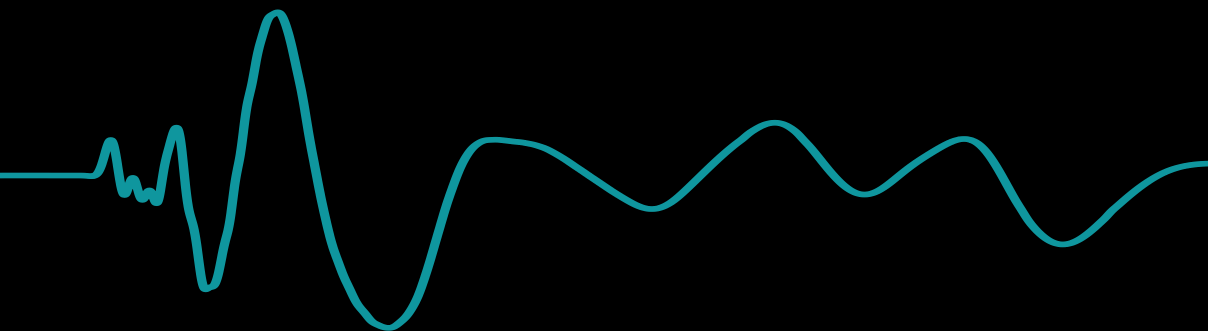
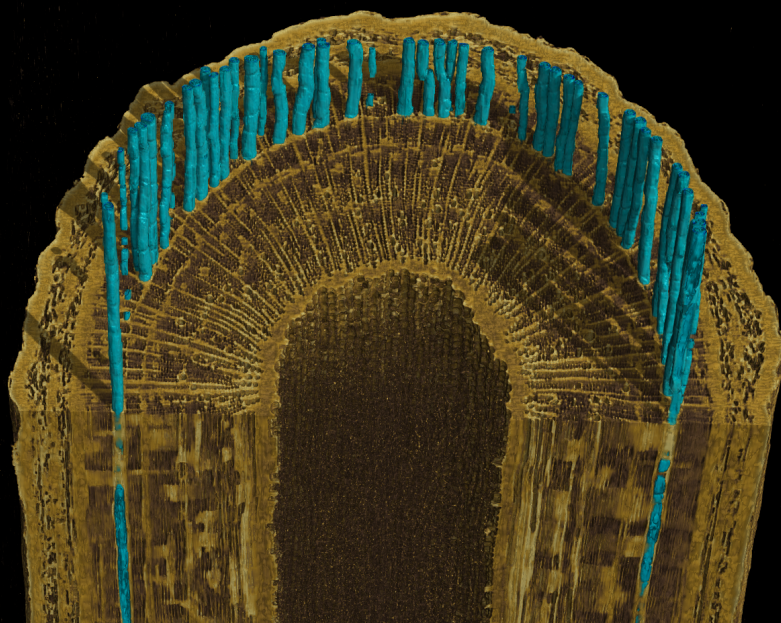




Investigation and application of the acoustic emission technique to measure drought-induced cavitation in woody plants



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Investigation and application of the acoustic
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cavitation in woody plants

Thesis submitted in fulfillment of the requirements for the degree
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Dutch translation of the title: Onderzoek en toepassing van de akoestische-emissietechniek voor het meten van droogte-geïnduceerde cavitatie in houtachtige planten

Cover: Two-years old stem of common ash (*Fraxinus excelsior* L.) with active xylem vessels indicated in blue. 3D visualization of μ CT scan by Manuel Dierick.

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Preface

Een doctoraat doen, dat betekende voor mij iets nieuws uitvinden. Maar hoe begin je hieraan? Het heeft me ongeveer vier jaar tijd gekost om dit uit te vissen. Zowel op wetenschappelijk als menselijk vlak heeft deze zoektocht me enorm verrijkt, ook al was het met vallen en opstaan. Dit hele avontuur zou niet doorgedaan zijn zonder Kathy. Bedankt om mij aan te moedigen tot het einde. Het doet me veel plezier dat het werk zal vervolgd worden in het lab. Niels, Célestin, Linus, ... ik wens jullie veel succes. Jullie worden vast een fantastisch team.

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Philip en Geert, jullie maakten met veel plezier en fijn detail de zotste proefopstellingen. Erik, dankzij jouw bakjes is dataloggen een fluitje van een cent. Jullie assistentie is zeer gegeerd en werd sterk geapprecieerd.

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Preface

Sabine, we share the same niche, but we reached a perfect symbiosis. Thank you for the nice collaboration and many interesting talks on conferences.

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Schellebelle, 2 september 2015

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List of abbreviations and symbols

Table 1 List of abbreviations

A-mode	anti-symmetric guided wave mode
AE	acoustic emission
AFM	atomic force microscopy
cum AE	cumulative number of AE signals
CWD	Choi-Williams distribution
FEM	finite element modelling
FC	frequency centroid (kHz)
HECTOR	high energy CT scanner optimized for research
L	longitudinal
LVDT	linear variable differential transformer
NMR	nuclear magnetic resonance
PF	peak frequency (kHz)
PLB	pencil lead break
PP1	partial power in the frequency range 0-100 kHz
PP2	partial power in the frequency range 100-200 kHz
PP3	partial power in the frequency range 200-400 kHz
PP4	partial power in the frequency range 400-800 kHz
PLC	percent loss of conductivity (%)
PVC	polyvinyl chloride
R	radial
S-mode	symmetric guided wave mode
SEM	scanning electron microscopy
T	tangential
VC	vulnerability curve
VWC	volumetric water content (kg m^{-3})
WPF	weighted peak frequency (kHz)
μ CT	micro-computed tomography

Table 2 List of symbols

ϕ	contact angle between air-water interface and pit pore wall
ρ	bulk wood density (kg m^{-3})
ρ_b	basic wood density (kg m^{-3})
σ	standard deviation
Ψ	water potential (MPa)
Ψ_o	osmotic water potential (MPa)
Ψ_g	gravimetric water potential (MPa)
Ψ_p	pressure water potential (MPa)
Ψ_{12}	water potential at which 12 % of the hydraulic conductivity is lost (MPa), also called air entry point (Domec & Gartner, 2001)
Ψ_{50}	water potential at which 50 % of the hydraulic conductivity is lost (MPa)
Ψ_{88}	water potential at which 88 % of the hydraulic conductivity is lost (MPa), also called full embolism point (Domec & Gartner, 2001)
τ	surface tension of water (0.072 N m^{-1} at 20°C)
C	hydraulic capacitance ($\text{kg m}^{-3} \text{ MPa}^{-1}$)
D	diameter of pit pore
Δd	branch diameter shrinkage (μm)
$\Delta d/d$	branch diameter strain ($\mu\text{m mm}^{-1}$)
E'_r	apparent modulus of elasticity in the radial direction (MPa)
Δt	difference in arrival time of signals of the same AE event at two different sensor locations
t_e	excitation time of AE source (μs)
P_c	capillary pressure (MPa)

Introduction and outline

Ever since plants left the ocean and invaded the land, they have had to cope with water shortage. Although plants are well adapted to the dehydrating land environment, they run the risk of air bubbles entering their water-conducting system. Especially during drought, vulnerability to air emboli formation in the xylem sap is a key aspect to understand plant survival or mortality (McDowell *et al.*, 2008). The formation of air bubbles may result in gas emboli that block sap flow in the xylem conduits. This process, called cavitation, reduces the hydraulic conductivity and therefore hampers the manifold vital functions of upward water flow through plants. Elevated temperatures and decreased rainfall are predicted in many regions on earth (IPCC, 2014). Moreover, precipitation is expected to occur more concentrated during extreme rainfall events. As a large part of the water is lost through run-off during extreme events, less water will be available for the vegetation (Zeppel *et al.*, 2014). These projections of increasing drought stress (Zeppel *et al.*, 2015), together with observations of increased drought-induced mortality due to climate change (Allen *et al.*, 2010; Matusick *et al.*, 2013; Williams *et al.*, 2013), raise questions about our common future. Plants are the basis of our food web. They provide medicines, materials and habitats. Their essential function in life triggers many fields of research to study plant survival in a changing environment with increasing droughts.

The standard cavitation measurement methodologies have come into question

As cavitation is a key process in plants during drought, cavitation measurements should be ubiquitous in drought research. However, measurements of cavitation have mainly been conducted by or in collaboration with highly specialized research groups with authorities in the field such as Frederick Meinzer, John Sperry, Melvin Tyree and Hervé Cochard (based on dataset in Choat *et al.* (2012)). The main reason for the absence of widespread cavitation measurements and large scale measurement campaigns is probably that many uncertainties persist about the cavitation process and the measurement methods involved. The origin of the air bubbles on one hand (Rockwell *et al.*, 2014; Schenk *et al.*, 2015), and the measurement methods of cavitation on the other hand (Jansen *et al.*, 2015), are currently a topic of fierce debate. In Chapter 1, an overview is given on the current knowledge and controversies on cavitation in plants. Usually, the degree of cavitation is measured by determining hydraulic conductivity loss. These measurements are quite burdensome and the sampling and measurement procedure is prone to artifacts. Therefore, we investigated the possibility to use the non-destructive and automatic acoustic emission (AE) technique for detecting cavitation in woody plants. Since the 60s, it has been known that the formation of air emboli is accompanied by the emission of pressure waves that can be detected with AE sensors. However, the reliability of the method was limited because AE signals have also been detected when no cavitation was expected to occur. Moreover, the actual sources of AE signals and the link with emboli formation is still under discussion. If these issues could be resolved, the AE technique could serve as a very powerful diagnostic tool in plant sciences.

Acoustic emission detection in a nutshell

The AE technique is especially applied in material testing. An ultrasonic sensor is used to listen to sounds coming from materials that undergo deformation and fracture processes. Typical industrial applications include the detection, location and assessment of flaws in structures made of metal, concrete or composites. The technique has its origins in acoustic detection of failure in the audible range,

which is a very basic warning system used by both humans and animals. The cracking sounds produced by stepping on a branch may warn animals of an approaching predator. During pottery making by early human beings, cracking sounds were indications of fast or non-uniform drying (Grosse & Ohtsu, 2008). In coal mines in the 19th and early 20th century, pine was used to support mine galleries because it audibly cracks before collapsing. However, internal material dislocations on the micro structural level also result in higher frequency sounds that cannot be detected by the human ear. These sound were called 'acoustic emissions' by Schofield (1961) (Ohtsu, 2008). The first engineering application of the AE technique was developed by Joseph Kaiser, and his doctoral dissertation (Kaiser, 1950) is often regarded as the start of AE science.

In forestry, fruit culture and horticulture, the application of the AE technique was rather limited up to now due to many methodological uncertainties. However, the acoustic detection of cavitation has a large potential to be used to support decision making with respect to aspects of the water balance. Sensors that are permanently attached to the plant may detect cavitation in real time and trigger an automatic irrigation system (Jackson & Grace, 1994). The vulnerability of certain species or cultivars to cavitation can be measured using AE sensors in a dehydration test. This test may be used as a guide to select the most suitable plants for breeding in a specific area. Moreover, plant breeders are interested in more drought-resistant plants that will perform well and sustain productivity under increasing droughts (Cochard *et al.*, 2007). Finally, continuous and non-destructive measuring of cavitation in plants will assist fundamental research into drought responses of plants and may improve our understanding of plant physiology in general.

Acoustic detection of cavitation reinvented

In this thesis, we investigated how the acoustic detection of cavitation may be improved in order to provide a reliable measuring method and increase the understanding of cavitation in plants. First, we introduced a protocol to investigate dehydration processes in woody branches. In these controlled dehydration experiments, a cavitation phase could clearly be distinguished, providing a solid framework to investigate the origin of the AE signals and identify the cavitation-

induced signals. A schematic of the experimental set-up is shown in Fig. 1. Throughout the different chapters we addressed two main questions:

- ▷ Can we identify different sources of acoustic emission?
- ▷ How can we use the acoustic emission method as a robust method to quantify vulnerability to cavitation?

In Chapter 2 we propose a new method to construct vulnerability curves and moisture release curves on grapevine branches (*Vitis vinifera*) using continuous measurements of AE, changes in branch diameter and weight loss. These curves characterize two important hydraulic features of the branch: vulnerability of the xylem to cavitation and available water resources in the different branch tissues. We observed that AE activity continued after all vessels were cavitated, indicating that other processes than cavitation may produce AE signals in branches of grapevine. However, by defining an appropriate endpoint, valid vulnerability curves could be developed.

We aimed to use the information contained in AE signals to unravel the AE sources. Through wave propagation and wave detection, the waveform is strongly changed, which complicates signal interpretation. Therefore, the whole AE detection chain was scrutinized from source to sensor. First, we selected the most appropriate sensor that measures actual surface displacement in a wide range of frequencies. As wave propagation through the branch from source to sensor has a large impact on the detected signals, this was studied using finite element modelling. The wave propagation model could be validated using a well-known artificial AE source, a pencil lead break. In Chapter 3, we explain how the branch geometry results in different guided wave modes and how these guided waves affect the AE signals.

In order to differentiate between different sources of AE signals in drought-stressed branches, we applied an automated clustering algorithm. Dehydration experiments as described in Chapter 2 were conducted with broadband sensors so that the obtained signal types could be linked with physiological processes by comparison with branch diameter strain, water potential and weight loss. In Chapter 4, we report on three clusters of AE signals in dehydrating branches of grapevine, of which one typified the cavitation phase.

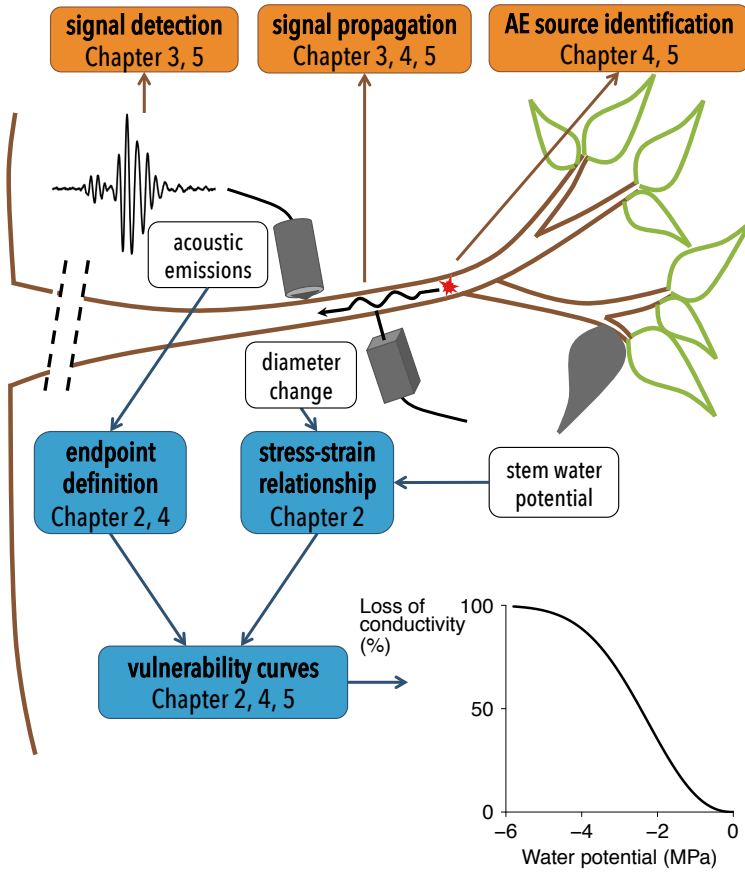


Figure 1 Schematic outline of the thesis showing the general set-up of the dehydration experiments and the main research topics. Throughout the different chapters, we addressed two questions. Orange boxes: 'Can we identify different sources of acoustic emission?' To this end, we scrutinized the full signal formation chain from acoustic emission (AE) source (red star), through signal propagation and signal detection. Blue boxes: 'How can we use the acoustic emission method as a robust method to quantify vulnerability to cavitation?' The proposed method uses continuous measurements of AE and branch diameter change. The linear relationship between relative diameter shrinkage (diameter strain) and stem water potential (measured on packed leaves) resulted in continuous water potential measurements. Cumulative acoustic emission was related to cavitation. After defining the point of 100% conductivity loss, a vulnerability curve could be constructed using both continuous measurements.

Finally, we used the insights on wave propagation and finite element modeling to explain the differences between the different signal types. Moreover, we investigated whether the identification of cavitation-type AE signals would be applicable for other branches and other species, with different diameter and wood properties. We selected an appropriate AE source model for cavitation, and used this model to investigate the influence of branch dimensions and elastic properties on the features of cavitation-related AE signals. These theoretical results, together with experimental results on white poplar (*Populus alba*) and common ash (*Fraxinus excelsior*), are presented in Chapter 5. In this Chapter, we also compared the applicability of different AE sensors to obtain valid vulnerability curves and for AE source identification.

1

Cavitation in plants: an overview of current knowledge and controversies

1.1 The peculiar path of water in trees

Trees abundantly extract water from the soil and the vast majority of all extracted water is transpired at leaf level. The amount of water that is transpired by the terrestrial vegetation in one year is double the total amount of water vapour present in the atmosphere today (Hetherington & Woodward, 2003). This seemingly wasteful water use is vital for plant functioning. Water is the transport medium that carries nutrients from the soil towards the plant organs and distributes generated carbohydrates throughout the plant. The evaporation of water in the stomata of leaves provides a cooling function, which is necessary to prevent overheating of leaves during sunny days. The water in living cells also provides a crucial role in the firmness and elasticity of soft tissues. The

positive pressure that is exerted on the cell walls, called the turgor pressure, is essential for growth (through cell growth and cell division) and fulfills the role of backbone in non-woody tissues such as leaves and petioles. Only a minor fraction of the transported water (< 1 %) is used to make new plant material through photosynthesis (Lambers, 1998).

Water is transported throughout the plant following a gradient in water potential (ψ), which consists of three parts: the osmotic (ψ_o), gravimetric (ψ_g) and pressure potential (ψ_p) (Eq. 1.1). The gravimetric water potential plays a role when water is transported over long vertical distances. The osmotic water potential is caused by solutes in the cell sap and is more negative for increasing solute concentrations. In sap in living cells, e.g. in the phloem, cambium and wood rays (Fig. 1.1), the concentration of solutes is much higher than in sap in dead xylem conduits (Fig. 1.1). The pressure potential can be positive or negative. In living cells, the pressure potential is positive, which is called the turgor pressure. The dead xylem conduits, in contrast, operate under negative pressure, or tension. According to the cohesion-tension theory (Dixon, 1914), the origin of this tension is the evaporation of water in the stomatal region (Fig. 1.2). When the stomata in the leaves are open, water is transpired due to the difference in water vapour pressure between the atmosphere and the substomatal cavity. As water evaporates into the air spaces in the leaf, water menisci in the small capillaries (nanometer scale) in adjacent cell walls are retracted and capillary forces (due to strong adhesion) pull the menisci back towards the surface. The network of many small capillaries in the cell wall acts thus as a wick for water rise (Nobel, 2005). Thanks to the strong cohesion between water molecules, the tension is transmitted downwards and water can be drawn towards the leaves. Under sufficient soil water availability, typical tensions in the sap-conducting conduits may be for example -1 MPa in the leaf conduits, -0.8 MPa in stems and branches and -0.6 MPa in the roots (Campbell *et al.*, 2008).

$$\begin{aligned}\psi &= \psi_o + \psi_g + \psi_p \\ &= -R \cdot T \cdot C + \rho_w \cdot g \cdot h + \psi_p\end{aligned}\tag{1.1}$$

R universal gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$)

T temperature (K)

C solute concentration (mol m^{-3})
 ρ_w density of water (kg m^{-3})
 g acceleration of gravity (9.81 m s^{-2})
 h height (m)

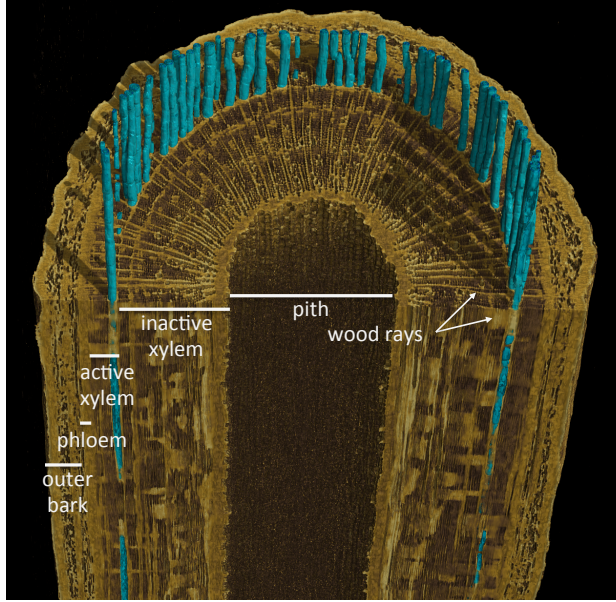


Figure 1.1 Anatomy of a two-years old stem of common ash (*Fraxinus excelsior* L.). The active xylem vessels are indicated in blue. In between the active xylem and the phloem is the vascular cambium. Note that the spongy tissue in the pith is not visualized in this μCT 3D-reconstruction. Credit: Manuel Dierick.

The tension in the xylem conduits may increase enormously when faced with a dry soil or with a great transpirational demand that exceeds the rate of water supply from the roots or from internal water reserves. This involves a risk of air bubbles entering the conduits, which may expand and quickly fill the whole conduit. The water released from the conduits during this process may contribute to the transpiration stream, but on the other hand, the water conducting system will be locally interrupted. Because adjacent conduits are interconnected via pits in the conduit walls (see Section 1.2), the sap may circumvent the embolized conduit. However, when too many conduits are embolized, this will impair plant functioning.

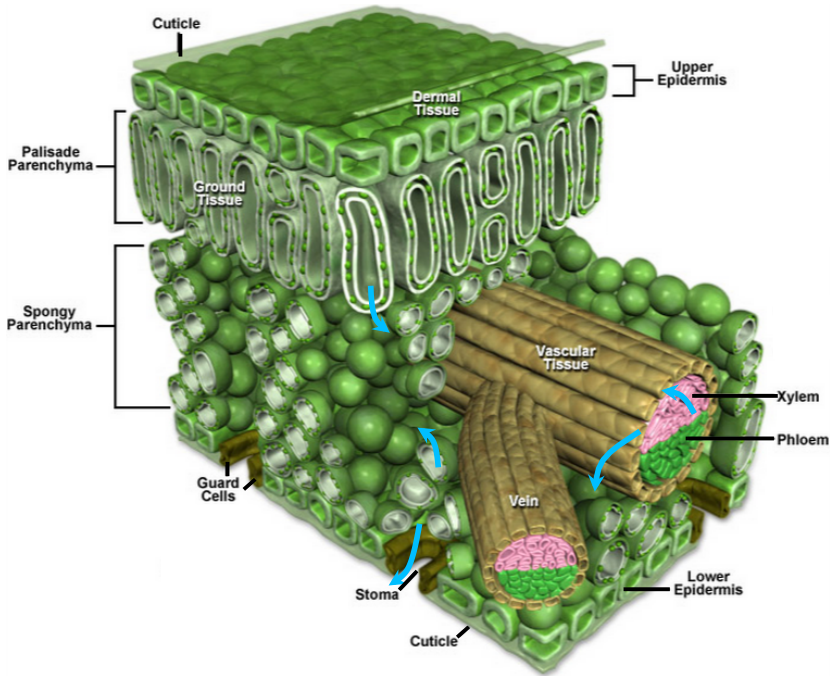


Figure 1.2 Schematic cross-section of a leaf, indicating the pathway of water molecules during transpiration (adapted from imgarcade.com).

1.2 Interruption of the water transport by cavitation

The formation of air emboli that block sap flow in xylem conduits is currently of high interest because it is one of the key processes leading to plant mortality during drought (McDowell *et al.*, 2008). The phenomenon was called cavitation, based on the analogy with unwanted air cavities formed near the surface of propeller blades due to low pressures (Jackson & Grace, 1994). However, cavitation in plants occurs in a different way. According to the current knowledge, the main cause of cavitation is the failure of a pit in the conduit wall to prevent air from entering the conduit at strong tension (Rockwell *et al.*, 2014), known as the air-seeding hypothesis (Zimmermann, 1983). Pits (Fig. 1.3) form the interconnection between adjacent conduits and between conduits and living cells.

At the pit location, the secondary cell wall that provides compression strength to the conduits is interrupted and the primary cell wall is chemically modified and thinned to increase lateral porosity (Ménard & Pesquet, 2015). The pits between conduits have a dual role: they have to maximize conductivity for sap flow and minimize the risk of air seeding at strong tension.

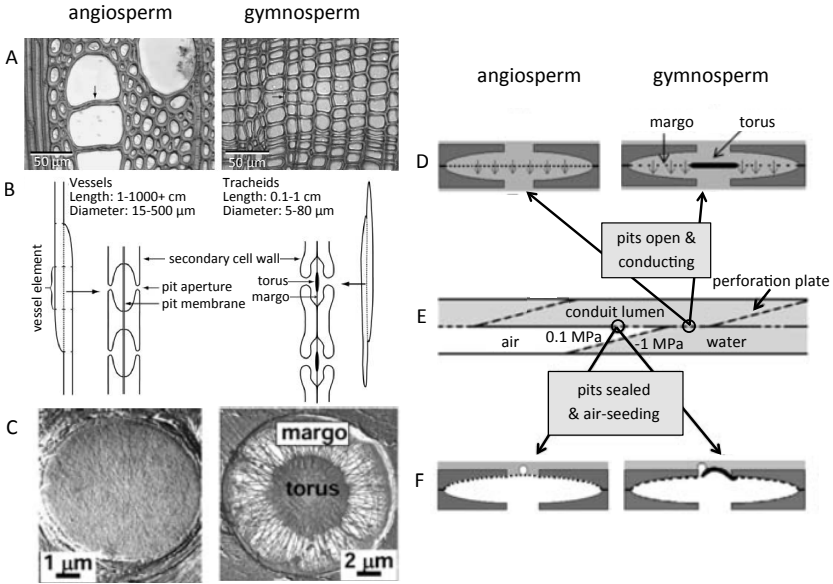


Figure 1.3 Differences in anatomy of the xylem conduits and pits between angiosperm and gymnosperm species. Transverse (A) and longitudinal (B) cross section of the xylem conduits. Vessels in angiosperms consist of connected vessel elements, of which the endwalls are degenerated (perforation plates). The xylem conduits in gymnosperms consist of tapered tracheids. Cross section of the inter-conduit pits (B) and cryo-SEM image of the pit membrane (C). In conducting state (D,E), sap moves through the pit margo in gymnosperms and through the pit membrane in angiosperms. Due to the tension difference between a cavitated and functional conduit (E,F), the impermeable torus is aspirated and seals the pit aperture to prevent spread of cavitation. Angiosperms do not have a torus and the semi-permeable pit membrane has to provide both hydraulic conduction and resistance to air-seeding. Figure adapted from Pittermann *et al.* (2005) and Choat *et al.* (2008).

In gymnosperm species, the pit membrane consists of a disk-shaped torus surrounded by a permeable pit margo that provides hydraulic conductivity between

conduits (Fig. 1.3). When an adjacent conduit is filled with air, the pressure difference between cavitated and functional conduit aspirates the torus towards the overarching secondary wall, sealing the pit aperture (Fig. 1.3F). Larger overlap between torus and aperture provides higher resistance to air seepage in between torus and overarching pit wall. This functional trait may explain the variation in the vulnerability to cavitation observed in gymnosperm species (Delzon *et al.*, 2010; Bouche *et al.*, 2014). However, a high margo elasticity or margo rupture could allow the torus to be pulled out through the pit aperture, leading to air seeding (Hacke *et al.*, 2004; Cochard, 2006; Delzon *et al.*, 2010). Another hypothesis states that a low margo elasticity could result in air seeding because the torus cannot be aspirated completely to seal the pit aperture (Hacke *et al.*, 2004; Cochard, 2006; Domec *et al.*, 2006).

Pits in angiosperm species, in contrast, do not have such a microscopic valve. The pit membrane in angiosperms is much less permeable than the pit margo in gymnosperms because it also has to provide resistance to cavitation. Moreover, how air may actually enter a conduit through the very small pores in the pit membranes is not completely understood (Rockwell *et al.*, 2014). The hypotheses regarding cavitation in vessels of angiosperm species can be divided into three categories: capillary seeding, rupture seeding and other pathways of air entry. In the research conducted during this doctorate, we focussed on angiosperm species. Therefore, the discussion in the remainder of this section is limited to cavitation in angiosperm species.

1.2.1 Capillary seeding

The capillary-seeding model states that air may pass a pore in the pit membrane (pit pore) when the pressure difference across the pit pore is larger than the capillary pressure in the pore (P_c , MPa). For a cylindrical pore, the Young-Laplace equation can be written as:

$$P_c = \frac{4 \cdot \tau \cdot \cos(\theta)}{D} \quad (1.2)$$

where τ is the surface tension of water (0.072 N m^{-1} at 20°C), θ is the contact angle between the air-water interface and the pore wall (assumed to be 0°) and D is the diameter of the cylindrical pore. This approximation is known as the

Laplace-Washburn equation (Schenk *et al.*, 2015). However, the observed pore diameters with scanning electron microscopy (SEM) are often much smaller (< 200 nm) than the theoretically required pore size that is required for capillary air seeding (Choat *et al.*, 2003; Jansen *et al.*, 2009; Lens *et al.*, 2011). Choat *et al.* (2003) suggested two explanations supporting the capillary-seeding model. First, stretching of the pore membrane due to aspiration (Fig. 1.3) may enlarge the pores. Second, large pores were present, but not detected because they are rare. Meyra *et al.* (2007) provided a third alternative by considering a non-zero contact angle in the pit membrane pores (Eq. 1.2). Including partial wetting in their model could explain the observed air seeding pressures. A fourth alternative is recently provided by Schenk *et al.* (2015). They included a pore shape correction factor, because pores in between microfibrils are slit- or V-shaped rather than circular. Moreover, they postulated that nanobubbles are snapped off during air seeding. These nanobubbles are stabilized by surfactants and may exist in the plant sap under tension. They may eventually result in an embolism when the size of the nanobubble exceeds a critical threshold due to increasing tension or when many nanobubbles coalesce.

1.2.2 Rupture seeding

When visualizing the surface of hydrated pit membranes using atomic force microscopy (AFM), the pit pores as observed with SEM could not be detected (Lee *et al.*, 2012). The measurements with AFM suggest that the porous network of microfibrils is embedded in a hydrogel matrix. These hydrogels are composed of pectins, which consist of various and complex galacturonic acid-rich polysaccharides and have been measured in the pit membrane (Maschek *et al.*, 2013; Herbette *et al.*, 2014). Sap movement between the conduits would occur thus through a hydrogel phase instead of a liquid phase and capillary seeding is not compatible with this model. Alternatively, air seeding may occur by rupture of the composite pit membrane when the pressure difference between the functional and air-filled conduit results in a mechanical stress that exceeds the membrane strength (Sperry & Hacke, 2004; Rockwell *et al.*, 2014). The cracks formed by breaking ionic cross links in hydrogels can be repaired after stress removal (Sun *et al.*, 2012). The hydrogel model may also explain the strong influence of ion concentration on hydraulic conductivity of the xylem (Zwieniecki *et al.*, 2001, 2003; Gascó *et al.*, 2006; Cochard *et al.*, 2010) by

affecting the thickness and permeability of the pit membrane (Lee *et al.*, 2012) (see Section 1.4.1).

