Monitoring of Freezing Dynamics in Trees: A Simple Phase Shift Causes Complexity[OPEN]

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During winter, trees have to cope with harsh conditions, including extreme freeze-thaw stress. This study focused on ice nucleation and propagation, related water shifts and xylem cavitation, as well as cell damage and was based on in situ monitoring of xylem (thermocouples) and surface temperatures (infrared imaging), ultrasonic emissions, and dendrometer analysis. Field experiments during late winter on Picea abies growing at the alpine timberline revealed three distinct freezing patterns: (1) from the top of the tree toward the base, (2) from thin branches toward the main stem’s top and base, and (3) from the base toward the top. Infrared imaging showed freezing within branches from their base toward distal parts. Such complex freezing causes dynamic and heterogenous patterns in water potential and probably in cavitation. This study highlights the interaction between environmental conditions upon freezing and thawing and demonstrates the enormous complexity of freezing processes in trees. Diameter shrinkage, which indicated water fluxes within the stem, and acoustic emission analysis, which indicated cavitation events near the ice front upon freezing, were both related to minimum temperature and, upon thawing, related to vapor pressure deficit and soil temperature. These complex patterns, emphasizing the common mechanisms between frost and drought stress, shed new light on winter tree physiology.

Plants in temperate biomes can contain water under its three physical states. Liquid water flows within the xylem according to the cohesion-tension theory (Dixon, 1896) and turns into vapor in leaves. During frost days, liquid water, at least partially, changes to ice. Alpine plants are an impressive example of plants coping with these phase shifts on a frequent basis, especially in late winter to early spring, when freeze-thaw cycles can occur daily (Mayr et al., 2006b).

Ice formation within plants influences their physiology mechanically, hydraulically, and at the cellular level (Cinotti, 1991; Charrier et al., 2013a, 2015b). Mechanical strain occurs as water expands during freezing (Kubler, 1983; Cinotti, 1991), and tension is induced in the remaining liquid-phase sap (Hansen and Beck, 1988; Charrier et al., 2014a; Charra-Vaskou et al., 2016). Xylem cavitation is initiated on freezing due to the low (i.e. negative) water potential of ice (Charrier et al., 2014a; Charra-Vaskou et al., 2016) and the low solubility of gases in ice (Robson et al., 1988; Sperry and Robson, 2001; Sevanto et al., 2012). Embolism subsequently develops on thawing when gas bubbles released from the ice may coalesce, expand, and embolize the complete conduit (Sperry and Sullivan, 1992; Lemoine et al., 1999; Pittermann and Sperry, 2003; Ball et al., 2006; Mayr et al., 2007; Mayr and Sperry, 2010; Sevanto et al., 2012; Charrier et al., 2014a; Charra-Vaskou et al., 2016). The low water potential of ice...
also generates an osmotic disturbance on macromolecule arrangement, which can lead to membrane breakage and cellular lysis (Pearce, 2001; Uemura et al., 2006; Ruelland et al., 2009).

When temperatures drop below 0°C, the xylem sap remains in a metastable, supercooled state (Wolfe and Bryant, 2001) until crystallization is initiated around a nucleus. Ice nucleation can occur when water molecules form long enough transient hydrogen bonds with each other, reaching a critical nucleus size of 1.13 nm, containing ~190 molecules (i.e. homogenous nucleation at temperatures below ~38°C), or onto a nucleating agent (heterogenous nucleation onto impurity, cell wall, or ice nucleation active bacteria; Sakai and Larcher, 1987).

In trees, ice nucleation generally is observed in the apoplastic compartment (Asahina, 1956) through intrinsic heterogeneous nucleation (Wisniewski et al., 1997; Pearce, 2001). Once started, ice propagates through the plant, radially and longitudinally (Neuner et al., 2010).

