follows: When sap freezes, air is released from solution and forms bubbles which become trapped in the ice. The risk of embolism formation will depend on the stability of these bubbles when released into the liquid phase upon thawing. This stability is mainly determined by the balance between the pressures exerted on the air-water meniscus: the xylem pressure potential (Px) and the bubble capillary pressure (Pc). If Px is greater than Pc then the bubble expands, if Px is less than Pc, the bubble contracts and vanishes. Good agreement has been found experimentally between conduit volume and vulnerability to freeze-thaw events with larger conduits being more vulnerable (Cochard and Tyree 1990; Wang et al. 1992; Sperry and Sullivan 1992; Lo Gullo and Salleo 1993). This may be explained if larger bubbles (lower Pc) form in larger conduits or if Px decreases faster in wide vessels.

Several studies have reported effects of freezing on xylem embolism in *Fagus sylvatica* L. (Borghetti et al. 1993; Magnani and Borghetti 1995; Hacke and Sauter 1996). The patterns of embolism development were typical of a diffuse-porous species with a graduated increase over winter (Sperry et al. 1988). However, no attention has been paid in these studies to within-branch variation on vulnerability. In a recent field survey (Cochard and Lemoine, unpublished data), we found during winter a higher degree of embolism in 1-year-old apical twigs compared to older shoots. This contradicts the theory that larger conduits are more vulnerable because vessels are narrower and shorter near the apices in *Fagus*. In this paper we report on two experiments undertaken to try to elucidate this anomaly in *F. sylvatica*. We tested the hy-

D. Lemoine · A. Granier · H. Cochard (✉)[1]
Unité d'Ecophysiologie Forestière, INRA Nancy,
F-54280 Champenoux, France

Present address:
[1] INRA-PIAF, Site de Crouël, F-63039 Clermont-Ferrand, France
e-mail: cochard@clermont.inra.fr; Fax: 33 4 73 62 44 54

pothesis that apical twigs were freezing and thawing initially in a branch and that the subsequent xylem water deficit would induce further embolism in the xylem sap near the apices. In the first experiment we simulated freeze-thaw cycles on cut branches and analyzed the patterns of ice formation and embolism development. In the second experiment, we analyzed the impact of shoot dehydration brought about by freezing on embolism formation.

Materials and methods

Plant materials

Most measurements were made on branches cut from 25-year-old beech (*Fagus sylvatica* L.) trees growing in a beech stand (Hesse state forest, 48°40′N, 7°05′E, France). Branches were collected from the sun-exposed part of the crowns with a pruning pole in January and February 1996. Branches were 4 to 6 years old, 1.5–2.0 m long and with a basal diameter of approximately 1.5–2.0 cm. In a preliminary experiment, the maximum vessel length was determined using the air perfusion method (Ewers and Fisher 1989). No vessels were cut open in the current year shoots but a few vessels were cut open in the 2- and 3- year-old growth increments. The cut ends were refreshed with a razor blade and the branches were pressurized with water (0.3 MPa, filtered through a 0.1 µm inline filter) over 4 h to dissolve any pre-existing embolism.

Experimental setup

The branches were then used for one of the two following experiments:

Experiment 1

Freeze-thaw cycles were simulated using a large temperature-controlled chamber in which intact branches could be wholly enclosed. Air temperature was gradually reduced from ambient (20°C) down to –10°C in 4 h (–7.5°C h^{-1}) and maintained at –10°C for 8–11 h. The maximum branch temperature variation rate observed in the field was close to –6°C h^{-1} (personal observation). Branches were then exposed to ambient temperature for the thawing phase. During this phase, a vacuum pump (–80 kPa pressure relative to atmospheric) was connected to the base of the branch in order to create a tension equivalent to the gravity tension occurring at the initial position above ground (ca. 8 m). For one branch, the pump was disconnected at thawing in order to infer the effects of such xylem tensions. After branches had reached ambient temperature, they were either sampled for hydraulic measurements (see below) or submitted to another cycle (up to five cycles). Because branches may have dehydrated and developed water-stress induced embolism during the thawing phases, one branch was used as control and left at room temperature for five cycles (i.e. 5 days). The branch was wrapped in a plastic bag during the freezing phases and air exposed during the thawing periods. The branch was also connected to the vacuum pump during the thawing procedure. The amount of embolism was measured on sub-branches excised before each freezing phase. A total of 15 branches were sampled for experiment 1.