1.2.3 Air entry prior or additional to air seeding

Air seeding by capillary or rupture seeding may only occur when a functional conduit is in contact with an air-filled conduit (Mayr *et al.*, 2006). This raises the question how air bubbles are initiated in the xylem of plants. The xylem is often damaged by pathogen infection, herbivory or nesting of various animals (Tyree & Sperry, 1989b; McElrone *et al.*, 2008), and branches may contain fractures due to snow load and wind (Liu *et al.*, 2003). These damages form a pathway for air entrance in the xylem. Internal tissue damage is also inherent to growth (Rockwell *et al.*, 2014). The soft tissues in the central pith (Fig. 1.1) are often ruptured and filled with air cavities. During growth, the protoxylem that surrounds the pith is stretched and may be ruptured, bringing the xylem into contact with air-filled protoxylem conduits (Rockwell *et al.*, 2014). Also, freezing of the xylem sap, which is almost fully saturated with gases, will force dissolved gases to come out of solution and form bubbles (Hacke & Sperry, 2001). The bubbles may expand upon thawing and result in air-filled conduits. Finally, heterogeneous cavitation may occur at air nuclei on hydrophobic patches in the conduit wall, when the strong tensions exceed the adhesive forces between sap and conduit wall (Pickard, 1981). Hydrophobic patches may consist of lignin depositions, which increase the compressive strength of the conduit wall (Zwieniecki & Holbrook, 2000), or cracks that expose cell wall lignin (Pickard, 1981). Moreover, Herbette *et al.* (2014) found evidence that the pit membrane in poplar xylem was mostly composed of cellulose embedded in a lignin matrix, and was thus hydrophobic.

1.3 How to cope with cavitation

As abundant water transport is vital for trees (Section 1.1), an excessive reduction in hydraulic conductivity by cavitation may cause irreversible damage and eventually lead to mortality (McDowell *et al.*, 2008). Therefore, it is essential

for trees to protect their xylem from excessive cavitation and to mediate or accommodate the adverse effects of cavitation.

1.3.1 Prevention is better than cure

1.3.1.1 Long-term protection

The anatomy of the xylem is optimized to prevent excessive cavitation under typical growth conditions that species experience in their natural habitats (Hacke & Sperry, 2001). The pit membranes are designed to prevent air entry for the usual range of tensions in the xylem sap. Features of the pit anatomy that have been related to high cavitation resistance include greater membrane thickness and smaller pit aperture fraction, which reduce the risk of air seeding (Lens *et al.*, 2011). Also, stretching of the membrane during aspiration, which could lead to rupture or larger pit pores, is reduced in resistant species by shallower pit chambers, a smaller pit aperture fraction and narrower slit-like apertures (Lens *et al.*, 2011). However, these features also pose a higher resistance to flow between conduits. A similar safety-efficiency trade-off (Tissier *et al.*, 2004; Manzoni *et al.*, 2013a) applies to the geometry of the conduits themselves. Shorter conduits that are manifold interconnected (Carlquist, 2009) increase the possibility to circumvent cavitation, but increase the resistance by increasing the number of pit passages (Lens *et al.*, 2011). Wide conduits would provide the highest hydraulic conductivity, but to withstand the strong tensions, the conduit wall has to be reinforced. As a consequence, a strong correlation exists between conduit thickness-to-span (inter-conduit wall thickness over conduit diameter) (Lens *et al.*, 2011; Hacke *et al.*, 2001; Jacobsen *et al.*, 2007), and thus wood density (Hacke *et al.*, 2001; Martínez-Cabrera *et al.*, 2009; Onoda *et al.*, 2010), and cavitation resistance. Also fibers have been found to provide extra mechanical strength to the conduits to prevent implosion (Jacobsen *et al.*, 2005). In addition to xylem anatomy, also the structure and functioning of the root system (David *et al.*, 2013) and the leaves (Franks & Beerling, 2009; Hao *et al.*, 2010; Markesteijn *et al.*, 2011) is fine-tuned to optimize water management.

1.3.1.2 Short-to mid-term protection

In a warm and dry period, low soil water availability and strong transpiration may induce very strong tensions that threaten to exceed the limits that the xylem was designed for. Then, cavitation can still be prevented through measures that reduce xylem tension. First, water extracted from internal water reserves may be released in the xylem conduits and thus prevent further lowering of the xylem water potential. In fact, this is a very common phenomenon that occurs on a daily basis and explains the typical diel pattern of shrinkage and swelling (Fig. 1.4) that can be observed in many stems, branches and leaves (Čermák *et al.*, 2007; Ishii *et al.*, 2014). The daily release of internally stored water into the transpiration stream allows a high rate of transpiration at the beginning of the day, despite a slower start-up of the water uptake by the roots (Schulze *et al.*, 1985). Usually, the water reserves are replenished at night. However, internal water reserves also have a vital function during more severe droughts as they may temper strong decreases in water potential (Meinzer *et al.*, 2009; Zhang *et al.*, 2013) and thus prevent excessive cavitation or at least retard this process (Phillips *et al.*, 2004). The capacity of a tissue type to store and release water is quantified by the hydraulic capacitance (C , $\text{kg m}^{-3} \text{MPa}^{-1}$), equal to the amount of water that can be released from the tissue for a unit decrease in water potential (Edwards & Jarvis, 1982). The role of C as a buffer to protect the xylem against excessive cavitation has been supported by many observations of a positive correlation between C and vulnerability to cavitation (Pratt *et al.*, 2007; Meinzer *et al.*, 2008; Sperry *et al.*, 2008; Meinzer & McCulloh, 2013; McCulloh *et al.*, 2014; Richards *et al.*, 2014). Meinzer & McCulloh (2013) suggested that species with low vulnerability to cavitation due to structural investments in the xylem have a lower hydraulic capacitance. They operate with a large safety margin, keeping the risk of reaching the cavitation-inducing water potential low. Species that are highly vulnerable to cavitation, operating with a small safety margin, would rely more on hydraulic capacitance. These species with a risky behaviour would also have a higher capacity to repair cavitation (Ogasa *et al.*, 2013; Meinzer & McCulloh, 2013) (see Section 1.3.2).

A mechanism that may prevent cavitation due to strong transpiration is stomatal closure (Tyree & Sperry, 1988; Cochard *et al.*, 1996; Sperry, 2000). By reducing the transpiration, further decline of the water potential is prevented and the xylem is preserved from cavitation. Partial stomatal closure is known to occur

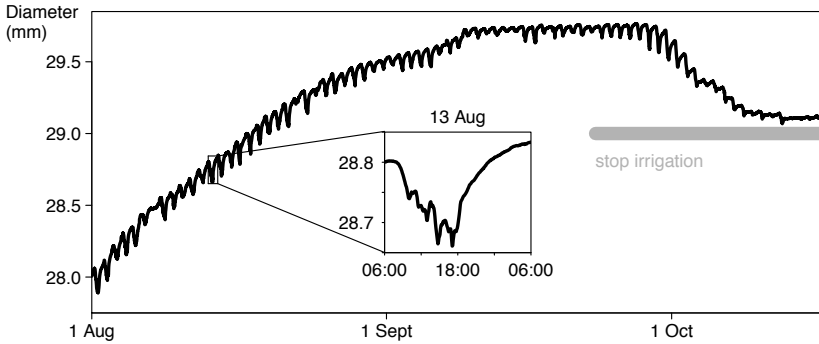


Figure 1.4 The diameter pattern (over-bark) of a common ash stem during summer growth followed by an artificial drought event illustrates the role of elastic water reserves during daytime water deficit and during drought.

under rather standard conditions. Between late morning and afternoon on sunny days, the stomatal conductance is often reduced with 50% (Klein, 2014). An important side effect of stomatal closure, however, is the reduced stomatal CO_2 uptake. Stomatal closure may thus impair the carbon balance due to reduced leaf photosynthesis. Although excessive cavitation can be prevented by stomatal closure, prolonged limitation of CO_2 uptake may eventually lead to mortality due to carbon starvation (McDowell *et al.*, 2008; Sevanto *et al.*, 2014). For short-term hydraulic control, however, stomatal regulation is an effective way to avoid xylem cavitation and mediate drought stress. Klein (2014) has found a linear relation between leaf water potential at which 50% stomatal closure and 50% conductivity loss occurs, supporting the hypothesis that stomata regulate leaf water potential to avoid xylem cavitation.

1.3.2 Intensive care?

Although measures are taken to prevent excessive cavitation, a certain degree of cavitation can not always be avoided. Moreover, many authors have identified a controlled level of cavitation as an extra internal water resource (Waring & Running, 1978; Tyree & Yang, 1990; Lo Gullo & Salleo, 1992; Meinzer *et al.*, 2001; Hölttä *et al.*, 2009; McCulloh *et al.*, 2014; Steppe *et al.*, 2015) that can be used to sustain transpiration and CO_2 uptake during drought events. Based

on reported measurements on a large range of woody species, it was hypothesized that species with a high vulnerability to cavitation may even encounter cycles of embolism formation and repair on a daily basis (Windt *et al.*, 2006; Ogasa *et al.*, 2013). Cavitation repair has been demonstrated by non-invasive imaging techniques (Holbrook *et al.*, 2001; Windt *et al.*, 2006; Scheenen *et al.*, 2007; Brodersen *et al.*, 2010), but no consensus has been reached about the mechanisms of refilling.

If embolism repair would be a passive process, like the long distance water transport in the xylem, refilling of emboli could only be possible when the pressure in the xylem would increase enough to force the gas back into solution. According to Yang & Tyree (1992), a xylem water pressure of at least -0.1 MPa would be needed for refilling to occur. For large trees, bubble solution would thus be impossible in branches at more than 10 m above ground without the assistance of active processes to overcome the gravitational water potential. However, evidence for refilling under tension is reported for a wide range of species in roots, stems, petioles and leaves (Clearwater & Goldstein, 2005). Although the validity of many of these studies was questioned due to possible experimental artifacts (Clearwater & Goldstein, 2005; Wheeler *et al.*, 2013), the active refilling of embolized conduits, with the aid of living cells, has been a popular topic of research.

Generally, the increase in osmotically active components is assumed to play a role in the active repair of cavitation. These components, which could be ions or low- or high-molecular weight carbohydrates, are released in the embolized conduit or in the neighbouring parenchyma cells. The osmotically-driven water flow directly fills the empty xylem conduit or pressurizes the adjacent parenchyma cells, so that water is pushed out of the cells into the conduits. These mechanisms (except for the case of high-molecular weight carbohydrates) require hydraulic isolation of the refilling conduit, to prevent that the solution is removed by the transpiration stream before refilling is completed. Also, the living cells of the axial parenchyma, wood rays and phloem (Salleo *et al.*, 2004; Hölttä *et al.*, 2006; Nardini *et al.*, 2011) need to be triggered to start starch conversion and to activate aquaporins and ion transporters (Secchi *et al.*, 2011; Secchi & Zwieniecki, 2012). Moreover, the movement of water and air in and out the refilling conduit should be facilitated. Possible mechanisms for these processes

are proposed by Zwieniecki & Holbrook (2009) (Fig. 1.5), but more research is necessary to elucidate the self-healing capacity of the xylem.

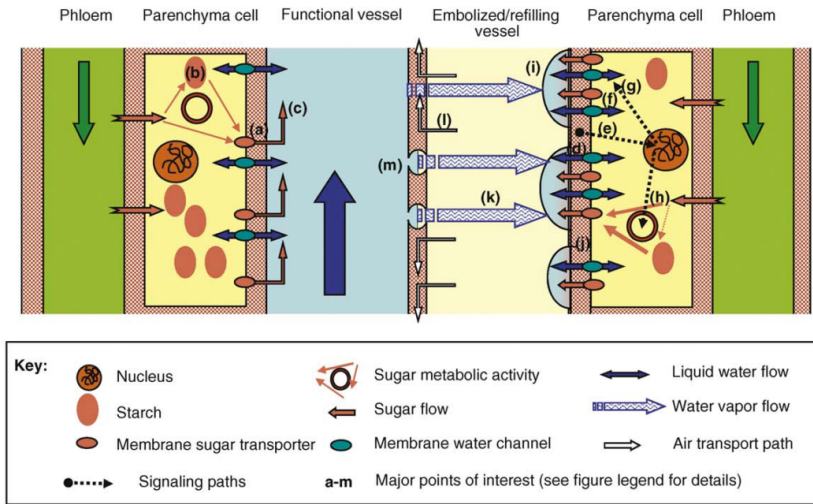
Moreover, the refilling mechanisms may be different depending on the location of the xylem conduits in the roots, stems, branches, petioles or leaf veins (Brodersen & McElrone, 2013). In the vicinity of the leaves, foliar water uptake (Limm *et al.*, 2009; McCulloh *et al.*, 2011; Mayr *et al.*, 2014b) has been hypothesized as a successful component of embolism repair, whereas root pressure (Sperry *et al.*, 1987; Cochard *et al.*, 1994; Wang *et al.*, 2011; Yang *et al.*, 2012) is likely to assist refilling in lower plant parts. In stems and branches, woody tissue photosynthesis can play a role in the local production of carbohydrates that are required for embolism repair (Schmitz *et al.*, 2012; Bloemen *et al.*, 2015; Steppe *et al.*, 2015).

In species that cannot rapidly refill their vessels, seasonal refilling may occur after the dry period (McCulloh *et al.*, 2011) or in the next spring (Sperry *et al.*, 1994; McCulloh *et al.*, 2011; Mayr *et al.*, 2014b; Laur & Hacke, 2014). Spring-refilling is also often observed in species with large vessels that are very susceptible to freeze-induced cavitation (Sperry *et al.*, 1987; Cobb *et al.*, 2007). Other species rely on the production of new conduits after drought (Brodribb *et al.*, 2010) or new earlywood next spring (Sperry *et al.*, 1994; Hacke & Sauter, 1996). Ring-porous species, having an annual ring with large vessels that are highly conductive and more vulnerable to cavitation, may rely on the narrower conduits that are produced later in the growing season for sustained hydraulic conductivity when soil moisture decreases (Cochard *et al.*, 1997; Klein, 2014).

1.4 Measuring cavitation

1.4.1 Hydraulic method

The degree of cavitation is most often measured as the resulting percent loss of hydraulic conductivity (PLC). This method was proposed by Sperry *et al.* (1988a) and consists of two consecutive measurements of hydraulic conductivity on a stem sample. A solution is forced under positive pressure (≤ 10 kPa) through the sample in native state and the flow is measured (Fig. 1.6). Then,



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Figure 1.5 Embolism refilling scenario reprinted from Zwieniecki & Holbrook (2009). (a) Living cells in contact with vessels release a small but steady amount of soluble carbohydrates into the xylem. (b) Starch stored in xylem parenchyma serves as a sugar capacitor. (c) These solutes are normally swept away by the transpiration stream, keeping concentrations at very low levels, (d) but accumulate in a vessel that has cavitated. (e) Sugar accumulation and the associated increase in apoplastic solute concentration triggers signaling pathways (f) for refilling that regulate sugar and (g) water membrane transport, as well as (h) sugar metabolic activity. (i) The accumulation of solutes results in water movement from xylem parenchyma cells by osmosis, forming droplets with high osmotic activity on internal vessel walls. (j) The partially non-wettable walls of xylem conduits prevent these droplets from being removed by suction from still-functional vessels. (k) Condensation of water vapour provides a second pathway by which water refills cavitated conduits, allowing adjacent conduits to provide water for refilling. (l) As the high osmotic droplets grow to fill the vessel, the embolus is removed both by forcing gas into solution and by pushing gas through small pores through the vessel walls to intercellular spaces. (m) The flared opening of the bordered pit chamber acts like a check valve until the lumen is filled, thus preventing contact with the highly wettable bordered pit membranes. Reconnection occurs once the pressure in the lumen exceeds that of the entry threshold into the bordered pit chambers; a hydrophobic layer within pit membranes might provide the needed simultaneity among multiple bordered pits.

the sample is flushed with water under high pressure (175 kPa, Sperry *et al.* (1988a)) or submerged in water under vacuum (Espino & Schenk, 2011) to remove all air bubbles from the xylem. The maximum hydraulic conductivity is measured again, on the saturated sample, and PLC is calculated as the ratio of both hydraulic conductivity measurements. Sperry *et al.* (1988a) pointed out, and this has been stressed by the work of Wheeler *et al.* (2013) and Torres-Ruiz *et al.* (2015), that care should be taken to minimize the introduction of additional air bubbles into the xylem during sampling. They recommended to excise a branch segment that was long enough so that the vessels that were cut open did not extend into the central part on which the measurement was done. Then, the tension in the branch was relaxed by soaking under water and the central sample was cut under water before connecting to the solution-filled tubing of the measurement system. The type of solution that is forced through the sample is another point of attention. The ion concentration and pH of the perfusing solution have been known to influence the measured flow rate (Melcher *et al.*, 2012). It was hypothesized that physicochemical changes of hydrogels change the resistance of the pit membrane (Zwieniecki *et al.*, 2001; Gascó *et al.*, 2006; Van Ieperen & Van Gelder, 2006), but also concentration-dependent bubble formation and coalescence in the solution under positive pressure was suggested to affect the flow rate (Espino & Schenk, 2011). Espino & Schenk (2011) recommended that degassing of the solution is essential for achieving stable flow measurement. Finally, the method requires that there is no net uptake of water by the sample. This so-called background flow is usually accounted for by a preliminary measurement of water uptake when both sample ends are connected to a water reservoir without pressure difference (Kolb & Sperry, 1999; Melcher *et al.*, 2012).

Although this method has long been established, it suffers from some major draw-backs. First of all, the method is destructive and very labour intensive, as many sample manipulations are needed for one measurement to be taken. Second, no consensus has been reached about which solution should be used to obtain accurate measurements. Third, the amount of water uptake (background flow) at positive pressure during measurements might not be correctly estimated if the background flow is measured under lower pressure (De Baeremaeker *et al.*, 2015). The fourth issue with the hydraulic method, which is currently a topic of fierce debate, is to find the most appropriate sampling method in order not to change PLC during sampling. Wheeler *et al.* (2013)

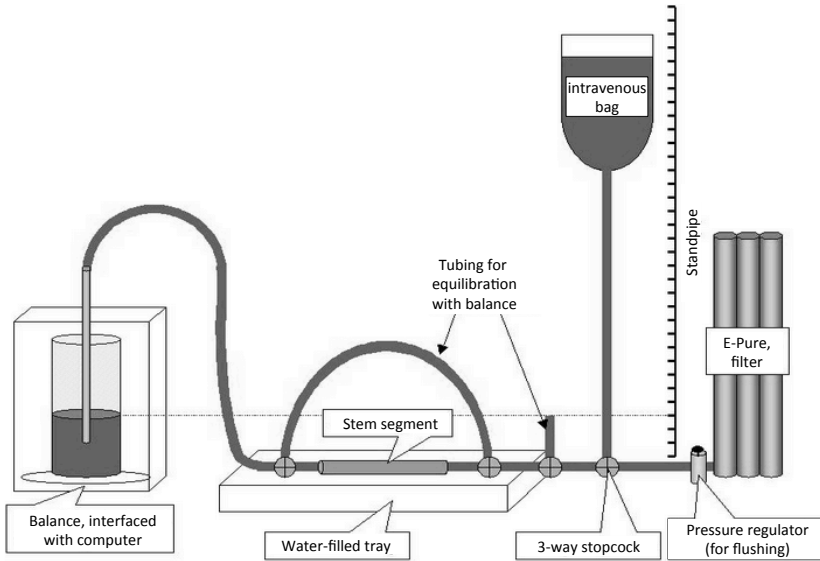


Figure 1.6 Schematic of the hydraulic conductivity apparatus by Uwe Hacke (Figure adapted from <http://biologylabs.utah.edu/sperry/methods.html>).

found that sampling under tension, without subsequent relaxation as recommended by Sperry *et al.* (1988a), may result in artificially high PLC values. As it is not clear in literature which studies have taken this measure, they suggest that observations of frequent cavitation during the day may be experimental artifacts. However, Trifilò *et al.* (2014) found that sample relaxation may enable embolism repair, which results in underestimations of PLC. They recommend to remove the bark at multiple locations along the branch (phloem girdling) 1 h prior to sampling, followed by xylem relaxation. Up to now, no consensus has been reached about how to obtain accurate cavitation measurements with the hydraulic method and the search for new reliable measurement techniques was encouraged at the International Workshop on Plant Hydraulic Techniques (Ulm University 2014) (Jansen *et al.*, 2015).

1.4.2 Visualization methods

Visualizations of air-filled vessels have been realized with cryo-scanning electron microscopy (cryo-SEM). In the cryo-SEM technique, a frozen xylem sample is fractured and the fracture surface imaged using SEM (Jackson & Grace, 1994; Canny, 1997; Hukin *et al.*, 2005; Johnson *et al.*, 2009; Bauerle *et al.*, 2011). Empty xylem conduits can be distinguished from ice-filled conduits on the cryo-SEM micrographs (Fig. 1.7). However, Cochard *et al.* (2000) pointed out that cavitation may be induced during freezing of xylem samples under tension by air expulsion from the xylem sap, overestimating cavitation measurements with cryo-SEM.

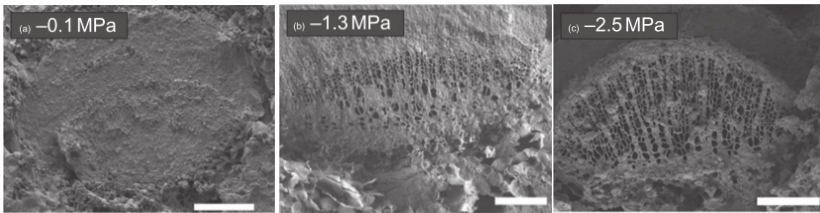


Figure 1.7 Cryo-SEM images of the cross-sections of leaf midveins of *Pieris japonica* at three different stages of dehydration. Scale bars represent 100 μm . Figure from Johnson *et al.* (2009).

Two non-destructive visualization technologies have been employed to measure xylem embolism on intact samples: nuclear magnetic resonance (NMR) imaging (Holbrook *et al.*, 2001; Choat *et al.*, 2010; Kaufmann *et al.*, 2009; Jones *et al.*, 2012) and X-ray micro-computed tomography (μCT) (Brodersen *et al.*, 2010, 2013; McElrone *et al.*, 2013; Cochard *et al.*, 2015; Choat *et al.*, 2015). X-ray μCT has been proposed as a reference technology for xylem embolism studies (Cochard *et al.*, 2015) because it offers sufficient spatial resolution (down to 1 μm is possible) and desktop-based μCT systems are currently affordable. NMR, in contrast, suffers from poor spatial resolution (down to 20 μm at best) and is not readily available (Cochard *et al.*, 2015). To date, *in vivo* visualization techniques are considered the only measurement techniques that can irrefutably prove or deny the existence of cavitation repair in xylem conduits (Brodersen *et al.*, 2010; Choat *et al.*, 2015) (Fig. 1.9).

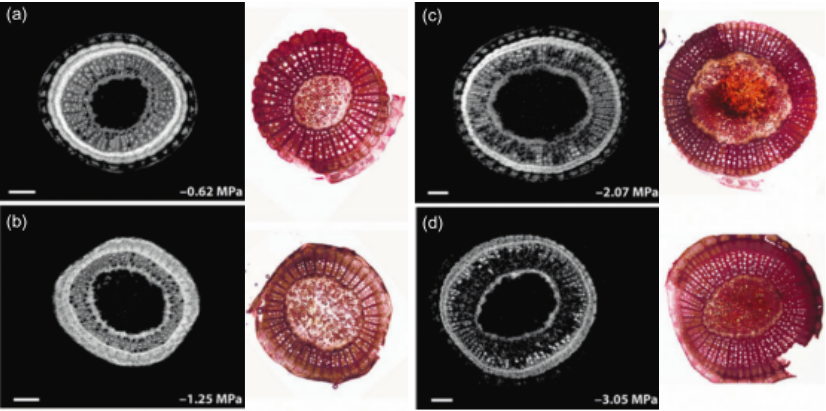


Figure 1.8 Increasing degree of cavitation induced by decreasing xylem water potential in stems of *Vitis vinifera* cv. Chardonnay is visualized with nuclear magnetic resonance (NMR) imaging (first and third column). The light microscopy cross sections (second and fourth column) were used to identify the corresponding anatomical features. Water filled xylem vessels can be seen as bright areas in the xylem on the NMR images, whereas cavitated vessels appear as black dots. Scale bars represent 1 mm. Figure from Choat *et al.* (2010).

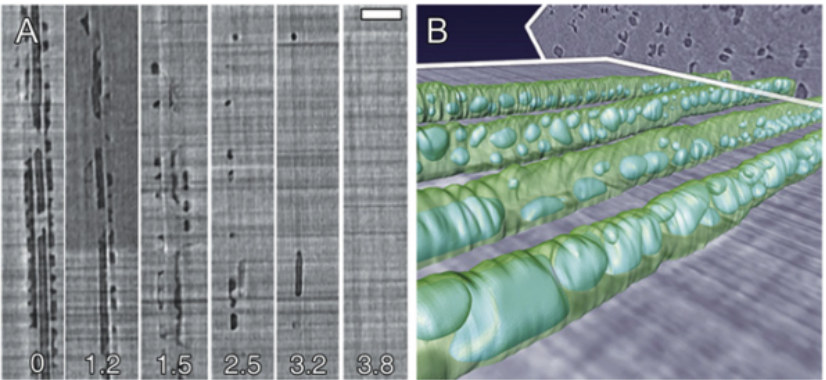


Figure 1.9 With X-ray μ CT, vessel refilling in *Vitis vinifera* cv. Chardonnay could be visualized. The time-lapse in (A) shows longitudinal sections of three adjacent vessels that undergo refilling in a period of 3.8 h. Bar = 200 μ m. The water droplets that appear in the vessels could be beautifully rendered in 3D (B). Figure from Brodersen *et al.* (2010).

1.4.3 Acoustic method

Next to vision, hearing is the second most important and developed human sense. It is thus not surprising that attempts have been made to listen to cavitation. In 1966, Milburn & Johnson recorded sounds in plants when they were subjected to dehydration. They detected audible vibrations in petioles of dehydrating leaves of diverse plant species by impaling the petiole on a phonograph pick-up needle (Fig. 1.10). Because measurements were often disturbed by environmental noise, the step was made towards acoustic emission (AE) detection in the ultrasonic frequency range by Tyree & Dixon (1983). This has facilitated various experiments because the problematic ambient noise in the audible range (< 20 kHz) could be filtered electronically. Based on the similar sound production by the rupture of plant sap under tension in glass tubes (Dixon, 1914; Temperley & Chambers, 1946), it was hypothesized that the rupture of sap in plant conduits produced the observed sounds during drying. Although extensive circumstantial evidence was provided to support this hypothesis (Milburn, 1973a,b; Tyree *et al.*, 1984a), it was realized that sounds may be produced by other mechanisms too. Sounds were observed during drying of plant tissues that do not contain conduits (Milburn, 1973a; Kikuta, 2003), during re-watering (Milburn, 1973b), and also during freezing (Raschi *et al.*, 1989; Mayr & Sperry, 2010; Charrier *et al.*, 2014) and thawing (Raschi *et al.*, 1989; Mayr & Sperry, 2010). The AE technique is also applied to monitor internal cracking of wood during drying (Moliński *et al.*, 1991; Rosner, 2007) and soaking (Moliński *et al.*, 1991). Moreover, Gagliano *et al.* (2012) speculated that plants may actively produce sounds for short-distance communication.

During the dehydration of fresh plant material, AE signal detection was found a valid method to measure cavitation in woody branches (Lo Gullo & Salleo, 1993), leaves (Kikuta *et al.*, 1997), herbaceous stems (Hacke *et al.*, 2000) and sap wood sections (Kikuta *et al.*, 2003), while others found a poor correspondence between hydraulic and acoustic measurements (Sperry *et al.*, 1988b; Cochard & Tyree, 1990). Especially in angiosperm species, the continued AE activity after the loss of most of the hydraulic conductivity was a great cause of concern (Tyree & Sperry, 1989b; Rosner, 2015). Other processes than cavitation in sap-conducting elements that could cause extra AE signals during drought stress have been suggested by various authors: cavitation of fibers, wood tracheids or ray cells (Sperry *et al.*, 1988b; Ritman & Milburn, 1988;

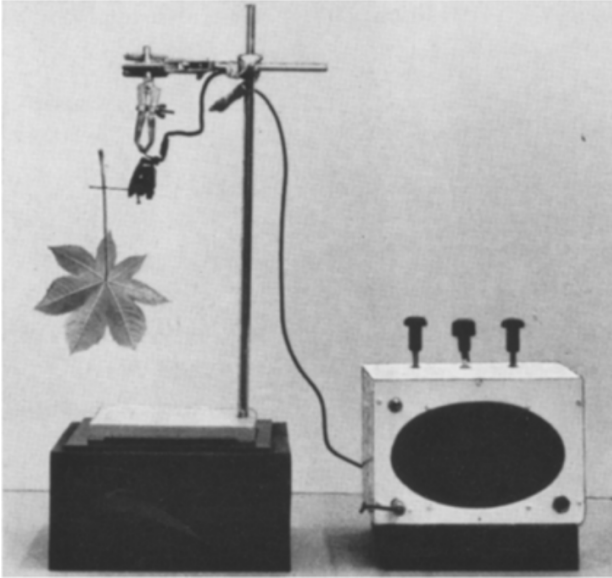


Figure 1.10 Picture of the vibration detector used by Milburn & Johnson (1966) to detect drying-induced sounds in leaf petioles.

Cochard & Tyree, 1990), mechanical strains (Milburn, 1973a; Jackson & Grace, 1994) and microscopic failure (Tyree & Dixon, 1983; Rosner *et al.*, 2010). Moreover, also the actual mechanism that causes cavitation-related AE signals is not exactly known. Different processes have been suggested: vibration of the conduit wall after sudden pressure release due to cavitation (Milburn & Johnson, 1966; Tyree & Dixon, 1983; Tyree & Sperry, 1989a), oscillation of hydrogen bonds in water after pressure release (Tyree & Dixon, 1983), conversion from liquid water to vapour during cavitation of the water (Jackson & Grace, 1994), pit membrane rupture (Rosner, 2012b), torus aspiration (in gymnosperms, Tyree & Dixon (1983)), the entry of an air bubble through a pore in the pit membrane (Jackson & Grace, 1994) and subsequent bubble oscillation (Ponomarenko *et al.*, 2014). Moreover, as the number of detected cavitation-related AE signals may be larger than the number of cavitated conduits (Kikuta *et al.*, 1997; Rosner *et al.*, 2006; Mayr *et al.*, 2014a), different AE-inducing processes might be involved during cavitation. Because gymnosperms have a more uniform and plain anatomy compared to angiosperms, the AE technique has been applied more often on gymnosperm trees.

Attempts have been made to use signal features in order to get more insights into the underlying processes. Ritman & Milburn (1988) suggested that vessel length has an influence on the cut-off frequency of the detected signals. They suggested that cavitation in long vessels produced broadband signals with frequencies down to 500 Hz, whereas cavitation in fibers and wood tracheids only produced higher frequencies (>100 kHz). Tyree & Sperry (1989a) observed that, when detecting AE signals in the frequency range 50-1000 kHz, the frequency spectra changed towards higher frequencies when the degree of cavitation increased in *Thuja*, *Pinus* and *Acer* stems. However, they stayed indecisive about the possible origin of the signals. Based on the waveforms, Laschimke *et al.* (2006) distinguished two types of AE signals in dehydrating leaves of *Ulmus glabra*: the abrupt disruption of the water column and an oscillating source that was related to the vibration of air bubbles during sap flow. It was also found that AE energy is a function of conduit size and xylem tension (Mayr & Rosner, 2011) and Rosner *et al.* (2006) found that this parameter might be a better measure for hydraulic conductivity loss than AE counts.

Although further research is necessary to develop reliable methods for AE data interpretation, this technique has a high potential for continuous long-term cavitation monitoring in the field (Tyree *et al.*, 1986; Takefumi & Ohtsu, 1992; Jackson & Grace, 1996; Perks *et al.*, 2004; Hölttä *et al.*, 2005). Already in 1983, Tyree & Dixon were looking forward to the scientific progress that could be realized with the AE method:

"If it can be proved that most ultrasonic AE are a result of cavitation events, then we will have a powerful diagnostic tool that may give us new insight into the water relations of trees and other plants."

