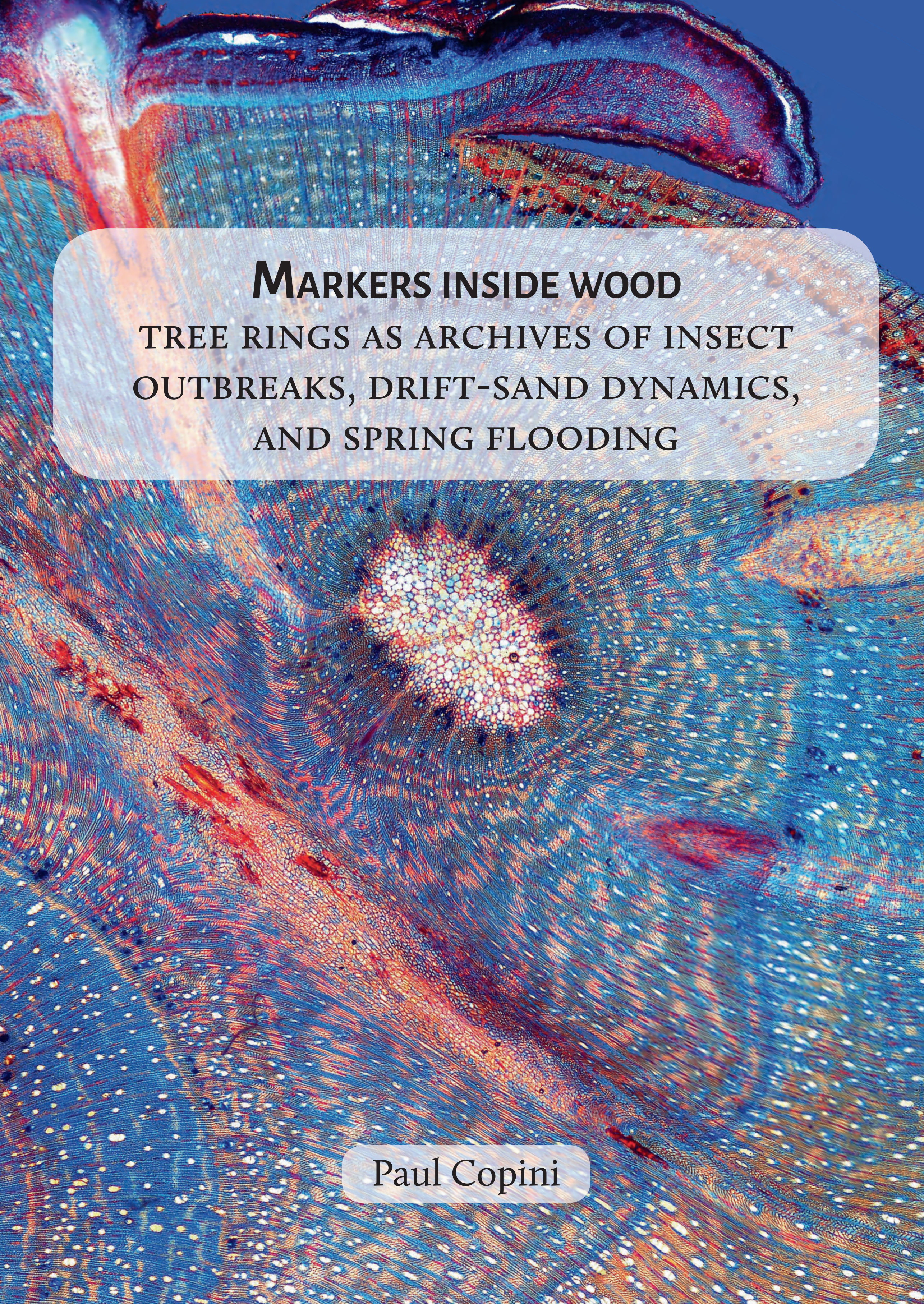

Markers inside wood

TREE RINGS AS ARCHIVES OF INSECT OUTBREAKS, DRIFT-SAND DYNAMICS, AND SPRING FLOODING

Paul Copini

Markers inside wood – tree rings as archives of insect outbreaks, drift-sand dynamics, and spring flooding

Paul Copini

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MARKERS INSIDE WOOD – TREE RINGS AS ARCHIVES OF INSECT OUTBREAKS, DRIFT-SAND DYNAMICS, AND SPRING FLOODING

Paul Copini

Thesis
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Paul Copini

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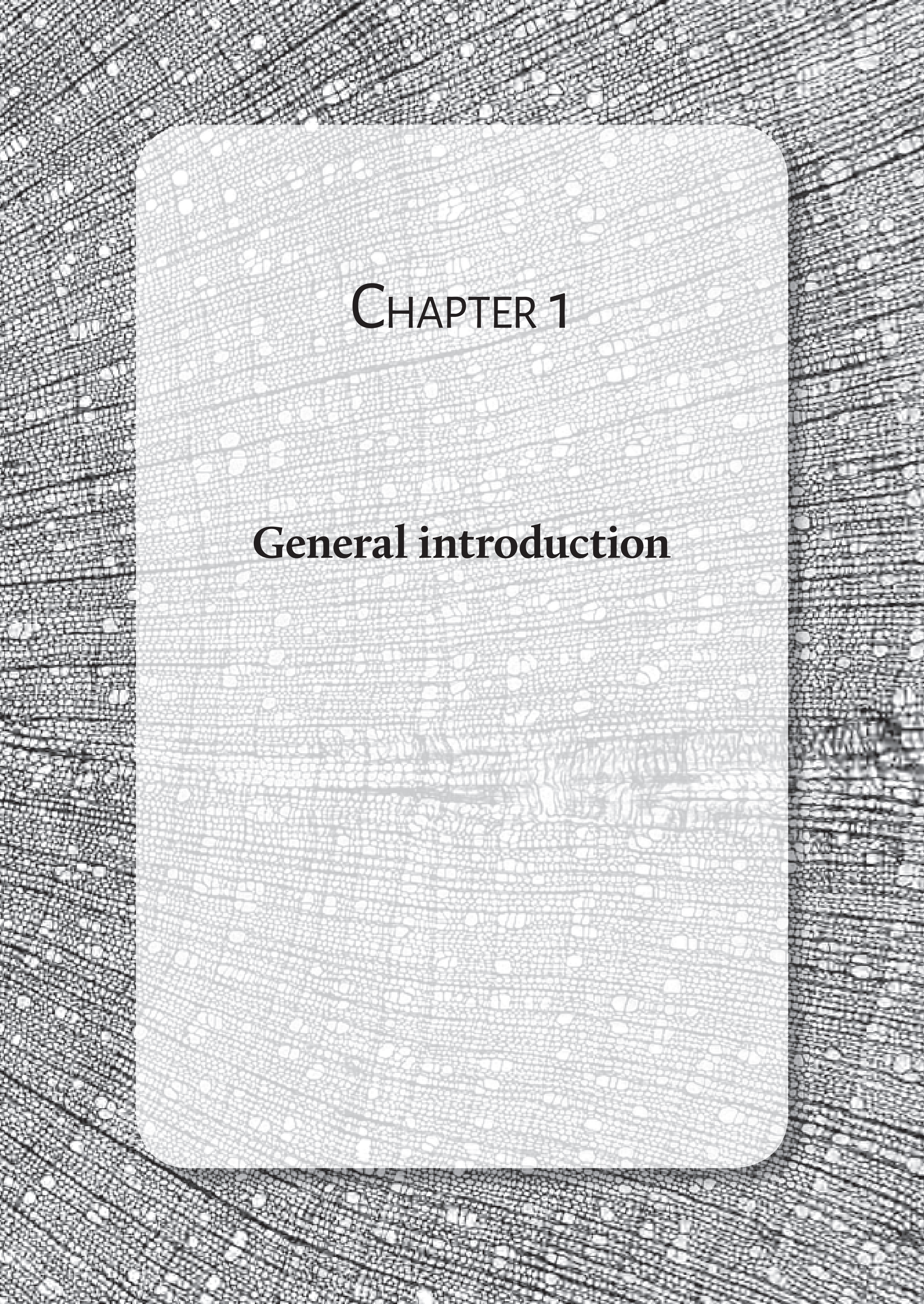

CHAPTER 1

General introduction

1.1 DATING CHANGES IN BIODIVERSITY, LANDSCAPES AND CLIMATE

During the Holocene, the period that started ca. 12000 years before present, huge changes have occurred in forest cover and climate, ***inter alia***, by the spread of agriculture and subsequent deforestation and increasing human population growth, and by a changing climate (Ruddiman, 2013). Especially during the past centuries, we have seen the effects of human-induced changes in biodiversity, landscape and climate (Thuiller et al., 2005; Ruddiman, 2013; Dirzo et al., 2014). Due to our global economy and lifestyle many species have been displaced around the globe including invasive ones (Mack et al., 2000; Hooper et al., 2005). Moreover cultural landscapes that have shaped our countries for centuries are now strongly diminishing or have disappeared (Rackham, 2003; Riksen et al., 2006). The number of extreme weather events such as extreme rainfall, heatwaves and storms seem to have increased and many meteorological records were broken recently, causing considerable damage to society (Coumou and Rahmstorf, 2012; Feyen et al., 2012). To understanding these changes in biodiversity, landscape and climate and the driving forces behind them, we frequently need to look beyond our instrumental records and written history. For this purpose scientists use different proxy records to reconstruct and understand changes and dating techniques such as radio carbon and luminescence to pinpoint them in time (Walker, 2005; Wallinga et al., 2007; Liritzis et al., 2013). While these techniques may be used over much longer timescales than the Holocene, their temporal resolution, especially of radio carbon dating, is relatively low compared to dendrochronology, the science that uses tree rings – dated to their exact year of formation – to analyse temporal and spatial patterns of processes in the physical and cultural sciences (Speer, 2010).