In order to assess the dynamics of sap freezing, one branch was equipped with copper-constantan thermocouples to detect the freezing exotherms. Ice crystal formation dissipated latent heat and the duration of the exotherm indicated the time required for the branch to freeze (Tyree 1983). Five thermocouples were placed on the bark of 1- to 3-year-old shoots and covered with foam. A second set of thermocouples measured the reference chamber air temperature at the same locations. The difference in temperature, Δt, between the two thermocouples characterized the freezing exotherm of each shoot. To standardize the values, we subtracted the temperature difference at the onset of freezing to Δt for each set of thermocouples. Data were logged every minute with a Campbell CR7 Data Logger. This branch was exposed to a first cycle as above, refilled with distilled water and exposed to a second cycle.

Experiment 2

This second experiment aimed at continuously following the water flow through a branch segment during freeze-thaw cycles. Because water was allowed to enter the segment at both ends, xylem dehydration was minimized in this experiment. The experimental set up was similar to that described by Cochard and Tyree (1990) for *Quercus rubra*. Seven 50–70 cm long, 1- to 7-year-old segments were excised under water from beech branches. Cut ends were refreshed under water with a razor blade and fitted to water-filled tubing connected to source and sink reservoirs. The shoots were placed in a 40 cm long insulated and temperature controlled box with both ends protruding outside. On two occasions, the reservoirs were placed on the scales of two computer-controlled balances located 90 cm (source) and 130 cm (sink) below the branch. The average water tension in the branches was then –11 kPa (relative to atmosphere). For the remaining experiments, the sink reservoir was connected to a vacuum pump. The depression was monitored with an electronic pressure transducer and adjusted between –65 and –75 kPa. The average water tensions in the branches ranged between –37 and –42 kPa. The branch temperature was monitored with thermocouples mounted on the bark. The branches were exposed to two or three consecutive freeze-thaw cycles with minimum branch temperatures respectively equal to –6, -11 and –16°C.

Embolism detection

Embolism was estimated via its effect on hydraulic conductivity (Sperry et al. 1988) in different segments of the branches (corresponding to different diameters and different ages). On each branch, usually 15 2-cm-long samples were cut under water with a razor blade. The hydraulic conductivity (K_{init}, Kg m s^{-1} MPa^{-1}) was measured by perfusing the samples with degassed distilled water at 6 kPa. Conductivity was restored by flushing with water pressurized to 0.1 MPa (K_{max}). A 15 min flush was usually sufficient to restore the full capacity of the xylem. However, a second flush was performed as confirmation. The degree of loss of hydraulic conductivity (PLC) was computed as:

$$PLC=100*(1-K_{init}/K_{max})$$

At the end of the measurements, we measured the mean xylem diameter, excluding bark, to compute the saturated xylem-area-specific hydraulic conductivity (K_s, Kg m^{-1} s^{-1} MPa^{-1}). K_s is an estimator of the xylem hydraulic efficiency and is mainly controlled by vessel size (Tyree et al. 1994).

Results

Experiment 1

The effects of one freeze-thaw cycle on the loss of hydraulic conductivity (PLC) in beech branches can be seen in Fig. 1. The *x* axis represents the sample specific hydraulic conductivity (K_s) which increases with sample diameter due to wider vessels in larger twigs (not shown). When no tension was applied upon thawing only apical shoots with low K_s exhibited significant degrees