However, Quarles (1990), who was especially interested in AE detection of fractures in wood, realized that deeper insights in the AE method would be essential for successful implementation:

"For successful implementation, it will be essential to understand how the propagating acoustic wave changes as a function of factors such as distance and propagating direction, between the acoustic source and the receiving transducer."

1.5 Establishing the vulnerability to cavitation

1.5.1 Vulnerability curve

Cavitation is one of the major processes leading to productivity loss and plant mortality (Choat *et al.*, 2012; Anderegg *et al.*, 2012), creating a high interest in quantifying the vulnerability to cavitation. As cavitation is known to occur due to strong tensions in the xylem, the vulnerability to cavitation is assessed by a so-called vulnerability curve, depicting percentage of cavitation, or percentage of hydraulic conductivity loss, as a function of xylem pressure potential (Fig. 1.11). Typically, a curve is fit through the data points and the water potentials at which 12, 50 and 88% of the xylem is cavitared (ψ_{12} , ψ_{50} and ψ_{88}) are used to compare the vulnerability of different species and to assess the safety margins at which plants operate. ψ_{12} and ψ_{88} are called the air entry point and full embolism point, respectively, and were defined by Domec & Gartner (2001) based on the tangent line through the bending point of the sigmoidal curve (Fig. 1.11, Eq. 1.3). These values are used to approximate the water potential at which cavitation starts and at which the whole xylem becomes non-conductive, respectively. Various safety margins have been proposed based on the difference between the minimum water potential observed in the field (Ψ_{min}) and one of the characteristic values of the vulnerability curve (Meinzer *et al.*, 2009). Also the slope of the curve, or the difference between ψ_{12} and ψ_{50} has been used as a safety indicator (Domec & Gartner, 2001). Recently, Skelton *et al.* (2015) proposed a more integrative safety margin based on both stomatal behaviour and hydraulic conductivity loss, $\psi_{g12} - \psi_{50}$, in which ψ_{g12} is the water potential at which 12% of the maximal stomatal conductance is reached.

$$PLC = \frac{100}{1 + e^{a(\Psi - b)}} \quad (1.3)$$

1.5.2 Induction of cavitation

The vulnerability curve can be established by combining different techniques to induce and measure cavitation. Cavitation measurement techniques have been

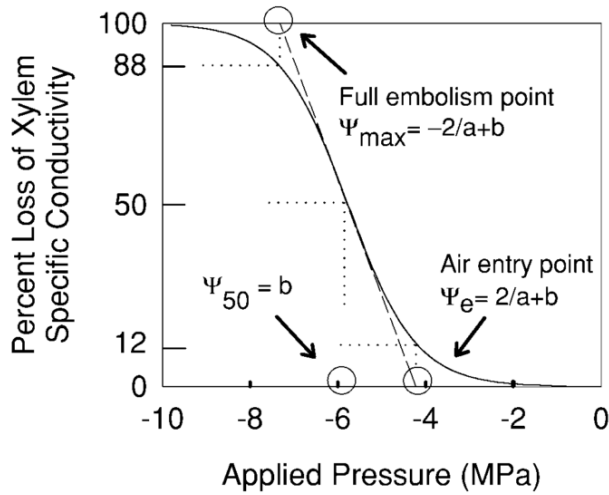


Figure 1.11 The vulnerability curve depicts percent loss of hydraulic conductivity versus xylem pressure potential. When a sigmoidal curve is fit to the data (Eq. 1.3), the air-entry point (ψ_{12}) and full embolism point (ψ_{88}) can be estimated based on the parameters a and b . Figure reprinted from Domec & Gartner (2001).

discussed in Section 1.4, so here will follow an overview of the commonly used techniques to induce cavitation in order to construct vulnerability curves.

The most natural way to induce cavitation is by imposing drought stress on potted or field-grown plants (Tyree *et al.*, 1992; Bréda *et al.*, 1993). More commonly, large branches are cut from a tree and dehydrated freely in the air. This so-called bench dehydration is preferred over in-situ dehydration because dehydration goes much faster (Cochard *et al.*, 2013) and the measurement set-up is less complicated without the need for greenhouses or rain-out shelters. According to the air-seeding hypothesis (Section 1.2), cavitation occurs due to the pressure difference between an air-filled conduit (at atmospheric pressure) and a functional conduit under tension. During (bench) dehydration, the tension builds up in the xylem conduits due to water loss through transpiration and evaporation. However, Pockman *et al.* (1995) demonstrated that a tension increase may also be realized by spinning a cut branch in a centrifuge. By altering the rotation speed, the xylem tension at the center of the spinning branch can

be adjusted (Alder *et al.*, 1997; Cochard, 2002). Moreover, it is possible to induce air seeding by increasing the ambient pressure (Cochard *et al.*, 1992; Salleo *et al.*, 1992) and thus pushing instead of pulling the air into a functional conduit. For this purpose, the cut branch is inserted in a double-ended pressure chamber, with the cut ends protruding outside the chamber. Usually, the bark is removed inside the chamber (Sperry *et al.*, 1994), or an incision is made in the branch segment (Ennajeh *et al.*, 2011) to facilitate air entry into the xylem. With both the centrifuge and the positive pressure method, any desired level of water tension (equal to the positive pressure applied) can be achieved very precisely within a short period of time. In the dehydration method, the xylem water potential has to be measured with a pressure chamber (Scholander *et al.*, 1964) on non-transpiring covered leaves, which are assumed to be in hydraulic equilibrium with the stem or branch, or with stem psychrometers.

2

Cavitation: a blessing in disguise? New method to establish vulnerability curves and to assess hydraulic capacitance of woody tissues

Redrafted from:

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2.1 Introduction

Species survival and fitness under changing environmental conditions with increasing droughts (Dai, 2011; IPCC, 2014) is currently of high interest (Allen *et al.*, 2010; Chopard *et al.*, 2011; Choat *et al.*, 2012). The hydraulic performance of trees and other woody species is a major issue in this debate because hydraulic failure (Brodribb & McAdam, 2013; Urli *et al.*, 2013) is one of the major causes of mortality during drought (Barigah *et al.*, 2013; Sevanto *et al.*, 2014). It is known that woody species have the capacity to mitigate periods of water shortage by using internally stored water. Different woody tissues have the capacity to store water and release it into the transpiration stream, dependent on the tissue's hydraulic capacitance (C , $\text{kg m}^{-3} \text{MPa}^{-1}$, see Section 1.3.1.2). During persistent water shortage, different organs and tissue types may sequentially release water into the transpiration stream and thus the total C may change with increasing drought stress. First, water release from elastic shrinkage of mainly living cells (Tyree & Yang, 1990) may temper strong decreases in water potential (Zhang *et al.*, 2013) and prevent the water-conducting conduits from cavitating or retard this process (Phillips *et al.*, 2004). Second, when cavitation occurs, water release from conduits by cavitation may contribute to the transpiration stream and buffer further decline in water potential (Waring & Running, 1978; Hölttä *et al.*, 2009). Tyree & Yang (1990) reported an increased C when cavitation occurred compared with C supplied by elastic shrinkage. Although sap-conducting conduits may ultimately serve as a water reserve, cavitation will hamper their primary function: conduction of sap. Loss in hydraulic conductivity as a function of decreasing water potential has been studied for many species (Choat *et al.*, 2012) using the so-called vulnerability curve (VC, see Section 1.5.1). The water potential at which hydraulic conductivity is reduced by 50%, Ψ_{50} , is derived from this curve and is used as an indication of vulnerability to cavitation. Both hydraulic capacitance and vulnerability to cavitation are inextricably linked and play a major role in hydraulic functioning of woody species.

Although the significant role of hydraulic capacitance has been widely accepted (Scholz *et al.*, 2007; Meinzer *et al.*, 2008; Zhang *et al.*, 2013; McCulloh *et al.*, 2014), quantitative estimates of this parameter are often lacking in studies on drought resistance. Moreover, no distinction is usually made between C from

elastic shrinkage and C supplied by cavitation. The contribution of cavitation to hydraulic capacitance is important to understand the balance between capacitive water release and loss in hydraulic conductivity (Hölttä *et al.*, 2009). In the synthesis of Choat *et al.* (2012), it is shown that many species (especially angiosperms) operate under water potentials below Ψ_{50} , which means that they take advantage of capacitive water release by cavitation and allow a substantial decrease in hydraulic conductivity. There is need for a more elaborate dataset of C-values of different species at a wide range of water potentials to feed functional plant models and to better understand the role and functioning of cavitation in woody species. In this chapter, we propose a new method to assess both the decrease in hydraulic conductivity and the change in hydraulic capacitance during bench dehydration of a whole-branch segment. We performed the experiments on grapevine (*Vitis vinifera* L. 'Johanniter'), a long-vesselled species for which some methods to obtain VCs have come into question (Choat *et al.*, 2010; Jacobsen & Pratt, 2012; McElrone *et al.*, 2012). We explored a new method using continuous measurements of acoustic emissions (AE), branch diameter shrinkage and gravimetric water loss. The use of cumulative AE signals to estimate the relative loss of hydraulic conductivity was validated by comparison with simultaneously obtained X-ray micro-computed tomography (μ CT) images. Branch diameter shrinkage was used to obtain continuous estimates of water potential. The benefits and drawbacks of both indirect measurements (AE and branch diameter shrinkage) are discussed. Finally, we demonstrate how monitoring gravimetric water loss during dehydration may be valuable to give a more profound insight into the consecutive phases of dehydration and into the different contributions to hydraulic capacitance.

2.2 Materials and methods

2.2.1 Bench dehydration experiments

Plant material was collected from 1-year-old grapevines (*V. vinifera*) that were grown outdoors (51°3'13.2"N, 3°42'22.7"E) in 50-l containers. The grapevines were watered and the leaves of three similar branches of 60 cm length and ~5 mm diameter were packed in an envelop of plastic and aluminium foil the evening before we harvested the branches. Early in the morning, before sunrise, the

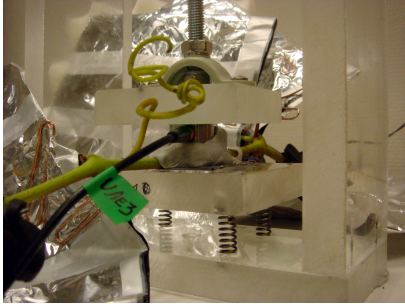


Figure 2.1 Acoustic emission sensors were attached to the branch using resin clamps with springs. Photo credit: Jan Bogaerts

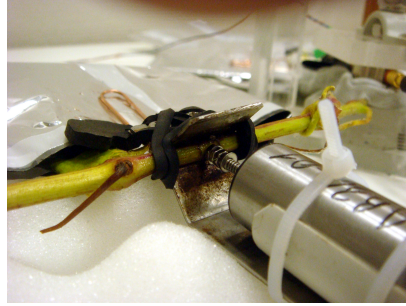


Figure 2.2 We installed LVDTs on the branch, using custom-made stainless steel holders, to measure branch diameter shrinkage. Photo credit: Jan Bogaerts

branches were excised under water and enclosed in a humid and light tight plastic bag for 1 h, the ends covered with parafilm. These precautions were taken to bring the branch segments in a hydraulic equilibrium state.

One branch segment without any sensor and stripped of all leaves was placed on a weighing scale (PS 4500/C/2, Henk Maas, Veen, The Netherlands). The wounds of the cut petioles were covered with petroleum jelly (Vaseline). The two other branches were equipped with an AE sensor (VS150-M sensor and ASCO-P signal conditioner, Vallen systeme, Icking, Germany) in the middle of the segment using a clamp (Fig. 2.1). Branch diameter shrinkage (Δd) was measured with a linear variable differential transformer (LVDT, DF/5.0, Solartron Mobrey, Brussels, Belgium) next to the AE sensor, on a small area where the outer bark was carefully removed and petroleum jelly was applied to prevent water loss through the bare xylem (Fig. 2.2). By only removing a small area below the LVDT tip, we minimized damage to the xylem. A custom-made stainless steel LVDT holder (Steppe & Lemeur, 2004) was used to mount the LVDT on the branch. The outer bark (dead cortex) of the grapevine branches was very stiff and was rapidly detached from the inner bark (phloem) upon dehydration, making over-bark measurements less useful. As long as there were leaves, xylem water potential (Ψ , MPa) was determined every 3 h on an excised leaf of the two equipped branch segments using a pressure bomb (PMS Instrument Company, Corvallis, OR, USA).

Continuous data logging of AE was achieved with a National Instruments acquisition system (Type NI USB 6009, National Instruments, Austin, TX, USA) and custom software developed in LabVIEW (LabVIEW 8.2, National Instruments). Every second, the maximum signal amplitude (APK) was logged and only the values >35 dB (noise level $+5$ dB) were retained. We used a data-logger (CR1000, Campbell Scientific, Logan, UT, USA) for the LVDTs and a custom-built acquisition board to monitor the weighing scale (with RS 232 communication port), both with a time resolution of 1 min. The data shown in this study are drawn from the first 6 days of two consecutive dehydration experiments, which started on 10 (Fig. 2.3a and b) and 25 (Fig. 2.3c and d) September 2012. Directly before and after the dehydration experiment, a wood sample of ~ 5 cm was taken from the end of each branch segment and oven dried until constant weight. The volume of the sample was approximated by the volume of a cylinder with diameter equal to the average of two perpendicular measurements. With these samples initial and final mass fraction (mass of water over mass of oven-dried wood) and initial basic wood density were calculated for each branch segment. Volumetric water content (VWC, kg m^{-3}) was calculated by multiplying mass fraction with initial basic wood density (ρ_b , kg m^{-3}). The actual change in VWC of each branch segment was estimated by rescaling the continuous weight data to the range of water contents between the initial and final water content in the samples. Data analysis and graphical display were performed using the R software (R version 3.0.1; R Core Team (2013)).

2.2.2 Quantification of Ψ_{50} and hydraulic capacitance

For each branch, we plotted the cumulative amount of AE signals and the calculated VWC as a function of relative diameter shrinkage ($\Delta d/d$, $\mu\text{m mm}^{-1}$), which is the ratio between diameter shrinkage and actual diameter (Fig. 2.3). In both curves, two breakpoints were determined with the segmented package in R (Muggeo, 2008). We defined two regions of interest, using the average breakpoints of both curves, and performed a linear regression analysis between VWC and $\Delta d/d$ in Phases I and II. With the point measurements of Ψ versus $\Delta d/d$, we defined the apparent modulus of elasticity in the radial direction (Irvine & Grace, 1997), E'_r , by fitting a linear regression to the stress-strain curve (Ψ versus $\Delta d/d$). We calculated the hydraulic capacitance for both phases with the slopes of segmented regression and E'_r :

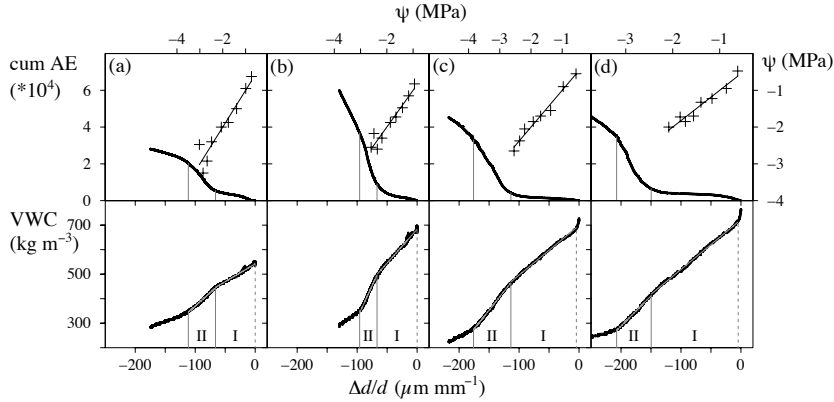


Figure 2.3 Curves of cumulative acoustic emission (cum AE, upper graph) and volumetric water content (VWC, lower graph) plotted against relative diameter shrinkage ($\Delta d/d$) during the dehydration of four branches of grapevine (*V. vinifera*) (a-d). Phases I and II are delimited by vertical lines. The dashed lines indicate the start of phase I, as the initial sharp drop (c, d) was excluded for the analysis. The full lines indicate the average of the breakpoints of the two curves. Point measurements of xylem water potential (Ψ) are shown on the second y-axis. Using the calculated E'_r (Eq. 2.1) and $\Delta d/d$ we added a second x-axis on top showing Ψ .

$$C = \frac{dVWC}{d\Psi} = \frac{dVWC}{d(\Delta d/d)} \left(\frac{d\Psi}{d(\Delta d/d)} \right)^{-1} = \frac{dVWC}{d(\Delta d/d)} (E'_r)^{-1} \quad (2.1)$$

We used the linear relationship between measurements of Ψ and $\Delta d/d$ to add a second horizontal axis on top (Fig. 2.3), expressed in Ψ , and calculated the values of Ψ for both breakpoints, as well as the dehydration times at which these points were reached. We assumed that the second breakpoint corresponded to 100 % conductivity loss and used the curve of cumulative AE to estimate Ψ_{50} and percentage loss of hydraulic conductivity (PLC) at Breakpoint 1.

2.2.3 Validation of acoustic cavitation detection with μ CT

We evaluated the AE method as an indirect measurement of hydraulic conductivity loss with HECTOR (Masschaele *et al.*, 2013), a custom-built μ CT scanner at the centre for X-ray CT scanning of Ghent University (UGCT), Belgium. A grapevine branch of 73 cm length and 7.8 mm diameter was excised under water before sunrise, stripped of all leaves, and wounds were sealed with parafilm for transport to the scanner.

The branch was then equipped with four AE sensors: two 150 kHz resonant sensors (indicated as 1 and 2, VS150-M sensor and AEP4 40 dB preamplifier, Vallen systeme) and two broadband point-contact sensors (indicated as 3 and 4, KRNBB-PC sensor (Glaser & Nelson, 1992) and AMP-1BB-J 35.6 dB preamplifier, KRN Services, Richland, WA, USA). Both sensor types differ in sensitivity and frequency response. Sensors 3 and 4 only detect vibrations in the direction perpendicular to the branch surface and have a flat frequency response in the range 20-1000 kHz, while 1 and 2 have a resonant response at 150 kHz and are also sensitive to vibrations parallel to the sensor surface. We installed sensors 1 and 3 opposite to each other at 3 cm below the midpoint of the scanned zone (Fig. 2.4). We used an acrylic resin holder and two 35.8 N extension springs to clamp the sensors to the branch surface and applied vacuum grease (High-Vacuum Grease, Dow Corning, Seneffe, Belgium) to ensure good acoustic transmission. A second pair of sensors (2 and 4) was installed in the same way 14 cm below the first pair. The AE signals were detected using two two-channel PCI-boards and the software AEwin (PCI-2, AEwin E4.70, MistrasGroup BV, Schiedam, The Netherlands). The signals were processed by a digital filter with 20-1000 kHz band pass and all AE signals above the noise level of 28 dB_{AE} were retained. We applied the lowest possible threshold to enable detection of low-amplitude signals that were attenuated by propagation along the branch. This is necessary for localization of the AE source (see below). From all available features in AEwin, we only used the time of detection (with 0.2 μ s time resolution) and peak amplitude for data analysis.

The whole set-up was installed on the rotation platform of HECTOR so that AE signals and μ CT images could be obtained simultaneously. Parafilm was removed

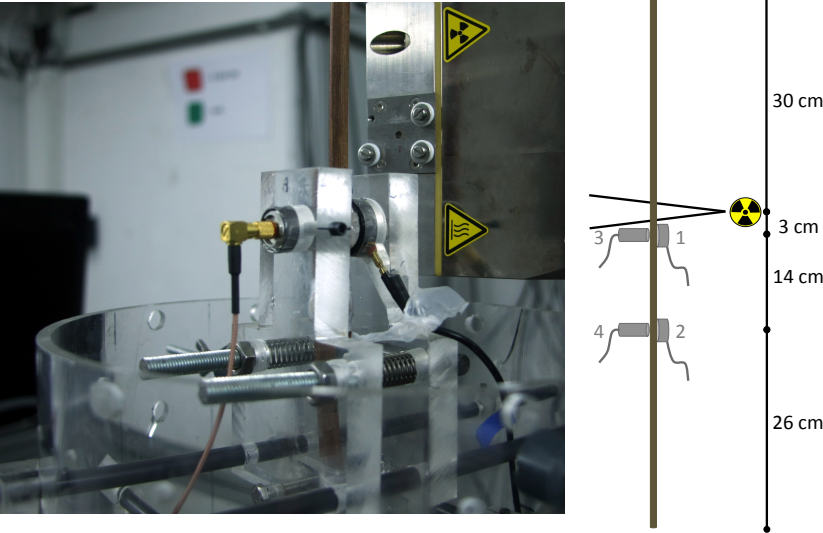


Figure 2.4 The X-rays leave the X-ray tube through a small hole above the sensors and are captured by a detector (not shown) at a distance of about 1.4 m on the other side of the branch. Sensor 1 (right) and 3 (left) are installed on the branch opposite to each other directly below the scanned zone. The other sensor pair is installed 14 cm lower on the branch and is not visible on the picture. Photo credit: Jan Bogaerts

from the upper cut surface and from the nodes above the upper sensor pair before the start of the 40 h dehydration period at constant temperature. Unfortunately, no images were available between 9.5 and 28.75 h after the first scan, due to breakdown of the X-ray source. The equipped branch was positioned vertically on a computer-controlled rotation platform at a distance of 1366 mm from the X-ray detector (with 200 μm pixel size) and at 58.6 mm from the X-ray source. A conical X-ray beam was generated with a voltage of 120 kV and a current of 83 μA and penetrated the branch at a distance of 30 cm below the upper branch end. To reduce beam-hardening effects (Dewulf *et al.*, 2012), we installed a 0.1 mm aluminium filter in the X-ray beam before the branch. During one rotation over 360°, 800 projections of 1421×2000 pixels were obtained with an exposure time of 500 ms. The scanner was programmed to scan continuously during three rotation cycles and then return to the original position to unravel the wires of the AE sensors. After the files were saved to the hard drive, this

process repeated itself. The branch cross-section at the middle of the beam was reconstructed for each scan using the Octopus software (Vlassenbroeck *et al.*, 2007) so that slices of 1421×1421 pixels were obtained with a voxel pitch of $8.6 \pm 0.2 \mu\text{m}$.

The μCT images were processed and analysed with the ImageJ software (Schneider *et al.*, 2012). We counted the cavitated vessels visually and measured the average inner-bark diameter on 15 reconstructed cross-sections throughout the dehydration period. The bright dead outer bark (dead cortex), which is clearly discernible on the μCT images (Fig. 2.5b-d), was again excluded because it was detached from the living inner bark (phloem) upon dehydration. The average inner-bark diameter was obtained as the average of two perpendicular measurements. In the same way, we measured mean vessel diameter for each cavitated vessel. With these data we constructed a similar curve as described above, with both cumulative AE signals and counted cavitated vessels as a function of relative diameter shrinkage. We applied a segmented regression analysis to define the breakpoint between Phases I and II based on the counted cavitated vessels versus $\Delta d/d$.

Before the start of the dehydration experiment, sensor installation and wave propagation were evaluated by eight pencil lead breaks (Pentel 2H 0.3 mm) on the cut surface at the upper end of the branch. Using the mean and standard deviation (σ) of the travel time (Δt) between both sensor pairs, we selected AE events that originated from the branch part in between the two sensor pairs. If the four sensors could simultaneously detect a real AE event from the region of the dehydrating branch in between both sensor pairs, the signals would arrive in all four sensors within a time frame smaller than $\Delta t \pm \sigma$. We counted the events that originated from the region within both sensor pairs by selecting these events that were detected by all sensors within a time frame of $\Delta t - 3\sigma$. A large margin (3σ) was used to exclude events originating from outside the region of interest.

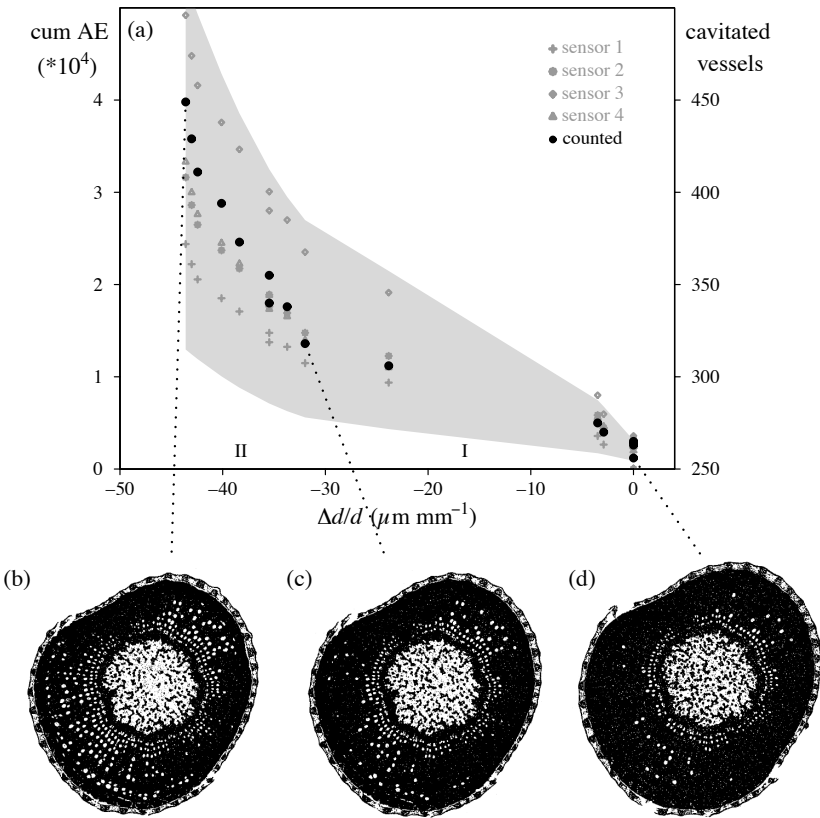


Figure 2.5 (a) Cumulative AE (cum AE, grey symbols, left y-axis) detected by the four sensors (1-4) with threshold 28 dB_{AE} showed a pattern similar to the number of cavitated vessels (black symbols, right y-axis) counted on μCT images when plotted against relative diameter shrinkage ($\Delta d/d$). The μCT cross-sections of the grapevine branch are shown for the beginning (d) and end (b) of the dehydration experiment and at the breakpoint between Phases I and II (c). The grey zone in (a) delimits the 99.7 % confidence interval around the mean cumulative AE curve.

2.3 Results

2.3.1 Vulnerability curves and hydraulic capacitances

Combining AE detection with branch diameter shrinkage and water loss revealed different phases during dehydration of the branches (Fig. 2.3). The first phase (Phase I) with few cavitations was associated with strong diameter contraction and linear decrease in VWC with $\Delta d/d$. A small increase in the number of cavitated vessels and strong shrinkage was also observed during Phase I in the μ CT experiment (Fig. 2.5a, c and d). A clear breakpoint in both curves of VWC and cumulative AE indicated the transition to a second phase during which cavitation exponentially increased and with a steeper decrease in VWC with $\Delta d/d$. The end of Phase II was defined by a second breakpoint in both curves. Acoustic emission activity and water loss decreased strongly with diameter shrinkage after Phase II. Assuming 100 % conductivity loss at the second breakpoint, we found an average $\Psi_{100} -3.39 \pm 0.36$ MPa (mean $\pm$ standard deviation, Table 2.1). The average Ψ_{50} of -2.86 ± 0.23 MPa was obtained from the curves assuming that the dehydration experiments in Fig. 2.3 started at full hydraulic conductivity. The first breakpoint, after which cavitation exponentially increased, was reached at an average Ψ of -2.45 ± 0.14 MPa. Based on the cumulative AE at this point (Fig. 2.3), we estimated a PLC of 21 ± 6 %.

Table 2.1 Values for relative diameter shrinkage ($\Delta d/d$), xylem water potential (Ψ), PLC and time at which both breakpoints were reached during dehydration of branches a-d in Fig. 2.3. Also the apparent radial modulus of elasticity (E'_r) and Ψ_{50} are derived from Fig. 2.3.

	E'_r (MPa)	Breakpoint 1		Time	PLC	Ψ_{50} (MPa)	Breakpoint 2		Time
		$\Delta d/d$ ($\mu\text{m mm}^{-1}$)	Ψ (MPa)				$\Delta d/d$ ($\mu\text{m mm}^{-1}$)	Ψ_{100} (MPa)	
a	26.2	-66.2	-2.32	18h35'	26.6	-2.77	-111.5	-3.50	45h10'
b	22.8	-66.9	-2.37	26h42'	24.6	-2.64	-95.8	-3.04	57h38'
c	19.2	-113.7	-2.64	25h14'	13.2	-3.19	-175.9	-3.84	87h10'
d	12.8	-149.7	-2.46	33h26'	18.7	-2.85	-207.3	-3.19	79h49'

We observed a wide range of values for E'_r and C in Phase I for the different branches, which could be related to the range of basic wood densities (ρ_b) (Table 2.2). Branch a, with high ρ_b , high E'_r and low initial VWC, showed the

lowest value for C, while the opposite was true for branch d. For Phase I, a correlation of 0.89 and 0.83 was calculated between C and ρ_b , and between E'_r and ρ_b , respectively. For all branches, C increased (with 22-92 %) as cavitation increased in Phase II (Table 2.2).

Table 2.2 Hydraulic capacitance (C), calculated with Eq. 2.1 for Phases I and II, together with the branch properties basic wood density (ρ_b), initial diameter (d_{init}) and initial VWC (VWC_{init}) for branches a-d in Fig. 2.3.

	C (kg m ⁻³ MPa ⁻¹)		Branch properties		
	Phase I	Phase II	ρ_b	d_{init}	VWC_{init}
a	55.4	83.7	633.9	5.7	546.3
b	120.5	233.2	492.6	5.5	687.4
c	105.4	160.1	455.8	4.5	720.4
d	158.9	193.2	441.3	4.3	758.1

2.3.2 Visual versus acoustic detection of cavitation

Comparing visual and acoustic cavitation detection (Fig. 2.5a), the general pattern is very similar. Despite Sensors 2 and 4 being of a different type, they detected the same amount of AE signals. The upper sensor pair, on the contrary, showed a very different response with more signals detected by Sensor 3 and less by 1. A closer comparison between visual and AE cavitation detection revealed a higher AE detection rate during Phase I compared with Phase II (Fig. 2.6a, Table 2.3). The AE amplitude distribution (Fig. 2.6b) of Phase I showed a higher abundance of low-amplitude signals compared with Phase II. When all signals with amplitude below 35 dB_{AE} were excluded, the difference in AE detection rate between both phases became less prominent (Fig. 2.7, Table 2.3).