1.2 TREES AS NATURAL ARCHIVES

Trees are long-living organisms that record ecologically relevant information in their xylem which can be accessed by dendrochronology (Schweingruber, 1996; Speer, 2010). In many climates, from the arctic to the tropics, tree rings are formed each year (Schweingruber, 1996; Blok et al., 2011; De Ridder et al., 2013; Groenendijk et al., 2014). By matching patterns of narrow and wide rings across trees at a site (crossdating) each tree ring can be assigned to the specific calendar year when it was formed (Douglass, 1941; Speer, 2010). This means that millennia-long chronologies can be built with an unrivalled annual resolution. Consequently, the study of tree rings is widely applied in e.g. climatology, ecology and archaeology (Friedrich et al., 2004; Sass-Klaassen and Hanraets, 2006; Büntgen et al., 2011; Domínguez-Delmás et al., 2014). Moreover, by using dendrochronology in combination with quantitative wood anatomy an even higher temporal resolution can be achieved (Fonti et al., 2010; Arbellay et al., 2012): by correlating time series of cells sizes, densities or tissue proportions to environmental factors (e.g. precipitation, temperature), new insights have been revealed especially in seasonal climatic conditions (Garcia-Gonzalez and Eckstein, 2003; Fonti and Garcia-Gonzalez, 2004; Fonti et al., 2010; Eilmann et al., 2011).

1.3 Wood-anatomical markers

Wood-anatomical markers, also known as wood imprints or signatures, have shown a high potential for studying specific environmental events with an annual or even intra-annual temporal resolution (Yanosky, 1983; Swetnam, 1993; Ortloff et al., 1995; Stoffel et al., 2005; Ballesteros et al., 2010). These markers include e.g. frost rings, fire scars, insect scars, flood and burial rings (Fig. 1.1), and have long been known. Already in 1737 both Henri Louis Duhamel du Moncieau and George Louis Leclerc du Buffon noted the 1709 frost ring which has been used frequently in dendrochronological research ever after (Speer, 2010). Similarly Ratzeburg (1871) described disorganised cells in tree rings of *Abies alba* and in *Fagus sylvatica* formed in 1866, and linked their occurrence to frost. Wieler (1891) described wood-anatomical changes in stem cuttings of *Salix* and *Populus* after burial and mentioned that below the soil, the wood anatomy became root-wood like. Similar observations were made when two-year-old stems of *Quercus* and *Robinea* were placed in water (Wieler 1891). In 1909, Mills described tree rings that contained, *inter alia*, fire scars and bullet holes in a yellow pine tree and concluded that fires had occurred in 1840 and 1859. Clements (1910) studied lodgepole pine (*Pinus contorta* Douglas) and dated forest fires using fire scars in tree rings. More recently these markers have been used to study fire frequencies in relation to El Niño and La Niña years (Swetnam, 1993) or frost events after volcano eruptions (LaMarche and Hirschboeck, 1984).

Based on their origin, wood-anatomical markers can be divided in two groups: (i) those originating by wounds or (ii) those originating from abrupt changes in the stem or root environment, such as sudden changes in soil pressure, aeration, moisture, light and temperature. The first group includes, *inter alia*, mechanical wounds caused by forestry operations (Vasiliauskas, 2001), fire scars (Swetnam, 1993; Ortloff et al., 1995), frost rings (Glerum and Farrar, 1966; LaMarche and Hirschboeck, 1984; Gurskaya and Shiyatov, 2002), ice scars (Tardif et al., 2010), and wounds caused by rockfall (Stoffel et al., 2005; Gärtner and Heinrich, 2009). While the second group comprises markers such as flood rings (Yanosky, 1983; St. George, 2010) and markers related to burial and exposure (Fayle, 1968; St. George and Nielsen, 2000; Gärtner et al., 2001; Friedman et al., 2005). Markers related to wounding are normally associated with the formation of callus tissue (traumatic parenchyma cells) and wound xylem, formed to compartmentalise wounds reaching into the cambial zone (Pearce, 1996; Fink, 1999; Smith and Sutherland, 1999) (Fig. 1.1A and 1.2A). Wood-anatomical markers formed in response to drastic changes in the stem or root environment are characterised by sudden changes in cell dimensions or structure. After exposure of coniferous roots, tracheid lumen area is reduced by 50% (Fig. 1.1E), whereas the opposite occurs after burial (Marin and Filion, 1992) (Cournoyer and Bégin, 1992; Gärtner et al., 2001). In diffuse-porous trees, significant increases in vessel area are found in response to burial. Conversely, in ring-porous species, burial leads to a significant reduction of earlywood-vessel area (Fayle, 1968; Cournoyer and Bégin, 1992; Friedman et al., 2005). In addition, ring-porous species show the tendency to appear as diffuse-porous, or root-like, after burial, and shift back to a ring-porous structure upon exposure (Fayle, 1968; Hitz et al., 2008a). Comparably, flooding leads to significantly reduced earlywood vessels in ring-porous trees (Yanosky, 1983; St. George and Nielsen, 2000; St. George, 2010) (Fig. 1.1F).

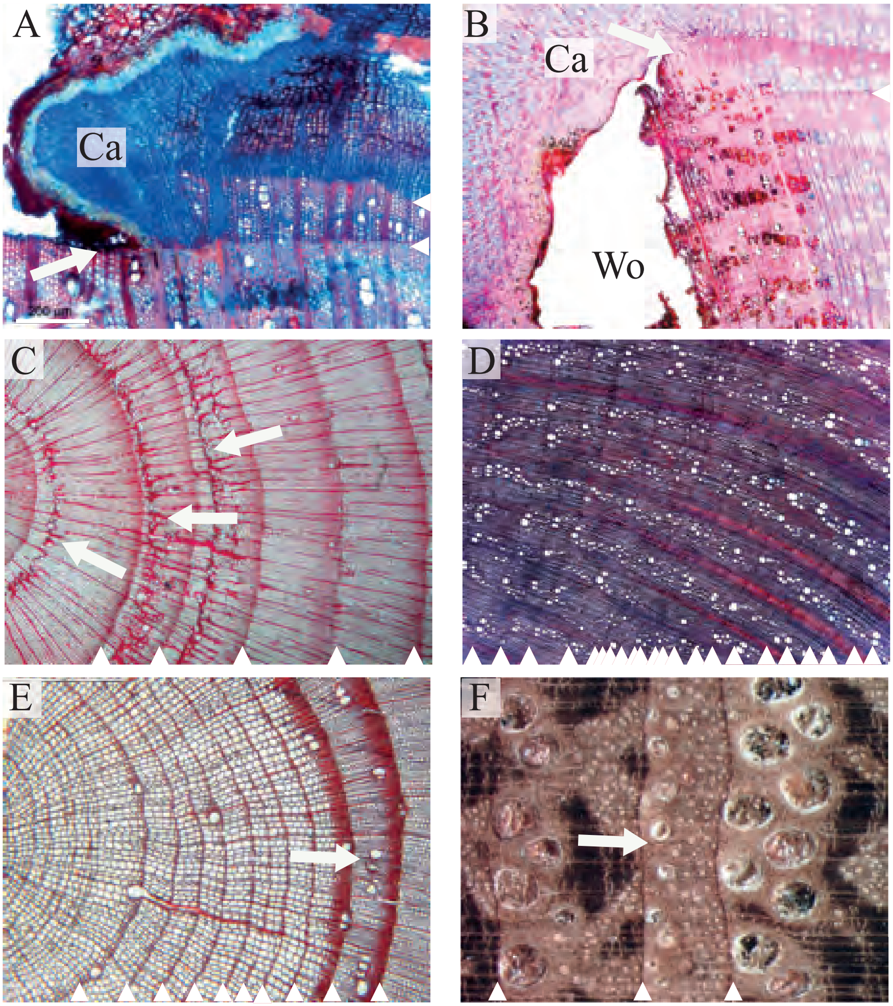

Figure 1.1 Transverse thin sections showing wood-anatomical markers. Growth occurs from bottom to top (A and B) or from left to right (C-F). Tree rings are indicated with white triangles. (A) mechanical wound in Japanese maple (*Acer palmatum*). The arrow indicates the position where the cambium was wounded and callus (ca) tissue started to overgrow the wound. (B) Wound (wo) caused by an invasive *Anoplophora* beetle in a maple tree (*Acer* sp). The arrow indicates the position of the cambium when the beetle formed this exit hole. (C) Three consecutive frost rings in Siberian larch (*Larix sibirica* Ledeb.). The arrows indicate collapsed tracheids and callus formation that occurred after frost during the growing season. (D): Root-like anatomy of a buried stem of pedunculate oak (*Quercus robur* L.) that was covered by two meters of sand. The ring-porous structure disappeared and growth is hampered. (E) Exposed root of European larch (*Larix decidua* Mill.) (Photo: H. Gärtner). The arrow indicates the tree ring that had formed after exposure; the tracheids are strongly reduced in size while growth has increased. (F) Flood ring in the 1826 tree ring in bur oak (*Quercus macrocarpa* Michx), characterised by anomalously narrow earlywood vessels (Photo: S. St. George).

1.4 Accuracy of wood-anatomical markers

The temporal accuracy of wood-anatomical markers depends on the period during which the cambium cells or the surrounding developing cells are receptive to environmental signals. In temperate climates this coincides with the growing season in which the cambium is active and division, differentiation, expansion, and maturation of xylem cells occur (Fink, 1999). This period normally starts in spring during the period of leaf unfolding: in ring-porous species leaf formation may start after or synchronously to the onset of tree-ring formation, whereas diffuse-porous species tend to start leaf unfolding before tree-ring formation in the main stem e.g. (Ladefoged, 1952; Aloni, 1991; Suzuki et al., 1996; Schmitt et al., 2000; Frankenstein et al., 2005a; Sass-Klaassen et al., 2011). The onset of tree-ring formation in temperate regions frequently starts in the second half of April or in the beginning of May and ends in August or in the beginning of September (Tucker and Evert, 1969, Lodewick, 1928, Ladefoged, 1952; Dujesiefken et al., 1991; Frankenstein et al., 2005; Hartig, 1860; Schmitt et al., 2000; Van der Werf et al., 2007). Maximum tree-ring growth occurs in May, June, and July but strong deviations have been found between years (Ladefoged, 1952). While the onset of tree-ring formation is more or less constant, tree-ring growth cessation might end in July for poorly growing trees while it can continue until September in healthy, good growing trees (Tucker and Evert, 1969; Marion et al., 2007). However, little is known about tree-ring growth cessation in relation to leaf phenology. In one of the rare studies, Michelot et al. (2012) found that *Fagus sylvatica* stopped tree-ring growth in August. To use wood-anatomical markers for intra-annual dating, it is crucial to obtain information on the formation and development of these markers and understand how and when they are stored within a tree ring.