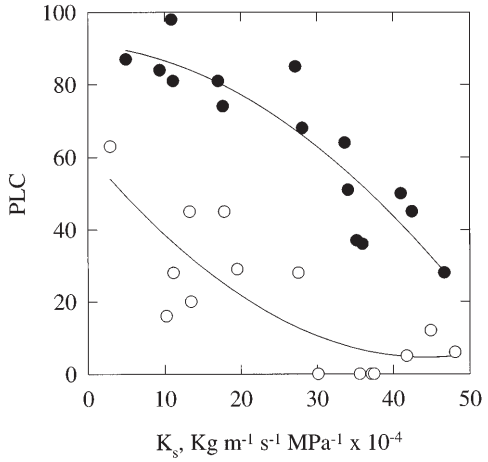

Fig. 1 Percent loss of xylem hydraulic conductivity (PLC, %) measured in internodes of two excised branches of *Fagus sylvatica* exposed to one freeze-thaw cycle. For one branch (*closed circles*) a vacuum pump was connected to the cut base upon thawing. The *x* axis represents the specific hydraulic conductivity (K_s) which is a measure of sample porosity. Each point represents an internode sampled. Xylem embolism decreased with K_s and increased when tensions were applied

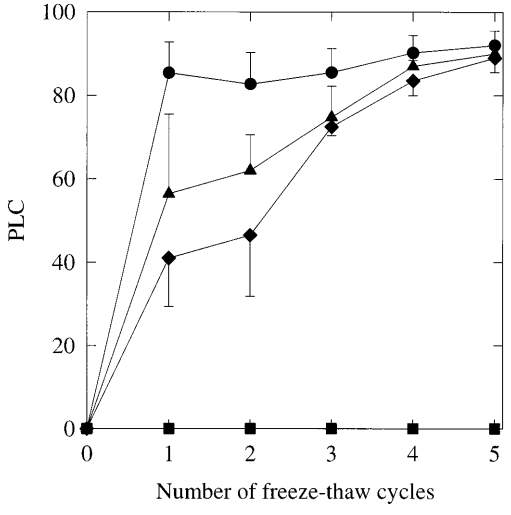

Fig. 2 Progression of embolism (PLC, %) in the xylem of cut branches exposed to successive freeze-thaw cycles (experiment 1). One branch (*squares*) was not frozen and used as a control. Xylem samples were averaged according to their K_s values in three classes (*circles*: <1.5 10^{-3}; *triangles*: 1.5–2.5 10^{-3}; *diamonds*: >2.5 10^{-3} Kg m^{-1} s^{-1} MPa^{-1}). *n*=15. *Error bars* represent one SD

of embolism (up to 60 PLC). On branches submitted to 80 kPa tension, a drastic increase in PLC (compare open and closed symbols) was measured.

Figure 2 shows the evolution of PLC with increasing number of freeze-thaw cycles. Because PLC were well correlated with sample K_s (Fig. 1) we distributed and averaged our data set for three conductivity classes. No significant water stress-induced embolism developed in the control branch at any stage. Branch segments with low K_s (apical) exhibited high PLC values (85%) after the first cycle with no subsequent significant increase in subsequent cycles (upper line on Fig. 2). The degree of PLC in samples with higher K_s values only reached 50% after the first cycle and steadily increased with repeated cycles up to 95%.

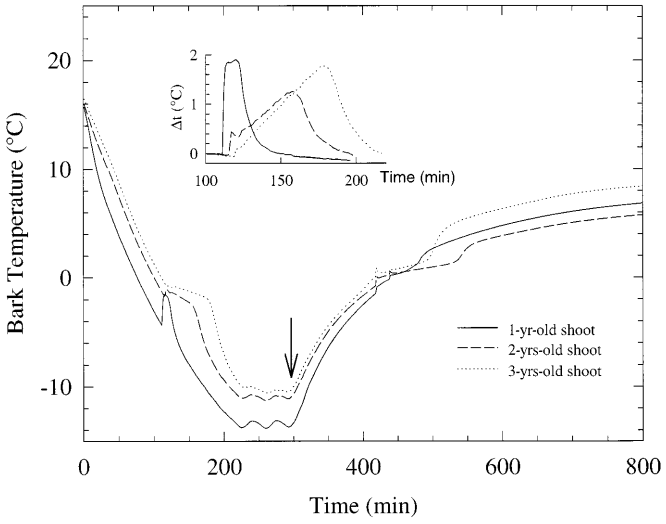

Fig. 3 Time course of bark temperature of an entire branch exposed to one freeze-thaw cycle (experiment 1). Different lines represent different 1- to 3-year-old internodes. The *arrow* indicates the onset of the warming phase. The time course of temperature differences between ambient air and the bark during the freezing exotherm is shown in the insert