Because of wave propagation along the branch, AE sensors may have detected cavitation events along the entire branch length, while the visually observed cavitations were counted in one cross-section. To compare the absolute numbers of acoustic and visual detection of cavitation, we have to estimate the total number of cavitated vessels in the whole branch. Using reported values for mean vessel length in *V. vinifera* of 11.6 cm (Jacobsen & Pratt, 2012) and 12.8 cm (Sperry *et al.*, 2005), we assumed a mean vessel length of ~12 cm. If we subtract one vessel length from the total branch length of 73 cm to account for

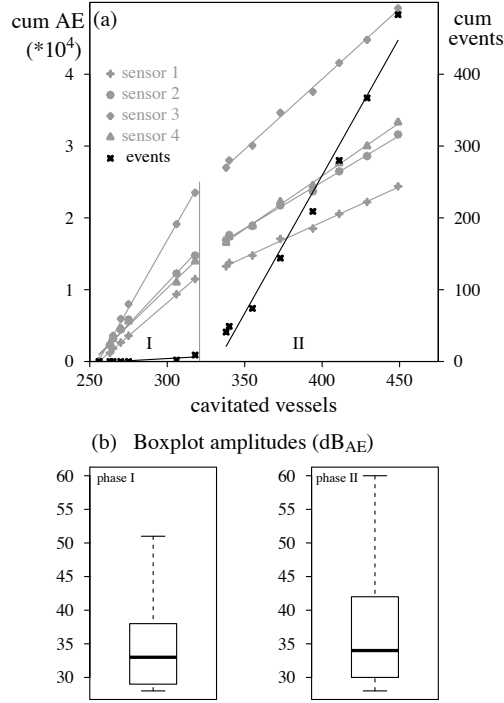


Figure 2.6 (a) Cumulative AE (cum AE, grey symbols, left y-axis) with threshold 28 dB_{AE} and cumulative number of localized events (cum events, black symbols, right y-axis) versus visual observation of cavitation on μ CT images. (b) Two boxplots show the amplitude (dB_{AE}) distribution of all AE signals detected in Phases I and II.

open vessels at the cut branch ends, the branch contains five full vessel lengths. Therefore, we estimated that the total number of empty vessels in the whole branch was five times higher than the number of empty vessels counted in one μ CT cross-section. However, the number of AE signals per observed cavitation was much higher than 5 (Table 2.3). Moreover, due to attenuation not all AE signals over the whole branch length could reach the sensors. There were thus much more AE signals than cavitated vessels in the dehydrating grapevine branches.

The travel time of the AE signals from pencil lead breaks on the upper branch end was $79.7 \pm 5.5 \mu$ s between both sensor pairs. Because of signal attenuation as

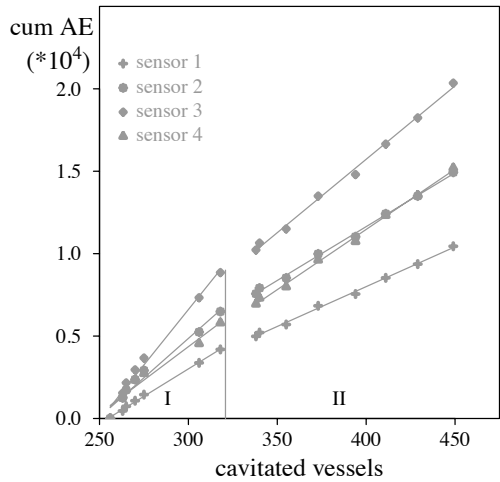


Figure 2.7 Cumulative AE (cum AE) with threshold 35 dB_{AE} versus visual observation of cavitation on μ CT images.

the wave travelled along the branch, peak amplitude was reduced between both sensor pairs, which resulted in a difference of 13.0 ± 1.5 dB between Sensors 3 and 4 and 29.6 ± 3.8 dB between 1 and 2. Using a time frame of 63.2 μ s (79.7–3 $\times$ 5.5 μ s), we detected ~ 4 AE events per cavitated vessel in Phase II (Table 2.3). During Phase I, we did not observe AE events in the zone between both sensor pairs (Fig. 2.6a, black symbols). Only near the end of Phase I could the first AE events be detected.

We did not observe a trend in the diameters of the cavitating vessels during the course of the experiment, but the vessels that were already empty at the beginning of the experiment were smaller (80 ± 27 μ m) than the ones that cavitated during the experiment (106 ± 30 μ m).

Table 2.3 Relationship between AE activity and visual observation of cavitation in μ CT images: number of AE signals above a certain threshold (28 or 35 dB_{AE}) detected by Sensors 1-4 per cavitated vessel, and number of localized events per cavitated vessel. The values represent the slopes and R^2 of the linear regressions depicted in Fig. 2.6a and 2.7.

	Threshold 28 dB _{AE}				Threshold 35 dB _{AE}				Events
	1	2	3	4	1	2	3	4	
Phase I	186	229	379	208	67	95	134	81	0
R^2 (%)	100.0	99.2	99.8	98.5	99.8	97.3	98.5	95.2	99.7
Phase II	98	129	196	148	48	65	89	73	4
R^2 (%)	99.7	99.7	99.7	99.7	99.7	99.7	99.6	99.7	98.0

2.4 Discussion

2.4.1 Acoustic detection of cavitation

Acoustic emissions have been proven useful as an indirect method to measure PLC in different organs of woody species; e.g., in twigs of *Quercus ilex* (Lo Gullo & Salleo, 1993), stems of *Chrysothamnus* shrubs (Hacke *et al.*, 2000), stems and leaf midribs of *Prunus laurocerasus* (Nardini *et al.*, 2001) and small sapwood beams of *Picea abies* (Rosner *et al.*, 2006). However, uncertainties about the sources of AE signals and the indirect approach discouraged the use of the AE technique. We compared AE detection by two spatially separated sensor pairs in grapevine branches with simultaneous observations of cavitated vessels in μ CT images of the branch cross-section (Fig. 2.5). Localized AE events were selected that originated from the region in between both sensor pairs, the length (14 cm) of which was close to the mean vessel length in grapevine (± 12 cm; Sperry *et al.* (2005); Jacobsen *et al.* (2012)). We could not detect localized AE events during Phase I (black symbols in Fig. 2.6a, Table 2.3), while 62 vessels were cavitated in the scanned cross-section during this period (Fig. 2.5c and d). Although the zone between the four sensors was located 3-16 cm below the scanned cross-section, we assume that the degree of embolism was similar. Probably AE events in this phase could not be localized because low tensions at the beginning of dehydration resulted in low-amplitude signals that, due to attenuation, might have fallen below the detection threshold when reaching the most distant sensors. Note that the frequency content also changes during

wave propagation, with stronger attenuation of higher frequencies (Feeney & Chivers, 2001). Therefore, the broadband Sensors 3 and 4 with higher sensitivity at low frequencies can better detect distant AE signals, which is reflected by the lower amplitude drop (13.0 ± 1.5 dB) for the lead break signals that propagated between 3 and 4. Resonant Sensors 1 and 2 are less sensitive at low frequencies and will observe distant signals as lower-amplitude signals, which explains the stronger amplitude drop between 1 and 2 (29.6 ± 3.8 dB).

In Phase II, the number of localized AE events increased strongly with increasing degree of embolism (Fig. 2.6a). Along the region, which contained approximately one vessel length, we detected on average four AE events per cavitated vessel in one cross-section. Stronger tensions in this phase could result in higher-amplitude signals, but also lower attenuation in drier wood (Bucur & Feeney, 1992) may result in better detection of weaker signals. We suggest repeated measurements of attenuation during dehydration by applying pencil lead breaks, and installation of the sensors at a closer distance for better localization of low-amplitude AE events.

The cumulative number of AE signals detected by each sensor provided a better measure for cavitation. Cumulative AE was linearly related to the number of cavitated vessels in both Phases I and II (Fig. 2.6a), but Phase I exhibited a higher AE rate than Phase II. Low-amplitude signals (Fig. 2.6b) caused by shrinkage processes (Wolkerstorfer *et al.*, 2012) may have contributed to the higher AE rate in Phase I with strong shrinkage and little cavitation. When a higher detection threshold of $35 \text{ dB}_{\text{AE}}$ was applied, the difference in AE rate between both phases was strongly reduced (Fig. 2.7, Table 2.3). The still higher AE rate during Phase I may tend to overestimate cavitation in this phase. However, the influence of this error is expected to be low when considering the entire VC because the amount of embolism formation and AE detection in Phase I was low compared with Phase II (Fig. 2.3). We conclude that the pattern of cumulative AE $>35 \text{ dB}_{\text{AE}}$ gave a good indication of the number of cavitated vessels. Because there was no significant trend in the diameters of the cavitating vessels over time, AE was valid as an indirect measure for PLC in grapevine in our experiments.

2.4.2 Diameter shrinkage instead of water potential measurements

We used relative diameter shrinkage to express the dehydration status (Fig. 2.3, 2.5), while VCs are usually plotted against water potential. Continuous measurements of water potential can be performed with a stem psychrometer, but complications with the brittle thermocouples during installation of the expensive psychrometer cups might involve a practical disadvantage. Therefore, we explored the opportunity to obtain continuous estimates of water potential using diameter shrinkage measurements. The combination of AE detection and radial xylem shrinkage has been explored by Hölttä *et al.* (2005) to construct a non-destructive vulnerability curve with continuous field measurements in which xylem diameter shrinkage was used to reflect the changes in water tension. We suggest that during controlled dehydration of an excised branch diameter shrinkage might even be a better indicator for water tension because volumetric changes due to growth and thermal expansion or contraction are eliminated. The observed shrinkage is thus mainly related to elastic shrinkage under tension. Water loss from parenchyma cells results in the release of turgor pressure and consequently elastic shrinkage of the cell walls. Also in dead xylem elements, increased water tension induces elastic shrinkage. The magnitude of shrinkage will depend on the modulus of elasticity, which has been studied for specific cell types (Murphy & Ortega, 1995; Takahashi *et al.*, 2012), tissues (Lee, 1981) and tree organs (Irvine & Grace, 1997; Offenthaler *et al.*, 2001; Alméras *et al.*, 2006). In plant organs such as the sapwood, or the whole branch, the apparent radial modulus of elasticity (E'_r) has been found constant over a range of moderate water potentials (Irvine & Grace, 1997; Offenthaler *et al.*, 2001; Alméras *et al.*, 2006). During Phase I of our dehydration experiments (Fig. 2.3), we could establish E'_r (Table 2.1) of the considered part of the branch between the LVDT holder and the LVDT contact point, including pith, xylem cylinder, phloem and the dead outer bark at the side of the LVDT holder.

We have no evidence that the linear relationship between $\Delta d/d$ and Ψ can be extrapolated during the cavitation phase (Phase II in Fig. 2.3), because all leaves were excised (Fig. 2.3c and d) or wilted (Fig. 2.3a and b) near the end of Phase I. The wilting of the leaves resulted in less reliable and more scattered values for Ψ , but the trend of Ψ during Phase II in Fig. 2.3a may suggest a continuing linear relationship. Upon cavitation, water loss from walls of emptied

vessels and also tissue ruptures may interfere with elastic shrinkage and distort the relationship between $\Delta d/d$ and Ψ . If these influences are small compared with the elastic shrinkage processes, extrapolating the linear relationship may be acceptable. The high abundance of parenchyma tissues in the xylem (wide rays) and phloem of grapevine may support the assumption that further elastic shrinkage outweighs other dimensional changes during Phase II. However, further experiments should be performed to examine E'_r during the cavitation phase. Offenthaler *et al.* (2001) used the stem psychrometer to establish E'_r but pointed at the limited measuring depth of the surface-installed psychrometer, while Ψ might vary along the radius of the trunk. However, for small-diameter branches the stem psychrometer could be used to study this relationship over a larger range of water potentials.

2.4.3 Cavitation: loss in hydraulic conductivity, gain in hydraulic capacitance

We constructed VCs using continuous measurements of AE and xylem shrinkage (Fig. 2.3). To translate cumulative AE into PLC, we defined the second breakpoint as the point of 100% loss of conductivity. The changes in both cumulative AE and VWC curves after this breakpoint suggest that cavitation was strongly reduced or had even ceased. First, the VWC curve levelled off, which indicates that the contribution of cavitating vessels to VWC probably disappeared. Second, the AE activity decreased, with the continuing AE activity after the second breakpoint probably being related to shrinkage processes. When all vessels are emptied, cell wall shrinkage may cause acoustic emissions due to fissures in or between the cell walls. By assigning the endpoint at the second breakpoint, the obtained VCs do not show a gradual saturation near the end, such as the commonly fitted sigmoidal curve (Domec & Gartner, 2001) or single or dual Weibull curve (Lens *et al.*, 2011; Cai *et al.*, 2014). Emerging AE activity due to shrinkage or fractures may affect the last part of the curve, but the cavitation process might also stop rather abruptly in the grapevine branches.

Assuming full hydraulic conductivity at the start of the experiments, we calculated Ψ_{50} (Table 2.1) based on the curves of cumulative AE. The parameters of the VCs were consistent with the field and greenhouse dehydration experiments on grapevines of Choat *et al.* (2010). They reported 88.9 and 90.3%

loss of conductivity at -3.5 MPa in a field and greenhouse dehydration experiment, respectively, and Ψ_{50} of -2.97 and -2.17 MPa. The latter value for Ψ_{50} and an estimation of -2.2 MPa based on μ CT measurements on *V. vinifera* 'Chardonnay' by Brodersen *et al.* (2013) were somewhat higher than our observation of -2.86 ± 0.23 MPa. In Phase I, down to -2.45 MPa, the AE activity was low and we estimated a PLC of $\sim 21\%$ at the first breakpoint. This is comparable to the low level of cavitation at -2.1 MPa observed with nuclear magnetic resonance (NMR) imaging by Holbrook *et al.* (2001), 30% cavitated vessels at -2 MPa by Choat *et al.* (2010) (NMR) and $<10\%$ at -1.22 MPa by McElrone *et al.* (2012) (μ CT). Note that in our μ CT experiment (Fig. 2.5d), the inner ring with smaller xylem vessels (80 ± 27 μ m diameter) was already cavitated before the experiment started. Choat *et al.* (2010) observed the same phenomenon with NMR imaging at Ψ above -0.3 MPa and assumed that the inner ring with primary xylem vessels is more vulnerable to cavitation due to only partial secondary thickening of the vessel walls. Also some larger vessels were already cavitated at the beginning of the experiment. Cavitation could have taken place during transport and installation in the μ CT scanner. Note that Brodersen *et al.* (2013) observed with *in vivo* μ CT measurements that the vessels surrounding the pith cavitated first. Therefore, the large empty vessels spread over the cross-section at the beginning of our experiment could have resulted from open vessels at the branch end after excision.

We calculated whole-branch capacitance for Phases I and II (Table 2.2) based on continuous measurements of whole-branch VWC and water potential estimated with continuous diameter shrinkage and E'_r . Note that in Fig. 2.3c and d, Phase I was preceded by an initial drop in VWC without shrinkage, probably caused by water loss from open vessels at the cut branch ends. We excluded the initial drop when applying linear regression so that this initial water loss did not contribute to hydraulic capacitance in Phase I. Because cavitation was low and water loss was related to elastic shrinkage, we could call the hydraulic capacitance in this phase the elastic C. We observed a large range in elastic C for the different branches, which could be explained by a difference in basic wood density (ρ_b) (Table 2.2). Branches with lower E'_r (Table 2.1) and lower ρ_b (Table 2.2) probably contained more living tissues, resulting in a larger shrinkage before the cavitation phase started (Fig. 2.3) and a larger C during Phase I. High sapwood density has also been associated with low sapwood C when comparing different species (Scholz *et al.*, 2007; Meinzer *et al.*, 2009; Barnard *et al.*, 2011). During

2

further dehydration, when cavitation increased exponentially, we observed an increase in whole-branch capacitance (Table 2.2) due to water release from cavitation in Phase II. The significant contribution of cavitation to the whole-branch hydraulic capacitance at low water potential has also been observed by Edwards & Jarvis (1982) and Tyree & Yang (1990). However, when C was measured on small sapwood samples in thermocouple psychrometer chambers Meinzer *et al.* (2009), sapwood C paradoxically decreased with decreasing Ψ (Meinzer *et al.*, 2003, 2008; Barnard *et al.*, 2011). We believe that experiments with whole-branch segments give a more profound insight into the mechanisms of water storage because the connections between different tissues are kept intact during the experiment and contributions of different tissues are included in the whole-branch C . Our results support the idea that cavitation may supply a significant contribution to hydraulic capacitance under conditions of drought stress (Tyree & Yang, 1990; Hölttä *et al.*, 2009).

2.5 Conclusions

We conclude that both continuous measurements of AE and branch diameter shrinkage were useful for constructing VCs in grapevine. The proposed method would gain credibility if further experiments could verify the validity of diameter shrinkage to estimate water potential over the whole dehydration period. We confirmed the validity of cumulative AE to measure cavitation, but we still do not understand the true nature of AE signals in dehydrating branches. However, the results of our VCs were in good agreement with the values from the literature that were obtained with the most reliable techniques (NMR, μ CT). Moreover, we could simultaneously obtain whole-branch hydraulic capacitance, an often-neglected hydraulic characteristic that is inextricably linked with vulnerability to cavitation. Our results showed that loss in hydraulic conductivity was accompanied by gain in hydraulic capacitance. Therefore, a certain degree of cavitation may be favourable for grapevine to survive periods of drought stress.

3

Deciphering acoustic emission signals in drought stressed branches: the missing link between source and sensor

Redrafted from:

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3.1 Introduction

For almost 50 years, scientists have been studying the sounds emitted by plant organs suffering from drought. The sources of the acoustic emissions (AE) have been under debate ever since, which complicates the interpretation of AE data in terms of plant physiological processes. Recently, Ponomarenko *et al.* (2014) demonstrated that nucleation of an air bubble inside a xylem conduit in a microscopic slice of conifer xylem can produce a detectable AE signal. However, other types of AE sources must exist in plants to explain the AE signals observed during dehydration coming from other tissues in addition to xylem (Milburn, 1973a; Kikuta, 2003), during drying of wood beyond the point when all xylem conduits are empty (Chapter 2), during re-watering (Milburn, 1973b), and also during freezing (Raschi *et al.*, 1989; Mayr & Sperry, 2010; Charrier *et al.*, 2014) and thawing (Raschi *et al.*, 1989; Mayr & Sperry, 2010). For small samples, it is possible to trace the AE source by simultaneous microscopic visualization at very high temporal resolution with multiple frames per second (Ponomarenko *et al.*, 2014; Mayr *et al.*, 2014a). However, when measuring on larger samples or actual plants, this approach is not feasible because the current visualization techniques do not enable combination of high spatial and temporal resolution on the scale of a macroscopic sample. Another method to trace the source of AE signals would be to extract the information that is included in the detected AE signals by analyzing the waveforms. Attempts have been made to interpret the waveforms (Milburn & Johnson, 1966; Tyree & Dixon, 1983; Rosner *et al.*, 2006; Laschimke *et al.*, 2006), but to date they remain indecisive about the actual AE sources. We believe that the major hurdle for interpreting AE signals is the general lack of knowledge about wave propagation in the measured plant organ, since the wave propagation has significant impact on the detected AE signals.

The choice of the AE sensor is also very important to properly understand and interpret the wave propagation features. Previous attempts to interpret whole waveforms were limited by the use of resonant sensors (Laschimke *et al.*, 2006) or broadband sensors with non-flat frequency response (Tyree & Dixon, 1983). These sensors strongly influence the frequency spectrum and the time domain of the detected AE signal. Moreover, common AE sensors are cylindrically shaped so that the captured signal is the average response over the disk-shaped sensitive

element. The detection of waves that do not arrive normal to the sensor face will be distorted by the large area of the sensitive element (so-called aperture effect), especially affecting frequencies where the wavelengths are smaller than the sensor diameter (McLaskey & Glaser, 2012). The aperture effect can be minimized by the use of point-contact sensors of which the diameter of the sensitive element is much smaller than the signal wavelengths (Sause *et al.*, 2012b). Finally, it is important to know the directivity of the sensor and the type of response. The point-contact sensor used in this study is only sensitive to out-of-plane displacements, perpendicular to the sensor face. Other sensors can have sensitivity to combinations of in- or out-of-plane displacement, velocity and acceleration (Ono *et al.*, 2008; McLaskey & Glaser, 2012). Such mixed sensitivity complicates signal interpretation.

In this study, we focus on wave propagation in woody branches. During propagation of acoustic waves through the branch, processes such as material attenuation, scattering, geometrical attenuation and dispersion change the waves and typically decrease the wave amplitude when it travels away from the AE source location (Bucur & Böhnke, 1994). Moreover, the majority of the signals have been reflected several times at the branch surface before they reach the AE detector. These reflections result in a reduced geometrical attenuation, which allows the waves to travel and be detected at longer distances than in an infinite medium. In addition, the interactions of multiple reflections by the geometric structure result in so-called guided waves (Rose, 2004). The fact that the branch acts as a waveguide strongly affects signal interpretation, but so far this has not been taken into account in the literature that we have found for AE studies in plants. The objectives of this chapter are twofold. First, we aim at understanding how acoustic waves are propagated in a branch with rod-type geometry and how source location may affect the composition of the detected waveform. Second, we use these insights to explain waveform features observed in drying-induced AE signals from desiccating branches and we provide a solid framework for further AE research in plant science.

3.2 Materials and methods

3.2.1 Pencil lead break as artificial AE source

In order to examine wave propagation and effects of source location, we used the well-known pencil lead break (PLB) source. The breakage of a pencil lead, according to ASTM Standard E976, is extensively used as an artificial source in AE technology (Tyree & Sperry, 1989a; Berthelot *et al.*, 1992; Hamstad *et al.*, 1994; Sause, 2011; Dehghan Niri & Salamone, 2012; Strantza *et al.*, 2014). When pressing the lead of a propelling pencil firmly against the surface, the lead will break and local stresses in the surface will be released so that the surface jumps back to its original position. This unloading force as a function of time $F(t)$ (N) can be described by a cosine bell function (Eq. 3.1) (Hamstad *et al.*, 1999; Sause, 2011), which is directed normal to the surface.

$$F(t) = \begin{cases} 0 & t < 0 \\ 0.5 - 0.5\cos\frac{\pi t}{t_e} & 0 \leq t \leq t_e \\ 1 & t > t_e \end{cases} \quad (3.1)$$

The excitation time t_e is dependent on the elastic properties of the surface material. This function is suitable to simulate the acoustic wave caused by a pencil lead break (Sause, 2011) in the far field. For a PLB on aluminium, the excitation time was found to be $1\mu\text{s}$ (Sause, 2011) and we use this value as a reasonable estimate for the excitation time on a PVC surface. The far field refers to distances from the AE source at which the signals have been significantly changed by the specimen geometry (Hamstad *et al.*, 1994) and guided waves can be observed. In a PVC rod, guided waves are already developed at a propagation distance of three times the rod diameter (Marvin Hamstad, personal communication).

We investigated the direct pressure wave (without reflections) that is created by a pencil lead break (2H, 0.3 mm, Pentel) directly on the sensor tip (KRNBB-PC sensor, KRN Services, Richland, WA, USA) and on a wooden board across from the sensor tip. For the latter, a sensor was installed in the middle of a board of dry Mahogany wood with dimensions $439 \times 122 \times 19.6\text{ mm}$ and the PLB

was made on the opposite side of the board, directly across from the sensor tip. Another PLB was carried out at 2 cm away from the center of the sensor.

3.2.2 Finite element modelling to investigate wave propagation

Wave propagation is a micro-mechanical process and can be seen as a series of stretching and relaxation of springs inside the material. We simulated wave propagation with finite element modelling of a linear elastic material, using the software COMSOL Multiphysics. First, a PVC rod was used for investigation of the wave modes because the simple geometry (8.5 mm diameter, 1 m length) and isotropic elastic properties facilitated the calculation of group velocity curves (explained below). We modelled the PVC rod as an isotropic cylinder with density 1500 kg m^{-3} , modulus of elasticity equal to 4.693 GPa and a Poisson's ratio of 0.3664 (Kaye & Laby Online, 2005). For modelling of a wooden rod, we used the elastic constants of ash (Table 3.1) from Dinckal (2011) and a fresh wood density of 1000 kg m^{-3} . The selected model was convergent with models of a higher resolution when applying a time step of $0.1 \mu\text{s}$ and a maximum mesh size of 1 mm. To enhance representation of fine details, mesh refining down to 0.01 mm was allowed with a maximum element growth rate of 1.5 per element. The far-field waveforms were simulated as displacements normal to the surface at 12 cm from the cylinder end (Fig. 3.1), where the pencil lead break was simulated. The force exerted by the simulated pencil lead break was implemented as a cosine bell function (Eq. 3.1), acting normal to the rod end. The surface displacements, which could be calculated at each point on the rod surface, represent the waveforms that would be detected by a broadband point-contact sensor with a near flat-with-frequency displacement response (Hamstad, 1997; McLaskey & Glaser, 2012; Sause *et al.*, 2012b). We examined the waveforms with respect to AE source location by first varying the distance from the PLB location to the center of the rod (0-3 mm off center). A second examination varied the angle (0-90°) between a radius to the PLB location and a radius to the detector location viewed from the end of the rod.

Table 3.1 Stiffness tensor (C , Voigt notation) with elastic constants (C_{ij} , GPa) of ash from Dinckal (2011), used for modelling the wooden rod. A cylindrical coordinate system was used with the first, second and third axis being aligned with the radial, tangential and longitudinal direction, respectively, of the internal wood structure.

$C_{11}=2.439$	$C_{12}=1.037$	$C_{13}=1.968$	$C_{14}=0$	$C_{15}=0$	$C_{16}=0$
$C_{21}=1.037$	$C_{22}=1.439$	$C_{23}=1.485$	$C_{24}=0$	$C_{25}=0$	$C_{26}=0$
$C_{31}=1.968$	$C_{32}=1.485$	$C_{33}=17.000$	$C_{34}=0$	$C_{35}=0$	$C_{36}=0$
$C_{41}=0$	$C_{42}=0$	$C_{43}=0$	$C_{44}=1.218$	$C_{45}=0$	$C_{46}=0$
$C_{51}=0$	$C_{52}=0$	$C_{53}=0$	$C_{54}=0$	$C_{55}=1.720$	$C_{56}=0$
$C_{61}=0$	$C_{62}=0$	$C_{63}=0$	$C_{64}=0$	$C_{65}=0$	$C_{66}=0.500$

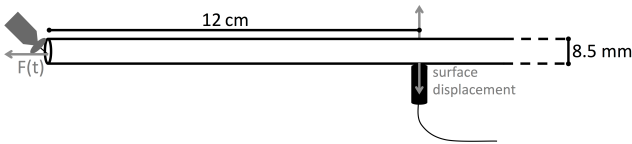


Figure 3.1 Schematic representation of the modelled PVC and wooden rod. Surface normal displacement is evaluated at 12 cm from the rod end, where a pencil lead break is made.

3.2.3 Verification of the model

Although the finite element method has been shown to be valid for calculating guided wave propagation (Sause *et al.*, 2013), we verified the integrity of our model by comparison with an experimental pencil lead break. We broke a pencil lead (2H, 0.3 mm, Pentel) of ~ 3 mm length on the end of the PVC rod at 1 mm from the rod axis towards the side of the sensor location (Fig. 3.1). We used a broadband point-contact sensor (KRNBB-PC sensor) that is sensitive to surface displacements in the frequency range 20-1000 kHz (Glaser & Nelson, 1992; McLaskey & Glaser, 2012). These sensors with conical piezoelectric elements have a very flat spectral response (Sause *et al.*, 2012b), which makes them most appropriate to experimentally verify the simulated signals. For detailed comparison with the simulations, the experimental signals were collected at much higher sample rate than usually required for AE monitoring in wood.

Signals with a length of 10240 samples were collected at $10 \text{ Msamples s}^{-1}$ with $250 \mu\text{s}$ pretrigger time. Both the simulated and the experimental signals were filtered with 7-100 kHz band-pass. We used 4th order Butterworth filters (Ligges *et al.*, 2015) for the 100 kHz low-pass filtering of both signals, since a valid comparison can only be made for both signals having the same frequency range. For the 7 kHz filtering, the experimental signal was filtered electronically, and for the simulated signal a 4th order Butterworth filter was used. The 100 kHz low-pass was necessary because we observed that frequencies above 100 kHz were strongly attenuated in the experimental signals at 12 cm source-sensor distance, while material attenuation was not included in the model. Even though there was significant material attenuation above 100 kHz in the experimental case, due to the wide dynamic range of the recorded signals it was still necessary to low-pass filter those signals. Based on the very good match of the model versus the experimental signals (see later section), we concluded that the experimental case did not experience significant material attenuation below 100 kHz. We applied a high-pass filter of 7 kHz because this was the lower limit of the frequency range of the preamplifier (model AMP-1BB-J, KRN Services, Richland, WA, USA).

3.2.4 Identification of the guided wave modes

The presence of different guided wave modes was investigated using the Choi-Williams distribution (CWD) of the simulated waveform (Hamstad, 2008), which is a spectrogram showing the wave intensity with a colour or varied black intensity scale on a plot of frequency versus time for the signal duration. This was carried out with the AGU-Vallen Wavelet software (www.vallen.de/downloads) using a frequency resolution of 1.221 kHz, 112 terms in the damping summation and an exponential damping parameter of 20. For the CWD representation we filtered the simulated signal with a 20-500 kHz band-pass in order to better visualize frequencies up to 500 kHz, which were otherwise overwhelmed in the CWD diagram by the high intensities at low frequencies.

All guided wave modes in a rod with a certain diameter, known density, and elastic properties can be characterized by so-called dispersion curves (Rose, 2004), which describe the relation between frequency and velocity. The group velocity at a certain frequency can be understood as the propagation velocity of wave packets containing frequencies in a narrow band around this value. Group

velocity dispersion curves were calculated for the PVC rod at a propagation distance of 12 cm, velocities were converted into arrival time and the curves were superimposed on the plot of the Choi-Williams distribution for comparison and identification of the wave modes. For the calculation of dispersion curves for cylindrical wave guides, an open-source Matlab program called PCDISP (Seco & Jiménez, 2012) is available.

3.2.5 Drying-induced AE signals

Excised branches of grapevine (*Vitis vinifera*), ash (*Fraxinus excelsior*), ivy (*Hedera helix*), poplar (*Populus × canadensis*) and common beech (*Fagus sylvatica*) with diameters of ~ 1 cm and ~ 1 m length were monitored with the broadband point-contact sensors during bench dehydration, without removing leaves or bark. On the branch of ash and grapevine we installed a pair of sensors opposite each other, while on the other branches only one sensor was installed. Vacuum grease (High-Vacuum Grease, Dow Corning, Seneffe, Belgium) was applied between wood and sensor tip to ensure good acoustic transmission by removing air pockets between the rough surface and the sensor face (Miller, 1987). Signals with a length of 4096 samples that crossed the threshold of 27 dB_{AE} were collected in the frequency range 20–1000 kHz at 5 Msamples s⁻¹ and with 250 μ s pretrigger time. The signal was amplified by an internal JFET amplifier inside the sensor (20 dB) and an in-line preamplifier (35.6 dB).

3.3 Results

3.3.1 Pencil lead break

A pencil lead break (PLB) directly on the sensor tip (Fig. 3.2A) resulted in a waveform with two sharp peaks, a fast loading (negative peak) and unloading (positive peak) of the sensor tip, followed by a damped oscillation. The waveform generated by a PLB on wood across from the sensor (Fig. 3.2B) also featured the initial two peaks, but the dynamics were slower (broader peaks). These differences are due to the more than ten times higher modulus of elasticity of the nickel faceplate of the sensor compared to wood, and also the strong attenuation of high frequencies on the path through the wood. Also, a reflection of the wave

from the opposite surface of the wood board arrived after about 10 μ s, which distorted the damped oscillation. When the pencil lead was broken 2 cm away from the sensor tip on the opposite side of the wood board (Fig. 3.2C), the initial part of the waveform changed substantially, which illustrates the need for understanding wave propagation before interpreting the detected signals.