Markers that are initiated in response to wounding are associated with cambial dieback, the formation of callus (traumatic parenchyma cells), and wound xylem to protect the tree's vital functions connected to transport and storage of water and assimilates (Shigo, 1984; Pearce, 1996; Yamada, 2001; Deflorio et al., 2009). Wound-related markers are normally recorded in the tree-ring structure within two weeks, if wounding occurs during the growing season (Glerum and Farrar, 1966; Grünwald et al., 2002; Frankenstein et al., 2006). Consequently, wound markers formed during the growing season are located within the tree ring (Fig. 1.2), whereas wounds occurring during winter dormancy are located at the tree-ring boundary (Schweingruber, 1996; Stoffel et al., 2005). Based on this method, Ortloff et al. (1995) and Kaczka et al. (2010) claim that fire scars in the gymnosperm *Pinus ponderosa* or wounds caused by debris flow events in *Abies balsamea*, can be dated with a monthly or biweekly accuracy respectively, although no study has yet been conducted to confirm these claims.

The formation of wood-anatomical markers occurring as a consequence of drastic changes in stem or root environment such as stem burial, root exposure or flooding are less understood and reports are often inconsistent or even contradictory. Wood-anatomical changes in response to burial have been observed within the year of burial in trees along rivers, (Sigafoos, 1964; Cournoyer and Bégin, 1992; Friedman et al., 2005). In dune areas, especially in conifer trees, wood-anatomical changes have been observed in the year of burial or exposure (Marin and Filion, 1992; Gärtner et al., 2001; Gärtner, 2007). However, other studies indicate that wood-anatomical changes may only occur in particular plant parts or can be substantially delayed (Knowlson, 1939; Bannan, 1941; Strunk, 1995; Heinrich and Gartner, 2008; Gärtner and Heinrich, 2009). The formation of flood rings seems to occur during the growing season when flooding occurs and depending on the timing of flooding within the growing season, wood-anatomical changes have been observed in the earlywood or in the latewood (Yanosky, 1983; St. George and Nielsen, 2003). A flood marker is probably formed in a direct reaction to flooding as it is only present in submerged basal stem parts (St. George and Nielsen, 2003).

1.5 Aim and objectives

Although wood-anatomical markers are amongst the most accurate dating tools, their application is hindered by a lack of fundamental studies that test their temporal accuracy by linking their occurrence to tree-ring growth (Fonti et al., 2010; Stoffel et al., 2010). In this thesis, we aimed to investigate the accuracy of wood-anatomical markers induced by wounding as well as by drastic changes in the environment of roots and stems. We expected that wood-anatomical markers both related to wounding and to environmental changes can be used with an intra-annual resolution.

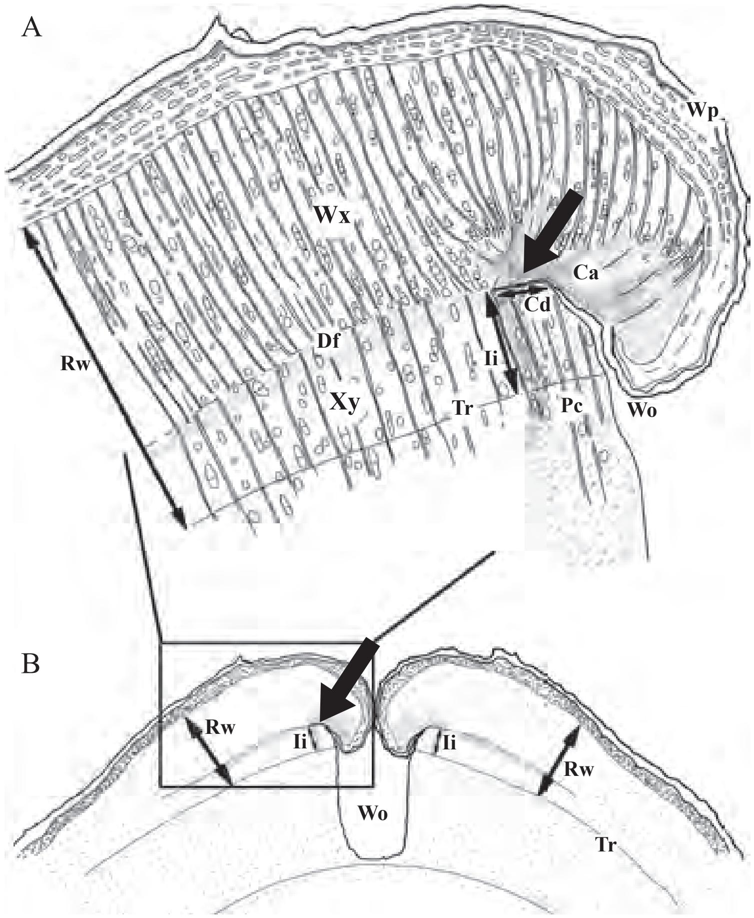

Figure 1.2 Schematic overview of dating wounds in tree rings. The arrow indicates the position of the wood-anatomical marker. (A) After a wound (Wo) occurs, the cambium dies locally (Cambial dieback, Cd), the tree forms callus (Ca), wound periderms (Wp), and wound xylem (Wx) that can be distinguished as a marker within the tree ring. (B) By relating the intra-annual increment before wounding (Ii) to the ring width (Rw) the relative position of the wound can be determined. Wounds that occurred during winter dormancy are located at the tree-ring boundary, while wounds occurring during the growing season – like in this case – are located within the tree ring. This approach allows dating of wounds of various origins with an intra-annual resolution.

First, we investigated the accuracy of wood-anatomical markers to date invasive *Anoplophora* outbreaks. *Anoplophora glabripennis* (Motschulsky) and *A. chinensis* (Forster) – naturally occurring in Asia – are two of the most destructive invasive insects found worldwide. These xylobiont insects are polyphagous but frequently infest maple trees (*Acer* sp) in which larvae feed in the xylem until they form characteristic exit holes that induce a wound marker in the tree ring (Cavey et al., 1998; Haack et al., 2010). *A. glabripennis* is mainly introduced through wooden packaging materials, whereas *A. chinensis* is generally introduced via import of ornamental trees such as the Japanese maple (Haack et al., 2010; Van der Gaag et al., 2010). As export of trees normally occurs during the dormant season there is a possibility that exit holes are formed during dormancy. Therefore, it was also relevant to study the possibility that wounds are recorded in the tree during dormancy. Accurate dating of *Anoplophora* outbreaks is crucial for taking customised eradication measures and to study population dynamics of these invasive insects over time.

Next, we studied the accuracy of dating drift-sand dynamics using the wood anatomy of pedunculate oak (*Q. robur*). During the past centuries, the area of active drift sand in Europe has decreased rapidly from approximately 800 km^2 in 1850 to 40 km^2 in 1980, and to only 15 km^2 in 2000 (Riksen et al., 2006). The future development of the current drift-sand areas or the potential of their successful restoration depend on a better understanding of the external factors that are driving the system: climate, landscape erodibility and land use (Koster, 1978; Bakker et al., 2003; Riksen et al., 2006). In active or formerly active drift-sand areas, pedunculate oak frequently occurs as shrubs or as multi-stemmed clusters which are partly covered by sand, and sometimes are exposed again (Tesch et al., 1926; Copini et al., 2005). Dendrogeomorphology, the study of tree rings to date geomorphic processes, may be used as an accurate technique to study sediment transport through time. We assessed drift-sand dynamics, using changes in ring width and wood anatomy of mature pedunculate oak (*Quercus robur* L.) in two drift-sand areas in the Netherlands. In addition, we performed a field experiment to test the temporal resolution of wood-anatomical changes related to burial.

Finally, we studied the formation of flood rings in pedunculate oak (*Q. robur*). The application of flood rings to reconstruct past flooding events is hampered by our limited understanding of the formation of this wood-anatomical marker, in the absence of experimental evidence (St. George, 2010). Firstly, spring reactivation of young pedunculate oak trees was studied in the absence of flooding, using non-invasive NMR imaging (Windt et al., 2006; Van As et al., 2009) and wood-anatomical research. Secondly, we conducted an experiment whereby various flooding treatments were applied to four-year-old pedunculate oak trees and studied the formation of flood rings in relation to leaf phenology and root dieback.

1.6 Thesis outline

In chapter 2 and 3, we investigated the accuracy of dating invasive *Anoplophora* outbreaks using wound reactions around exit holes of Japanese maple (*Acer palmatum* Thunb.). In chapter 2, we tested the accuracy of dating invasive *Anoplophora* outbreaks by simulating the formation of exit holes through cambial wounding in *Acer palmatum* trees. In chapter 3, we tested how dormant *Acer palmatum* trees respond to wounding during winter dormancy in relation to two contrasting temperature regimes.

In chapter 4 and 5, we assessed the temporal precision of dating burial events in drift-sand areas. In chapter 4, we demonstrated the use of dendrogeomorphology as a tool to date burial or exposure events in drift-sand areas. After this, in chapter 5, we studied the precision of wood-anatomical changes in response to stem burial in mature pedunculate oak (*Q. robur*) in an field experiment in the 'Loonse en Drunense Duinen'.

In chapter 6, we assessed the formation of earlywood vessels in relation to phenology and water transport using non-invasive Magnetic Resonance Imaging (MRI) and wood-anatomical research. Next, in chapter 7, we investigated the formation of flood rings and root development by experimental flooding of four-year-old pedunculate oak trees at different phenophases and flooding durations.

We conclude this thesis with a general discussion (Chapter 8) in which the main findings are discussed for the study of dating insect outbreaks, drift-sand dynamics and flooding events.


G

CHAPTER 2

Precision of dating insect outbreaks using wood anatomy: the case of *Anoplophora* in Japanese maple

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Frits Mohren & Antoon Loomans

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2.1 ABSTRACT

To control invasive *Anoplophora* outbreaks, it is crucial to accurately date infestation dynamics. Dating of *Anoplophora* outbreaks is possible as these xylobiont insects induce wounds in living trees by forming e.g. exit holes. This study investigates to what precision these wounds can be dated with dendrochronological techniques. In an experimental setting, we studied the precision of wound dating on *Acer palmatum*, an ornamental tree occasionally containing larvae of *A. chinensis*. We studied the development of wound reactions at the beginning, during and after the growing season, both in relation to leaf phenology and intra-annual tree-ring growth. We found that the precision of dating exit holes is limited due to the highly variable intra-annual tree-ring growth whereby only an accurate distinction can be made between wounds originated during, or after tree-ring formation. The resolution was improved using local growth—the local reactivation of xylem growth around the wound—as a marker for wounds that occurred at the end of the growing season. We conclude that the intra-annual precision of dating *Anoplophora* outbreaks in *Acer palmatum* in the temperate North-western European climate is limited to three distinct phases: (i) The period of dormancy and leaf emergence (ca. October until April/beginning of May), when the wounds are located at the tree-ring boundary (ii) The period of tree-ring growth in which wounds are located within the tree ring (ca. end of April/beginning of May until late August/beginning of September), (iii) end of growing season (ca. end of August/September) in which local growth occurs.