The physical transition from a disordered liquid state to an ordered crystal structure is an exothermic reaction, which releases latent heat (334 J g⁻¹; Dereuddre and Gazeau, 1992) and entraps gas bubbles emerging from solution (Sperry and Robson, 2001; Sevanto et al., 2012). Therefore, a freezing exotherm can be detected using thermocouples (Mayr et al., 2006b; Pramsohler et al., 2012) or infrared imaging (Wisniewski et al., 1997; Hacker and Neuner, 2007; Charrier et al., 2015b). Exotherm monitoring is widely used to detect freezing in plants, but this technique is very limited at detecting much slower phenomena such as thawing. At thawing, xylem sap becomes liquid through an opposite endothermic reaction.

Further insights into freezing and thawing processes were enabled by dendrometers (Zweifel and Häslar, 2000) and ultrasonic emission (UE) analysis (Charrier et al., 2015b). Dendrometers are commonly used to monitor growth and drought stress in woody plants (Reineke, 1932; Daubenmire, 1945; Deslauriers et al., 2007; de Swaef et al., 2015). However, upon freezing, a sharp decrease in diameter is observed in the bark of acclimated plants, which is probably caused by water fluxes toward the ice nucleation point in intercellular spaces (Zweifel and Häslar, 2000; Ball et al., 2006; Charra-Vaskou et al., 2016). On thawing, the initial diameter is restored, but only partly in plants that are not fully acclimated (Améglio et al., 2001). The proportion of irreversible diameter shrinkage, therefore, can serve as an assessment of frost-induced damage to living cells (Lintunen et al., 2015). UE (Mayr et al., 2007) analysis is a nondestructive technique that detects spontaneous liquid-to-vapor phase transitions (i.e. cavitation events) under drought (Milburn, 1966; Tyree and Dixon, 1983; Mayr and Rosner, 2011; Nolf et al., 2015; Vergeynst et al., 2015) or frost stress (Kikuta and Richter, 2003; Mayr et al., 2007; Charrier et al., 2014a). The latter is likely based on bubble formation due to the low water potential of the xylem sap near growing ice fronts. When the water potential reaches the cavitation threshold, gas bubbles form, and the sudden relaxation of water columns generates UEs. Recently, the UE technique was used to monitor ice propagation within branches in laboratory experiments (Charrier et al., 2015b), and UE velocity and attenuation were shown to enable the detection of ice in woody stems (Charrier et al., 2014b). UE also was observed during thawing, in relation with embolism formation according to the thaw-expansion hypothesis (Mayr and Sperry, 2010).

In trees, the dynamics of freezing and the resulting occurrence of ice in the axis system have a crucial effect for hydraulics (e.g. increased levels of frost-induced embolism in distal parts of branches; Lemoine et al., 1999). Frozen xylem in the trunk blocks water uptake from the soil and thus supply to distal parts such as the leaves of evergreens. Ice in branches disconnects the hydraulic continuum within the plant and thus prevents equilibration of water potentials, which may cause complex within-plant variation in drought stress intensities (Mayr and Charra-Vaskou, 2007), especially when high evaporative demand and soil frost are concomitant (Mayr et al., 2002, 2006a, 2014). This complexity is further increased by the spatiotemporal pattern of ice and respective frost damage in dead (xylem embolism) and living (cell damage) tissues.

Ice nucleation usually occurs where the local (organ) temperature reaches the freezing point. Under field conditions, air temperature is often considered as a proxy for organ temperature. However, temperature varies spatially, being lower close to the ground in an open environment (Leuning and Cremer, 1988; Jordan and Smith, 1994; Battany, 2012) or, in more closed stands, at the air-plant interface (Tappeiner, 1985; Winkel et al., 2009). Compared with air temperature, plant organ temperature exhibits a buffered response due to thermal inertia of the local wood section (proportional to stem diameter) and isolating layers (e.g. bark). The actual temperature in plant tissues, therefore, is the result of the energy balance driven by net radiation, air temperature, humidity, and soil calorific capacity (Cellier, 1984, 1993; Leuning, 1988; Jordan and Smith, 1994). Thus, freezing and thawing patterns are complicated by temperature variation (e.g. due to microclimatic factors), topography and crown architecture, or the latent heat released by ice formation. Additionally, tissues with more negative osmotic potentials exhibit lower freezing points (Sakai and Larcher, 1987; Yelenosky and Guy, 1989), which, in turn, influences the spatial pattern of potential ice nucleation.