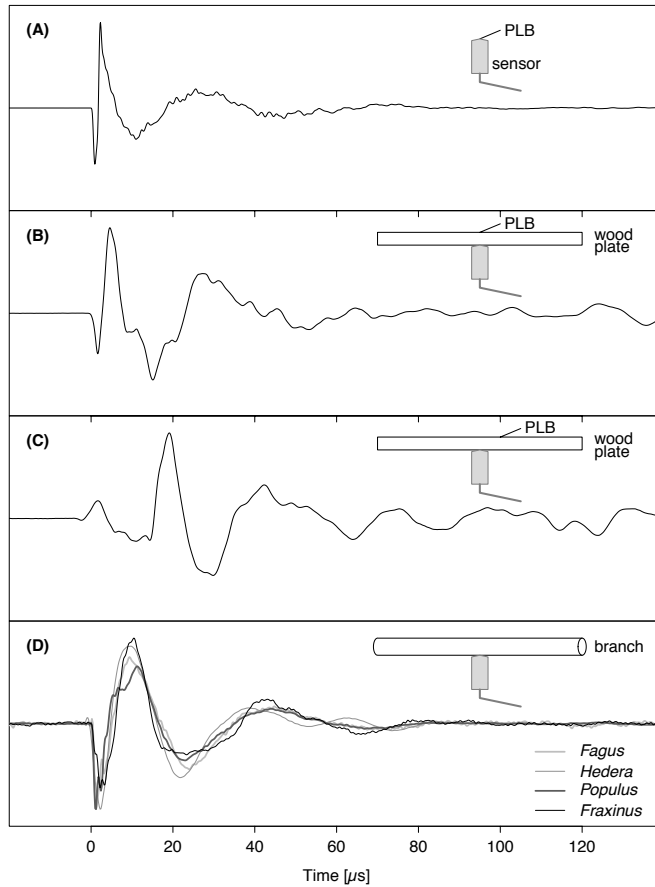


Figure 3.2 Comparison between signals caused by a pencil lead break (PLB) (A–C) and nearby drying-induced AE signals from dehydrating branches (D). (A) PLB on sensor tip, (B) PLB on wood directly opposite to the sensor and (C) signal from PLB on the opposite side of the wood at 2 cm from sensor location.

3.3.2 Guided waves

Good correspondence between simulated surface displacement and experimentally obtained waveform at 12 cm from the PLB at the end of the PVC rod (Fig. 3.1) confirms that the model configuration was valid (Fig. 3.3A). Comparing simulated signals on both sides of the rod (Fig. 3.3A-B), we can identify a first arrival of a symmetric wave at around $70\text{ }\mu\text{s}$, due to the simultaneous out- and inward displacement at opposite sides of the rod. Subsequently, an anti-symmetric wave with lower velocity arrives at around $110\text{ }\mu\text{s}$, with outward displacement at one side of the rod occurring simultaneously with inward displacement at the opposite side. A schematic representation of both wave modes is shown in Fig. 3.3C. From the velocity dispersion curves of a cylindrical rod (Fig. 3.3C) it can be seen that these arrival times correspond to the arrival of the fundamental symmetric (S0) and anti-symmetric (A0) wave mode, respectively. The good correspondence between CWD and the dispersion curves confirms that we are dealing with the S0- and A0-mode and provides added validation of the correctness of the finite element model.

3.3.3 Influence of source location

When increasing the angle between the AE source (PLB) and the sensor, the amplitude of the first-arriving S0-mode was not affected (Fig. 3.4A, 3.5A). The A0-mode amplitude, in contrast, decreased with increasing detection angle, until it completely disappeared at 90° . The A0 amplitude decrease was proportionate to the cosine of the detector angle, but because of the anisotropy of wood, the decrease in A0 amplitude deviated somewhat from the cosine function for the wooden rod (Fig. 3.5A). The distance of the AE source from the axis of the rod has a strong influence on the amplitude of the A0-mode as well (Fig. 3.4B and 3.5B). With constant angle between source and detector (0°), the A0-mode amplitude decreased linearly with decreasing distance between the source and the center of the rod.

3.3.4 Drying-induced AE signals

In the whole range of AE signals collected from dehydrating branches, we selected some typical near- and far-field signals based on characteristic AE features.

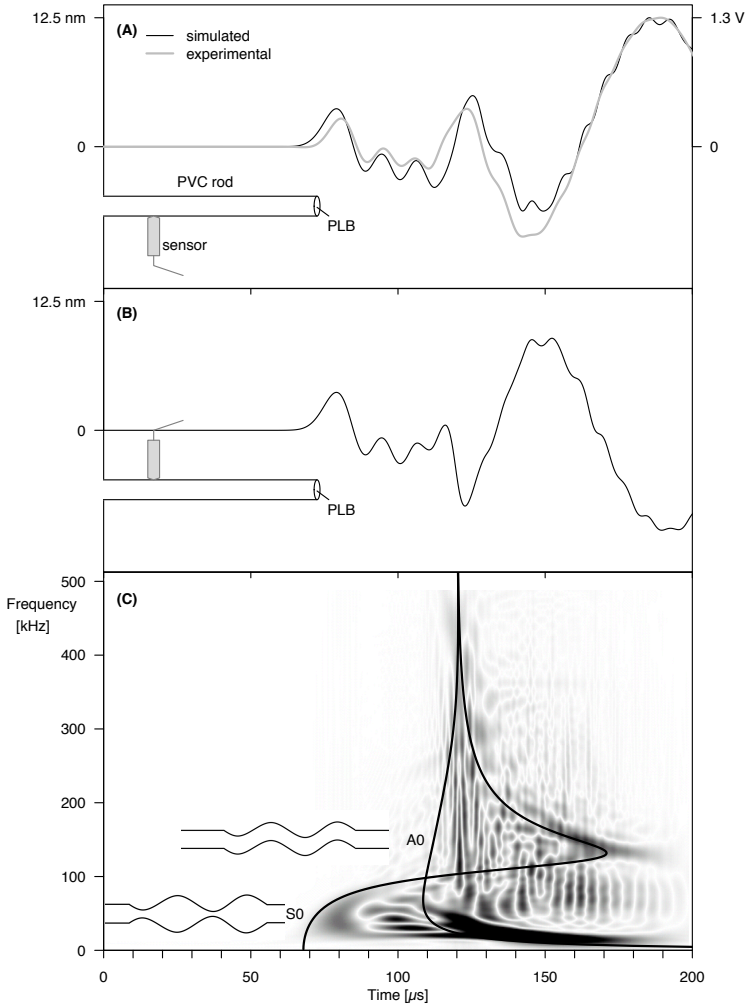


Figure 3.3 Surface displacement at 12 cm from the end of a PVC rod, resulting from a pencil lead break (PLB) at the rod end, at 1 mm off center: (A) simulated (black) and experimental (grey) waveforms at 0° from the PLB location, (B) simulated waveform at 180° and (C) Choi-Williams transformation of the simulated signal in (A) with velocity dispersion curves for the S0- and A0-mode.

The near-field signals (Fig. 3.2D) were characterized by a small time to reach the peak amplitude and fast decay. The far-field signals (Fig. 3.6) did not

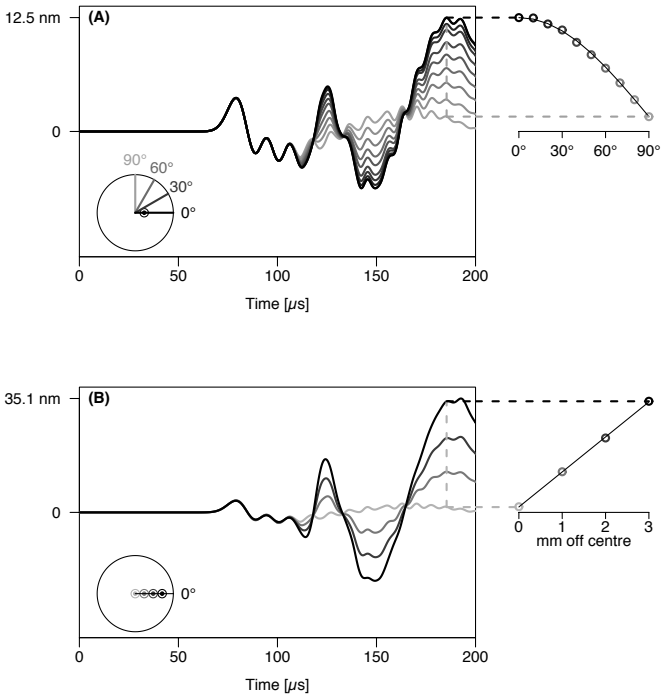


Figure 3.4 (A) Waveforms simulated at 12 cm from the end of the PVC rod at an angle of 0-90° with the location of the pencil lead break, which was made at 1 mm off center on the rod end. (B) Waveforms simulated at 12 cm from the end of the PVC rod, with pencil lead break at 0-3 mm from the center of the rod end. The schematic at the bottom-left of the graphs shows the location of sources and sensors, when viewed perpendicular to the rod end.

feature the initial sharp peaks, but were smeared in time and were attenuated due to the propagation along the branch. The waveforms of the far-field signals could be divided into two parts based on the signals detected at both sides of the branch. At the beginning of the waveforms (first vertical line), both signals were symmetric, with positive peaks co-occurring at the same times on opposite sides of the branch. After some time (second vertical line) both signals became anti-symmetric, with positive peaks co-occurring with negative peaks at the same times on the opposite side of the branch. The difference in arrival time between the symmetric and anti-symmetric wave modes was larger for signal (B) than (A), two examples from a grapevine branch, and larger for signal (D)

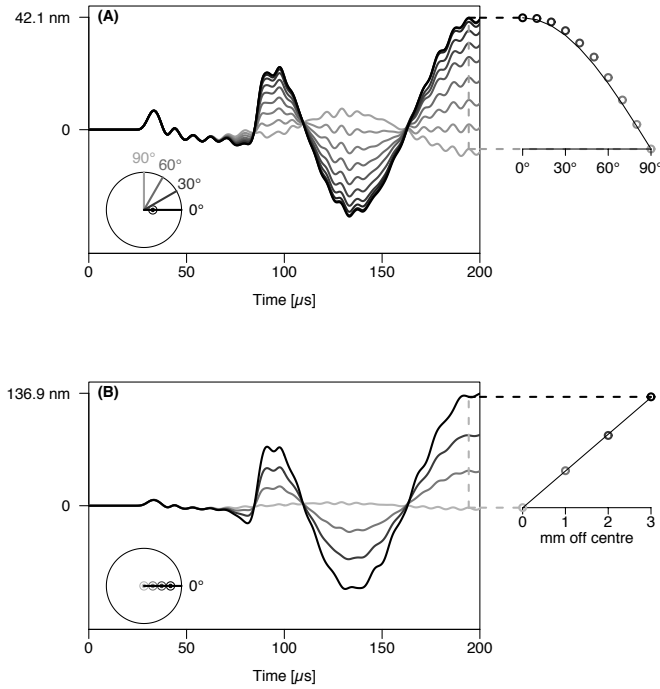


Figure 3.5 Same set-up as Fig. 3.4 on anisotropic wooden rod shows similar behavior of A- and S-modes.

than (C), two examples from an ash branch. This suggests that for the signals on grapevine, the AE source was located closer to sensor set (A) and likewise closer to sensor set (C) for the ash examples.

3.4 Discussion

3.4.1 Behaviour of guided waves in a branch

Acoustic emission signal analysis requires a thorough understanding of wave propagation from source to sensor. When a displacement wave propagates along a waveguide, the shape of the initial displacement wave (Fig. 3.2B) may become distorted beyond recognition (Fig. 3.2C, Fig. 3.3). However, this dis-

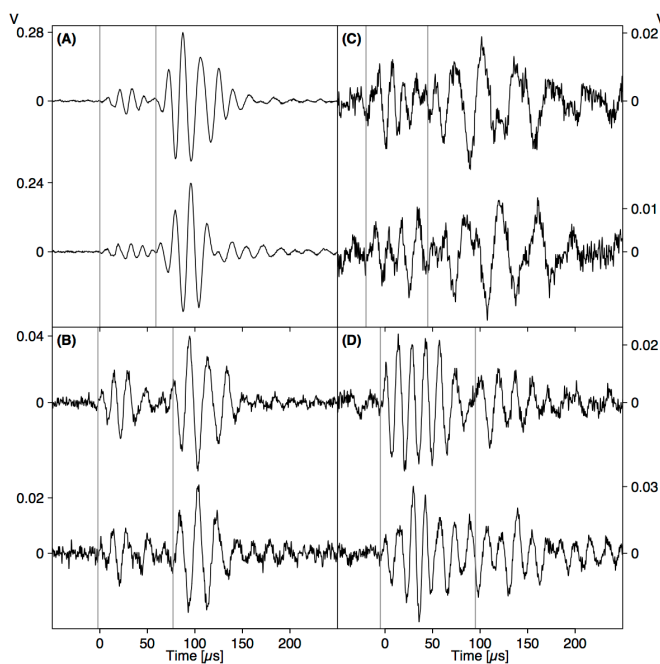


Figure 3.6 Two examples of AE events detected simultaneously by two sensors installed opposite each other on dehydrating branches of grapevine (*Vitis vinifera*) (A, B) and ash (*Fraxinus excelsior*) (C, D). The vertical lines indicate the arrival of the symmetric (first line) and anti-symmetric (second line) wave modes.

tortion is not random and after propagating some distance away from the source, guided wave modes are developed. The finite element model, which was proven to simulate far-field AE signals correctly (Fig. 3.3), was used to investigate wave propagation between source and sensor. The basic principles of guided wave modes that were investigated on the isotropic PVC rod also applied on the wooden rod (Fig. 3.4, 3.5), and thus on cylindrical woody branches. Experiments with a PVC rod as model system, with well-known properties and simple geometry, are therefore helpful to investigate the principles of wave propagation in branches.

From the surface displacements on both sides of the PVC rod (Fig. 3.3A,B), the fundamental symmetric (S0) and fundamental anti-symmetric (A0) mode

with lower frequency were clearly discernible in the waveforms. The fundamental wave modes are the modes with the lowest frequency of an infinite series of symmetric and anti-symmetric modes that can be stimulated in a waveguide. The absence of higher modes, which contain higher frequencies, is explained by the nature of the PLB source. Since the PLB signal typically has the highest amplitude at low frequencies (Breckenridge *et al.*, 1990), the higher modes are not stimulated. Also the amplitude of the A0-mode with lowest frequencies that arrives after 110 μ s is much stronger than the S0-mode amplitude for this reason. In the remaining part of the signal that is not shown in Fig. 3.3, the low frequencies of the A0-mode continue for a while and are gradually dampened. A third type of wave mode that could exist in a cylindrical rod is the torsional mode (Bischoff *et al.*, 2014). According to theory (Rose, 2004), torsional modes consist of displacements in the tangential direction. Because we can only observe radial displacements (normal to the rod surface) with the point-contact sensor, the torsional guided wave modes are not detected by our sensor system. To investigate the torsional guided wave modes, a wafer-type AE sensor could be used (Giurgiutiu, 2008), which is sensitive to in-plane waves.

By varying the angle between source and sensor, we shed light on the behaviour of the symmetric and anti-symmetric wave modes in a cylindrical branch. The amplitude of the anti-symmetric wave mode observed normal to the rod surface decreased according to the cosine of the angle between the radial line through the sensor and the radial line through the source (Fig. 3.4A, 3.5A). As a consequence, the A0-mode was not observable when the angle between source and sensor was 90°. When the wave propagates down the rod in this mode, the rod is put in localized oscillating bending (as schematically shown in Fig. 3.3C) in the plane defined by the source location and the rod axis. The amplitude of the symmetric mode (S0-mode), in contrast, is equal in all directions (Fig. 3.4A, 3.5A) and independent of source-to-sensor angle (Fig. 3.4B, 3.5B). In the symmetric mode, the rod is subjected to sequential symmetric expansion and contraction of the cross sections (Fig. 3.3C). When changing the depth of the source below the branch surface, the balance between A- and S-mode changed. The amplitude of the A0-mode increased linearly with increasing distance between the source location and the rod axis (Fig. 3.4B, 3.5B), whereas the S0-mode was not affected. The more asymmetric the source location (further from the rod axis), the more the anti-symmetric wave mode prevails. As the two modes have distinctly different frequencies (Fig. 3.3C), the source location also influences

the frequency content of the detected signals. Thus, signals from a particular source mechanism will show some variation in frequency features due to variation in their distance from the rod axis as well as changes in the angle between the source location and the sensor.

3.4.2 Deciphering drying-induced AE signals

The direct pressure wave caused by an abrupt AE source (Fig. 3.2B) is clearly discernible from the far-field signal with guided wave modes (Fig. 3.3). Based on the gained insights in wave propagation, we were able to distinguish near- and far-field signals in dehydrating branches. The signals depicted in Fig. 3.2D were caused by nearby AE sources. As we observe the waves very close to the source, without superimposed reflections from the rod surface, we detect the direct bulk compressional waves. The nearby direct bulk waves will be similar for AE sources with similar source functions. Therefore, similarities between PLB signals (Fig. 3.2B) and near-field drying-induced AE signals indicate the presence of similar AE source dynamics. Given the step-like force function of a PLB, the drying-induced signals in Fig. 3.2D were most likely also caused by a sudden displacement in the close surrounding of the sensor. In the AE signals from the far field (Fig. 3.6), we could distinguish a separated symmetric and anti-symmetric mode in the signals from two opposite sensors. Although these longer signals may evoke the impression that the underlying AE sources were of oscillating nature, we know that the elongated shape is caused by wave dispersion. These results suggest that displacements during bubble growth, rather than superimposed small bubble oscillations of high frequency (Vincent *et al.*, 2014), may be a source of AE signals.

A large portion of the signals detected in the dehydrating branches fell in between both categories of signals that we have discussed, showing mixed characteristics. These AE signals may have resulted from a relatively close AE source from which the wave modes were not yet fully developed. Because of the difference in wave mode velocity, both modes are better separated when the propagation distance is larger, so that we can clearly distinguish them in the signals at a certain distance from the source (Fig. 3.6). When the AE source mechanism was similar, we could extract some information on the source location. For example, based on the smaller time lag between the arrival times of both wave modes in Fig. 3.6A,

we could deduce that this AE event took place closer to the sensor pair than the event in Fig. 3.6B. We also observe a higher amplitude ratio between A- and S-mode in Fig. 3.6A compared to Fig. 3.6B. This might indicate that the AE source in Fig. 3.6A was located closer towards the surface of the branch, or that the angle between source and sensor was larger in Fig. 3.6B.

However, different AE sources may stimulate the S- and A-modes with different degree. It has been shown that different source excitation time (time period in which the displacement takes place in a microscopic volume) can stimulate the S- and A-modes with different degree, due to activation of different frequencies (Hamstad, 2010). Moreover, different orientations of the major displacements at the AE source (source geometry) can change the balance between S0- and A0-mode (Downs *et al.*, 2003). In other words, distinction between the different modes may help to identify different source types (with certain source excitation time and source geometry) when the source location is constant, and vice versa.

3.4.3 Challenges for the AE technique in plant science

Because wave propagation along the branch has a large effect on the observed waveform characteristics, it is essential to understand the principles of guided wave modes in order to make any progress in signal interpretation. We focused our study on wave propagation in young branches. Being extremities of the hydraulic pathway, young branches are more prone to drought stress than the trunk and larger branches (McCulloh *et al.*, 2014). Moreover, compared to leaves and petioles, measurements on branches can continue after wilting of the leaves. Branches are thus convenient study objects in terms of drought stress. However, when measuring AE signals on other plant organs, guided wave modes may be involved too. Note that the frequencies of the guided waves are inversely proportional to the lateral dimensions of the waveguide (Rose, 2004). Both leaf petioles and large trunks will act as a cylindrical waveguide, but the frequencies of resulting guided waves will be much higher or lower, respectively, than those in young branches. Leaves, having a plate-like geometry, will also guide typical symmetric and anti-symmetric wave modes. The behavior of guided wave modes in plates, called Lamb modes, has been extensively studied in the context of structural health monitoring (Hamstad, 2010; Sause *et al.*, 2013; Park *et al.*, 2014). When aimed at signal analysis of other plant parts, with

different geometry, the behavior of guided waves will thus need to be considered too.

To make advances in AE research on plants, it is essential to choose appropriate AE equipment. Sensors should be sensitive to surface displacement in a broad frequency range in order to obtain results that can be compared to calculated waveforms. The point-contact sensor used in this study is very appropriate for this purpose, as it has a very flat spectral response over a broad frequency range (20-1000 kHz). Moreover, we switched from parameter-based to signal-based AE analysis. Instead of focusing on a few waveform features (Rosner, 2012b; Wolkerstorfer *et al.*, 2012), we considered the whole waveform of the detected signal. Currently, high performance acquisition systems are available that are able to record and store waveforms from multiple channels at high sampling frequencies. This allows post-processing of the data and thorough analysis with an unlimited number of the signal features.

The combination of state-of-the-art AE measurement techniques and finite-element modelling of wave propagation is essentially new in plant science, and could be the first step towards revealing the secrets behind AE signals in plants. Dynamic finite-element modelling is increasingly used in modern AE work to simulate the AE sources and the subsequent propagation of the displacement waves (Hamstad, 2007; Aggelis & Matikas, 2012; Sause *et al.*, 2013; Ge *et al.*, 2014). When the geometry and elastic properties of the considered plant part are known, the AE signal that results from a certain AE source type can be simulated at the detector location using FEM (Sause & Richler, 2015). The combination with the well-known pencil lead break is of great value since all source characteristics are known and pseudo sensors (model simulations) are perfect point-contact sensors. As this approach has proven successful in the field of material science (Sause *et al.*, 2012c) to distinguish between different types of AE sources, AE signal analysis may provide a promising avenue also in the field of plant science.

The ability to use the wider frequency information of this new approach to identify AE sources in experiments where multiple source types are present is a significant advancement. Thorough understanding of the AE signals may lead to the identification of the underlying sources. This may greatly improve the reliability of the AE measurements for the detection of drought-induced cavita-

tion (Chapter 2), which will facilitate research into drought responses of plants, our ultimate goal. Especially the identification of cavitation-induced AE signals would make this method very suitable for non-destructive and automated detection of gas emboli formation in the xylem under drought. These measurements could deliver valuable information for forest management, irrigation strategies and selection of plants for breeding under water-limited conditions.

3.5 Conclusions and future perspectives

In this work we investigated how guided wave modes are developed in rod-like branches. These insights made us realize that the drought-induced AE signals probably originate from sudden abrupt AE sources, rather than oscillating sources. We introduced a new framework for deciphering AE signals from plants based on broadband point-contact sensors, high sampling rate signal recording, time-frequency analysis and FEM. More detailed AE source models could be developed to approach the actual microstructure of AE sources in plants. We believe that signal-based AE analysis supported by FEM could lead to a breakthrough in the current controversy about the actual sources of AE signals in drought-stressed plants.

4

Clustering reveals cavitation-related acoustic emission signals from dehydrating branches

Redrafted from:

Vergeynst LL, Sause MGR & Steppe K Clustering reveals cavitation-related acoustic emission signals from dehydrating branches. Tree Physiology (under revision).

4.1 Introduction

We need accurate quantification of the vulnerability to cavitation, not only for predicting plant responses to a changing climate, but also as a tool to select and breed suitable, drought tolerant plants for production. Several techniques (Cochard *et al.*, 2013) have been developed to quantify vulnerability to cavitation, based on the reduction in hydraulic conductivity with respect to increasing xylem tension (Chapter 1). Acoustic emission (AE) sensors can be used to measure the loss in hydraulic conductivity (Chapter 2). This technique is gaining increasing attention (Rosner, 2015) because it is non-destructive and can be applied without cutting the xylem conduits open, which is currently facing a barrage of criticism because of the risk of artificial embolisms (McElrone *et al.*, 2012; Sperry, 2013; Martin-StPaul *et al.*, 2014; Torres-Ruiz *et al.*, 2015).

Despite the practical advantages of the AE technique, being automatic and labour extensive, the indirect measurement of hydraulic conductivity loss is considered a major drawback. The actual nature of the AE signals, and thus its link with hydraulic conductivity loss, is not fully understood, hampering its interpretation. It has been demonstrated that cavitation in xylem conduits induces a pressure wave that is detectable with AE sensors (Ponomarenko *et al.*, 2014) in dehydrating microscopic xylem slices of the gymnosperm *Pinus sylvestris*. However, when measuring on larger samples (Rosner *et al.*, 2006) or whole branches (Chapter 2), the number of measured AE signals may largely exceed the number of xylem conduits. Also, AE activity is observed to continue beyond the point of 100 % loss of conductivity (Tyree & Sperry, 1989b; Wolkerstorfer *et al.*, 2012, Chapter 2). This implies that cavitation cannot explain all detected AE signals, and that most likely other sources of drying-induced AE signals exist. To become confident in the use of the AE technique for measuring hydraulic conductivity loss, it is imperative for us to elucidate the different sources that produce AE signals in drought-stressed plants. If different AE sources are active, they should be distinguishable based on the waveforms of the detected AE signals (Sause *et al.*, 2012c; Aggelis *et al.*, 2013). Therefore, we recorded the whole waveforms of AE signals from dehydrating grapevine branches (*Vitis vinifera* L. 'Chardonnay') with broadband point-contact sensors. We searched for different types of AE signals using an automated clustering algorithm based on a set of seven frequency features. During branch dehydration, we also recorded diameter shrinkage, gravimetric water loss and xylem water potential. In

this chapter, we explain the meaning of different types of AE signals by relating their activity to the different phases observed during bench dehydration as given by the acoustic vulnerability curve (Chapter 2).

4.2 Materials and methods

4.2.1 Measurements during branch dehydration

Plant material was collected in September 2014 from fruit-bearing wood of grapevine (*Vitis vinifera* L. 'Chardonnay ') in a vineyard located in Lede, Belgium. The day before cutting the branches, the leaves of three branches of ~ 100 cm length and 7.5 ± 1.1 mm diameter were packed in aluminium foil. A broadband point-contact acoustic emission (AE) sensor (KRNBB-PC, KRN Services, Richland, WA, USA) and a holder for the diameter dendrometer (DD-S, Ecomatik, Dachau, Germany) were installed on the branches at distances of ~ 30 cm and ~ 22 cm, respectively, from the top of the branch. The AE sensor was pressed to the branch surface using a compression spring (D22050, Tevema, Amsterdam, The Netherlands) in a small PVC tube (Fig. 4.1). A droplet of vacuum grease (High-Vacuum Grease, Dow Corning, Seneffe, Belgium) was applied between sensor tip and wood to ensure good acoustic contact. The next day, before sunrise, the branches were excised under water and the cut surface was kept under water in plastic test tubes. Packed in an opaque plastic bag, the branches were transported to the lab. In the laboratory, the dendrometers were installed after removing the bark at the dendrometer contact area with a scalpel and smearing petroleum jelly to prevent direct evaporation through the wound (Fig. 4.2).

We cut a piece of ~ 40 cm at the lower side of each branch and placed it on an electronic balance ($2 \times$ DK 6200 with 0.01 g accuracy and $1 \times$ PS 4500/C/1 with 0.1 g accuracy, Henk Maas, Veen, The Netherlands), stripped of all leaves. The wounds of the cut petioles were covered with petroleum jelly (Vaseline). A wood sample of ~ 4 cm was taken from the cut end of the other branch part. Also after the 4.5 days dehydration period, a wood sample was taken from the middle of both branch parts. We determined the volumetric water content (VWC, kg m^{-3}) of the samples and used these measurements, together with



Figure 4.1 The point-contact sensor was installed normal to the branch surface using a custom-made PVC holder with spring inside. Photo credit: Sim Minnaert

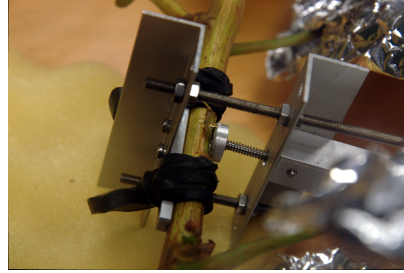


Figure 4.2 We used dendrometers from Ecomatik in combination with the broadband sensors as they do not produce acoustic noise. Photo credit: Sim Minnaert

the continuous measurements of the balance, to calculate continuous VWC throughout the dehydration period (details in Chapter 2).

Every three hours, we measured xylem water potential (Ψ , MPa) on an excised leaf with the pressure bomb (PMS Instrument Company, Corvallis, OR, USA). We determined the linear stress-strain relationship (Irvine & Grace, 1997) for each branch and calculated continuous xylem water potential as described in Chapter 2.

The dendrometers and the electronic balances were read out each minute using custom-built acquisition boards. The AE signals were amplified by 35.6 dB (AMP-1BB-J, KRN Services, Richland, WA, USA) and waveforms of 7168 samples length were acquired at 10 MHz sample rate. An electronic band pass filter of 20-1000 kHz was applied and all waveforms above the noise level of 28 dB_{AE} were collected.

4.2.2 Feature extraction and automatic clustering algorithm

To investigate the presence of different AE sources, we first grouped the signals based on the similarity of their frequency features using an automatic clustering algorithm. Then, we investigated whether these clusters or signal types belong

to a certain physiological process during dehydration. For each waveform, a set of seven frequency features and four waveform features were calculated in the software NOESIS (Envirocoustics S.A., Athens, Greece). Feature extraction was conducted on the initial 50 μ s after the time of arrival (500 samples) and frequencies below 50 kHz were filtered with an 8th order Butterworth filter. The following waveform features were calculated: amplitude (dB), rise time (μ s), rise angle (rad) (Fig. 4.4) and absolute energy (aJ) (Sause, 2010). From the frequency spectrum (Fig. 4.4), we extracted peak frequency (PF), frequency centroid (FC), weighted PF (WPF, geometric mean of PF and FC) and the partial powers of the following frequency ranges: 0-100 kHz, 100-200 kHz, 200-400 kHz and 400-800 kHz (PP1-4) (Sause, 2010).

We applied an automated clustering algorithm as described in detail in Sause *et al.* (2012a) to identify natural clusters of acoustic emission signals. A flow chart of the clustering algorithm is shown in Fig. 4.3. We screened the dataset for the presence of two to four clusters using each possible combination of four to seven frequency features. The algorithm gives as output the ranking of the 192 clustering results (64 features combinations $\times$ three numbers of clusters) according to four cluster indices: the Tou-index, Rousseeuw's silhouette value, the Pearson's Gamma statistic, and the Davies-Bouldin-index. For each cluster index, the 25 best partitions get a score of 25 to 1, and the partitions are ordered according to the sum of the four scores. Because the calculation of the cluster indices is computationally intensive for very large datasets (in our case in the order of magnitude of 10^5 AE signals), we used a random sample of 4000 AE signals for each of the 64 feature combinations. After preliminary analysis this sample size was found to be representative for the whole dataset. The automated clustering algorithm and graphical display were performed using the R software (R version 3.0.1; R Core Team, 2013).