Keywords: *Acer palmatum* • *Anoplophora chinensis* • Dendrochronology • Invasive species • Japanese maple • Wound dating

2.2 Introduction

The citrus longhorned beetle, *Anoplophora chinensis* (Forster) and Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) are two of the most destructive and invasive xylobiont insects found worldwide (Haack et al., 2010). *A. glabripennis* and *A. chinensis*, both naturally occurring in Asia, were accidentally introduced to the USA, Canada and Europe where they cause serious damage to a wide variety of angiosperm host species, especially maple trees (*Acer* spp.) (e.g. Nowak et al., 2001; MacLeod et al., 2002; Hérard et al., 2006; Haack et al., 2010). *A. glabripennis* is mostly introduced through wooden packaging materials whereas *A. chinensis* is generally introduced via import of ornamental trees, such as the Japanese maple (*Acer palmatum* Thunb.) (Haack et al., 2010; Van der Gaag et al., 2010). Both species weaken their host trees by causing wounds during all stages of their 2–3 years life cycle: (i) after hatching, larvae tunnel through the cambial region into the sapwood and sometimes into the heartwood, (ii) after larvae pupate, the beetles leave their host tree by making exit holes through the sapwood, cambial zone, and bark (Fig. 2.1a, b) and (iii) subsequently feed on the bark of twigs and on foliage during maturation. During ovipositioning (iv) the insects make cavities in the phloem (*A. chinensis*) or cambial region (*A. glabripennis*; Fig. 2.1c) in which eggs are deposited (Haack et al., 2010).

When these insects wound the cambial zone by forming exit holes or oviposition pits, cambial cells surrounding the wound die. The surrounding cells, inter alia, start forming traumatic parenchyma cells (referred to as callus), wound xylem and wound periderms, which are preserved as scars in tree rings (e.g. Sass-Klaassen and Copini, 2008; Copini et al., 2010). These scars can be dated to the exact year of origin by applying dendrochronological techniques (Sawyer, 2007; Sass-Klaassen and Copini, 2008; Van der Gaag et al., 2010). Moreover, the position of a scar within a particular tree ring can provide an estimate of the period when wounding occurred (Ortloff et al., 1995; Schweingruber, 1996; Swetnam et al., 1999; Schneuwly and Stoffel, 2008). The precision of intra-annual dating has hardly been studied and relies on the rate at which wound reactions develop, and on radial intra-annual tree-ring growth (Schweingruber, 1996). Although Ortloff et al. (1995) claim that fire scars in the gymnosperm *Pinus ponderosa* can be dated to the exact month of wounding, no study has yet been conducted to validate this claim.

In this paper, we study the precision of wound dating in young diffuse-porous *Acer palmatum*—a small ornamental tree that is exported from Asia in bulk quantities and occasionally contains *A. chinensis* larvae (Van der Gaag et al., 2010). In an experimental setting we assessed (I) the temporal succession of wound reactions in relation to timing of wounding, e.g. during or outside the growing season and (II) the intra-annual radial growth dynamics of *A. palmatum* trees to determine the precision of intra-annual wound dating. We hypothesised that (i) the formation of callus, wound periderm and wound xylem, only occurs during tree-ring formation (Grünwald et al., 2002; Frankenstein et al., 2005b). Conversely, cambial dieback is always recorded instantly, independent of season. We expected that (ii) cambial dieback is higher during the dormant season as in this period trees are physiologically restricted in their response to wounding (Dujesiefken and Liese, 1990; Dujesiefken et al., 1991). As concerning intra-annual tree-ring growth, we expected that in the Netherlands (iii) *A. palmatum* starts growing around the end of April, beginning of May, reach their highest growth activity during the middle of the growing season (June/ July) and stop radial growth by the end of August or beginning of September, as has been found for many other temperate tree species (e.g. Ladefoged, 1952; Schmitt et al., 2000; Frankenstein et al., 2005a; Michelot et al., 2012).

2.3 MATERIALS AND METHODS

Plant material

We used 100 six-year-old Japanese maples (*Acer palmatum* Thunb.) with an average stem diameter between 3 and 6 cm at 20-cm stem height and an average height of 165 ± 24 cm (mean ± standard deviation). The trees were imported from China and were grown for ca. 3 years at a Dutch nursery where they were planted in 65l pots in 2009. The ca. 6-year-old potted trees were placed in a 2 x 2 m grid in a garden experiment in Wageningen, the Netherlands (51.9884°N, 5.6644°E) in March 2010. The trees were watered using a semi-automatic irrigation system, simultaneously supplying nutrients.

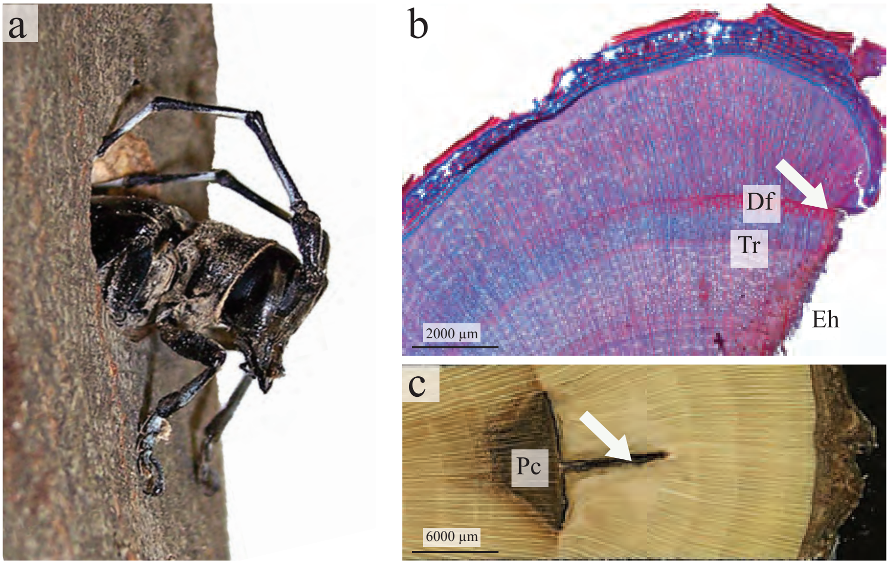

FIGURE 2.1 Exit holes and oviposition pits of *Anoplophora glabripennis* and *A. chinensis*. (A) An emerging *A. glabripennis* beetle formed an exit hole in a tree, thereby injuring the cambial zone and leaving a scar in a particular tree ring that can be dated with dendrochronological techniques (photo adapted from the USDA). (B) Transverse thin section showing wound reactions on the left side of an *A. chinensis* exit hole (Eh) in *Acer palmatum*. The arrow indicates the position of the cambium at the moment of exit-hole formation in the most recent tree ring. Normally trees form density fluctuations (Df) around exit holes. (C) Transverse section through an *A. glabripennis* oviposition pit that was formed in 2005 in *Acer pseudoplatanus*. The dish-shaped dark discoloured area consists of death xylem in which vessel elements are frequently blocked and phenolic compounds (Pc) are deposited. The dark horizontal fissure (arrow) consists of bark remains

Wounding experiment

At six different moments throughout the year (W1–W6), we created artificial wounds in 15 randomly selected trees by slowly drilling 1-cm-diameter holes at least 2.5 cm into the western side of the stem at approximately 25-cm stem height (Table 2.1). The diameter of the holes was comparable to exit holes of *A. glabripennis* and *A. chinensis* (Haack et al., 2010). The first wounds were made on 19 Mar. 2010, when the trees were still dormant (Table 2.1). After the trees started flushing in April, we repeated this treatment approximately every 46 days until 15 Oct. 2010, when the leaves were almost shed (Table 2.1). From each series, five trees were harvested 2 and 4 weeks after wounding, respectively. The remaining five trees from each series were harvested at the end of the growing season (12 Nov. 2010) (Table 2.1). Stem sections containing the wounds were stored in a 50% ethanol solution at room temperature. We scored leaf phenology on the upper part of each tree at the time of tree wounding. For the classification of spring phenology, we used information provided by Kriebel and Wang (1962) and Norby et al. (2003) and defined the following phases: dormant, buds swelling, buds just opening, leaves expanding, leaves fully expended, autumn colours and leave abscission.

Assessment of intra-annual tree-ring growth

To trace radial tree-ring growth during the growing season of 2010, we applied the pinning technique (e.g. Wolter, 1968; Kuroda and Shimaji, 1984; Schmitt et al., 2004). The cambium of ten randomly selected trees was marked with a 0.8-mm-diameter pin on the south side of the trunk. The timing of cambial marking largely coincided with the wounding experiment and was supplemented by three additional pinning moments in between wounding dates to enhance the resolution. Pinning started on 12 Apr. 2010 and was repeated every 23 days until 15 Oct. 2010 (Table 2.1). The first cambial marking was carried out at approximately 20-cm stem height and the subsequent markings were made using a zigzag pattern 2.5 cm higher up the stem. The trees were harvested at 12 Nov. 2010 during bud dormancy. Stem sections containing the pinning marks were stored in a 50% ethanol solution at room temperature. Simultaneously, we scored leaf phenology every time the trees were marked, distinguishing the same phenological phases as in our wounding experiment. The course of intra-annual radial growth was compared to hourly temperature data measured at 10 and 130 cm height above the soil from the nearby (2.5 km) Wageningen University, Haarweg climate station (www.maq.wur.nl). We calculated daily average temperatures based on the 130 cm temperature and daily minimum temperature based on the 10 cm temperature measurements.

Sample preparation

All stem sections were sawn transversely, through the middle of the experimentally induced wound so that the wood and bark anatomy surrounding the wounds became visible. Transverse sections with a thickness of about 25 lm were prepared using a sliding microtome (G.S.L.-1 microtome, WSL, Birmensdorf, Switzerland). All sections were stained with a safranine/astrablue solution for 5 min. This treatment stains lignified cells red and non-lignified cells blue. Following dehydration in graded series of ethanol (50–95–100%), the samples were rinsed with xylol, mounted in Canada balsam and dried at 60 °C for 15 h. We took photos with a digital camera (DFC 320, Leica, Cambridge, UK) mounted on a microscope (DM2500, Leica, Cambridge, UK) using Leica imaging software (version 3.6.0).