We hypothesized that late winter environmental conditions result in disturbances in the plant hydraulic system, which generate ice formation and propagation from the coldest organs of the tree (e.g. the apex or thin side branches) toward the base. To test this hypothesis, we monitored ice formation and propagation within the trunk and crown of Picea abies trees growing near the alpine timberline using nondestructive methods (thermocouples, infrared imaging, and UE analysis; Fig. 1). It is known that timberline trees are exposed to numerous and intense freeze-thaw cycles (see above) and thus provide an ideal system with which to study the corresponding dynamics. Furthermore, an analysis
RESULTS

During four successive late winter periods, we recorded 15 independent freezing processes within the tree crown via exotherm detection in the xylem (thermocouples). Three different patterns of freezing exotherms within the crown, with respect to temporal and spatial courses, were observed: (1) from the top downward to the basal part of the tree (~20% of observations; Fig. 2, A and B, March 19, 2013); (2) from the base upward to the tree top (~30% of observations; Fig. 2, C and D, March 22, 2013); and (3) from side branches to the stem and upward and downward (~50% of observations; Fig. 2, E and F, March 13, 2015). Ice propagated at ~1 to 4 mm s⁻¹ in upward and downward directions.

Infrared image analysis allowed us to map changes in surface temperature across a large part of the tree, highlighting a more complex freezing pattern than indicated by exotherm analysis based on single, individually placed thermocouples. For instance, on March 14, 2015 (Fig. 3; Supplemental Video S1), before freezing occurred (17:00), a vertical gradient in surface temperature was observed along the trunk from the middle (T3; ~−2°C) to the apex (T1; ~−2.5°C) and side branches (B; ~−3°C). From 17:46 to 18:15, exotherms indicated that freezing started from the branch base and moved toward the distal parts of branches, respectively. However, the pattern within the crown appeared as spatially erratic, with seemingly independent exotherms detected in different side branches. Overall, branches at middle height froze before higher and lower branches. Although the increase in trunk surface temperature was too low to be detected visually on the video, temperature dynamics extracted from infrared image analysis (Supplemental Fig. S1, A and B) were in agreement with the dynamics observed via thermocouples (Supplemental Fig. S1, C and D). On side branches, exotherms were recorded earlier with thermocouples than with infrared imaging, because the latter recorded temperature changes at the surface while thermocouples were installed directly in the xylem. In the apical and middle parts of the trunk (T1–T3), exotherms were detected concurrently with thermocouple and infrared imaging, indicating a stronger exotherm signal based on a much larger volume of freezing wood. The freezing patterns of a second tree (tree 2, at the right in Supplemental Video S1) and during other freezing events (March 25–27, 2014 or March 13–14, 2015) were similar, with side branches at middle height freezing before branches near the apex or the base.

Upon freezing, UEs were detected concurrently with exotherm formation within the trunk as measured by thermocouples (Fig. 4; March 7, 2012): from the lower position (T5; UE, 17:48; thermocouple, 17:57) to the upper portion of the trunk (T2; UE, 18:00; thermocouple, 17:57). While the onset of UEs thus clearly corresponded to freezing, consecutive acoustic activity did not exhibit a clear spatial pattern, and UEs were detected long after the exotherm had dissipated (Fig. 5). UEs exhibited two phases of activity within the daily freeze-thaw cycle: a phase from the onset of freezing (indicated by a freezing exotherm) until sunrise and a second phase after the xylem had thawed in the morning until sunset (Fig. 5; March 12–13, 2015). Of the high number of UEs recorded during the different monitoring periods (~760,000 UEs), 52% were recorded in the side branch (B), 29% in the middle of the trunk (T3), 15% in the upper portion of the trunk (T2), and only 3.5% in the lower part (T5), with similar trends for daytime and nighttime activities.