4.2.3 Endpoint of the acoustic vulnerability curve and transition point between shrinkage and cavitation phase

For each of the obtained clusters and for all the AE signals together, we constructed an acoustic vulnerability curve in order to compare the appearance of

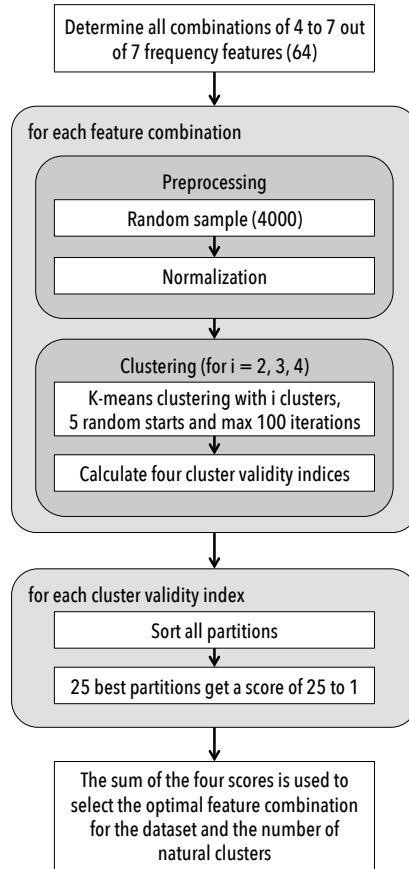


Figure 4.3 We searched for the amount of natural clusters and the optimal feature combination using an automated clustering algorithm. The flow chart of Sause *et al.* (2012a) is adapted here for our specific application.

the clusters with other plant physiological measurements. For the calculation of the characteristic points of the curves, the endpoints were defined by the end of the AE activity peaks. We determined the endpoint of the curves as the point at which the decrease in AE activity, following the AE activity peak, decreased most strongly. Mathematically, this point can be found where the third derivative of the curve of cumulative AE versus time reaches a local maximum (Fig. 4.5). The AE activity (first derivative) was calculated numerically over a time interval of 15 min, while a time interval of 12 h was used for the second

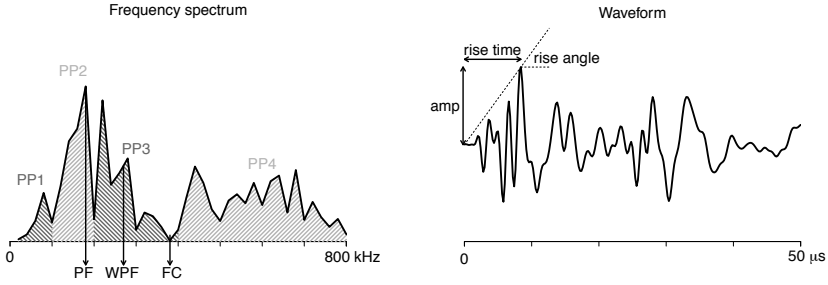


Figure 4.4 The frequency features peak frequency (PF), weighted peak frequency (WPF), frequency centroid (FC) and four partial powers (PP) were used for the automated clustering algorithm. Waveform features rise time, amplitude and rise angle describe the shape of the AE signal.

and third derivative. The latter interval was chosen based on the time scale at which the AE peak occurred. When dehydration occurs much faster, in different samples or under different conditions, this time scale should be adapted in order to obtain meaningful results. For the transition point between the shrinkage and cavitation phase, we searched for a minimum in the second derivative to the curve of VWC versus water potential. This is the point where the hydraulic capacitance (first derivative to the curve) increased most strongly.

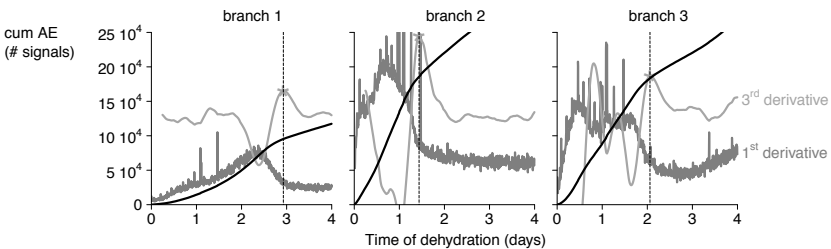


Figure 4.5 We calculated the point of 100 % loss of hydraulic conductivity as the endpoint of the AE activity (1st derivative of the curve of cumulative AE) peak, where the 3rd derivative of the curve of cumulative AE signals in time reached a local maximum, as indicated by the vertical dashed line. Here, the curves of total AE are shown.

4.3 Results

4.3.1 Clustering of AE signals reveals three clusters

A first application of the automated clustering algorithm (Table 4.1) suggested an optimal partition based on the frequency features (Fig. 4.4) frequency centroid (FC), peak frequency (PF), weighted peak frequency (WPF) and partial power in the range 400-800 kHz (PP4) (Fig. 4.6A,B). The clustering resulted in a small group containing signals with high PP4 and high WPF. The other group contained 98-99 % of all signals and showed a large variation in the frequency range 100-200 kHz. A second clustering of this predominating cluster (Table 4.1) resulted in a third cluster (Fig. 4.6C,D), based on the frequency features PF, WPF and partial powers in the frequency ranges 0-100 kHz (PP1) and 100-200 kHz (PP2). Based on their frequency content, the three clusters were labelled as the low-, mid- and high-frequency cluster. High PP1 and PP2 were typical for the low- and mid-frequency cluster, respectively (Fig. 4.7). The high-frequency cluster was characterized by a high PP4, PF, FC and WPF. These signals were slightly larger in amplitude and had a shorter rise time, resulting in the largest rise angles. The low-frequency cluster also contained signals with small rise time, but from the violin plots it is clear that a large portion has longer rise times and small rise angles. The average absolute energy was highest for the low-frequency cluster and lowest for the mid-frequency cluster (Fig. 4.7).

Table 4.1 The cluster indices are a measure of the clustering quality. The second clustering is based on the data from the lowest frequency cluster that resulted from the first clustering approach.

	Davies-Bouldin index	Tou-index	Rousseeuw's silhouette value	Pearson's Gamma statistic
1 st clustering	0.564	2.568	0.786	0.746
2 nd clustering	0.784	2.032	0.594	0.752

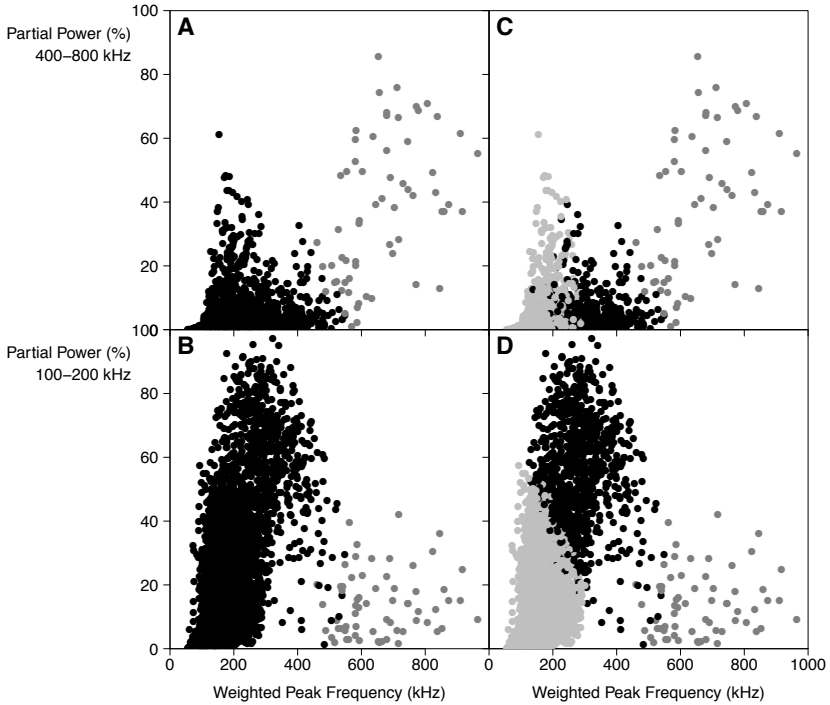


Figure 4.6 The first clustering of the whole dataset (all AE signals from three branches) resulted in two clusters (A, B). When removing the high-frequency cluster (grey) from the dataset, a second clustering split the lower-frequency cluster into the low- (light grey) and mid- (black) frequency cluster (C, D).

4.3.2 Vulnerability curves

The vulnerability curve based on all AE signals (Fig. 4.8A) contained an initial shrinkage phase, followed by a cavitation phase with higher capacitance (Fig. 4.8B, Table 4.2). The vulnerability curves based on the low- and mid-frequency cluster were quite similar to the curve that included all AE signals (Fig. 4.8C, Table 4.3). However, the activity of the low-frequency cluster was high after the endpoint of the curve (Fig. 4.8D), whereas the activity of the mid-frequency cluster reached zero. The high-frequency cluster, in contrast, was mainly active during the shrinkage phase with fast shrinkage (Fig. 4.8E), and reached its endpoint far before the two other clusters.

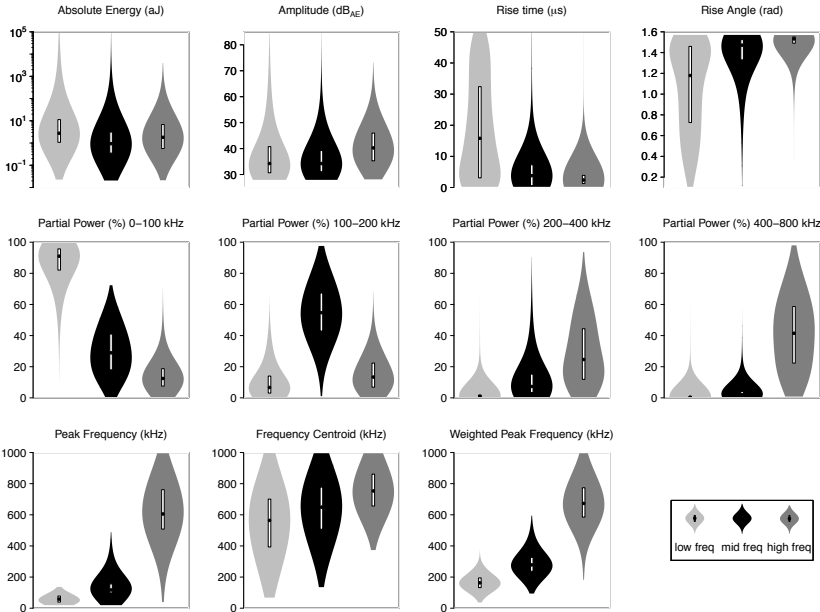


Figure 4.7 The distributions of the signal features for the three frequency clusters (low, mid and high) are visualised by violin plots (Adler, 2005). The box plot indicates the lower hinge, the median and the upper hinge. Overlaid on this box are two mirrored graphs, showing the distribution of the data by using the kernel density estimation (smoothed histogram).

4.4 Discussion

4.4.1 Vulnerability curves based on AE activity peak

Defining the endpoint of the cavitation phase, when the percentage loss of conductivity (PLC) reaches 100 %, is crucial to develop a meaningful acoustic vulnerability curve (VC), but has always been a main difficulty with the AE technique, and particular in angiosperms (Tyree & Sperry, 1989b; Rosner, 2015). In Chapter 2, this endpoint has been defined as the average of the second break-points of the VC and the desorption curve (VWC versus Ψ). In this definition, the endpoint is also influenced by the behaviour of VWC, while PLC should by definition be only linked to cumulative AE. Moreover, a specific AE-inducing

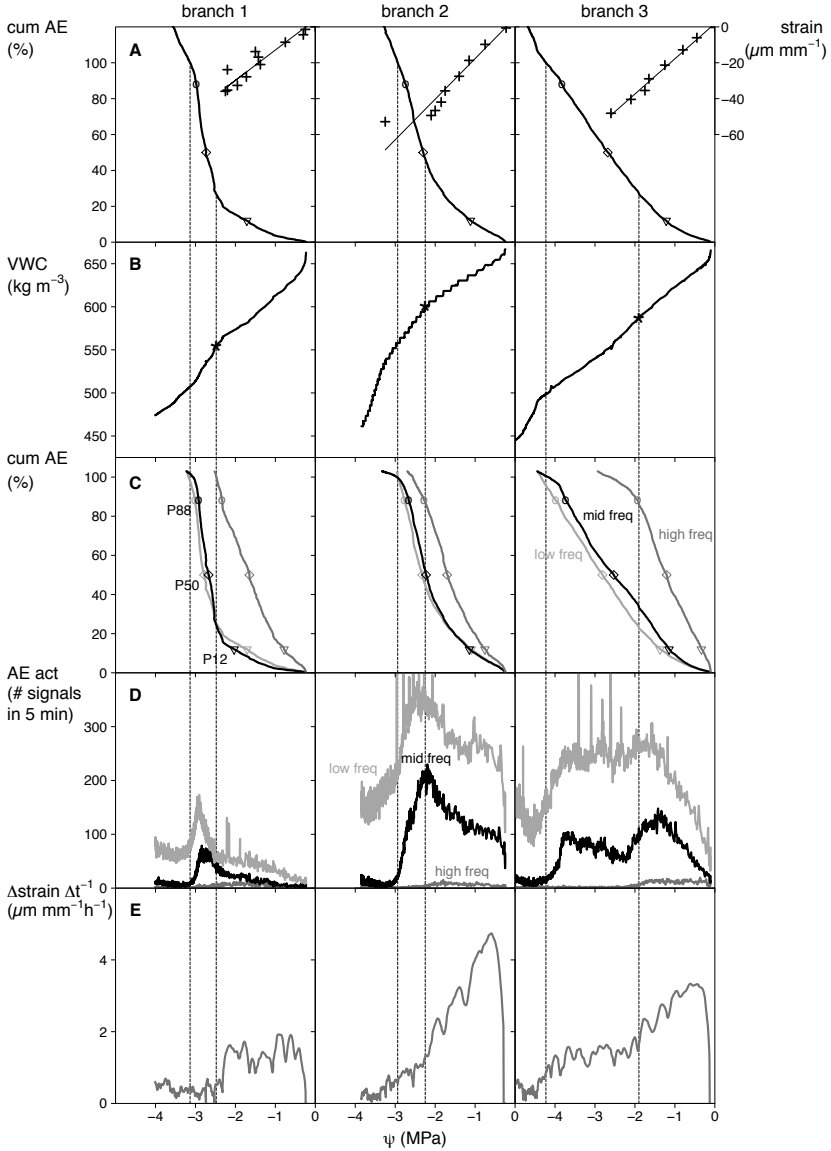


Figure 4.8 The left vertical dashed line indicates the endpoint of the vulnerability curve of all AE signals (A). The transition between shrinkage and cavitation phase based on changing hydraulic capacitance (B) is indicated by the right vertical dashed line. Based on the AE activity peak of the different signal types (D), we constructed three vulnerability curves (C) with Ψ_{12} , Ψ_{50} and Ψ_{88} indicated by triangles, diamonds and circles, respectively. The shrinkage rate ($\Delta\text{strain } \Delta t^{-1}$) was highest during the shrinkage phase (E).

Table 4.2 Parameters derived from the dehydration curves: apparent radial modulus of elasticity (E'_r) (Fig. 4.8A), water potential at transition point between shrinkage and cavitation phase (Fig. 4.8B) and hydraulic capacitance (C) for both phases.

	E'_r (MPa)	transition point (MPa)	C (kg m ⁻³ MPa ⁻¹)	
			shrinkage phase	cavitation phase
branch 1	59.1	-2.48	39.1	73.3
branch 2	44.2	-2.25	30.4	59.5
branch 3	51.0	-1.90	37.7	36.2

mechanism usually results in an AE activity peak (Aggelis *et al.*, 2010). Therefore, we propose a new definition, based on the behaviour of the AE activity peak and its return to zero, which corresponds with the local maximum of the third derivative of the curve of cumulative AE versus time (Fig. 4.5). This makes it possible to define a clear endpoint, even in situations where the VC does not clearly level off due to strong decrease in diameter shrinkage, e.g. for branch 3 in Fig. 4.8A, and also when continuous measurements of water potential are not available. The transition between shrinkage and cavitation phase (Fig. 4.8B) delimits two phases with different hydraulic capacitance. We therefore consider it most meaningful to define the transition at the point where the capacitance changes most strongly. We calculated the point of the dehydration curve where the hydraulic capacitance increased most strongly, instead of taking the average of the breakpoints of both curves as defined in Chapter 2. The re-definition of the endpoints does not alter the results previously presented, but we propose to use these new definitions because they are linked to a physiological meaning.

Although the curve of total cumulative AE signals resulted in plausible values for Ψ_{50} (Table 4.3) when compared with other methods (Brodersen *et al.*, 2013), the total AE activity still continued beyond 100 % PLC (Fig. 4.8A, Fig. 4.5). Acoustic emission sources that were not related to cavitation most likely caused this additional AE activity. We tried to filter out these different AE sources by searching for natural clusters in the AE signals. For composite materials, which also include the natural composite wood (Baensch *et al.*, 2015), frequency features have proven to be useful for the identification of different AE sources (Sause & Horn, 2010). Our observation of three different clusters of AE signals

Table 4.3 Characteristic water potential values of the vulnerability curves using all AE signals (Fig. 4.8A), compared to the three clusters (low, mid and high frequency) of AE signals (Fig. 4.8C). The cumulative number of AE signals at 100 % PLC is indicated for each curve.

		Ψ_{12} (MPa)	Ψ_{50} (MPa)	Ψ_{88} (MPa)	Ψ_{100} (MPa)	cum AE ₁₀₀ (10 ³ signals)
branch 1	all signals	-1.71	-2.73	-2.98	-3.13	95
	low-freq	-1.71	-2.78	-3.01	-3.17	65
	mid-freq	-2.03	-2.67	-2.92	-3.06	26
	high-freq	-0.78	-1.65	-2.33	-2.48	2
branch 2	all signals	-1.12	-2.30	-2.74	-2.94	186
	low-freq	-1.09	-2.32	-2.77	-2.93	125
	mid-freq	-1.14	-2.23	-2.66	-2.96	54
	high-freq	-0.76	-1.70	-2.28	-2.56	2
branch 3	all signals	-1.22	-2.68	-3.83	-4.23	183
	low-freq	-1.37	-2.81	-3.98	-4.35	139
	mid-freq	-1.15	-2.53	-3.74	-4.17	43
	high-freq	-0.34	-1.21	-1.93	-2.62	2

during dehydration of the branches strongly suggested the presence of at least three types of AE sources. The activity of the mid-frequency cluster became almost zero at the VC endpoint (Fig. 4.8D). This AE signal type was thus better related to the expected definition of emboli formation in the xylem than the low-frequency cluster, with continued activity after the endpoint, and the high-frequency cluster, which was mainly active during the shrinkage phase (Fig. 4.8E).

4.4.2 AE features support AE source identification

Beside AE activity, also AE signal features (Fig. 4.7) may support the identification of different AE sources in dehydrating branches. In general, AE signals are caused by sudden microscopic displacements, followed by damped vibration of the surfaces involved. These may include the surfaces of newly formed air bubbles in a xylem conduit as well as crack surfaces in or between the cell walls. The displacements are propagated away from the source as macroscopic elastic

waves (pressure waves and shear waves), of which the frequencies are influenced by the macroscopic elastic properties of the surrounding wood. When the initial displacement is large enough and when the detector is sensitive for the propagated frequencies, a signal can be measured at the branch surface. The frequency content of the detected signal is related to the frequency spectrum of the source vibration (Sause & Horn, 2010).

The early activity of high-frequency AE signals (Fig. 4.8D) was probably caused by micro-cracks, due to fast contraction during the initial shrinkage phase (Fig. 4.8E). This type of signals containing high frequencies, with relatively high peak amplitude, short rise time and thus large rise angle (Fig. 4.7) has also been related to surface checking in drying oak (*Quercus variabilis*) by Kim *et al.* (2005). The signals originate from very fast movements of the microscopic fracture surfaces in or between the cell walls (Čunderlik *et al.*, 1996) being pulled apart. In the field of fracture mechanics, this is called tensile cracking (Aggelis *et al.*, 2010). The micro-cracks may act as precursors for the formation of macro-cracks, which may occur at increasing drought stress levels. The formation of macro-cracks is associated with shear stresses and the so-called shear cracking is accompanied by AE signals with high energy, long rise time and low frequencies (Aggelis *et al.*, 2010), comparable with the low-frequency cluster that we observed (Fig. 4.7). Beyond 100 % PLC, when tension in the xylem conduits is released by cavitation, the stress field changes and existing fractures may grow by shear stresses leading to strong low-frequency signals. However, we distinguish a second possible origin for the low-frequency signals beyond 100 % PLC. At the moment when all hydraulic conductivity is lost, the volumetric water content is still far above the fibre saturation point. Free water that is still left in the wood, and which is disconnected from the transpiration stream, may move under influence of a combination of viscous, capillary and gravity forces (Løvøll *et al.*, 2005). The movement of the drainage front through a porous medium is characterized by rapid meniscus displacements, called Haines jumps (Haines, 1930). These rapid rearrangements of the air-water interfaces have been related to acoustic emission signals in the frequency range 50-250 kHz (Chotard *et al.*, 2007), 10-30 kHz and below 3.75 kHz (DiCarlo *et al.*, 2003) and could occur e.g. when a meniscus passes a perforation plate of a xylem vessel. Also during the cavitation phase, movement of water that was disconnected from the transpiration stream by gas emboli may result in these low-frequency signals. However, high activity of the low-frequency cluster during the cavitation phase

may also partially be explained by overlapping clusters (discussed in Section 4.4.3).

The formation of a gas bubble (Vincent *et al.*, 2012, 2014) in a xylem conduit under tension is most probably a more violent event than drainage of free water because of the large pressure drop involved. Faster movements at the AE sources result in higher frequencies, explaining the higher frequencies of the mid-frequency cluster related to cavitation. Vincent *et al.* (2014) found that the bubble vibration frequency is inversely proportional to the bubble radius. This might result in a certain frequency range of cavitation-induced AE signals, dependent on the bubble size. However, the total number of AE signals in the mid-frequency cluster (Table 4.3) exceeds the number of xylem vessels that might be expected in a 36 cm-long branch (Chapter 2) by one or two orders of magnitude. On the one hand, the boundaries of this cluster, obtained with the clustering algorithm, might be too wide and might include signals that belong to one of the other AE sources. On the other hand, with the current limited mechanistic understanding of the cavitation process (Rockwell *et al.*, 2014) in angiosperms, it is not self-evident that one embolized conduit results from the birth of one air bubble. The nanobubble theory (Schenk *et al.*, 2015) states that nanobubbles may coalesce in the pit chamber to form an air bubble, without resulting in embolism formation. The process of bubble coalescence and subsequent bubble collapse may produce numerous AE signals before finally resulting in an emboli.

Note that changes in water content during dehydration may influence wave propagation and thus the frequency features of the measured signals. Lower water contents result in lower attenuation and higher wave velocities (Sakai *et al.*, 1990). Lower attenuation could result in the detection of more high-frequency signals, which are strongest attenuated. Higher wave velocities would result in guided waves with higher frequencies (Rose, 2004). However, the signals with highest frequencies occurred mainly at the beginning of dehydration and at the end only the lowest-frequency signals remain (Fig. 4.8). The decreasing water content of the branch during the experiment can thus not explain the occurrence of different types of AE signals.

4.4.3 Differences, but also overlap due to wave propagation

Although only the mid-frequency cluster complied with the features expected upon the occurrence of cavitation, the shape of the VC (Fig. 4.8C) and calculated Ψ_{50} (Table 4.3) were very close when either the low- or mid-frequency cluster were used. The main reason for these similarities is probably the overlap between clusters caused by wave propagation. The bulk of the AE signals have propagated some distance along the branch before reaching the sensor, which gives rise to guided waves. Differences in source location (Chapter 3) may influence the frequency content and thus the location in the scatterplots (Fig. 4.6). Therefore, a particular AE source results in a point cloud, which may show some overlap with adjacent point clouds. Moreover, in wood, higher frequencies are attenuated stronger than lower frequencies. Acoustic emission sources of higher frequency may fall into a lower-frequency cluster due to a frequency shift by attenuation. Part of the activity of the mid-frequency cluster during the initial shrinkage phase thus probably resulted from high-frequency AE sources, related to shrinkage at a longer distance from the detector. This could result in a risk of overestimating PLC at low tensions. However, given that the high-frequency signals were much lower in abundance than the mid-frequency signals (Fig. 4.8D), this overestimation is probably small. The mid-frequency cluster, in contrast, showed a high activity during the cavitation phase which was in the same order of magnitude as the activity of low-frequency signals beyond 100 % PLC. Therefore, a large part of the low-frequency signals before the endpoint of the VC could have originated from cavitation-related AE signals that experienced a frequency shift by attenuation, resulting in similar VC features. In this case, the low-frequency AE sources, which were still active beyond 100 % PLC, probably had a relatively low or constant activity during the shrinkage and cavitation phase, and therefore did not strongly change the shape of the VC. On the other hand, movement of water that was disconnected from the transpiration stream by gas emboli might result in low-frequency signals co-occurring with cavitation. To reduce the overlap between AE signal clusters, source localization would be helpful to account for frequency changes due to attenuation.

In general, even when no distinction would have been made between different AE sources (Fig. 4.8A), the VC parameters (Table 4.3) did not change substantially compared to the filtered mid-frequency VC in our experiment. We explain this by the very low activity of high-frequency AE sources and a relatively constant or simultaneous activity of the low-frequency AE sources during the shrinkage and cavitation phase. However, when measuring on other branches, other species, and in different environments, the coordination between the different AE sources might change, necessitating the filtering of the specific cavitation-induced AE signals as proposed in this chapter. This clustering approach has great potential to also detect non-destructively cavitation in the field by considering only the mid-frequency signals. This would be of great use to clarify the current controversy about diurnal emboli formation and repair (Sperry, 2013).

4.5 Conclusion

Three clusters of AE signals were active during the dehydration of grapevine branches. Based on differences in AE features and AE activities during the consecutive dehydration phases, we concluded that the mid-frequency signal type with high intensity at 100-200 kHz was related to cavitation. High-frequency signals probably resulted from micro-fractures due to shrinkage, while low-frequency signals were most likely caused by macro-fractures or Haines jumps. In our experiment, the filtered vulnerability curve based on mid-frequency signals did not strongly differ from the curve based on total AE activity. However, for reliable widespread application of the AE technique, and for *in vivo* measurements, distinction between the different types of AE signals is imperative. Moreover, the information contained in the AE signals should be used as a precious resource to support important on-going discussions on the processes behind hydraulic failure.

5

Investigation of cavitation-related acoustic emission signals in various woody branches using finite element modelling and experiments

5.1 Introduction

During the International Workshop on Plant Hydraulic Techniques (Ulm University 2014), no consensus could be reached about the validity of the common methods to establish vulnerability to cavitation (Jansen *et al.*, 2015). Novel techniques to measure vulnerability curves were encouraged, such as acoustic emission, X-ray microtomography, magnetic resonance imaging and sap flow

methods. Actually, the acoustic emission (AE) technique is not new in plant science as it was first applied on plants in 1966 (Milburn & Johnson). However, it is still very challenging, with the major challenge being the understanding of cavitation-related sounds in terms of plant physiological processes.

In Chapter 4 we managed to separate cavitation sounds from other signals in dehydrating branches of grapevine, based on the frequency spectrum of the detected signals. Signals of high frequency were identified as micro-fractures, whereas signals of very low frequency were attributed to macro-fractures or movements of water that was disconnected from the transpiration stream. The mid-frequency signals typified cavitation. The signals were measured with a point-contact broadband sensor, which, to our knowledge, had not been used in plant science before. More commonly, a sensor with resonance frequency at 150 kHz is used (Tyree & Sperry, 1989a; Rosner *et al.*, 2006; Mayr & Rosner, 2011; Wolkerstorfer *et al.*, 2012; McCulloh *et al.*, 2014; Bloemen *et al.*, 2015). We found that for grapevine branches, a 150 kHz resonant sensor was suitable to construct vulnerability curves, provided an endpoint is defined (Chapter 2). Moreover, we could identify cavitation-related signals by the high partial power in the frequency range 100-200 kHz (Chapter 4). The question arises whether these findings are valid for woody branches in general. Can we pinpoint cavitation signals in branches of other species as well? Are 150 kHz resonant sensors generally applicable to construct VCs?

To this end, we determined the differences between AE sources of the various AE signal types found in grapevine using finite element modelling combined with AE signal clustering, and we selected an appropriate AE source model for cavitation. As branch diameter and wave velocity influence the dispersion curves of the guided waves, we investigated the influence of these parameters on the frequency features of cavitation-related AE signals. Second, we conducted a dehydration experiment on common ash (*Fraxinus excelsior* L.) and white poplar (*Populus alba* L.), two angiosperm species with ring- and diffuse-porous wood anatomy, respectively. We measured AE signals with a 150 kHz resonant sensor as well as a multi-resonant broadband sensor (100-1000 kHz) and determined continuous water potential using diameter shrinkage measurements in order to construct AE-based vulnerability curves.

In this chapter we elaborate on the difference between clusters of AE signals detected on dehydrating branches. We use finite element modelling to support signal interpretation and to investigate the possibility to identify cavitation sounds in branches with different diameter and elastic properties and with different AE sensors.

5.2 Materials and methods

5.2.1 Sensitivity study of AE source characteristics with FEM

We studied the influence of AE source characteristics on the measured AE frequency features using a finite element model (FEM) of a cylindrical woody branch with diameter 6 mm and theoretically infinite length in the software environment COMSOL Multiphysics. To reduce computational load, only 25 cm of the branch was modelled (Fig. 5.1). We mimicked the infinite branch length by applying a low-reflecting boundary condition at one end, to avoid any incident reflections on this boundary, and a symmetry plane at the other end of the branch model. By using a symmetry plane at the cross-section where the AE sources were modelled, the model size could be reduced by half. To model wave propagation in the orthotropic wood, 9 independent values of the stiffness tensor are needed (Chapter 3). Since no stiffness tensors for fresh wood were reported in literature, we approximated the properties of the branch by using elastic properties of dry ash wood (Dinckal, 2011; Table 3.1) with a dry wood density of 600 kg m^{-3} . A numerically convergent solution of the FEM model was reached with a $5 \times 10^{-8} \text{ s}$ time step and a mesh size of $1\text{-}100 \times 10^{-5} \text{ m}$ applying a maximum element growth rate of 1.5 to increase mesh density at regions of narrow details.

At the sensor position, AE signals were evaluated as surface displacements normal to the surface (Fig. 5.1; black arrows), which is in agreement with the detection principle of the point-contact sensor that was used on grapevine in Chapter 4. The AE sources, which could be movements of air-water interfaces, bubble formation and cell wall cracks, were modelled as two parallel forces acting at two points separated by $200 \mu\text{m}$ (represented by a single symbol in Fig. 5.1)

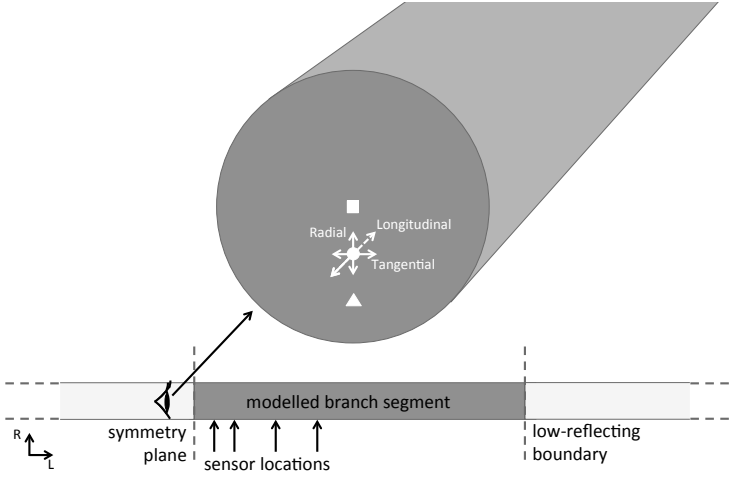


Figure 5.1 Schematic of acoustic emission source model and modelled branch segment with finite element modelling in Comsol Multiphysics.

and acting with the same force function in opposite directions. These point couples, called dipoles, are often used to simulate AE sources (Hamstad, 2010; Sause *et al.*, 2013) and study the three main AE source characteristics: location, source geometry and source dynamics. The source dynamics are represented by a source function (Eq. 5.1), describing force as a function of time. We used a linear force pulse (Sause *et al.*, 2013), with varying excitation time (t_e) and F_{max} equal to 3 N:

$$F(t) = \begin{cases} F_{max} \frac{t}{t_e} & t \leq t_e \\ F_{max} - 2 \cdot F_{max} \frac{t - t_e}{t_e} & t_e < t \leq 1.5t_e \\ 0 & t > 1.5t_e \end{cases} \quad (5.1)$$

The excitation time represents the duration of the sudden volume change that causes the acoustic emission. The dipole is a simplified representation of an AE source with main displacements in one orientation (Sause & Richler, 2015). By varying the dipole orientation along the three main directions radial (R), tangential (T) and longitudinal (L) (Fig. 5.1), we explored the effect of different source geometries. Variations in signal features due to different source locations

were examined by changing source depth and source-sensor distance along the branch (Fig. 5.1; 1.5, 3, 6 and 9 cm). In Chapter 3 we found that different source-sensor angles, as well as different source depths, changed the balance between symmetric and anti-symmetric guided wave modes. Because the effect of source depth was stronger than source-sensor angle (Fig. 3.4 and 3.5), we only investigated the influence of source depth on AE frequency features in this study, keeping the source-sensor angle 0° . We modelled sources at depths of $2/3$ and $1/3$ of the branch radius and at $100\text{ }\mu\text{m}$ from the centre (Fig. 5.1; triangle, circle and square), as a perfectly symmetric position of an AE source is rarely found in practice.