TABLE 2.1 Experimental setup of the wound and pinning experiment in relation to leaf phenology and presence of wound markers *W* wounding, *P* pinning

Treatment	Date of treatment wounding (*n* = 15) pinning (*n* = 10)	Leaf phenology	Harvest dates wounded trees (*n* = 5) (P all harvested on 12 Nov. 2010)	Av. cambial dieback in lm (s.d.)	Callus and wound Periderm (# oftrees)	Wound xylem (# of trees)
W1	19 Mar.	Dormant buds	2 Apr.	706 (577)	0	0
			18 Apr.	510 (256)	5	5
			12 Nov.	702 (360)	5	5
W2, P1	12 Apr.	Leaves expanding	26 Apr.	883 (425)	5	5
			10 May	501 (196)	5	5
			12 Nov.	820 (383)	5	5
P2	5 May	Leaves fully developed				
W3, P3	29 May	Leaves fully developed	12 Jun.	462 (144)	5	5
			26 Jun.	1,036 (573)	5	5
			12 Nov.	936 (252)	5	5
P4	21 Jun.	Leaves fully developed				
W4, P5	14 Jul.	Leaves fully developed	28 Jul.	934 (371)	5	5
			11 Aug.	762 (216)	5	5
			12 Nov.	542 (247)	5	5
P6	6 Aug.	Leaves fully developed				
W5, P7	30 Aug	Leaves fully developed	13 Sep.	520 (96)	5	5
			27 Sep.	756 (312)	5	5
			12 Nov.	641 (583)	5	5
P8	22 Sep	Autumn colours				
W6, P9	15 Oct.	Leaf abscission	29 Oct.	627 (345)	0	0
			12 Nov.	526 (128)	0	0
				Total: 699 (361)		

Measurements

To study the development of wound markers, we scored the presence of callus tissue (traumatic parenchyma cells), wound periderm and wound xylem (Fig. 2.2a) in all samples using light microscopy (DM2500, Leica, Cambridge, UK). To study whether the extent of cambial dieback can be used to improve intra-annual dating, we measured tangential cambial dieback as the distance between the bore hole and newly formed callus tissue (Fig. 2.2a), using the digital images of the transverse thin sections in combination with Image J software (version 1.44) (Rasband, 1997-2012). The intra-annual increment was measured as the distance between the 2009/2010 tree ring and pinning-induced cambial dieback (Fig. 2.2) (Sass et al., 1995). Tree-ring widths were measured at approximately 1 cm to the left and right of the pinning hole so that locations with wound xylem were avoided. To reconstruct tree-ring growth over the season and allow comparisons between the sample trees that differed in growth level, the relative intra-annual increment was calculated as intra-annual increment/tree-ring width x 100% (Grotta et al., 2005; Fig. 2.2). When wounds were located at the 2010/2011 tree-ring boundary, the relative intra-annual increment was set to 100%. To show the precision of intra-annual wound dating, we made use of all samples of W1–W6 (Table 2.1) that were harvested on 12 Nov. 2010 and we measured the relative wound position within the 2010 tree ring, using the same method as in our pining experiment (Fig. 2.2).

Statistical analyses

All statistical analyses were performed with the SPSS statistical package, version 19 (SPSS Inc., Chicago, IL, USA), using a significance level of 0.05. The presence or absence of callus, wound periderms, wound xylem in relation to the moment of wounding and moment of measuring were tested using Pearson Chi-square tests. The response of cambial dieback in relation to the time of wounding and time of measuring was analysed after a square root transformation to obtain normality. We used all samples that were harvested on 12 Nov. 2010 to test for differences in cambial dieback in response to the moment of wounding using a one-way ANOVA in combination with a Tukey post hoc multiple comparison tests. The samples we collected 2 and 4 weeks after wounding were used to study the rate of cambial dieback using a GLM univariate procedure in which both the moment of wounding, and the moment of measuring and their interaction were evaluated.

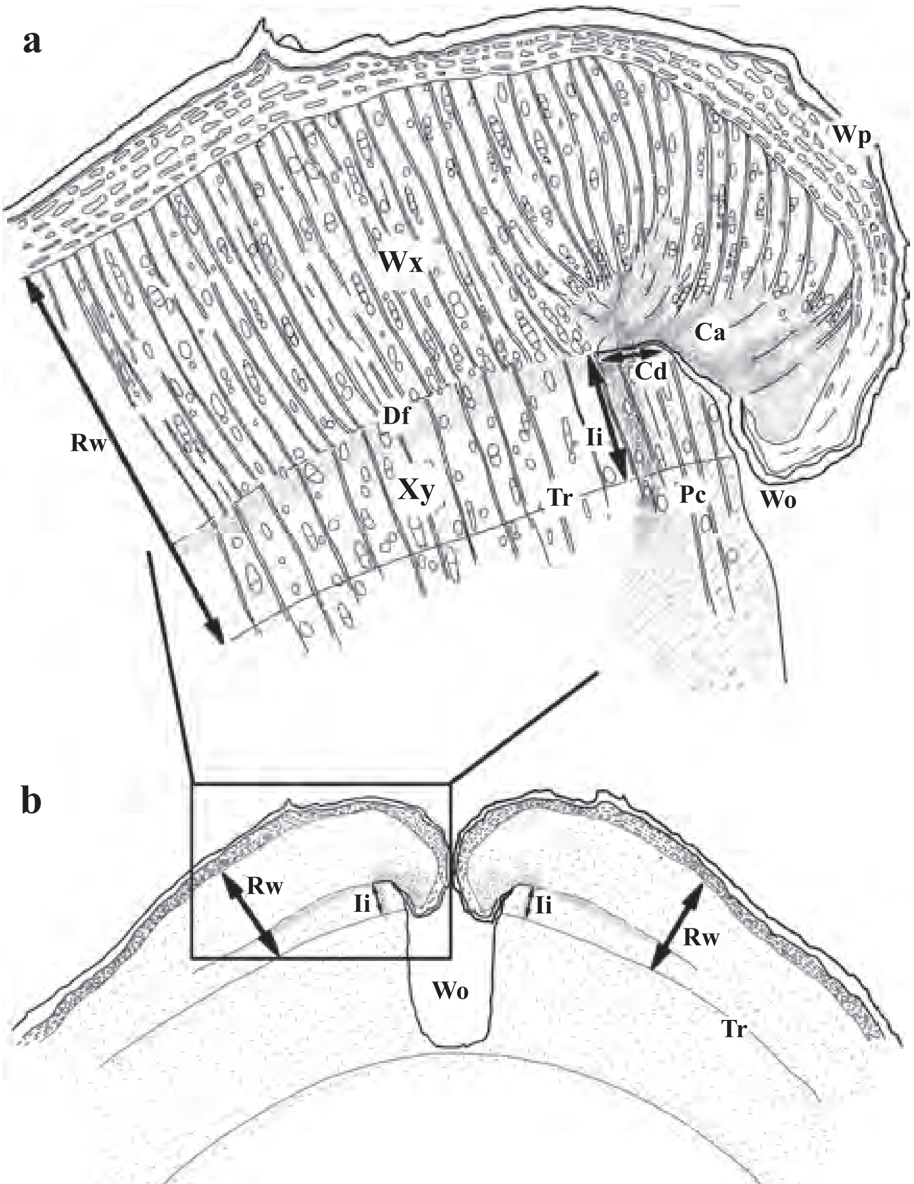

FIGURE 2.2 Schematic overview of a transverse section around a wound (Wo) in a tree stem. (A) To analyse the development of wound markers, we scored the presence of cambial dieback (Cd), callus (Ca), wound periderms (Wp), and wound xylem (Wx). In addition, we measured the tangential width of cambial dieback as the distance between the wound and the callus tissue as is indicated with the horizontal arrow. (B) To determine the relative wound position, both the intra-annual increment (Ii) and the 2010 tree-ring width (Rw) were measured on both sides of the wound.

2.4 RESULTS

Development of wound markers

Cambial dieback from the wound edge along the tangential plane around the stem (Fig. 2.3a) occurred in all wounded trees. Cambial dieback was highly variable, and extended 699 ± 361 lm (mean ± SD) after the removal of four outliers (Table 2.1). No significant differences in cambial dieback were observed in relation to the timing of wounding ($F_{5,24} = 1.033$, $p = 0.421$). No significant differences were found between 2 and 4 weeks after wounding ($F_{1,44} = 0.001$, $p = 0.993$) nor between the interaction between the moment of wounding and the moment of measuring ($F_{5,44} = 2.098$, $p = 0.084$). Callus tissue was always surrounded by a (developing) wound periderm (Fig. 2.3e). Significant differences in presence of callus tissue and related wound periderms were observed between wounding treatments ($P < 0.001$). Callus and related wound periderms were found in treatments W1–W5 (Table 1.1) and were always present already after 2 weeks except for W1 where they were visible after 4 weeks. In all samples of W1–W5 that were harvested at 12 Nov. 2010, the wound periderms were merged with the original periderm (Fig. 2.3c). Wound xylem (Fig. 2.3a, c, e) is characterised by more ray parenchyma and smaller vessels that are more frequently clustered in radial direction than in normal xylem. Significant differences in presence of wound xylem were found between treatments (P \ 0.001). Wound xylem was observed in treatments W1–W5. In W1, wound xylem was observed after 4 weeks, during the period in which leaf unfolding had occurred, while in the remaining treatments it was visible after 2 weeks. At the end of the growing season, all trees, except for W6 (Fig. 2.4f), contained wound xylem. Increased radial growth was observed close to wound margins as compared to the average ring width; in slow growing individuals a ca. fivefold increase could be observed.

Peculiarities: local growth and density fluctuations

In trees of treatments W1, W2 and W5, i.e., during the beginning and end of the growing period, local growth was frequently observed. We defined local growth as local xylem growth that was restricted to the wound margins and occurred before or after normal tree-ring formation (Fig. 2.3b, d). Within the W1 treatment, all trees showed local growth 4 weeks after wounding, during the period when leaf expansion had occurred. In the W2 treatment, local growth was observed in only three trees: in two trees after 2 weeks and in one tree after 4 weeks.

In the remaining trees of this treatment, growth started close to the wound margins as well as in other places, asynchronously around the circumference. In W5 all trees showed local xylem growth (Fig. 2.3d). Whilst local growth in W5 remained stored in tree rings, local growth in W1 and W2 could only be observed 2 or 4 weeks after wounding as local growth was indiscernible after full development of the tree ring. In one tree of treatment W3 and all trees of W4, density fluctuations were observed in the xylem of the 2010 tree ring (Fig. 2.3c). These density fluctuations occurred as tangential bands of thick-walled fibre cells, initiated at the exact radial position in the ring where the cambium was wounded. Most density variations were local and extended several centimetres left and right from the wound. In some trees, however, they continued almost around the entire circumference of the tree.