The two phases of acoustic activity were remarkably associated with decreasing trunk diameter, although to different extents (Fig. 5; March 12–13, 2015). Daytime UE activity (~0.07 UEs s⁻¹) and decreasing trunk diameter (~100 µm, ~0.01 µm s⁻¹) were observed as long as temperature remained above 0°C (16:00). After sunset, the second phase of UE activity (0.45 UEs s⁻¹) started once the freezing exotherm reached a plateau (19:30), concurrent with diameter shrinkage (~175 µm, ~0.12 µm s⁻¹). Finally, another peak in UE activity (2.25 UEs s⁻¹) was recorded when the xylem differential temperature (xylem temperature − air temperature;
dT_{xylem-air} = +2.7°C at 0:00) started to decrease, again associated with trunk diameter shrinkage (−400 μm, −0.12 μm s⁻¹; Fig. 5). During the intermediate periods of low UE activity (e.g. plants thawing from 8:20 until 10:20 or at the onset of freezing from 17:00 until 19:30), the stem recovered its initial diameter.
From March 12 to 17, 2015, we observed a period of pronounced daily temperature fluctuations. Temperatures exhibited high diurnal amplitudes of 10 to 15 K as well as increasing minimum and maximum temperatures and corresponding vapor pressure deficit (VPD; Fig. 6A). After March 14, minimum temperature increased progressively, resulting in lower UE activity and higher minimum diameter at night (Fig. 6B). However, UE activity during the daytime increased over this period in relation to temperature (higher than 5°C) and VPD (higher than 500 kPa). Interestingly, nighttime freezing generated ~10 times higher diameter shrinkage than daytime thawing, whereas maximum UE activity was 3 times lower at night than during the day (Fig. 6B). Furthermore, UEs were restricted almost completely to periods of diameter shrinkage (~87% of total recorded UEs).

Nighttime UE activity and diameter shrinkage were negatively correlated with minimum air temperature ($r^2 = 0.509$, $P < 0.001$ and $r^2 = 0.410$, $P = 0.042$ for UEs and diameter, respectively; Fig. 7A; Table I). During the day, UE activity and diameter shrinkage were correlated with minimum soil temperature ($r^2 = 0.303$, $P < 0.001$ and $r^2 = 0.559$, $P = 0.008$ for UEs and diameter, respectively; Fig. 7B). However, daytime and nighttime UE activities were related to temperatures via exponential functions, whereas diameter shrinkage was related to temperatures via linear functions. Consequently, UE activity was exponentially correlated to diameter shrinkage ($r^2 = 0.233$, $P = 0.031$; Fig. 7C), with no significant difference between day and night ($P = 0.124$). Correlations between maximum VPD and UEs ($P = 0.049$) and between diameter shrinkage and minimum air temperature were also observed ($P = 0.002$). Furthermore, the

![Figure 3. Spatial temperature patterns in a P. abies tree at the alpine timberline at the time of freezing (after sunset; onset at ~17:55) monitored via infrared imaging. Letters (B and T1–T4) indicate sensor positions (Fig. 1), and arrows indicate additional positions with very pronounced changes. Note the different time scales in the top row versus the bottom row. Top images (A–E) show temperature changes over 2 h within the crown. Bottom images (F–J) illustrate the period when xylem exotherms were detected (17:55–18:05). This time frame corresponds to the onset of freezing, and areas with pronounced events are highlighted in the thermograms.](image-url)
maximum VPD observed during the same night was correlated with both nighttime UEs ($P = 0.015$) and diameter ($P < 0.001$) shrinkage.

