Acoustic Emission Related to Drought Stress Response of Four Deciduous Broad-Leaved Woody Species

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Abstract

The aim of the present study was to test if acoustic emission (AE) detected on leaves and stem surfaces of young trees gives information about stomatal closure and thus about a tree’s performance under drought stress. Two diffuse-porous (conduit size shows little variation within an annual ring) and two ring-porous (conduits in earlywood are much bigger than in latewood) species were tested for their reaction to drought stress. In both diffuse (Populus alba L., Fagus sylvatica L.) and ring-porous (Fraxinus excelsior L., Quercus pubescens WILLD.) species, AE activity of leaves ceased when stomatal conductance reached minimum values. In some species, AE rate and amplitude distribution can be a useful parameter to discriminate between AE resulting from conductivity loss and from other processes such as shrinkage and wilting. AE activity of stem wood showed a clear pattern with two amplitude peaks in ring-porous species whereas no such pattern was found in diffuse-porous species. The results are discussed with respect to the species-specific reaction to drought stress.

Keywords: Drought stress, stomata response, stomatal closure, vulnerability to cavitation

1. Introduction

Knowledge about the hydraulic performance of long-living plants under drought stress is of high importance with respect to global change. Water is transported from the roots to the leaves of the crown in a metastable state (i.e. under tension) [1]. Due to this metastable state the water column inside the small conduits is likely to break when the water tension increases above a certain level. Breakage of the water columns (cavitation) produces acoustic emission (AE) in the high frequency range [2, 3]. Cavitation induces a reduction in the hydraulic conductivity of the plant, which impairs water supply of the transpiring leaves. Cavitation would result in total loss of hydraulic conductance in the absence of any regulative mechanisms of transpiration [4]. Plants therefore respond by closing their stomata, which should reduce transpiration and keep the water potential (i.e. tension) above the species-specific cavitation threshold value, preventing further loss of conductivity [1]. Limitation in gaseous exchange due to stomatal closure restricts, however, simultaneous carbon assimilation by the plant and thus growth [5].

Drought stress induced stomatal behavior differs widely among species and may be a critical factor in the regulation of survival and mortality during extended drought periods and thus for species distribution [6]. Plants fall into two categories according stomatal regulation of transpiration; “drought avoiders of the saving type” (isohydric plants) and “drought avoiders of the spending type” (anisohydric plants) [7]. Isohydric plants maintain high leaf water potentials during the day for extended periods of drought, thus preventing cavitation. Anisohydric species let midday leaf water potentials decline as soil water potential declines with drought. They can keep their stomata open because their xylem is either less prone to cavitation or they are able to compensate rapidly for water loss, due to a deep and large root system [6, 8]. Ring-porous woody species, with a band of wide vessels in earlywood followed by much narrower vessels in latewood should be successful if plants maintain similar water status throughout the season. This strategy would
however be less essential for diffuse porous species, with narrower conduits, packed more densely per unit wood area [10].

The control of stomata under conditions of drought is achieved due to chemical or hydraulic signaling processes [4, 11]. ABA (abscisic acid) is the most likely candidate for chemical root-to-shoot signaling [12]. It might however also be possible that a combination of both hydraulic and chemical signaling is important in the stomatal regulation in plants [4, 9, 13]. Stomata have to respond directly to some component of the leaf water status because a lack of response to distal shoot water potentials would be dangerous and potentially fatal to plants. Moreover, ABA transport will be limited under severe drought by increased hydraulic resistance in the xylem [14]. Stomata might not respond to leaf water potential per se [15], but to factors affecting leaf water status, such as cavitation, which decreases the hydraulic conductivity in stems [13, 16] and leaves [17, 18]. The onset of stomatal closure is in some species reported to coincide well with the onset of cavitation [19-21], whereas in other species no such relationship was found [16]. AE counting combined with water potential measurements is a reliable method to detect cavitations in different plant organs, but provides little information on the loss in hydraulic conductance [16, 22]. If conductivity loss is a trigger for stomatal closure [13, 18-20], AE testing with subsequent feature analysis could give additional information on stomata’s responses to drought stress [22].

Within this study, the response of stomata to drought stress induced cavitation in leaves and in stem wood was tested in two ring-porous and two diffuse-porous woody species with different demands on water availability. The aim was to test if AE testing combined with AE feature analysis gives information about the species-specific strategy to avoid severe losses in hydraulic conductivity.