Tree-ring growth

Tree-ring formation did not yet occur on 12 Apr. 2010, when all trees showed bud break and a few leaves were expanding. The first xylem growth was visible on 5 May, when the leaves were fully expanded (Fig. 2.4). Tree-ring growth was characterised by a slow start in spring and maximum activity in July and August (Fig. 2.4). Only 16% of the tree ring was formed halfway the growing season, by the end of June (P4). The 2010/2011 tree-ring boundary was completed in all trees on 30 Aug. 2010 when the leaves were still fully developed. Autumn colouration and leaf abscission occurred on 22 Sep. 2010 (P8) and 15 Oct. 2010 (P9), respectively. All trees were dormant when they were harvested on 12 Nov. 2010. In total, tree-ring growth lasted for approximately 141 days, while the period between bud break and leaf abscission was approximately 187 days. Local growth—cambial activity left and right to the pin-mark—was observed after pinning at the end of August (P7) when the leaves were fully developed and at the end of September (P8) when the leaves started autumn colouration. While, after P7, local growth occurred over a tangential width of 1.2–3.0 mm, this was reduced to 1–1.2 mm after P8.

The growing season in 2010 was characterised by a relative warm April month, followed by an extremely cool May month—especially the first 19 days of the month, with many nights with frost at the soil level. June and July were warm; while August and September were relatively cool (Fig. 2.4).

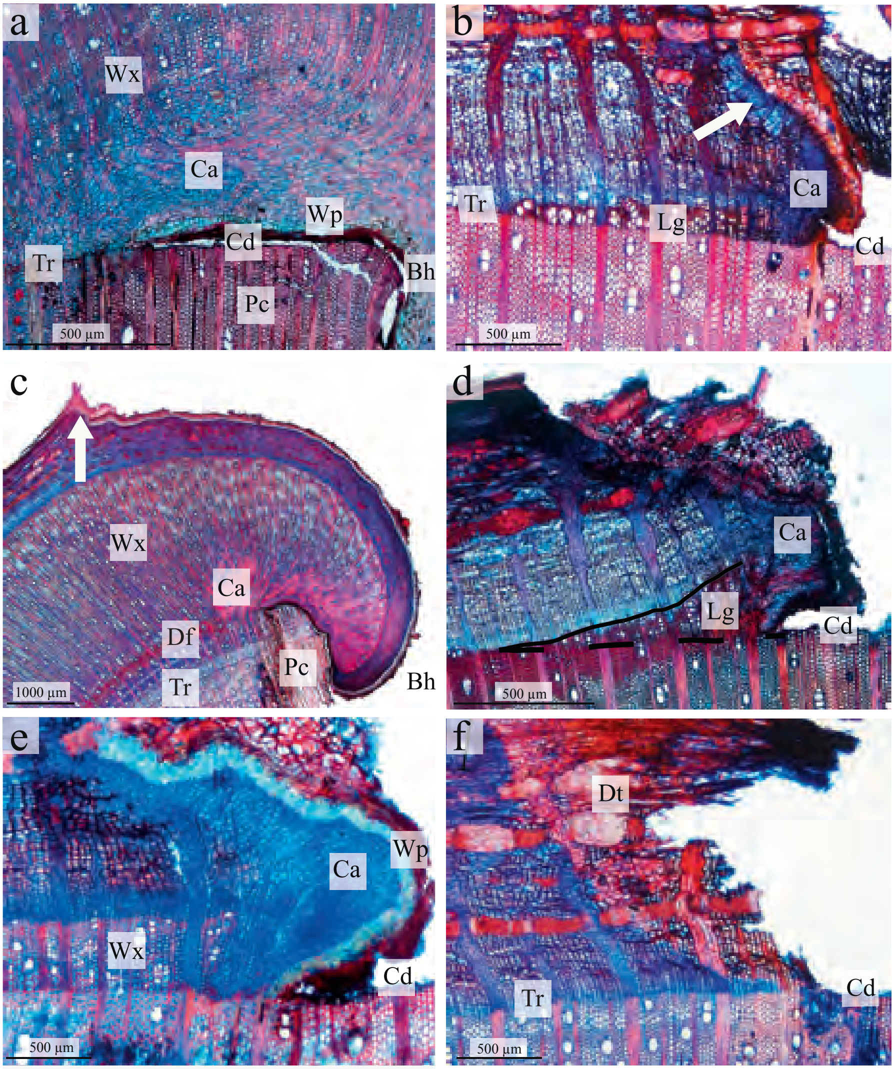

Figure 2.3 Wound reactions on transverse thin section of Japanese maple (*Acer palmatum)* after experimental wounding. (A) Wound reactions around a bore hole (Bh) of the W1 treatment showing Cambial dieback (Cd), Callus tissue (Ca), Wound-periderm formation (Wp), wound xylem (Wx) and deposition of Phenolic compounds (Pc). On 12 Nov. the bore hole (Bh) was partly filled with callus tissue. Cd is located at the 2009/2010 tree-ring boundary (Tr). (B) Wound reactions 4 weeks after the W2 treatment. Xylem growth starts locally close to the wound margin. The *arrow* indicates the onset of wound-periderm formation. Cd is located at the 2009/2010 tree-ring boundary (Tr) (C) Wound reactions after the W4 treatment (harvested 12 Nov. 2010) showing a typical density fluctuations (Df). The *arrow* indicates the area where the wound periderm merges with the original periderm. (D) Local growth (Lg) after the W5 treatment. The *dashed line* represents the tree-ring boundary that was deposited before wounding. The *solid line* indicates the locally present tree-ring boundary that was

formed after wounding. (E) Wound reaction in the cambial zone after the W5 treatment showing Cd, Ca, Wp, Wx. (F) Wound reactions 4 weeks after the W6 treatment are characterised by the absence of Ca, Wp and Wx; only dead bark tissue (Dt) and cambial dieback (Cd) can be observed. Cd is located at the 2010/2011 tree-ring boundary (Tr)

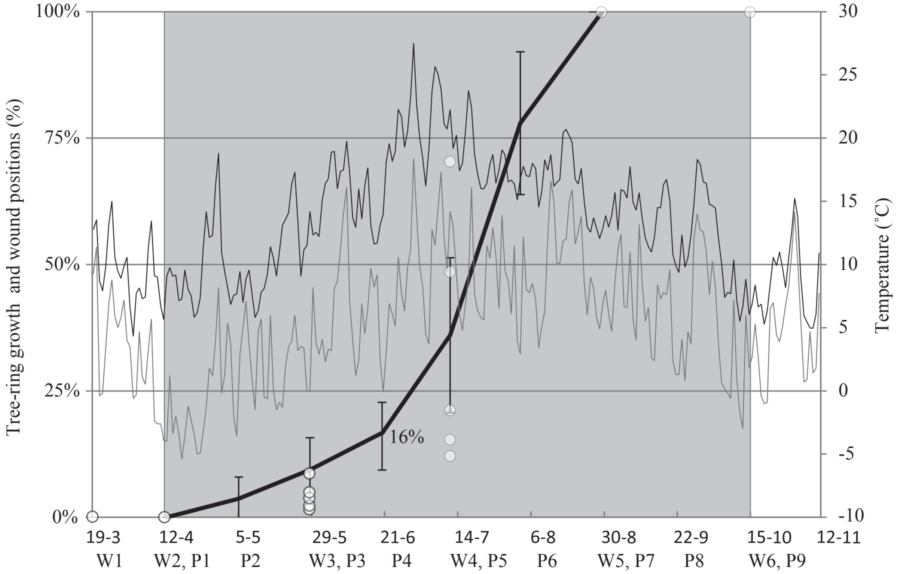

Figure 2.4 Relative intra-annual tree-ring growth (P1–P9) in relation to leaf phenology, temperature and wound positions of Japanese maple (*Acer palmatum*) during the growing season of 2010. The ***grey*** background indicates the period between leaf expansion and leaf abscission and shows that tree-ring growth is preceded by leaf expansion in spring whereas xylem growth stops long before leave abscission in autumn. Halfway the growing season, on June 21, on average only 16% of the tree ring had been formed. The ***black temperature line*** represents average daily temperature measured at 130 cm whereas the ***grey line*** shows the minimum air temperature measured at 10 cm. After leaf expansion, many nights with frost occurred. The ***open dots*** indicate the relative wound positions of 30 wounds in 30 trees that were made at six different moments through the year. All wounds (W1–W6) that were set before tree-ring formation, during dormancy and leaf expansion, had a relative wound position of 0% and were thus located at the 2009/2010 tree-ring boundary. All wounds, except one, that were set during tree-ring formation were located in the first half of the tree ring. Wounds that were made after tree-ring formation, during the period of leaves colouring and abscission had relative wound positions of 100% and were located at the 2010/2011 tree-ring boundary

Precision of wound dating

All wounds that were initiated during the dormant season (W1 and W6) were located at the tree-ring boundary of 2009/2010 or 2010/2011 and thus had a relative wound position of 0 or 100% of the tree-ring widths, respectively (Fig. 2.4). Wounds that were initiated during leaf expansion (W2), before the start of tree-ring formation, were located at the 2009/2010 tree-ring boundary as well

(Fig. 2.4). Wounds in trees that were injured on 29 May (W3), during the first half of the growing season, were located within the beginning of the tree ring with an average relative wound positions of 8 ± 3% ($n = 5$) (Fig. 2.4). Wounds that were made on 14 Jul. 2010 (W4) during the second half of the growing season, had an average relative wound position of 33 ± 25% ($n = 5$) and were thus mostly located within the first half of the tree ring (Fig. 2.4). All wounds that were made on 30 Aug. (W5) were located at the tree-ring boundary and were surrounded by local xylem growth; between 1.5 and 3 mm was added in tangential direction (Fig. 2.3d).

2.5 Discussion

Occurrence of wound markers

By comparing experimentally induced wounds in *Acer palmatum* that were made at six different moments through the year, we found that callus tissue, wound periderms and wound xylem were formed within 2 weeks during the growing season (W2–W5). In contrast, cambial dieback occurred within 2 weeks both at the beginning, during and after the growing season (W1–W6). This shows that the precision of wound dating is not limited by a delay caused by wound-marker formation. These results are in accordance with the general view that the cambium around a wound margin collapses immediately, independent of the season, while the formation of callus, wound periderm and wound xylem is limited to the growing season (e.g. Dujesiefken et al., 1991; Oven et al., 1999; Grünwald et al., 2002; Schmitt et al., 2004; Frankenstein et al., 2005b).