**DISCUSSION**

This study shows that freezing plant tissues do not just undergo a simple phase shift. Instead, UEs and stem diameter variation revealed that ice nucleation is only the onset of a complex series of internal changes that continue several hours beyond the initial exotherm formation (Figs. 5 and 8). These changes may promote sap cavitation upon freezing and thawing in environmental conditions typical for early spring (i.e. low air and soil temperature, high VPD). Remarkably, even once most of the sap was in solid state, the frozen branch underwent changes, as indicated by subsequent radial stem (i.e. bark) shrinkage and increasing UE activity with decreasing air temperatures (Figs. 6 and 7; Table I). Both of these effects were correlated with soil temperature and VPD and likely resulted from the low water potential of ice, which depends on the degree of supercooling ($-1.16 \text{ MPa} \cdot \text{K}^{-1}$; Hansen and Beck, 1988).

Upon freezing, radial water fluxes and related cavitation events increased the heterogeneity in water distribution within the tree crown. Such conditions are expected to favor embolism development on thawing, when the root system cannot compensate for evaporative water losses (Fig. 8).

The remarkable correlation between diameter shrinkage and UE activity (Figs. 5–7) indeed indicates that water fluxes are generated toward the ice nucleation point (Cavender-Bares, 2005; Ball et al., 2006). These water fluxes resulted in bark shrinkage and increased the tension in the remaining liquid xylem sap (Charra-Vaskou et al., 2016). When the cavitation threshold was reached, UEs were generated (Charrier et al., 2014b) in relation to freezing velocity (Charrier et al., 2015b). Furthermore, cavitation-related UEs may be generated when air is expelled from the crystal lattice during freezing and gets pulled through pits (Mayr et al., 2007; Charrier et al., 2014b, 2015b). Such air bubbles can then expand at thawing and, especially while the xylem sap is under tension (Mayr and Sperry, 2010), fill and therefore embolize the whole conduit (Ball et al., 2006; Charra-Vaskou et al., 2016).

**Figure 4.** Sequence of freezing exotherms observed using thermocouples (differential thermal analysis [dT], xylem – air temperature; A) and accumulated UEs (B) in a *P. abies* tree during a freezing event. Arrows indicate the onset of exotherms at different sensor positions (B, T2, T3, and T5; Fig. 1).

**Figure 5.** Ultrasonic activity (A) and stem diameter changes (B) in a *P. abies* tree during a freezing event. Temperatures are given as absolute (T3, air; A) and differential thermal analysis (dT; xylem – air temperature; B) values. Stem diameter is given as differential with respect to initial diameter (i.e. day 1, 12:00) to highlight dynamic changes. Vertical dashed lines indicate the onset of the exotherms as observed using thermocouple data.
While ice nucleation and its related effects typically occur at night, UE activity and decreasing stem diameter also were observed during the day (Figs. 5 and 6; Table I). Daytime UE activity increased with VPD and decreasing soil temperature. The observed diameter shrinkage indicates that the water supply could not compensate the evaporative demand, resulting in increasing drought stress during sunny days (winter drought; Tranquillini, 1979; Sakai and Larcher, 1987), as described previously for mountainous areas (Michaelis, 1934a, 1934b; Henson, 1952; Larcher, 1957, 1977; Wardle, 1971; Tranquillini, 1979). Cavitation events thus occurred not only on freezing but also on thawing, which, over the course of many freeze-thaw cycles, can lead to high levels of winter embolism (Mayr et al., 2003, 2006a). Daytime and nighttime environmental conditions, therefore, interact in water redistribution within the tree crown, which exponentially increases the probability of cavitation events (Fig. 7). Embolism repair and hydraulic recovery, which do not produce UEs, may mitigate this hydraulic limitation and, indeed, have been observed in *P. abies* even while the soil was frozen (Mayr et al., 2006a, 2014). Furthermore, insulation from VPD due to snow cover helps small trees and shrub species to avoid or reduce drought stress during winter months (Larcher, 1963).