Time course of bark temperature during one freeze-thaw cycle is shown in Fig. 3a. Three shoot internodes of different ages are shown. Freezing exotherms are shown in Fig. 3b. The onset of freezing was clearly identified by a rapid increase of bark temperature (up to 2°C). The end of the exotherm was arbitrarily taken when Δt returned to zero. Freezing occurred within a few minutes in the different parts of the branch but lasted much longer in bigger and older stems (from 40 min for 1 year old twigs to 100 min for 3 year old twigs).

Experiment 2

The results for one experiment for a branch segment with both ends connected to a balance can be seen in Fig. 4. The water flow measured by the lower balance (downstream) progressively decreased during the cooling phase due to changes in water viscosity (Fig. 4b). When water started to freeze, as shown by the onset of the exotherm (Fig. 4a arrows) the water flow rapidly increased corresponding to water expulsion from the branch. Upon thawing the opposite phenomenon was noticed, the water entering the branch induced a negative flow. Figure 4c shows the algebraic summation of water weights on the two balances. This sum represents the variation of water stored in the branch and the tubing. Because the ambient air temperature was constant during the experiment, this graph mostly indicates the variation in the water content of the branch itself. The variations in branch temperature produced minor changes in water content due to change

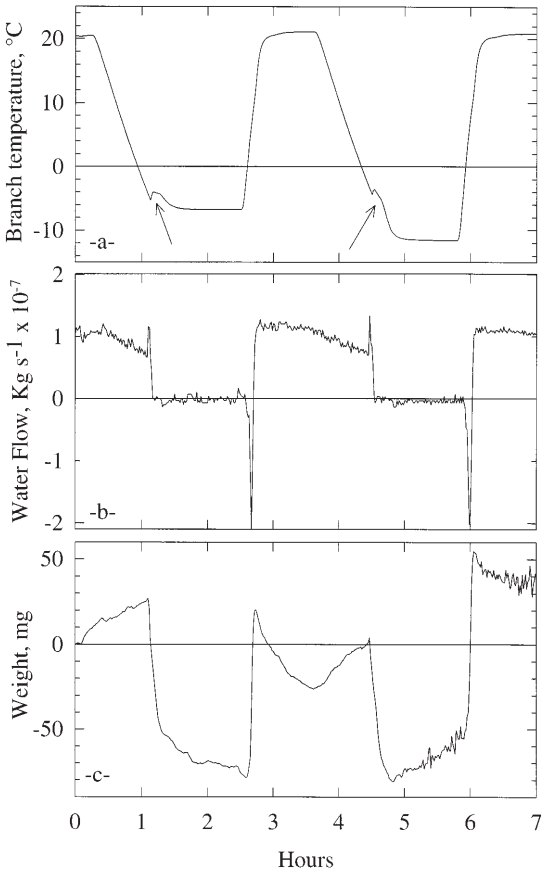

Fig. 4 Representative results from experiment 2. A 60 cm long branch segment with each cut end connected to an analytical balance was exposed to two successive freeze-thaw cycles. **a** time course of bark temperature. Note the two freezing exotherms (*arrows*). **b** Time course of water flow through the branch as measured by the downstream balance. **c** Variation of branch water content. Water was exuded out of the branch during freezing