Fig. 1. Species investigated were European ash (Fraxinus excelsior L.) (A), Downey oak (Quercus pubescens WILLD.) (B), silver poplar (Populus alba L.) (C) and European beech (Fagus sylvatica L.) (D).
2. Experiment

2.1 Plant Material

Young trees (2-3 years) grown in the botanical garden at BOKU, Vienna, were harvested during the morning hours and re-hydrated for 24 hours in the dark in order to refill all sapwood conduits with free water (Fig. 1). Species used within this study were European (common) ash (*Fraxinus excelsior* L.), occurring in riparian mixed forests along big rivers in central Europe, Downey oak (*Quercus pubescens* WILDL.), a submediterranean species native to southern and eastern Europe, silver poplar (*Populus alba* L.), a relatively drought tolerant riparian pioneer species, and European (common) beech (*Fagus sylvatica* L.), which can be found in pure as well as in mixed stands across central and northern Europe [23]. Common ash and Downey oak are ring-porous woody species, whereas silver poplar and beech are diffuse-porous woody species. In ring-porous wood, wide conduits are produced in the early growing season and narrow conduits during the rest of the growing season. In diffuse-porous species, all water-conducting elements have quite similar diameters and are evenly spread across the annual ring.

2.2 Bench drying experiment

Whole above-ground biomass of young trees was dehydrated at the laboratory bench (“bench drying”) at ambient conditions (25°C, 50% r.h.) for 24 h. During dehydration, AE from leaves and the main trunk as well as changes in water potential and stomatal conductance of leaves were acquired. Prior to the dehydration experiment, cut ends of the small trees were tightly wrapped in Parafilm® (Pechiney Plastic Packaging, Chicago, IL, USA).

AE testing was performed with the μDiSP™ digital AE system from Physical Acoustics Corporation (PAC, Princeton Jct, USA). Preamplifiers (40 dB) were used in connection with WD transducers (100 to 1000 kHz). One WD transducer was positioned on the upper side of the leaf, at the proximal third of the leaf midrib [24], except in common beech, where better results were obtained when the sensor was positioned on the lower side of the leaf. AE signals were recorded with a detection threshold of 32 dB (0 dB = 1 μV input). Data analysis was done with Vallen VisualAE™ software (Vallen Systeme GMBH, Munich, Germany).

Bark was thereafter removed from the young stems and the second WD transducer was positioned directly on the wood surface (Fig. 2). Silicone grease served as a coupling agent (Wacker, Burghausen, Germany). Open cut faces, which were not covered by the transducer, were sealed.
with Parafilm®. All preparation steps were performed while the main parts of the small trees were covered by dark polyethylene sheets in order to avoid moisture loss due to transpiration.

During dehydration, water potential was measured at intervals of 10-120 min by means of a plant water status console (“Scholander pressure bomb”, Soilmoisture Equipment Corp. Santa Barbara, CA, USA). Continuous changes in stomatal conductance in leaves were measured with the AP4 leaf porometer (Delta-T Devices Ltd, Cambridge, GB) in intervals of 1-20 min.

2.3 Anatomical investigations

Wood sections and sections from the midrib of leaves (20 µm) were produced on a sliding microtome (Jung Reichert, Germany). Sections were stained with Astrablue/Safranin dye and mounted in Entellan (Merck, Darmstadt, Germany). Microscopic views of tree stems and leaf midribs were produced by means of a Leica DM4000M microscope interfaced with a digital camera and Leica image analysis software (Leica Microsystems Wetzlar GmbH, Germany).

3. Results and Discussion

3.1 AE characteristics of tree stems

AE from tree stems of ring-porous species (ash and oak) showed different amplitude distribution compared to diffuse-porous species (poplar and beech) (Fig. 3). In ash and oak, a distinct amplitude cluster could be detected, whereas poplar and beech wood had a similar amplitude distribution throughout the whole dehydration experiment. The amplitude cluster of ring-porous species coincided with their first AE rate peak. A distinct rate peak was also found in poplar but not in common beech. It is suggested that AE of the first peak were produced from the breakage of the water columns in conducting elements, whereas AE detected thereafter represents sound produced from capillary action in wood fibers (non-water conducting wood elements), wood shrinkage or crack formation [25]. In Fig. 3, AE results are shown only for the first 10 hours of dehydration, since AE production thereafter is of no physiological relevance, meaning that most of the hydraulic conductivity was lost already by then.

Dehydration of whole plants on the laboratory bench simulates extreme drought stress. Out in the field, all these reactions will proceed much slower and plants will have the chance to refill emptied conduits during the nighttime provided that water availability from the ground is sufficient. Nevertheless, AE monitored from living, extremely drought stressed trees should show a similar pattern. If it is assumed that almost all hydraulic conductivity is lost when the AE rate reaches again very low values after the first AE rate peak, decreasing AE rates indicate a beginning of dramatic loss in hydraulic conductance. Common ash and especially silver poplar developed sufficient mechanisms to prevent further cavitation in stem wood, when most of the hydraulic conductivity was lost already, whereas Downey oak and common beech exhibited quite high AE rates after the first rate peak.