The extent of cambial dieback did not significantly vary between the moments of wounding, even when comparing wounds initiated within and outside the growing season. This is in contrast to earlier research on cambial dieback. Dujesiefken et al. (1991) wounded *Acer pseudoplatanus* trees five times between August and April, and found that cambial dieback was lowest during spring wounding and highest during winter wounding. Dujesiefken and Liese (1990) wounded *Fagus sylvatica* in the beginning of October, December, February and April, and found highest tangential dieback during December wounding, while after April almost no dieback occurred. On the other hand, our results are in agreement with Dujesiefken et al. (2005) who found no differences in tangential cambial dieback after wounding *Fagus sylvatica*, *Quercus robur* and

Q. rubra trees in October, December, February and April. As tangential cambial dieback is highly variable and does not show differences between the time of wounding, we conclude that the tangential extent of cambial dieback is not suitable to enhance the precision of dating *Anoplophora*-induced wounds.

Local growth and density fluctuation

We observed local xylem growth around wounds that were made before or after tree-ring formation, while the leaves were absent (19 Mar.), expanding (12 Apr.), still fully developed (30 Aug.), or started to show their autumn colours (22 Sep.). Local growth is only preserved as an anomaly and thus can be used to enhance the precision of dating when occurring at the end of the growing season. The occurrence of local growth at the beginning of the growing season was only visible when samples were taken 2 and 4 weeks after wounding because later in the season it is imperceptible as it merges with successive xylem formation. This phenomenon of local growth has been observed in the evergreen gymnosperms *Picea abies* and in several angiosperm trees after wounding toward the end of the growing season in late summer (Dujesiefken et al., 1991). Copini et al. (2010) and Copini and Sass-Klaassen (2010) assumed that some *A. chinensis* and *A. glabripennis* exit holes were formed at the very end of the growing season based on the presence of local double tree rings. The current study supports this view. As local growth after tree-ring formation remains stored in the xylem, it can be used as a specific marker indicating wounding at the very end of the growing season. We found density fluctuations, i.e., tangential bands with thick-walled fibre cells left and right to the wound, after wounding that occurred during high radial growth activity (14 Jul.) (Fig. 2.1b). Such density fluctuations have been found in the xylem surrounding wounds in both gymnosperms (Forster et al., 2000) and angiosperms (Schmitt et al., 2000; Frankenstein et al., 2006) and were also found surrounding *A. chinensis* exit holes in *Acer palmatum* (Fig. 2.1b) (Sass-Klaassen and Copini, 2008; Copini et al., 2010). Sometimes density fluctuations impede wound dating due to their resemblance with tree-ring boundaries (Copini et al., 2010). Most likely, density fluctuations consist of cells that were developing at the moment when wounding occurred (Frankenstein et al., 2006). It is unclear why in some trees density fluctuations develop only locally and in other trees cover most of the circumference.

Dynamics of tree-ring formation

We observed tree-ring growth in the period between Mid-April/early May until the end of August and found that only 16% of the tree ring had been formed halfway the growing season (21 Jun.; P4 in Fig. 2.4). The observed period of tree-ring growth is in accordance with the general view that diffuse-porous trees in Western Europe start xylem reactivation, after leaf unfolding, normally between Mid-April till Mid-May and stop tree-ring growth before autumn colouration, usually between mid-August and mid-September (e.g. Ladefoged, 1952; Schmitt et al., 2000; Frankenstein et al., 2005a; Michelot et al., 2012). However, the slow tree-ring growth in the first half of the growing season is uncommon for diffuse-porous trees (e.g. Ladefoged, 1952; Schmitt et al., 2000; Werf et al., 2007; Michelot et al., 2012). This slow tree-ring growth in our study might be related to factors, such as horticultural treatments or site-related growing condition. Since temperatures during the first 19 days of May 2010 were far below average and among the coldest measured since weather records of the Royal Netherlands Meteorological Institute (KNMI) began, we assume that unfavourable weather conditions in 2010 strongly decreased cambial activity in our experimental trees. Research on tree-ring growth in *A. pseudoplatanus* showed that the amount of tree-ring formation until June can vary between 38 and 49% (Hartig, 1860; Ladefoged, 1952). In *Fagus sylvatica*, Schmitt et al. (2000) found only 10% completion of the 1996 tree ring before 21 Jun., while van der Werf et al. (2007) and Michelot et al. (2012) found for the same species about 50% completion in the Netherlands at 30 Jun. 2003, and in France at 11 Jun. 2009, respectively. This confirms that intra-annual tree-ring growth is highly variable, which has strong implications for the precision of dating wounds that were initiated during the growing season.

Precision of dating exit holes

We found that all wounds made before and after the growing season (W1 and W6) were located at the tree-ring boundary which is in agreement to the general view on wound dating (e.g. Ortloff et al., 1995; Schweingruber, 1996; Stoffel et al., 2005). In addition, we found that all wounds made during leaf expansion (W2) were located at the tree-ring boundary as well, which is in line with the general tendency of diffuse-porous trees to start leaf emergence before tree-ring formation as described above.

We expected that wounds that were made in the first half of the growing season (W3) were located in the first half of the tree ring, while wounds made in the second half (W4) were located in the second half of the tree ring. Surprisingly, almost all wounds (9 of 10) were located in the first half of the 2010 tree ring (Fig. 2.4). These results are in line with the results of our pinning experiment in which we showed that only 16% of the tree ring had been formed halfway the growing season (Fig. 2.4). This means that wounds that are located in the first half of a tree ring could, in years with a slow initiation of tree growth, actually have been formed in the second half of the growing season. Therefore, only with additional knowledge on intra-annual tree-ring growth of a particular year, a further indication can be given whether exit holes are formed in the first half or second half of the growing season. This is in contrast to Ortloff et al. (1995) who claim, albeit in gymnosperm trees with a clear distinction between earlywood and latewood, that wounds originated during the growing season can be dated to the exact month. Most likely, this is because Ortloff et al. (1995) used intra-annual tree-ring growth as a static phenomenon, while in fact this can be highly dynamic as also found for other gymnosperm species (e.g. Dünisch and Bauch, 1994; Grotta et al., 2005; Deslauriers et al., 2008; Eilmann et al., 2011).

We found that all wounds of the W5 treatment, which were made when the leaves were still fully developed, were located at the tree-ring boundary and surrounded by local growth. This local growth is preserved as a marker in the tree rings and indicates wounding at the end of the growing season, between tree-ring growth cessation and leave abscission. Local growth, therefore, enables us to improve the resolution of wound dating.

Our results are relevant to the study of dynamics in *Anoplophora* outbreaks. Accurate dating of *Anoplophora* outbreaks provides a background for eradication measures to be taken. For instance, dating of exit holes in imported trees can substantiate whether expensive eradication measures are necessary in the country of import. In addition, by studying population dynamics on an intra-annual scale, the success of populations can be related to the timing of insect emergence. With proper knowledge on tree-ring formation, exit-hole dating is possible in all host species which forms annual tree rings and is applicable to many other xylobiont species with a comparable life cycle.

2.6 CONCLUSIONS

We conclude that due to highly variable intra-annual tree-ring growth, the resolution of intra-annual dating of exit holes in *Acer palmatum* in the temperate North-western European climate is limited to three distinct phases: (i) the period before tree-ring growth that includes the period of dormancy and leaf emergence. In this period (ca. October till April/beginning of May) wound markers are located directly at the tree-ring boundary. (ii) The period of tree-ring formation (ca. end of April/beginning of May till late August/beginning of September), in which wound markers are located within the tree ring, (iii) end of season (ca. end of August and September) in which local growth occurs around the wound. More exact dating of wound formation during the growing season is possible only when the intra-annual growth pattern is known for that particular year.

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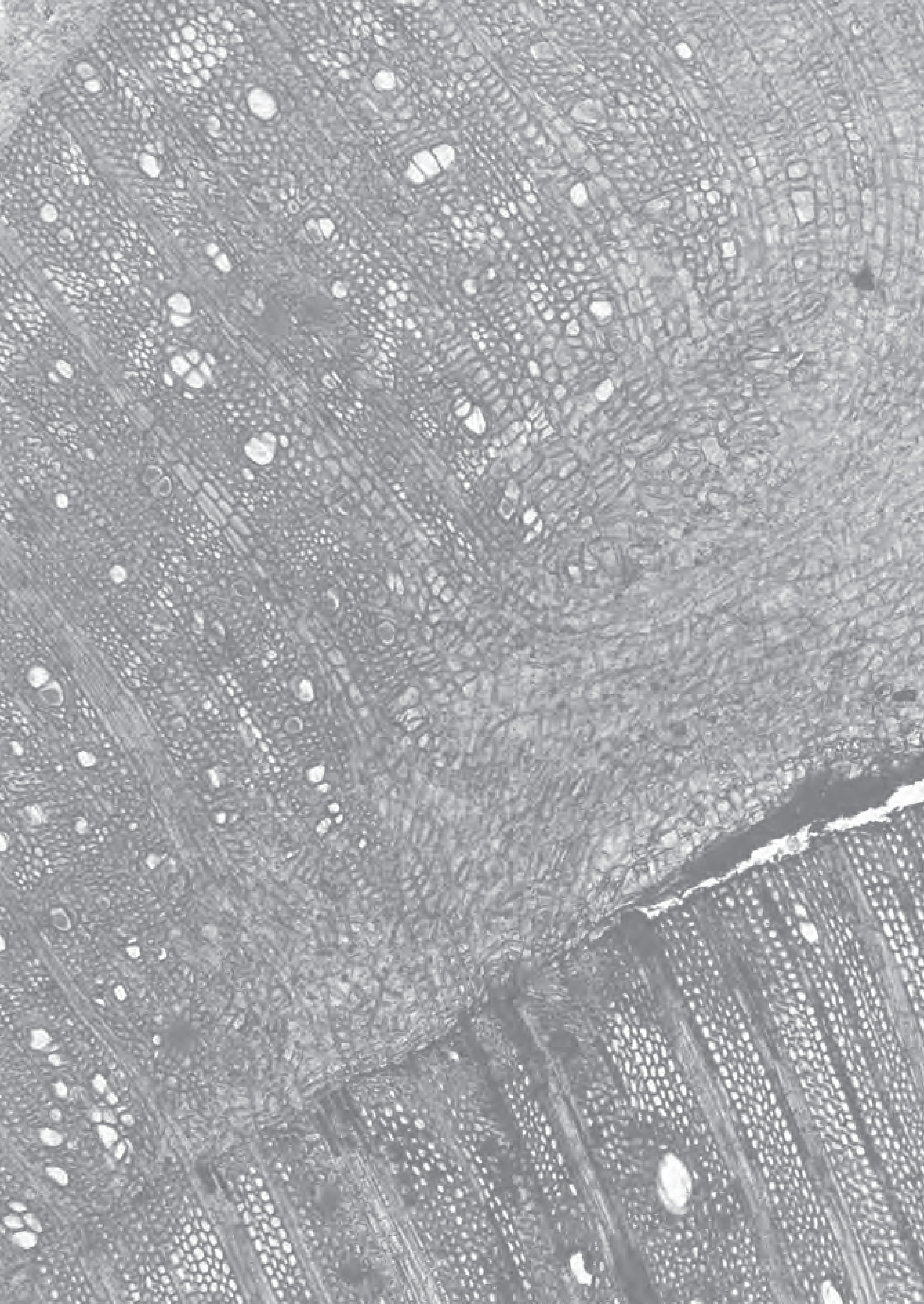