5.2.2 Influence of branch properties on guided wave modes

Because most of the measured AE signals have propagated some distance along the branch before being detected, guided wave modes develop upon propagation (Chapter 3). Each wave mode propagates at a typical frequency-dependent velocity, which is described by a dispersion relation. These dispersion curves are dependent on the diameter of the branch and the bulk wood acoustic velocities (pressure and shear waves). Wave velocity is inversely proportional to $\sqrt{\rho}$ (ρ is bulk wood density, kg m^{-3}), when the elastic properties are kept constant (Newton-Laplace equation). However, the elastic properties are also dependent on dry wood density (Yang *et al.*, 1999) and moisture content (Sakai *et al.*, 1990). In a study on longitudinal pressure wave velocity in wood of 9 different species and with moisture contents ranging from dry to saturation (Sakai *et al.*, 1990), the wave velocity at full saturation was $\sim 20\%$ lower than in dry condition. As our model consisted of dry wood, we changed the wood density from 600 to 1000 and 1500 kg m^{-3} , keeping the elasticity values constant, to mimic the behaviour of fresh wood. This decreased the wave velocities with 23 and 37%, respectively. The influence of branch diameter was investigated by increasing the diameter from 6 mm to 10 and 20 mm. We do not have access to a suitable model to calculate the theoretical dispersion curves for the anisotropic wood, but to give an idea on the behaviour of the guided wave modes in a rod, we calculated the curves for an isotropic PVC rod using the software package PCDISP (Seco & Jiménez, 2012). We plotted the Choi-William distributions of simulated signals as described in Section 3.2.4 to visualize the dispersion behaviour of different woody branches.

5.2.3 Bench dehydration and vulnerability curves

One-year old shoots of common ash (*Fraxinus excelsior* L.) and white poplar (*Populus alba* L.) of ~ 1.5 m length came from plants grown in the botanical garden at BOKU, Vienna. Shoots were cut early in the morning at late summer (8–11 Sep 2014) and were transported to the lab with their cut ends in water and their leaves preserved by an opaque plastic bag. One ash shoot of 12.5 mm diameter and three poplar shoots of 9.3 ± 0.2 mm diameter were equipped with AE sensors at 34 ± 4 cm from the cutting location 24 h before the experiment started. On each shoot, two different sensors were installed at a distance of 4 cm. We used multi-resonant wideband sensors (WD, 100–1000 kHz) and 150 kHz resonant narrow band sensors (R15C, 50–200 kHz), both from Physical Acoustics Corporation (PAC, Princeton Jct, USA). Below the sensors, the bark and cambium were carefully removed using a fine-tooth wood rasp file to achieve a flat and polished surface. On the surface, silicone grease (Wacker, Burghausen, Germany) was applied to enhance the acoustic coupling between surface and sensor. Diameter shrinkage was measured with a DMS load cell (Type 8416–5500, range 0–500 N; amplification with an inline amplifier for DMS, Type 9235; Burster, Gernsbach, Germany) that was positioned upon the AE sensor in a holder assembly as described in Rosner *et al.* (2009). After sensor mounting, shoots were allowed to rehydrate and equilibrate in the plastic bag for 24 h at ambient temperature, with their cut ends in water. Directly before the dehydration experiment started, the plastic bag was removed and the cut end of the shoot was tightly wrapped (under water) in Parafilm (Pechiney Plastic Packaging, Chicago, IL, USA) in order to minimize moisture loss through the cut surface.

We measured xylem water potential 1–2 times per hour using a pressure bomb (Scholander-type, Soilmoisture Equipment Corp. Santa Barbara, CA, USA) on leaves wrapped at least 30 min before sampling in aluminium foil. We calculated 1 min data for xylem water potential using the linear relationship between diameter strain and xylem water potential (Chapter 2).

The acoustic emission signals were recorded in the frequency range 20–1000 kHz (WD sensor) and 20–400 kHz (R15C sensor) using a PCI-2 AE data acquisition system and the software AEwin (PAC) with a sampling rate of 2 Msamples s^{-1} and applying a detection threshold of 33 dB_{AE}. The signals were amplified by

40 dB preamplifiers in the frequency range 100-1200 kHz (2/4/6, PAC) for the WD sensor and 100-300 kHz (IL40S, PAC) for the R15C sensor. We analysed the AE signals obtained with the WD sensor and calculated peak frequency (PF), frequency centroid (FC), weighted peak frequency (WPF) and partial powers in the frequency ranges 0-100 kHz, 100-200 kHz, 200-400 kHz and 400-800 kHz (PP1-4) (NOESIS, Envirocoustics S.A., Athens, Greece). These frequency features were used to detect natural clusters of AE signals as described in Chapter 4. The clustering results were then compared with the AE signal types found in grapevine with the point-contact broadband sensor (Chapter 4).

We constructed AE-based vulnerability curves with the endpoint being defined by the local maximum of the third derivative of the curve of cumulative AE (Fig. 4.5). For the numerical calculation of the third derivative, we used a time interval of 3 min for the first derivative (AE activity) and 1 h for the second and third derivative of the curve of cumulative AE as a function of time. Smaller time intervals were used than in Chapter 4 because the dehydration was much faster in the experiments with ash and poplar branches (100% point after ~2.5 h) than in the experiments with grapevine branches (2-3 days, Table 2.1). For each curve, we calculated Ψ_{50} and Ψ_{88} , the water potential at which 50 and 88 %, respectively, of the cumulative AE signals was reached relative to the endpoint.

5.3 Results

5.3.1 Sensitivity study AE source modelling with FEM

The sensitivity study with finite element modelling of AE signals in branches revealed that the excitation time of the AE source has the largest impact on the frequency features of the detected AE signal. At constant source location (at 2/3 from the centre) and with the same dipole orientation (T), the data points in the scatterplots (Fig. 5.2a) were ordered from low to high weighted peak frequency according to excitation time and formed a scatterplot of typical shape as observed in the grapevine data of Chapter 4 (Fig. 5.2d). Faster dynamics at the AE origin thus result in AE signals of higher frequency. Different source-to-sensor pathways, i.e. different depths (Fig. 5.2b, for 5 μ s excitation time) and distances along the branch (decreasing symbol size in Fig. 5.2), modify the signals so that a certain AE source type manifests itself in a scattered point

cloud. Next to excitation time, the source geometry is an intrinsic characteristic of an AE source. Variations in source geometry, e.g. by changing the orientation of the dipoles, introduced extra variation in the point cloud of 5 μs excitation time at constant source depth (Fig. 5.2c). The data points resulting from L-oriented dipoles were shifted towards lower frequencies in the scatterplot, while the difference between R- and T-orientation was found to be rather small. This is probably caused by the strong anisotropy of wood, with elastic properties in longitudinal direction, along the grain, being very different from the elastic properties in radial and tangential direction.

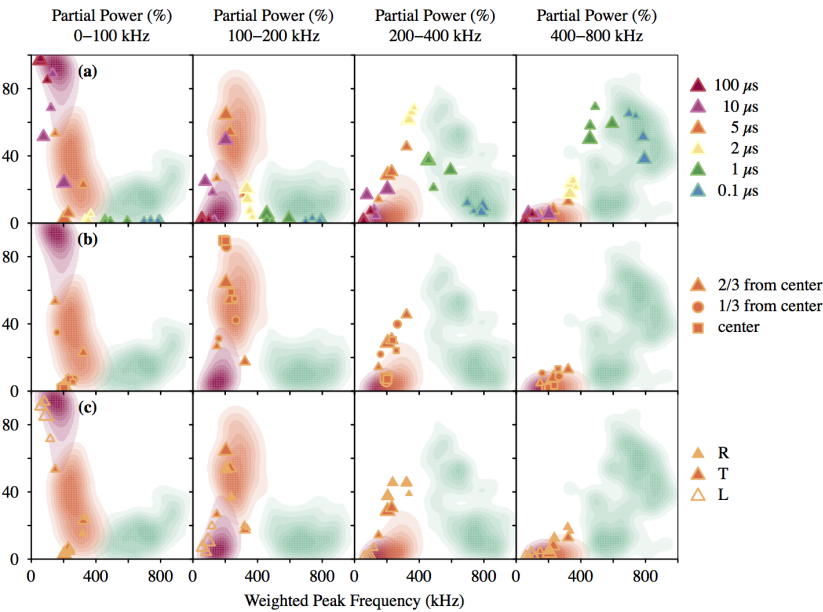


Figure 5.2 The density plots in the background show the location of the signal clusters observed in dehydrating grapevine branches (data from Chapter 4, Fig. 4.6 C,D), with higher colour intensity at higher point density. Frequency features of simulated AE signals with different excitation time (a) form a scatterplot with typical shape, as observed in the signals from grapevine. Different depths (b) and orientations (c) result in a scattered point cloud when keeping excitation time constant (5 μs). Data points of simulated signals at 15, 30, 60 and 90 mm from the dipole location are shown with decreasing symbol sizes for longer propagation distances.

5.3.1.1 Guided wave mode identification

The peaks in the scatterplots of partial powers could be related to the activation of certain guided wave modes in the branch. Based on the shape of the theoretical dispersion curves of a PVC rod (Fig. 5.3), we identified the wave modes in the modelled branch (Fig. 5.4), since the overall shape is typically retained despite of the change in material properties. We found that L-oriented dipoles located close to the centre did not excite anti-symmetric wave modes and thus modelled these dipoles with varying excitation time to visualize the symmetric wave modes S0-S2 (Fig. 5.4b-e). The anti-symmetric wave modes were excited by T-oriented dipoles at 2/3 from the branch axis (Fig. 5.4f-j). AE sources with 10 μ s excitation time activated the lowest anti-symmetric wave mode (A0, Fig. 5.4f). This translates into a high PP1 for this dipole, and even higher for the dipole with t_e 100 μ s (Fig. 5.2a). Even slow AE sources close to the centre activated this mode (Fig. 5.4a), due to the absence of symmetric wave modes at low frequencies (Fig. 5.3). The horizontal part of the second A-mode (A1), around 150 kHz (Fig. 5.4f-g), was activated by excitation times 5-10 μ s and shorter excitation times activated higher A-modes that were difficult to distinguish (Fig. 5.4h-j). The S0-mode was stimulated by dipoles with excitation times 1-5 μ s, but for 1 μ s t_e , also S1 and S2 were stimulated, resulting in a broad frequency range for this signal type. Shorter excitation times stimulated mainly S2 (Fig. 5.4e). The activation of the A1- and S0-mode resulted in a high PP2 (Fig. 5.2a), whereas the higher modes corresponded to high PP3 (especially A-modes) and PP4 (also S-modes).

5.3.1.2 Influence of branch diameter and wave velocity on guided wave modes

With a constant source model, we changed the diameter and wave velocity of the woody branch. The tip of the S0-mode shifted from $\sim$ 200 kHz to 100-200 and below 100 kHz when increasing the diameter from 6 mm to 10 and 20 mm (Fig. 5.5b,c). This is according to the theoretical inverse relationship between frequency and diameter. In the same way, the horizontal part of the A1-mode should thus shift from $\sim$ 150 kHz (Fig. 5.5f) to $\sim$ 90 and $\sim$ 45 kHz. However, higher A-modes were activated when the diameter increased (Fig. 5.5g-h). In the branches with lower wave velocities, the S0- and A1-mode also shifted

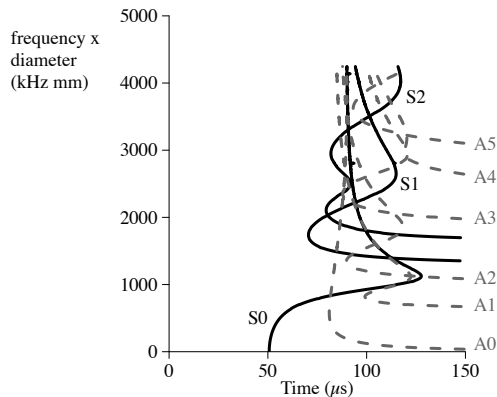


Figure 5.3 Dispersion curves S0-2 and A0-5 for a PVC rod (Chapter 3) at a propagation distance of 9 cm.

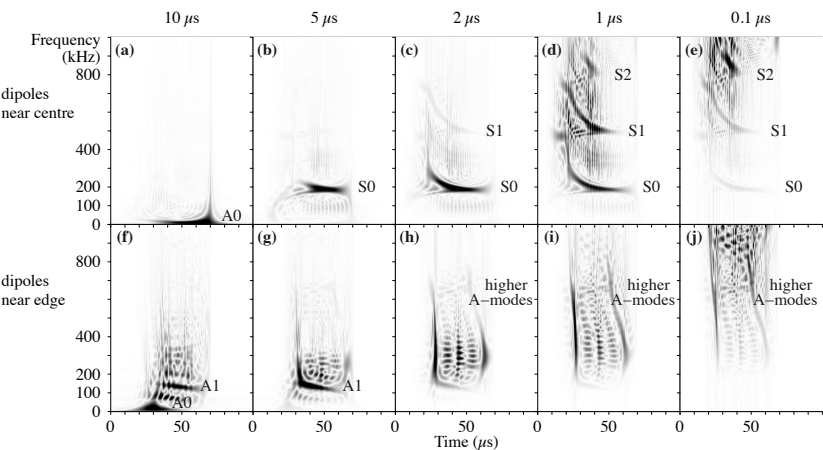


Figure 5.4 Choi-Williams distributions of the simulated signals in the wooden rod at 3 cm propagation distance from L-oriented dipoles close to the centre (a-e) and T-oriented dipoles at 2/3 from the rod axis (f-j). Different excitation times stimulate different guided wave modes, which were identified based on the theoretical dispersion curves in a PVC rod (Fig. 5.3). The greyscale intensity represents the signal intensity.

downwards (Fig. 5.5 d-e and i-j) and due to the lower velocity, the modes arrived later.

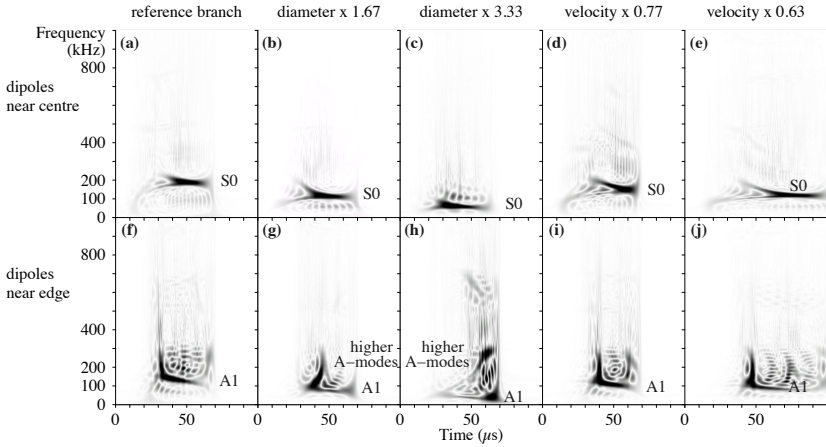


Figure 5.5 Choi-Williams distributions similar to Fig. 5.4, but with constant excitation time ($5 \mu\text{s}$) and varying rod diameter and wave velocity. Increasing the diameter of the rod from 6 (a,f) to 10 (b,g) and 20 mm (c,h), shifts the guided wave modes towards lower frequencies. Decreasing wave velocity with 23% (d,i) and 37% (e,j) also decreased the frequencies, but to a lesser degree. Because the signals arrived much later with slower velocities, we calculated the first $100 \mu\text{s}$ of the waveform for (e) and (j).

5.3.2 Signal clustering and vulnerability curves of common ash and white poplar

Automated clustering of the AE signals from drying branches of common ash and white poplar resulted in two clusters, with a separation at $\sim 360 \text{ kHz}$ WPF (between cluster 2 and 3 in Fig. 5.6a,b). As the left cluster (cluster 1 and 2 in Fig. 5.6a,b) visually contained two structures, a second clustering was applied on this cluster which resulted in clusters 1 and 2. Clusters 1, 2 and 3 were characterized by high partial powers in the frequency ranges 100-200 (PP2), 200-400 (PP3) and 400-800 kHz (PP4), respectively. The cluster positions did not directly correspond to the clusters found in grapevine (Fig. 5.6c). After filtering the grapevine data in the frequency range 90-550 kHz (68% contour lines in Fig. 5.6c), the low-frequency cluster shifted to the right and largely merged with the mid-frequency cluster. The high-frequency cluster shifted to-

wards lower WPF and part of the signals even fell into the WPF range of the mid-freq cluster (second concentration of data points at 200–400 kHz WPF).

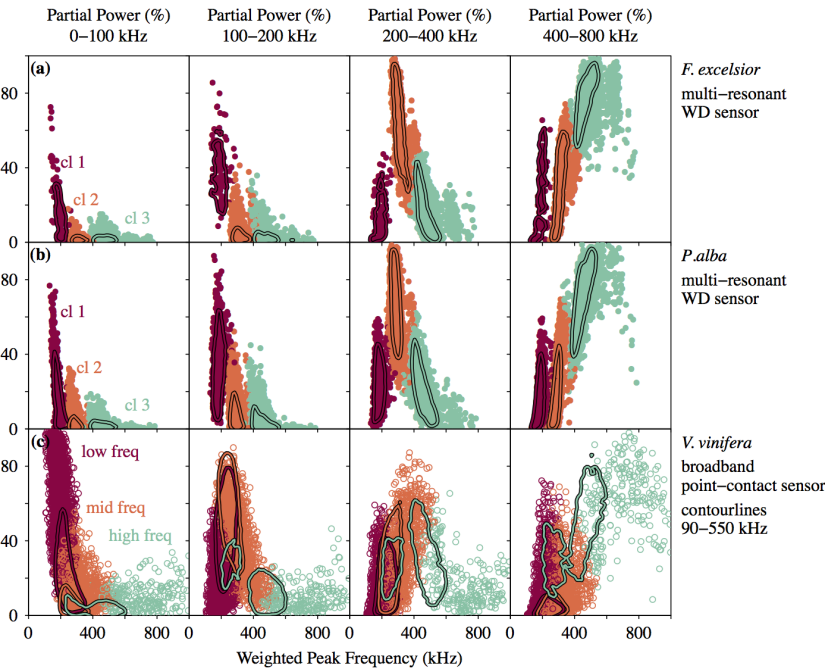


Figure 5.6 Scatterplots showing frequency features PP1–4 versus WPF for AE signals from dehydrating branches of common ash (a), white poplar (b) and grapevine (data from Chapter 2) (c). The signals were measured with a multi-resonant broadband sensor (full symbols) in (a) and (b) and with a flat-with-frequency broadband sensor (empty symbols) in (c). The different clusters as obtained with the automated clustering algorithm are indicated by different colours. The contour lines demarcate the region with highest density, enclosing 68% of the data points. The 68% contour lines in (c) indicate the position of the clusters after applying a bandpass filter of 90–550 kHz.

For white poplar, cluster 1 was most representative of the cavitation phase as the activity of this signal type became close to zero after Ψ_{88} (Fig. 5.7a) whereas clusters 2 and 3 reached a minimum but continued AE activity. All clusters reached a zero activity at the end in the ash branch, but the activity of clusters 2 and 3 was better related to the shrinkage rate (Fig. 5.7b) than cluster 1. Also in the poplar branches, the continued AE activity of clusters 2 and 3 beyond

Ψ_{88} was related to the continued shrinkage rate. As cluster 2 and 3 behaved very similar, we merged these clusters for constructing the vulnerability curves. The activity of the signals detected with the R15C sensor (Fig. 5.7c) showed a similar pattern as the signals of the WD sensor (Fig. 5.7a). We did not apply clustering on these signals, given the very small bandwidth of the R15C sensor and single resonance at 150 kHz which strongly influence the frequency spectrum. The vulnerability curves constructed with the signals of cluster 1, the signals of cluster 2+3 and the signals detected with the R15C sensor were very close (Fig. 5.8a). The variation between the curves and values of Ψ_{50} (Table 5.1) due to different sensor- or signal-types was not larger than the variation between branches.

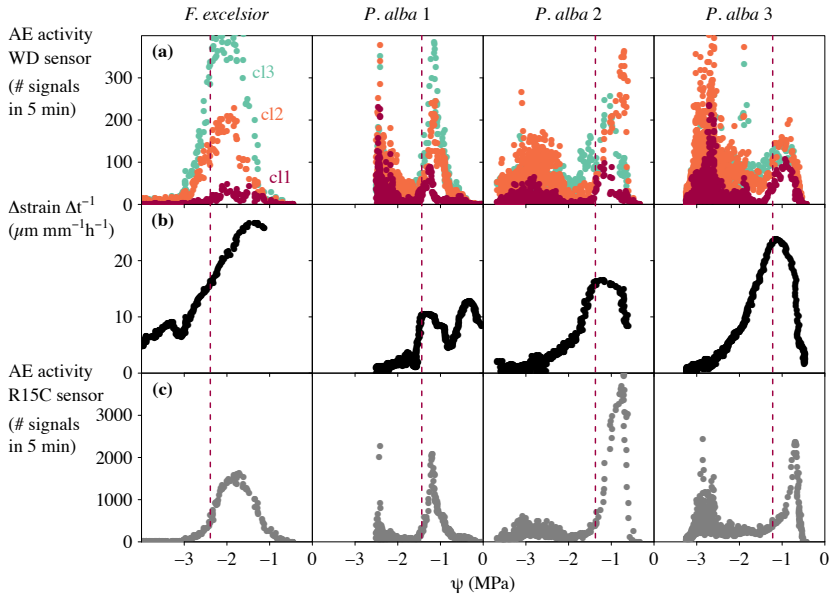


Figure 5.7 Activity of the AE signals for three clusters measured with the WD sensor (a), shrinkage rate (b) and activity of the signals measured with the R15C sensor (c) are plotted versus water potential. The vertical dotted line indicates Ψ_{88} of the vulnerability curve based on cluster 1.

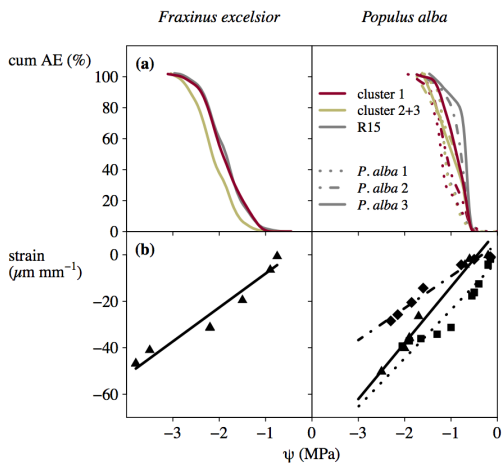


Figure 5.8 Vulnerability curves (a) constructed with cumulative AE signals from cluster 1 and cluster 2+3, measured with the WD sensor, and signals from the R15C sensor were very similar. For continuous water potential measurements, we used the linear relationship between water potential and diameter strain (b).

Table 5.1 Ψ_{50} values for the four branches, as obtained from the vulnerability curve based on signals from cluster 1 and cluster 2+3 detected with the WD sensor and signals from the R15C sensor (Fig. 5.8a). Also the apparent radial modulus of elasticity E'_r is listed, together with the determination coefficient for the linear relationship between water potential and strain (Fig. 5.8b).

	E'_r (MPa)	R^2	Ψ_{50} (MPa)		
			WD	WD	R15C
			cluster 1	cluster 2+3	
<i>F. excelsior</i>	68.5	0.95	-1.96	-2.14	-1.84
<i>P. alba</i> 1	48.0	0.97	-1.18	-1.13	-1.13
<i>P. alba</i> 2	72.7	0.91	-1.16	-0.89	-0.70
<i>P. alba</i> 3	41.4	0.91	-0.91	-0.93	-0.69

5.4 Discussion

5.4.1 Differentiation between AE sources in branches supported by FEM

We used finite element modelling of AE sources and AE wave propagation in a branch to further support the differentiation between the different AE sources found in grapevine (Chapter 4). The scatterplots of partial powers versus WPF (Fig. 5.2) revealed a good match between the signals of 5 μ s excitation time and the cavitation-related mid-frequency cluster in grapevine with high PP2. A high PP2 is explained by the activation of the S0 and A1-modes (Fig. 5.4). The high-frequency cluster linked to micro-fractures due to shrinkage corresponded well with the modelled dipoles with shortest excitation time (0.1-1 μ s), that stimulated higher S- and A-modes. The low-frequency cluster observed in grapevine corresponded with modelled dipoles with longest excitation time and with the activation of the A0-mode. The sensitivity study (Fig. 5.2) confirmed that the excitation time of the AE sources explains the different frequency features of the three clusters, while different source locations and orientations result in a scattered point cloud for a certain source excitation time.

Because of the good correspondence between the simulated signals of dipoles with 5 μ s excitation time and the measured cavitation signals, we used these AE sources to investigate the influence of wave velocity and branch diameter on the frequency content of the cavitation signals (Fig. 5.5). These two parameters affect the position of the dispersion curves of the guided wave modes in a rod and are expected to differ between different branches and between species. The frequencies of the guided wave modes are inversely proportional to the branch diameter. This means that the wave mode frequencies decrease when the diameter increases, and that the wave mode frequency positions come closer to each other. As a consequence, higher A-modes were activated when the diameter increased and the activated wave modes in a 10 mm branch (Fig. 5.5b,g) were still in the range 100-200 kHz for signals of the cavitation type. However, when increasing the diameter to 20 mm, the activated wave modes fell mainly above 200 kHz (higher A-modes) or below 100 kHz (S0 and A1-mode) and the characterization of cavitation signals based on high PP2 might need revision for branches of this diameter and larger.

Guided wave mode frequencies are also dependent on wave velocity. As our model consisted of a dry branch, the wave velocities were at the higher end of possible acoustic velocities expected for living wood. In retrospect, the model with 23% lower velocity more closely resembled a living branch. The activated S0- and A1-modes still fell within the frequency range of PP2. The frequencies of the guided wave modes decreased proportional to the decrease in wave velocity, meaning that a decrease in wave velocity of more than 50% would shift the S0-mode below 100 kHz. As a maximum change in longitudinal wave velocity of 20% was observed by Sakai *et al.* (1990) from dry to full hydration, we expect that changes in properties of the branch during dehydration, and during daily cycles of shrinkage and swelling, will be negligible and will not affect the high PP2 of cavitation-related AE signals. Also variations in acoustic wave velocities between different woody species are expected to be small as Sakai *et al.* (1990) found very similar wave velocities at saturation for 9 different gymnosperm and angiosperm species. The identification of cavitation-related AE signals based on a high 100-200 kHz frequency content (PP2) will thus likely be valid for a large variety of woody branches, but might need revision for larger diameter branches.

5.4.2 Signal types detected with multi-resonant sensor

The multi-resonant broadband WD sensor used in this study was fundamentally different from the broadband point-contact sensor used in the previous chapter on grapevine. On one hand, the point-contact sensor has a flat response from 12 kHz to 960 kHz (McLaskey *et al.*, 2007), whereas the WD sensor has a non-flat response with multi-resonance behaviour. On the other hand, the WD sensor has a smaller sensitivity range and according to the sensor specifications delivered by the manufacturer the sensitivity decreases steeply below 100 kHz and above 800 kHz. We found that the frequency range of the WD signals could be approximated by applying a bandpass filter of 90-550 kHz on the signals detected with the broadband sensor on grapevine (Fig. 5.6). The bandpass filter for the broadband signals was obtained through trial and error and is mainly used to illustrate the possible effects of reduced bandwidth. However, this may suggest that the WD sensor has a strongly reduced sensitivity above 550 kHz when coupled on wood, which should be further investigated.

Based on the FEM sensitivity study, we expected again a high PP2 for cavitation signals in the ash and poplar branches with diameter 9-12.5 mm. The signals of cluster 1, of which the activity pattern was in accordance with the expected cavitation pattern, indeed had the highest PP2. However, looking at the scatterplots (Fig. 5.6), cluster 1 would at first sight correspond to the low-freq cluster observed in grapevine. Probably, the low- and mid-frequency cluster were merged due to the reduced bandwidth of the WD sensor (Fig. 5.6c). The low-frequency AE sources in white poplar and common ash were thus much less abundant than in grapevine. If macro-fractures resulting from separation of the outer bark caused the low-frequency activity in grapevine, this might explain the absence of this AE source in poplar and ash branches. However, at more negative water potentials, beyond the endpoint of cavitation, there was again some activity of cluster 1 in the poplar branches (Fig. 5.7a), which might be related to the formation of macro-fractures emitting strong low-frequency signals.

The signals of cluster 3 corresponded to the shrinkage-related high-frequency cluster in grapevine, that was shifted towards lower WPF due to the reduced bandwidth (Fig. 5.6). Indeed, the activity pattern of cluster 3 corresponded to the shrinkage rate (Fig. 5.7), but also cluster 2 followed this pattern. When mimicking the WD response by filtering the broadband signals in Fig. 5.6c, there is no concentration of data points at the location of cluster 2 (at 200-400 kHz WPF, with high PP3). We assume that a strong sensor resonance frequency was present in this frequency range. This resulted in an aspiration of data points towards these frequencies. Probably, cluster 2 consists of data points from both the mid-freq and high-freq type, but the predominance of high-frequency signals may explain the correspondence between the activity of cluster 2 and 3. Compared to grapevine, there were much more shrinkage-related signals in ash and poplar. Because ash and especially poplar have less living tissue in the xylem (Wagenführ, 2000; Rosner, 2012a), this is somehow contrary to what we expected. However, the shrinkage-related AE activity probably originates mainly from tissues with stiff secondary cell walls that experience changing tensions due to shrinkage, rather than from the living tissue itself. As the woody tissue of climbers such as grapevine does not have to deliver the same mechanical support as in trees, the strength properties are expected to be rather different. This might explain the different fracture behaviour between the grapevine branches, and the poplar and ash branches.

5.4.3 Vulnerability curves

When defining an appropriate endpoint, the total AE activity detected with (multi-resonant) broadband as well as 150 kHz resonant sensors could be used to construct vulnerability curves in grapevine (Chapter 2 and 4), common ash and white poplar (Fig. 5.8). For grapevine, this was due to the simultaneous activity of the co-dominant low-frequency cluster with the cavitation-related mid-frequency cluster during the cavitation phase (Chapter 4). For common ash and white poplar, the high-frequency signals co-occurred with the cavitation-related signals (Fig. 5.7). The simultaneous occurrence of different drying-induced AE sources is not surprising as the processes are strongly interlinked, but the coordination between different processes seems to be species-dependent. Moreover, experiments on living plants should be conducted to investigate the interplay between elastic shrinkage and cavitation during daily cycles and during drought events. In this way, the role of capacitive water release in the prevention and/or repair of cavitation could be investigated (Meinzer & McCulloh, 2013). Note that a flat-with-frequency broadband sensor is most appropriate for this purpose as we have seen that the clustering results and comparison with model simulations are complicated by the multi-resonant sensor with reduced bandwidth (Fig. 5.6).