3.2 AE characteristics of leaves

Stomatal closure and cavitation started right from the beginning of the drought stress experiment [19-21]. AE rate of drought stressed leaves showed a distinct peak during the first two hours of dehydration [26], which coincided with a first AE amplitude cluster in both ring- and diffuse-porous species and the course of stomatal conductance (Figs. 4 and 5). At the AE rate peak, single hits with highest amplitudes were detected. Highest amplitudes were found in oak leaves, which might be explained either by bigger lumen diameters of the sound producing sources, the xylem conduits [22, 27, 28], or by less attenuation of the (denser) plant tissue.
Fig. 3. AE amplitudes and AE rates/10 min (red lines) received from stems of different broad-leaved species (common ash, Downey oak, silver poplar, common beech plotted against time. Microscopic views of transverse cuts (20 µm) of the same stems where the AE transducers were positioned are show below the AE plots. The reference bars represent 250 µm.
Fig. 4. AE amplitudes (blue dots) and AE rates/10 min (red lines) received from leaves of two different ring-porous species plotted against time. The courses of the water potential (green line) and the stomatal conductance (pink line, in % of the maximum value measured during dehydration) and a microscopic view of the leaf midrib (where the transducer was positioned) for each species are shown below the corresponding AE plots. On the left side, results for common ash (*Fraxinus excelsior* L.), and on the right side, results for Downey oak (*Quercus pubescens* WILLD.) are presented.

(Total) stomatal closure is reached when stomatal conductance approaches towards minimum percentage values. Thereafter, AE rate decreased rapidly, but increased again when leaves started to wilt. Most of the hydraulic conductivity was probably lost already by then. In common ash and silver poplar, stomatal closure proceeded very fast and was quite efficient; after the first peak, AE rate did not increase within the next 6 hours. In common beach and Downey oak, however, AE rate increased again after another hour of dehydration. These results coincided with the drop in the water potential: whereas Downey oak and common beech reached quite negative water potentials between -5 and -4 MPa, water potentials of silver poplar and common ash did not drop below -2.5 MPa, although leaves started to wilt already.
Fig. 5. AE amplitudes (blue dots) and AE rates/10 min (red lines) received from leaves of two different diffuse-porous species plotted against time. The courses of the water potential (green line) and the stomatal conductance (pink line, in % of the maximum value measured during dehydration) and a microscopic view of the leaf midrib (where the transducer was positioned) for each species are shown below the corresponding AE plots. On the left side results for silver poplar (*Populus alba* L.), and on the right side results for common beech (*Fagus sylvatica* L.) are presented.

Thus, a decline in the AE rate of leaves after a pervious rise indicates that plants reacted to drought stress by closing their stomata. Common ash and silver poplar had the most efficient strategy to prevent transpiration, since water potential reached a quite stable high (less negative) value; furthermore, in silver poplar no increase in AE rate of stems could be detected after total stomatal closure.
3.3 Drought avoiders of the spending or of the saving type?

Based on these preliminary results, common ash and silver poplar would be classified as isohydric species; as “drought avoiders of the saving type”, whereas common beech and Downey oak as anisohydric species; as “drought avoiders of the spending type” [7].

Isohydric plants have a high degree of stomatal control enabling them to maintain high leaf water potentials during the day for extended periods of drought, thus preventing cavitation. Anisohydric plants can keep their stomata open because their conducting elements in the xylem are either less prone to cavitation or they compensate for water loss due to a deep and large root system [6, 8, 9]. A deep root system is typical for oak species [23]; however, ring-porous species, with a band of wide vessels in the earlywood followed by much narrower vessels in latewood, should show in theory isohydric behavior of stomatal control, i.e., a tighter stomatal regulation of transpiration to extremes in vapor pressure than diffuse-porous species and conifers [15, 29-32].

Although isohydry would be less beneficial in diffuse-porous species [10], silver poplar exhibited an efficient stomatal control, thus a near-isohydric behavior in order to avoid cavitation and keep the water potential at high (less negative) values. It has been reported in literature that diffuse-porous species with a tendency to half-ring-porosity, such as beech (Fagus sylvatica L.) and poplars (Populus spp.), might exhibit isohydric [33-35] as well as anisohydric behavior [30].

4. Conclusions

At least in some broad-leaved species, AE rate and amplitude distribution of dehydrating stems can be a useful parameter to discriminate between AE resulting from conductivity loss and from other processes such as cavitation from non-conducting wood elements, wood shrinkage and wilting. The pattern of AE activity of dehydrating stem wood was strongly related to wood anatomy, where ring-porous species show clear clusters, which can be separated by their amplitude characteristics. AE activity of dehydrating leaves gives information on the (a) onset of stomatal closure and (b) achievement of maximum stomatal closure. Together with water potential measurements, AE activity and peak amplitude analysis should give thus a quite good insight into the species-specific strategy to survive drought stress.

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References