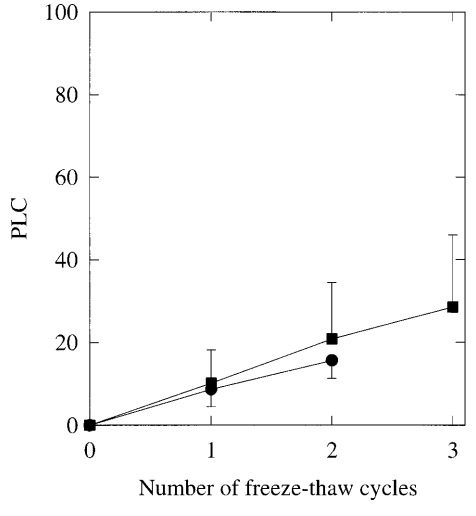

Fig. 5 Progression of embolism (PLC, %) in the xylem of branch segments exposed to successive freeze-thaw cycles (experiment 2). Contrary to experiment 1, xylem dehydration was prevented upon freezing. Water tension in the branches was ca. –11 kPa (*circles*, *n*=2) or ca. –40 kPa (*squares*, *n*=5). *Error bars* represent one SD

in water density. When the branch was frozen, its water content was clearly reduced. It can be seen (Fig. 4b) that the water flow through the branch segment under ambient temperature was barely reduced by two freeze-thaw cycles. The same pattern was observed when branches were placed under higher tensions (ca. –40 kPa). In other words, under the conditions of experiment 2, the freeze-thaw treatments had much lower impact on the development of embolism than under the conditions of experiment 1 (Fig. 5).

Discussion

The experiments conducted, under controlled conditions, on *F. sylvatica* branches may explain why, in apparent contradiction to the theory of larger conduits being more vulnerable, apical shoots with smaller vessels are the more vulnerable to freeze-thaw events. Results from experiment 1 confirmed field observations and emphasized the role of xylem tension in the mechanism of embolism induction. Furthermore, the analysis of the freezing exotherms revealed that smaller shoots were entirely frozen before the remainder of the branch. Results of experiment 2 suggested that upon freezing water was exuded as a consequence of water volume expansion. Water exudation was probably the triggering factor for embolism formation in the shoot apex because when water was allowed to freely enter the shoot upon thawing, the embolism developed little (experiment 2). From these observations we can conclude that water exudation upon freezing in intact shoots probably lowered the water content of the smaller shoots and thus induced higher xylem tensions upon thawing near the apex. Although bubbles were likely to be smaller in these terminal shoots, the capillary pressure they developed was not high enough to compensate for the decrease in xylem pressure.

These results largely agree with the mechanism proposed by Sucoff (1969) for conifers. Sperry et al. (1998) proposed a different mechanism for *Acer*. According to their observations, winter embolism in sugar maple was probably caused by ice sublimation. Terminal shoots were more vulnerable because they were more exposed to the sun and exhibited higher surface-to-volume ratio. Ice sublimation can occur under field conditions but was unlikely during our experiments. Acer is also unusual because its xylem uptakes water upon freezing (Tyree 1983). Consequently, xylem tension is considerably reduced or even positive upon thawing which lowers the risk of embolism formation.

Was the higher vulnerability of *Fagus* terminal shoots only incidental or could it benefit the trees? Sucoff (1969) suggested that upon thawing in conifers, only a few tracheids with big air bubbles cavitate. As these bubbles expand, tension is released which allows bubbles in the surrounding tracheids to dissolve. A similar phenomenon appears to be plausible for *Fagus*. The embolisation of distal vessels may protect the older parts of the branch from freezing damage. However, results from experi-

ment 1 and other experiments (Sperry and Sullivan 1992; Magnani and Borghetti 1995) suggest that this effect is cumulative and after several freeze-thaw cycles the whole xylem reaches a high degree of embolism.

It is likely that the mechanism we describe is exacerbated under field conditions. This results because at dusk, tree apices will be exposed to lower ambient air temperature and freeze earlier, whereas in the morning they will be the first to be rewarmed by the sun.

In conclusion, within a branch of *F. sylvatica*, the likelihood of embolism formation is more dependant on the dynamics of sap freezing than xylem characteristics. Because terminal shoots freeze first, they are more prone to embolism even though they exhibit smaller vessel size.

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