Freeze-thaw cycles in winter and early spring are common in temperate to boreal and mountainous regions. The study site at the timberline and the late winter season were chosen as the ideal place and time to observe them in situ, with broad daily thermal amplitudes due to topography, high elevation (1,680 m above sea level), southeast exposure, and an open stand (Blennow, 1998; Blennow and Persson, 1998). Such conditions are particularly favorable for radiative freezing events to occur in late winter (Monteith and Unsworth, 1990; Lindkvist et al., 2000; Charrier et al., 2015a). During this period, when the sky is clear, the energy balance is usually positive at daytime (solar radiation > 600 W m⁻²) and negative at night (long-wave infrared radiation dissipated to clear sky; Nobel, 1987; Snyder and Melo-Abreu, 2005). Air temperature during the study period thus alternated on average from -4.4°C (nighttime) to +7°C (daytime), but extreme daily thermal amplitudes can reach up to 30 K in alpine conditions (Mayr et al., 2006b). Local sunset at the study site was between 16:00 and 17:00 (surrounding mountains ~20° above horizon), resulting in a negative net energy income of the ecosystem. Therefore, a rapid decrease in air temperature (6.3 K h⁻¹ on average) led to fast freezing events. In the morning, the temperature increased at an even faster rate (8.1 K h⁻¹ on average), and thus temperature changes were overall faster than generally reported (~5 K h⁻¹; Levitt, 1980; Sakai and Larcher, 1987). During the monitored period, the fastest rates of temperature change were similar for cooling (~14.5 K h⁻¹; April 7, 2015) and warming (~15.8 K h⁻¹; March 7, 2012). Cooling, however, was
far slower than the rates at which intracellular freezing and thus cellular damage were reported (−48 K h⁻¹; Levitt, 1958; Siminovitch and de la Roche, 1978). Accordingly, stem diameters recovered fully to initial values after thawing (Figs. 5 and 6), indicating that living tissues were still intact (Améglio et al., 2001; Lintunen et al., 2015; Charra-Vaskou et al., 2016). Also, minimum temperatures were not critical, as plants can usually suffer temperatures as low as −40°C without significant cell damage (Christersson, 1978; Repo, 1992; Charra-Vaskou et al., 2012) before deacclimation during the spring period.

The delay between the onset of exotherm formation (17:00) and diameter shrinkage (20:35), as well as the two phases observed in diameter shrinkage (before/after 21:50), suggest that the spatiotemporal pattern of freezing was heterogenous (Fig. 5). Although exotherms observed via thermocouples revealed freezing of xylem tissue, the diameter remained unaffected for 1 to 3 h (Figs. 5 and 6). As long as the stem remained warmer than the air, the ice water potential did not compensate the intracellular osmotic potential (Cavender-Bares, 2005; Charrier et al., 2013b) or reach the cavitation threshold (Charrier et al., 2015a). However, as the exothermal heat dissipated, liquid water was pulled from the cytoplasm toward the apoplasm, leading to cell plasmolysis (Uemura et al., 2006; Ruelland et al., 2009), diameter shrinkage (Charra Vaskou et al., 2016), and UEs (Charrier et al., 2015a; Kasuga et al., 2015).

Because of their higher elasticity, bark tissues exhibit more pronounced thickness changes upon freezing than xylem (Zweifel and Häsler, 2000; Améglio et al., 2001). The extracellular freezing of the bark tissues was probably the trigger of water fluxes outward from the xylem, and the resulting tension generated UEs. Other elements than the xylem conduits have been shown to generate UEs on freezing in angiosperms, such as bark (Kikuta, 2003) or xylem parenchyma (Kasuga et al., 2015).

Ice nucleation is partly a stochastic process (i.e. freezing depends on the interaction between low temperature and the presence of a nucleus). In our study, the lowest temperatures were generally observed in the apex and branches, whereas, surprisingly, freezing sometimes also started from the base of the trunk (Figs. 2 and 3). We observed three distinct freezing patterns in the same P. abies tree (Figs. 2 and 8). (1) Ice propagated from the base, when this part remained frozen during the entire day while the upper crown thawed in the sun. (2) Ice nucleation occurred in the apex, and the ice then spread downward. (3) Freezing started in branches, with the ice front seemingly moving toward the main axis. These last two freezing patterns suggest that the distal parts of the tree (apex or branches) experienced lower temperatures, which, therefore, had increased probability for nucleation. However, infrared image analysis showed a more detailed picture: ice nucleation in branches occurred close to the base of the branch and propagated toward the distal ends (Supplemental Video S2). These observations show not only that the presence of metallic sensors in contact with the xylem did not induce artificial ice.