The average Ψ_{50} of -1.1 MPa obtained with cluster 1 for white poplar branches (Table 5.1) can be regarded as a realistic value, as poplars are known to be quite vulnerable to drought (Awad *et al.*, 2010; Choat *et al.*, 2012; Fichot *et al.*, 2015). These values were somewhat higher than the reported -1.5 MPa obtained on shoots of this species with hydraulic conductivity measurements after air injection (Hukin *et al.*, 2005) or bench dehydration (Nolf *et al.*, 2015). The deviating pattern in dehydration rate of the first poplar branch (Fig. 5.7b), with a period of decreased shrinkage rate, may explain the deviation in the relationship between water potential and diameter strain (Fig. 5.8a). Pressure bomb measurements are taken over the whole branch length, while diameter shrinkage and AE signals are measured locally, at a distance of 4 cm. Therefore, we believe that water potential values based on strain measurements give a better indication of the local water potential near the AE sensor.

Common ash is less vulnerable to drought (Stöhr & Lösch, 2004; Scherrer *et al.*, 2011) and the Ψ_{50} obtained for this branch was -1.96 MPa (Table 5.1).

Cochard *et al.* (1997) reported a Ψ_{50} of -2.5 MPa measured with the pressure chamber technique on an upper crown branch, but common ash is known to adapt to a broad range of wet to dry environments (Marigo *et al.*, 2000) and branches of the upper crown might be less vulnerable to cavitation than young shoots.

5.5 Conclusions

In this chapter it has been demonstrated that the cavitation-related signal type as found for grapevine in Chapter 4 could also be identified in white poplar and common ash. This signal type, with typical high partial power in the frequency range 100-200 kHz, could be simulated by a dipole AE source with 5 μ s excitation time. Based on a sensitivity study with FEM, we concluded that the identification method might apply on small branches of a large range of woody species. Although the cavitation signal-type could also be identified with a multi-resonant broadband sensor, we recommend using a sensor with flat frequency response when signal classification and comparison with FEM is aimed for. For the construction of AE-based vulnerability curves, the differentiation between different clusters might not be necessary when an appropriate endpoint is defined at the end of the AE activity peak. Even a 150 kHz resonance sensor with narrow frequency range resulted in valid vulnerability curves. However, this should be confirmed by an extensive measurement campaign covering a wide range of woody species.

6

General discussion and conclusions

What happens inside plants when they suffer from drought? In view of the threat of increasing droughts in many regions on earth, this question is ubiquitous. Despite the great importance of forests and other ecosystems, little is known about their resilience to climate change. Appropriate measurement methods are the base of effective research that enables to make great strides in the comprehension of plant responses to drought. Especially the cavitation phenomenon in water conducting tissues is poorly understood due to the lack of appropriate measurement techniques. In this doctoral research, we investigated how the acoustic emission (AE) technique can be successfully applied for the detection of cavitation in woody plants. We developed an alternative method to quantify the vulnerability to cavitation and the role of xylem conduits as extra internal water supply. Moreover, important steps were made towards understanding the different sources of AE signals during drought. Continuing

developments in the AE detection technique are promising for application of this non-destructive method in plant sciences.

6.1 Acoustic emission: from past to present

Today, the AE technique is widely applied for material testing and structural health monitoring on the engineering materials concrete (Robeyst *et al.*, 2009; Ohtsu, 2015), metal alloys (Jayakumar *et al.*, 2005) and fiber composite materials (Hamstad, 2000). However, the oldest reported scientifically planned AE experiment *avant la lettre* was performed on wood in 1933 by Fuyuhiko Kishinouye (Drouillard, 1990; Kishinouye, 1990; Ohtsu, 2008), long before the term acoustic emission was introduced by Schofield in 1961 (Ohtsu, 2008). In 1966, Milburn & Johnson used a similar measurement set-up as Kishinouye to detect for the first time AE signals in plants (Fig. 1.10). It was not possible to record the full waveforms of the signals at that time, because electronic components were not fast enough to record data at such a high frequency (MHz). Therefore, the AE technique was limited to parameter-based analysis. This is contrary to the history of seismology, where full waveform detection and analysis has been performed since the end of the 19th century (Agnew, 2002). During the next couple of decades, rapid developments in microelectronics have resulted in great advances in the AE technique. Currently, high performance acquisition systems are available that are able to record and store waveforms from multiple channels at high sample frequencies. Unfortunately, the full potential of these state-of-the-art measurement systems had not been used in plant science so far.

6.2 What causes AE signals in drought-stressed branches?

The interpretation of AE signals in terms of the underlying sources is not straightforward as the signal is being shaped not only by the AE source, but also by the propagation medium and the detector. The influence of the detection step can be minimized by using broadband sensors with very flat frequency response. Signal propagation can be accounted for by finite element modelling

of the propagation medium, which is the woody branch in this study. Using the combination of FEM and broadband waveform acquisition (Chapter 3), we could investigate the relationship between AE source characteristics and the measured AE signals (Chapter 5). As this approach was introduced and had already paid off in the field of material science (Sause *et al.*, 2012c), it is sensible to pursue cross-fertilization with this field. Recently, deeper insights have been obtained into AE sources of tensile cracks in epoxy resin by combining AE sensors with measurements of electromagnetic emissions (EME) (Gade *et al.*, 2014). EME signals could be measured with capacitive sensors with a very flat frequency response in the range 1-1000 kHz. A large advantage of this technique is that the signals are not changed by damping, dispersion and reflections at the branch surface through signal propagation from source to sensor. Moreover, capacitive sensors can easily be fabricated in the lab and they can be connected to the same AE signal acquisition system (Gade *et al.*, 2014). The potential of EME detection combined with AE measurements for further unravelling of the cavitation phenomenon in plants is worth exploring.

Based on the frequency spectrum of the drought-induced AE signals, we could distinguish three types of AE sources. The signals that typified the cavitation phase were characterized by a high intensity at 100-200 kHz. Another signal type, with high intensity in the range 400-800 kHz, was strongly related with branch shrinkage and probably originated from micro-fractures. We also observed a low-frequency signal type (high intensity below 100 kHz) that was attributed to macro-fractures or free drainage of water through the porous wood medium. The identification of cavitation-related AE signals is a great advance and may offer a tool to investigate the occurrence of cavitation in plants in their native environment. Moreover, other sources of AE such as micro- and macro-fractures may not longer disturb cavitation measurements, but may rather be used as additional information on other drought-induced processes. Shrinkage-induced AE signals could for example provide information on the use of elastic water reserves, or on mechanical stresses in the plant.

It is often assumed that one AE signal represents cavitation of a single water column (Haack & Blank, 1990). However, except from the observed one-to-one relationship by Tyree *et al.* (1984b) and Lewis (1987) on small sapwood samples of two gymnosperm species, the number of AE signals was either lower (Sandford & Grace, 1985; Ritman & Milburn, 1991; Kikuta *et al.*, 2004; Ponomarenko

et al., 2014) or higher (Kikuta *et al.*, 1997; Rosner *et al.*, 2006) than the number of cavitating conduits in further experiments. In our measurements on grapevine, the number of cavitation-related AE signals exceeded the number of vessels in the branch by one or two size orders (Chapter 2). Moreover, the amplitude distribution with maximum signal density near the detection threshold of 28 dB_{AE} (Fig. 4.7) suggests that a considerable part of the signals could not be detected because their amplitude fell below the detection threshold, and that even more AE signals are produced during xylem cavitation. Possibly, the use of less sensitive measurement systems, or higher detection threshold settings, might explain cases where less AE signals than cavitating conduits were observed.

One reason for this high number of signals could be the overlap between adjacent cluster types (Chapter 4), so that part of the cavitation-related signal type possibly originated from other co-occurring AE sources, such as micro-fractures or water drainage. A second hypothesis would be that the cavitation process generates many AE signals. According to the nanobubble theory of Schenk *et al.* (2015), many nanobubbles may be formed and exist in the xylem before they coalesce and form an embolism. Coalescence of these nanobubbles and subsequent bubble collapse may result in much more AE signals than cavitating conduits. Alternatively, fissures in the stretched pith membrane or rearrangements of the cell wall layers due to pressure redistribution after cavitation may cause many AE signals per cavitating conduit. Further research and more detailed modeling of the actual micro-mechanical processes at the AE source such as bubble formation and coalescence may hopefully throw light on the cavitation-related AE sources and, accordingly, on the processes behind emboli formation.

6.3 In vivo measurements

The commonly applied hydraulic method (Chapter 1) to measure cavitation in plants may suffer from the observer effect (Jansen *et al.*, 2015) because the degree of cavitation may change during sampling and measuring. Cutting branches under tension may enable air entrance in the cut vessels, even when cutting under water (Wheeler *et al.*, 2013; Torres-Ruiz *et al.*, 2015). To prevent artificial emboli, a sample should be taken from a larger branch that was relaxed under water (Torres-Ruiz *et al.*, 2015) and precautions are necessary to prevent

embolism repair during relaxation (Trifilò *et al.*, 2014; Torres-Ruiz *et al.*, 2015). The measurement of hydraulic conductivity has to be repeated after all emboli are removed by flushing (Sperry *et al.*, 1988a) or refilling under vacuum (Espino & Schenk, 2011) to obtain percentage loss of hydraulic conductivity. The whole protocol of sample preparation and hydraulic conductivity measurements is not only tricky because of many possible artifacts due to sample manipulation and measurements under positive pressure with a solution that is foreign to the xylem tissue (Chapter 1). The method is also very labour intensive. Non-destructive and very reliable observations of cavitated vessels are possible with μ CT, but this is limited to small potted plants. Depending on the μ CT device and the required level of detail (McElrone *et al.*, 2013), a single scan may take 5 min to more than half an hour (Brodersen *et al.*, 2013; Cochard *et al.*, 2015; Torres-Ruiz *et al.*, 2015; Vergeynst *et al.*, 2015). Although μ CT is proposed as a reference method for the validation of other techniques (Cochard *et al.*, 2015), also this method is too cumbersome for routine measurements of cavitation. This is where the AE technique comes into play. Enabling non-destructive, automated and continuous measurements of cavitation, the AE technique is very promising in this context.

When using the AE technique on living plants in lab or field conditions, we recommend to use a broadband sensor (20-1000 kHz) with flat frequency response in order to differentiate between different AE sources (Chapter 4). The AE measurement system used in this thesis requires a PC in the proximity of the sensors to record and store the waveforms, which may involve a practical issue in field conditions. However, wireless monitoring systems have been developed (Grosse *et al.*, 2007, 2010; Girard *et al.*, 2012; Dong *et al.*, 2015), consisting of robust portable AE signal acquisition units that remotely collect AE data and send real-time measurements to the computer. Limited data transfer and storage of current systems will require parameterization of the signals, instead of full waveform storage. Based on preliminary analysis and classification, a minimum set of signal frequency features may be selected (weighted peak frequency, frequency centroid and partial powers Chapter 4 and 5) that enable to discriminate between different AE sources. A second point of attention for *in vivo* measurements is the particular nature of cavitation detection. Contrary to the hydraulic method and visualization methods, the AE technique measures changes in the degree of embolism rather than the degree of embolism itself. This principle should be kept in mind when preparing and interpreting AE

measurements of cavitation. If the initial state of xylem embolism is known, cumulative AE measurements might result in the degree of embolism if cavitation has not been repaired in the measurement period. This will require a reference number of AE signals at 100% embolism, which may be obtained in a vulnerability curve or at the end of a drought experiment. We believe that combining broadband AE measurements with other non-destructive measurements of e.g. sap flow, water potential, water content and xylem/phloem shrinkage (Steppe *et al.*, 2015) may yield the largest insight into the water dynamics of the plant.

6.4 A new standard method to measure vulnerability to cavitation?

Vulnerability curves and especially the resulting water potential values that characterize vulnerability to cavitation are of high interest in plant science (Lens *et al.*, 2013; Jansen *et al.*, 2015). Strongly related with this trait in the hydraulic functioning of the plant is the hydraulic capacitance of plant tissues (Meinzer *et al.*, 2009; Meinzer & McCulloh, 2013), which mediates transpiration-induced changes in the xylem tension. On one hand, these data may feed mechanistic plant models that provide deeper insights into plant functioning (Tyree & Ewers, 1991; Hölttä *et al.*, 2009; Manzoni *et al.*, 2013b; Matheny *et al.*, 2014; Manzoni *et al.*, 2014; Baert *et al.*, 2015) and, by extension, also vegetation, hydrological and climate models (Sus *et al.*, 2014; Sheil, 2014; Anderegg *et al.*, 2015; Bernacchi & VanLoocke, 2015). On the other hand, it may provide highly valuable information for decision support in forestry and crop production. Water use may be optimized when including vulnerability to cavitation and hydraulic capacitance in irrigation strategies (Jones, 2007; Torres-Ruiz *et al.*, 2013; Sperling *et al.*, 2015). Forest managers and plant breeders may select the most appropriate species or genotypes to anticipate climate changes (Saxe *et al.*, 2001; Cochard *et al.*, 2007; Allen *et al.*, 2010; Wortemann *et al.*, 2011; Eilmann *et al.*, 2013; Comas *et al.*, 2013; Tramontini *et al.*, 2013; Fichot *et al.*, 2015) or restore the ecohydrological function of natural forests in de- or reforested landscapes (Bleby *et al.*, 2012). Eventually, actual hydraulic properties of the plants may be used as a guide for thinning stands to reduce competition for water (Allen *et al.*, 2010; Martinez-Vilalta *et al.*, 2012). Traditional methods for the establishment of VCs (Cochard *et al.*, 2007, 2013) and moisture release curves

(Meinzer *et al.*, 2003) are time consuming and unsuitable to obtain large data-sets in a short amount of time. Moreover, the reliability of VC protocols based on hydraulic measurements of cavitation have been questioned (Wheeler *et al.*, 2013; Sperry, 2013; Torres-Ruiz *et al.*, 2015). The new method presented in this thesis, based on automated sensor measurements, may fulfill these needs in the future.

A convenient method for widespread application in research on drought responses in plants is preferably easy, fast, cheap and automatic. The use of automated measurement techniques (AE, strain, weight) strongly reduces the number of manual manipulations (Chapter 2). When using the diameter strain as a proxy for xylem tension, minimum two water potential measurements (sufficiently spread in time) are needed to establish the apparent radial modulus of elasticity. A full automatic system would be possible using stem psychrometers, but this increases the costs and installation time. The cost of the AE equipment used in this study was rather high, but recent technological advances in the fabrication of MEMS (microelectromechanical systems) provide a promising avenue (Glaser & Pescovitz, 2002; Ozevin *et al.*, 2006; Hutchins *et al.*, 2011; Harris *et al.*, 2011; Saboonchi & Ozevin, 2013) as MEMS-type AE sensors can be inexpensive in large scale production (Ozevin *et al.*, 2006; Hutchins *et al.*, 2011). These MEMS-type sensors are typically resonant sensors sensitive in a narrow frequency range (Ozevin *et al.*, 2006). We observed that 150 kHz resonant sensors were suitable to construct vulnerability curves in *Populus alba*, *Fraxinus excelsior* and *Vitis vinifera*. This suggests that resonant AE sensors might be satisfactory for this application when the endpoint is determined at the end of the AE activity peak as described in Chapter 4. The time efficiency of the method is largely dependent on the dehydration time. In our experiments on cut branches, the dehydration time varied from a couple of hours (Chapter 5) to more than a day (Chapter 2 and 4), but dehydration could eventually be accelerated in a climate chamber. With the available knowledge and techniques it is possible to fabricate a system that automatically combines the sensor data into vulnerability curves and moisture release curves, which may greatly improve the ease-of-use of this method. However, also for this application parallel fundamental research with broadband AE sensors is favorable as the distinction between different drying-induced processes may contribute to a more comprehensive understanding of drought stress.

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Summary

Cavitation in woody plants

Drought stress is a major cause of plant death. In a changing climate with increasing droughts in many places on earth, the hydraulic performance of plants during water shortage is an important trait for plant survival. As the health state of forests has a strong impact on our climate and on human life, there is a large interest to understand drought responses of woody species. The formation of air emboli in xylem conduits, called cavitation, is considered one of the key processes leading to plant mortality. The air-filled conduits reduce upward movement of sap in the xylem and hamper many vital functions of water in the plant. However, the process of emboli formation is not fully understood and measurement methods to detect air emboli have come into question. Moreover, cavitation is accompanied by the release of internally stored water, which may be used as an extra water source during drought. Little is known about this trade-off and quantification of the contribution of cavitation to total water use is sparse.

The sound of cavitation

In this thesis, the focus was on the development and application of the acoustic emission (AE) method for drought-induced cavitation detection in woody species. Although the first application of this method dates back to the 60s, its full potential had not been used in plant sciences so far. The AE signals contain information on the AE sources, but effects of signal propagation and

Summary

signal detection need to be accounted for to decode the measured signals. In this research, three main steps were taken to unravel the AE sources in drought-stressed woody branches.

- ▷ First, a new type of AE sensor was introduced, the point-contact broadband sensor, which detects surface displacements in a wide frequency range. Whereas commonly used AE sensors influence the frequency content of the signals, this broadband sensor measures the actual AE waves.
- ▷ Second, the AE signals were fully used and analysed in both the time and frequency domain. Usually, the AE signals are only counted, or a limited set of waveform features is extracted. Natural clusters of signals were distinguished using an automated pattern recognition algorithm, which resulted in three types of AE signals in dehydrating grapevine branches: the low-, mid- and high-frequency signal type.
- ▷ Third, finite element modelling (FEM) was used to simulate AE sources, wave propagation and AE signal detection. This allowed to make the link between the three AE signal types and the corresponding AE source properties. The excitation time of the AE sources, which is the duration of the microscopic volume change, was found to have the largest impact on the AE signal frequency content.

The low-, mid- and high-frequency signal types originated from distinct AE sources with decreasing excitation time. The low-frequency signal type, with high 0-100 kHz frequency content was hypothesized to originate from macro-cracks or drainage of water that was disconnected from the transpiration stream. Brittle micro-cracks due to shrinkage probably resulted in the high-frequency signal type with high intensity in the range 400-800 kHz. The cavitation-related mid-frequency signal type with high 100-200 kHz frequency content could result from bubble formation, coalescence and/or collapse in the xylem conduits.

Vulnerability to cavitation and hydraulic capacitance

This research also resulted in a new method to estimate vulnerability to cavitation and hydraulic capacitance. To this end, two curves were constructed.

- ▷ The vulnerability curve (VC) depicts percent loss of hydraulic conductivity versus decreasing xylem water potential. From this curve, the vulnerability to cavitation can be characterized by the water potential at which 50% of the conductivity is lost.
- ▷ The moisture release curve depicts volumetric water content versus xylem water potential. The slope of this curve represents the water buffering capacity of the branch and is called hydraulic capacitance. Combining this curve with the VC enables us to differentiate between the contribution of elastic shrinkage and cavitation to hydraulic capacitance.

To construct these curves we used continuous measurements of acoustic emissions, branch diameter strain and gravimetric water loss. Diameter strain was used as a proxy for xylem water potential, after a linear relationship was established. This resulted in continuous measurements of xylem water potential. Hydraulic conductivity loss was estimated from the cumulative number of AE signals. It was important to define an appropriate endpoint at the end of the AE activity peak because total AE activity often continued after the end of the cavitation phase. For the three investigated species (*Vitis vinifera*, *Populus alba* and *Fraxinus excelsior*), the resulting VC based on total AE activity did not strongly differ from the curve based on cavitation-related AE signals. If this would hold for woody species in general, a very robust and automated VC-generating system could be fabricated. At the same time, more fundamental research using broadband AE detection, combined with FEM, is encouraged to further investigate the different drying-induced processes.

Samenvatting

Cavitatie in houtachtige planten

Droogtestress is een belangrijke oorzaak van plantensterfte. In een veranderend klimaat met toenemende droogtes in veel regio's op aarde is de hydraulische performantie zeer belangrijk voor de overlevingskansen van planten. Gezien het grote belang van bossen op aarde is er een sterke wetenschappelijke belangstelling om de reactie van houtachtige planten op droogte beter te begrijpen. De vorming van luchtemboli in xyleemvaten, ook wel cavitatie genoemd, wordt beschouwd als één van de belangrijkste processen die leiden tot plantensterfte. De vaten die gevuld zijn met lucht verminderen het opwaarts transport van sap in het xyleem en hinderen op die manier vele vitale functies die water vervult in de plant. Het cavitatieproces is echter nog niet goed begrepen en de methoden om cavitatie te meten staan momenteel ter discussie. Cavitatie gaat bovendien gepaard met het vrijkomen van een hoeveelheid water uit het vat, dat kan gebruikt worden als extra watervoorraad tijdens droogte. Er is weinig geweten over deze trade-off, en metingen van de bijdrage van cavitatie in de waterbalans zijn schaars.

Het geluid van cavitatie

In deze thesis lag de focus op de ontwikkeling en toepassing van de akoestische-emissietechniek om droogte-geïnduceerde cavitatie te meten in houtachtige planten. Reeds in de jaren 60 werden akoestische metingen gedaan op planten, waarbij werd verondersteld dat men cavitatie kan horen. Vaak zijn er echter veel

meer geluiden dan gecaviteerde vaten, wat de toepasbaarheid van de akoestische-emissietechniek (AE techniek) in vraag stelt. De geluiden (AE signalen) bevatten informatie over de AE bronnen, maar om de gemeten signalen te kunnen interpreteren, moeten signaalvoortplanting en signaaldetectie in rekening worden gebracht. In dit onderzoek hebben we drie belangrijke stappen genomen om de geluiden in planten tijdens droogte beter te kunnen begrijpen.

- ▷ Vooreerst werd een nieuwe AE sensor gebruik, de punt-contact breedbandsensor, die bewegingen van een oppervlak kan detecteren in een breed frequentiebereik. Terwijl de standaard AE sensoren bij detectie de frequentie-inhoud van het signaal wijzigen, kan deze breedbandsensor de werkelijke AE golven detecteren.
- ▷ De informatie in de signalen werd vervolgens volledig benut en de signalen werden geanalyseerd in zowel het tijds- als frequentiedomein. Gewoonlijk worden de AE signalen enkel geteld, of wordt er gewerkt met een beperkt aantal golfvormkarakteristieken. Met behulp van een automatisch clustering algoritme werd gezocht naar verschillende types van signalen. In druiventakken konden we op die manier drie verschillende signaaltypes onderscheiden.
- ▷ Tenslotte gebruikten we de eindige-elementenmethode om AE bronnen, signaalvoortplanting en signaaldetectie te simuleren. Hierdoor konden we de link leggen tussen de drie signaaltypes en de eigenschappen van de bron waar deze signalen ontstaan. De excitatietijd van de AE bron bleek de grootste impact te hebben op de frequentie-inhoud van de resulterende AE signalen. Dit is de duur van de initiële microscopische volumeverandering bij het ontstaan van een AE signaal.

De drie verschillende signaaltypes, met lage, gemiddelde en hoge frequentie, konden dus verklaard worden door verschillende AE bronnen met toenemende excitatietijd. Het lage-frequentietype, met hoge amplitude bij 0-100 kHz is vermoedelijk afkomstig van macroscopische breuken of van het bewegen van water dat uit de gecaviteerde vaten draineert. Brosse microscopische breuken door krimpprocessen resulteren waarschijnlijk in de hoge-frequentiesignalen, met hoge amplitude bij 400-800 kHz. De cavitatie-gerelateerde mid-frequentie signalen, met hoge amplitude bij 100-200 kHz, gaan vermoedelijk gepaard met het ontstaan, samenvoegen of uiteenbarsten van luchtbellen in xylemvaten.

Vatbaarheid voor cavitatie en hydraulische capaciteit

Dit onderzoek resulteerde ook in een nieuwe methode om de vatbaarheid voor cavitatie en de hydraulische capaciteit te meten. Hiervoor werden twee curves opgesteld.

- ▷ De vatbaarheidscurve (VC) geeft het procentuele verlies aan hydraulische geleidbaarheid weer ten opzichte van een afnemende xyleemwaterpotentialiaal. Op basis van deze curve kan de vatbaarheid voor cavitatie worden gekarakteriseerd door de waterpotentialiaal waarbij de hydraulische geleidbaarheid met 50 % is verminderd.
- ▷ De uitdrogingscurve geeft het volumetrisch watergehalte weer in functie van xyleemwaterpotentialiaal. De helling van deze curve is een maat voor het waterbufferend vermogen van de tak, wat we de hydraulische capaciteit noemen.

Om deze curves op te stellen maakten we gebruik van continue metingen van AE signalen, radiale krimp en gravimetrisch waterverlies. De lineaire relatie tussen xyleemwaterpotentialiaal en relatieve radiale krimp werd gebruikt om continue metingen van xyleemwaterpotentialiaal te bekomen. Het verlies aan hydraulische geleidbaarheid werd geschat op basis van cumulatief aantal AE signalen. Aangezien er na de cavitatie fase, met 100 % verlies aan hydraulische geleidbaarheid, nog steeds AE signalen werden gemeten, werd een gepast eindpunt van de curve gedefinieerd. Voor de drie onderzochte soorten (*Vitis vinifera*, *Populus alba* en *Fraxinus excelsior*), kwam de VC bekomen met cavitatie-gerelateerde AE signalen goed overeen met de VC op basis van alle AE signalen samen. Indien deze bevinding zou gelden voor houtachtige planten in het algemeen, zou de AE techniek kunnen worden gebruikt in een zeer robuuste methode om vatbaarheidscurves op te stellen. Verder fundamenteel onderzoek met breedband AE sensoren, gecombineerd met de eindige-elementenmethode, wordt evenwel aangemoedigd om de verschillende droogte-geïnduceerde processen in planten verder te onderzoeken.

Curriculum Vitae

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Education

2011-2015	PhD in bioscience engineering: Investigation and application of the acoustic emission technique to measure drought-induced cavitation in woody plants. promoter: prof. dr. ir. Kathy Steppe Doctoral school of bioscience engineering, Faculty of Bioscience Engineering, Ghent University, Belgium
2006-2011	Bachelor and master in bioscience engineering, Land & Water Management, Faculty of Bioscience Engineering, Ghent University, Belgium Thesis: Changes in temperature and stem water content evoke erroneous sap flux density estimates with Thermal Dissipation Probes. promoter: prof. dr. ir. Kathy Steppe

Grants

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| Sep 2014 | Grant from the Commission for Scientific Research (Faculty of Bioscience Engineering, Ghent University) for the participation in the 31 st Conference of the European Working Group on Acoustic Emission, Dresden, Germany. |
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| Oct 2011 | PhD fellowship from the Research Foundation - Flanders (FWO - Vlaanderen) for the PhD research at Ghent University, Belgium |
| Jul 2010 | Grant from the Commission for Scientific Research (Faculty of Bioscience Engineering, Ghent University) for the research visit at the University of Georgia, USA. |

Research stays abroad

- | | |
|--------------|--|
| Jan-Apr 2014 | Research stay at the University of Augsburg, Germany, to be instructed in acoustic emission signal analysis and finite element modelling of acoustic emission signals with dr. Markus G. R. Sause. |
| Aug-Sep 2010 | Research stay at the University of Georgia, Athens, USA, to conduct a field experiment in the context of my master thesis with prof. dr. Robert O. Teskey. |

Teaching and coaching activities

Guidance of practical courses and technical lectures for the courses Ecophysiology, Plant-Water Relations, Ecology, Meteorology and Hydrology, and Meteorology and Climatology.

Tutor of the M.Sc. thesis of Jan Bogaerts (New type of dehydration curve gives insights into the internal water reserves of grapevine), Eline Meyvis (Ra-

diaal transport en opslag van water in de bast van *Quercus agrifolia* tijdens droogtestress) and Fran Lauriks (Bast als waterreservoir: dynamisch radiaal watertransport tussen bast en xyleem in *Quercus agrifolia*).

Coach of four groups of PhD-students in effective scientific communication: presentation skills (courses by Jean-Luc Doumont).

Publications

International publications with peer review

Vergeynst LL, Sause MGR, Steppe K Clustering reveals cavitation-related acoustic emission signals from dehydrating branches. *Tree Physiology* (under revision)

Vergeynst LL, Sause MGR, Hamstad MA, Steppe K (2015) Deciphering acoustic emission signals in drought stressed branches: the missing link between source and sensor. *Frontiers in Plant Science* 6:494

Vandeghechuchte MW, Bloemen J, Vergeynst LL, Steppe K Woody tissue photosynthesis in trees: salve on the wounds of drought? *New Phytologist* doi: 10.1111/nph.13599

Bloemen J, Vergeynst LL, Overlaet-Michiels L, Steppe K How important is woody tissue photosynthesis in poplar during drought stress? *Trees - Structure and function* doi: 10.1007/s00468-014-1132-9

Vergeynst LL, Dierick M, Bogaerts JAN, Cnudde V, Steppe K (2014) Cavitation: a blessing in disguise? New method to establish vulnerability curves and assess hydraulic capacitance of woody tissues. *Tree Physiology* 4:400-409

Vergeynst LL, Vandeghechuchte MW, McGuire MA, Teskey RO, Steppe K (2014) Changes in stem water content result in underestimated sap flux density with thermal dissipation probes. *Trees-Structure and Function* 28:949-955

Vergeynst LL, Bogaerts J, Baert A, Kips L, Steppe K (2013) New type of vulnerability curve gives insight in the hydraulic capacitance and conductivity of the xylem. *Acta Horticulturae* 991:341-347

Oral presentations

Vergeynst LL, Brunner AJ, Sause MGR (2015) FEM simulation of acoustic emission signals from tensile tests on miniature spruce wood specimens for comparative signal classification using pattern recognition. 20. Kolloquium Schallemission, Garmisch-Partenkirchen, Germany, 18-19 June 2015

Brunner AJ, Vergeynst LL, Sause MGR, Baensch F, Niemz P (2015) Pattern recognition for classification of acoustic emission signals from tensile tests on miniature specimens made from spruce wood: comparison of waveforms from finite element modelling and experiment. DACH-Jahrestagung, Salzburg, Austria, 11-13 May 2015

Vergeynst LL, Sause MGR, Ritschel F, Brunner AJ, Niemz P, Steppe K (2014) Finite element modelling used to support wood failure identification based on acoustic emission signals. COST - Timber Bridges Conference, Biel, Switzerland, 25-26 Sept 2014, pp 141-146

Vergeynst LL, Sause MGR, Steppe K (2014) Acoustic emission signal detection in drought-stressed trees: beyond counting hits. 31st Conference of the European Working Group on Acoustic Emission, Dresden, Germany, 3-5 Sept 2014, Fr.3.A.4

Steppe K, Vergeynst LL, Dierick M, Cnudde V (2013) Use of plant sensors to establish vulnerability curves and assess water storage capacity. Plant Biology, Providence, Rhode Island, US, 21 Jul 2013

Vergeynst LL, Bogaerts J, Baert A, Kips L, Steppe K (2013) New type of vulnerability curve gives insight in the hydraulic capacitance and conductivity of the xylem. 9th International Workshop on Sap Flow, Ghent, Belgium, 5 Jun 2013

Vergeynst LL, Steppe K (2013) Leer luisteren naar de ultrasone taal van bomen. Studiedag Starters in het natuur- en bosonderzoek, Brussels, Belgium, 15 Mar 2013

Poster presentations

Bloemen J, Overlaet-Michiels L, Vergeynst LL, Steppe K 2013 Understanding plant responses to drought: how important is woody tissue photosynthesis? 9th International Workshop on Sap Flow, Ghent, Belgium, 4-7 June 2013

Vergeynst LL, Vandegehuchte MW, McGuire MA, Teskey RO, Steppe K (2013) Changes in stem water content result in underestimated sap flux density with thermal dissipation probes. 18th National Symposium on Applied Biological Sciences, Ghent, Belgium, 6 Feb 2013

Vergeynst LL, Steppe K (2012) Can we grasp the ultrasonic language of living trees? 30th European Conference on Acoustic Emission Testing - 7th International Conference on Acoustic Emission, Granada, Spain, 12-15 Sept 2012

Vergeynst LL, Vandegehuchte MW, McGuire MA, Teskey RO, Steppe K (2011) Changes in temperature and stem water content evoke erroneous sap flux density estimates with Thermal Dissipation Probes. 8th International workshop on sap flow, Volterra, Italy, 8-12 May 2011