**Figure 7.** Average ultrasonic activity (black circles) and diameter shrinkage (white circles) observed per night (net radiation < 10 W m⁻²) depending on average air temperature (A) or per day depending on average soil temperature (B). Average ultrasonic activities depending on diameter shrinkage (C) are presented with no significant difference between day (white diamonds) and night (black diamonds). Note that y scales are logarithmic; r² values apply to exponential fits (UE activities) or linear fits (diameter shrinkage).
nucleation. They also suggest that a lower temperature in branches (compared with the trunk), combined with larger conduits (at the branch base compared with distal branch sections), may increase the probability for ice nucleation (Asahina, 1956; Lintunen et al., 2013) and, in consequence, make the base of branches a likely ice nucleation site. Additional freezing experiments in the laboratory, where the direction of ice propagation was determined in excised branch segments, could not confirm this pattern consistently (data not shown). However, these laboratory experiments could not simulate the microclimatic effect of dense needle cover in the distal portion of the branch, which might have buffered the temperature decrease.

Once nucleated, the ice front can propagate after the exothermal heat has dissipated through the bark (Shibkov et al., 2003), whereby propagation rates within the water column are proportional to the degree of supercooling (Langer et al., 1978; Rauschenberger et al., 2013). In excised branches of *P. abies*, propagation rates similar to those found in this field study were observed (2 and 4 mm s\(^{-1}\) for 1.5 and 2 K of supercooling, Table I.

Because of the particular local conditions, night-day shift was selected based on a threshold in net incoming radiation equal to 10 W m\(^{-2}\). Different symbols refer to P values: ***, \(P < 0.001\); **, \(P < 0.01\); *, \(P < 0.05\); and ns, not significant.

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Figure 8. Generalized scheme of observed patterns and underlying water fluxes in a *P. abies* tree during a freeze-thaw cycle in late winter. Shown are the initial equilibrated state (A), ice nucleation and propagation with associated water fluxes, stem shrinkage, and UEs (B), ultrasonic activity after exotherm dissipation (C), stem diameter increase and developing water potential gradient during thawing (D), and stem shrinkage and high ultrasonic activity during daytime transpiration with frozen soil (E).
respectively; Charrier et al., 2015b). Ice propagation toward the distal end of a branch can generate two opposite effects on sap water potential: (1) the density of ice with respect to liquid water (917 versus 1,000 kg m\(^{-3}\) at 0°C, respectively) induces an ~9% increase in local pressure (Hare and Sorensen, 1987; Holten et al., 2012); and (2) the tension induced by ice is ~1.16 MPa K\(^{-1}\) (Hansen and Beck, 1988). Thus, ice nucleation in the basal part of the branch can pull liquid sap from the distal end, which redistributes water toward nucleation sites (Ball et al., 2006; Charra-Vaskou et al., 2016), explaining the gradient in water potential and higher embolism observed under similar conditions (Mayr and Charra-Vaskou, 2007) and the higher number of UEs in branches than in any other part of the trunk. Nevertheless, during the daytime, distal ends most likely thawed first and, hydraulically disconnected from the rest of the plant, would subsequently experience higher levels of drought stress and embolism compared with basal parts (Lemoine et al., 1999). Such a spatial distribution of embolism within the plant may prevent embolism from spreading toward the base when the soil is frozen.

This study reveals an enormous complexity in freezing dynamics and ice distribution within trees (Fig. 8). We showed that ice fronts may diverge into different directions within a stem or collide in other stem sections, which may cause separation of the hydraulic continuum unless ice blockages dissolve during thawing. The combined use of several sensing technologies enabled us to demonstrate not only contrasting ice propagation patterns but also the link between ice formation, diameter changes due to water shifts, and ultrasonic activity due to cavitation. These processes are highly relevant for plant hydraulicities and freezing stress, and their understanding is a prerequisite for studies on winter stress not only in timberline species but also for temperate trees in general.

MATERIALS AND METHODS

Study Site and Tree Description

Study trees were growing naturally in Praxmar (Tyrol, Austria; 47°09’N, 11°07’E; 1,680 m above sea level) close to the timberline. Two Picea abies (Norway spruce) trees, located on the edge of the closed forest, were monitored during late winter. One tree was completely isolated from surrounding trees (thereafter called tree 1), whereas the other one was located closer to surrounding trees (thereafter called tree 2). Tree 1 was monitored repeatedly during 4 successive years from 2012 until 2015, whereas tree 2 was monitored only during late winter 2015. Both trees were similar in dimension although growing during the four monitored years: height from ~2.5 to 4 m, and diameter at breast height from 5 to 10 cm. The monitoring period ranged from early March until late April, at a period when frequent diurnal freeze-thaw cycles are known to occur. At that time, a snow cover was still present and the soil was frozen. Freezing events were monitored with different sensors (thermocouples, UE sensors, and dendrometers) at six positions along the main axis, from trunk base to top, and on a side branch (Fig. 1). Depending on the availability of equipment, installations were made in different combinations.

Temperature Measurements

Xylem and air temperature were monitored using copper-constantan thermocouples connected to a data logger (1000 Series Squirrel Meter/Logger; Eltek) at 30- to 60-s intervals. Thermocouples were installed in the xylem at 0.5- to 1-cm depth at six positions, along the main trunk (apex [T1] to base [T5]) and on a side branch (B), and attached using Terostat putty (Henkel).

Infrared Imaging

Surface temperature was recorded using a thermal infrared camera (Jenoptik VarioCam HR; InfraTec) at ~7 m distance from the trees. This camera is sensitive to radiation at 7.5 to 14 μm wavelength of the electromagnetic spectrum with a temperature resolution of less than 0.08 K. Thermal images (thermograms) were recorded at 0.025 s focal length, resulting in 384 × 288-pixel images at ~0.01 m² pixel\(^{-1}\) spatial resolution. Thermograms were recorded every 5 s and processed using IRBIS 3 plus software (InfraTec).

UE Analysis

UEs were monitored on tree 1 with a four-channel USB-based system (1283 USB AEnode, 18-bit A/D, 20 MHz) and 150-kHz, 26-db preamplified resonance sensors (PK15I; all components from Physical Acoustics) set to a 20-dB threshold. On tree 2, an autonomous AE system device was installed, also equipped with four 150-kHz, 26-dB preamplified resonance sensors (PK15I; all components from Mistras Holdings). Registration and analysis of ultrasonic events were performed with AEwin software version E.4.40 (Mistras Holdings). Acoustic sensors were attached using spring-loaded clamps at three positions along the trunk (T2, T3, and T5) and on a side branch (B; Fig. 1) after removing ~1 cm² of bark and covering the exposed xylem with silicone grease to ensure acoustic coupling and prevent dehydration. Acoustic coupling was tested with lead breaks (Hsu-Nielsen method; Kalyanasundaram et al., 2007; Charrier et al., 2014b) at a distance of 1 cm from each sensor, and sensors were reinstalled when the signal amplitude was below 90 dB.

Microdendrometers

Diurnal and longer term variations in stem diameter were monitored using dendrometers on both trees during late winter 2015. A PepsLAF system (Forest Future) was installed in the upper third of the main axis (between T2 and T3; Fig. 1) of each tree. Each unit contains a linear variable differential transformer sensor (sensitivity, ±1 μm) connected to a data logger recording at 5-min intervals.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Freezing patterns monitored via infrared imaging and thermocouples.

Supplemental Video S1. Temporal progression of differential surface temperatures of a P. abies tree and the surrounding snow cover, recorded by infrared imaging on March 14, 2015.

Supplemental Video S2. Example of poikilothermic versus homothermic regulation in alpine winter conditions, recorded on the evening of March 3, 2011.

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