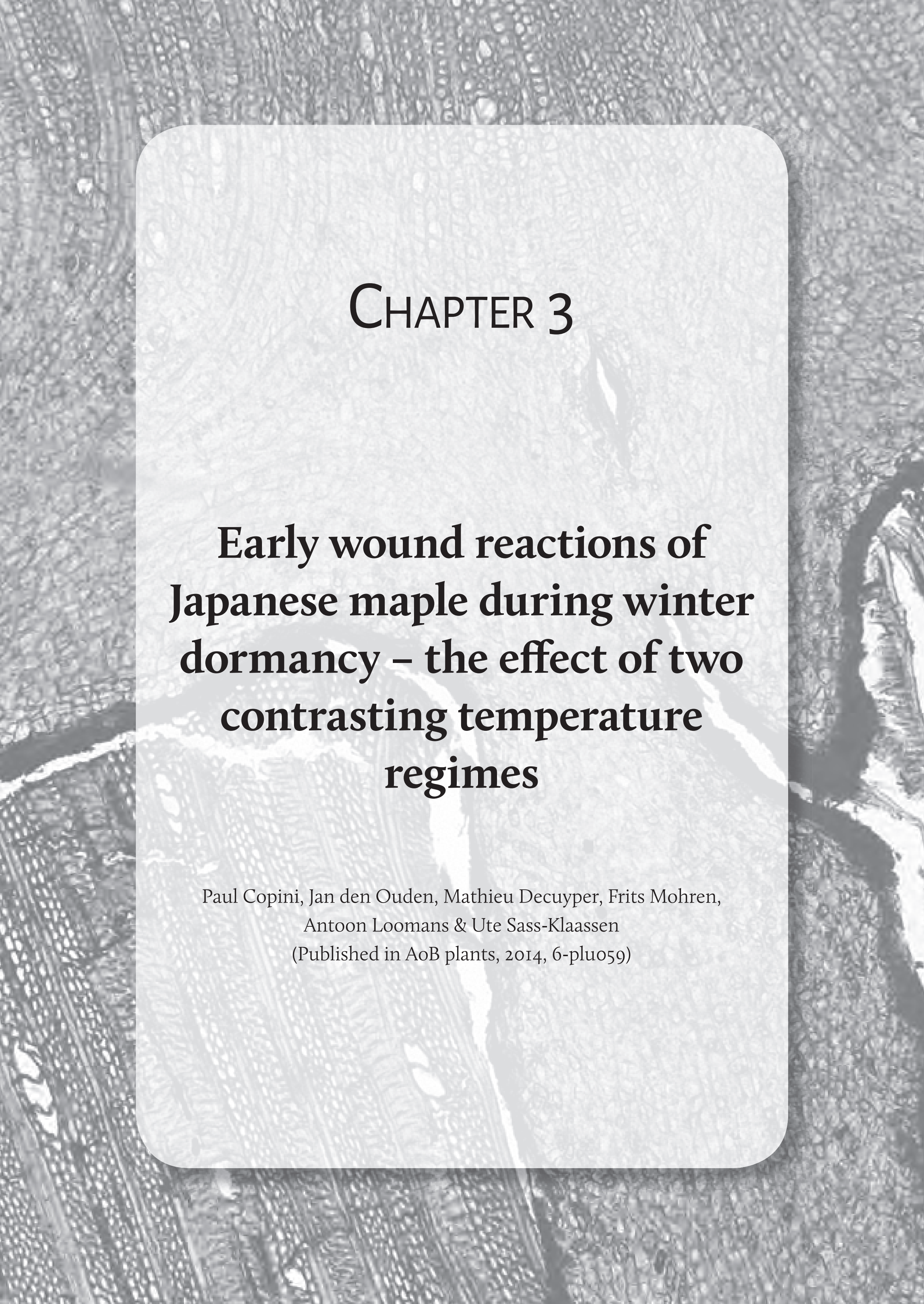

CHAPTER 3

Early wound reactions of Japanese maple during winter dormancy – the effect of two contrasting temperature regimes

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3.1 ABSTRACT

During winter dormancy, temperate trees are capable of only a restricted response to wounding. Depending on the ambient temperature during winter dormancy, wounded trees may start compartmentalization, e.g. by producing inhibitory compounds, but it is thought that processes involving cell proliferation, such as the formation of callus and wound xylem, are delayed until the next growing season. We investigated the effect of two contrasting temperature regimes on early reactions of *Acer palmatum* trees to wounding during winter bud dormancy. Stems of *A. palmatum* trees were wounded and stored under an ambient temperature of 4 or 15 °C for 3 weeks during winter bud dormancy. We then studied wound reactions in the living bark, cambial region and xylem. In the 4 °C treatment, wound reactions were virtually absent. In the 15 °C treatment, however, trees reacted to wounding by dieback of the cortex and phloem and by the formation of ligno-suberized layers. In the cambial zone, cambial dieback occurred and callus tissue and wound xylem were formed locally, close to the wound margins. In the xylem, compartmentalization took place by deposition of inhibitory compounds in fibre cells and vessel elements. We conclude that temperature is an important factor in wound reactions during winter dormancy, and may even induce proliferation of callus and wound xylem within a 3-week period. It therefore seems likely that trees that have been wounded during dormancy in areas with mild or warm winters might cope better with wounding, as unlike trees in cold environments, they may compartmentalize wounds even during winter dormancy.

Keywords: *Acer palmatum* • Japanese maple • local xylem growth • temperature • winter dormancy • wound reactions.

3.2 Introduction

Trees have evolved effective defence mechanisms to protect their physiologically active xylem and phloem after wounding (Shigo, 1984; Pearce, 1996; Frankenstein et al., 2005b; Frankenstein et al., 2006; Deflorio et al., 2009). Whatever be the size or nature of the wound, the tree tends to react by forming boundary layers to compartmentalize the wound (Shigo, 1984; Bostock and Stermer, 1989; Pearce, 1996; Deflorio et al., 2009). When the living bark (cortex and phloem) is wounded, cells directly adjacent to the wound release inhibitory compounds and then die. Cells further away start forming a ligno-suberized layer, after which a wound periderm develops (Biggs, 1986; Oven et al., 1999; Renzi et al., 2012). If the cambial zone is affected by wounding, cambial cells around the wound die and adjacent intact cells react by forming callus tissue (traumatic parenchyma cells) and wound xylem to overgrow the wound (e.g. Schmitt and Liese, 1992; Grünwald et al., 2002; Stobbe et al., 2002; Dujesiefken et al., 2005; Frankenstein et al., 2005b; Copini et al., 2014). If wounds reach into the sapwood, parenchyma cells secrete inhibitory compounds, and distinctly coloured boundary layers start to form in the axial, radial and tangential directions (Shigo, 1984; Schmitt and Liese, 1992; Shortle et al., 1995; Pearce, 1996; Dujesiefken et al., 2005; Deflorio et al., 2009). In the discoloured zone, vessel elements may be blocked by tyloses, or by secretion of inhibitory compounds known as vessel plugs or gels (Murmanis, 1975; Bauch et al., 1980; Schmitt and Liese, 1992).

Wound reactions are temperature dependent and therefore there are clear differences between reactions that occur during the growing season and those occurring during winter dormancy (Murmanis, 1975; Armstrong et al., 1981; Shigo, 1984; Dujesiefken et al., 1991; Schmitt and Liese, 1992; Barnett and Miller, 1994; Liese and Dujesiefken, 1996; Dujesiefken et al., 2005; Copini et al., 2014). In temperate deciduous hardwood trees, wound reactions that entail cell dedifferentiation and cell proliferation (such as the formation of wound periderms, callus cells and wound xylem) occur only during the growing season (Trockenbrodt, 1991; Grünwald et al., 2002; Frankenstein et al., 2005b; Copini et al., 2014). In addition, whereas inhibitory compounds begin to be deposited in the xylem and living bark soon after wounding during the growing season (Schmitt and Liese, 1993; Fink, 1999; Grünwald et al., 2002; Frankenstein et al., 2005b; Copini et al., 2014), if this reaction occurs after wounding during winter dormancy, smaller amounts of compounds are involved and the deposition tends to be restricted to the wound margins (Jurásek, 1958; Murmanis, 1975; Schmitt and Liese, 1992; Copini et al., 2014). Regardless of the season of wounding, the cambium around the wound usually dies back; dieback tends to be more severe if the wound was incurred during winter dormancy (Smith 1980; Dujesiefken and Liese 1990; Dujesiefken et al., 1991, 2005; Copini et al., 2014). The development of cambial dieback over time has hardly been studied. So far, it has been shown that cambial dieback occurs within 2 weeks shortly before, during and just after the end of the growing season (Copini et al., 2014).

Here we report on an investigation of early wound responses of Japanese maple trees (*Acer palmatum* Thunb.) that were wounded and then exposed to a short period of mild temperature during winter dormancy. Japanese maple is native to Japan, Korea and China and is exported as an ornamental tree (e.g. to the USA and Europe). During export or storage and after planting in temperate or subtropical climates, the trees may experience contrasting temperatures during winter dormancy (Copini et al., 2010). We hypothesized that wound responses entailing cell proliferation such as formation of callus, wound xylem and wound periderms are absent during winter dormancy (Fink, 1999; Begum et al., 2007; Begum et al., 2013), while cambial dieback and deposition of inhibitory compounds followed by discolouration may occur during winter dormancy (Schmitt and Liese 1992; Copini et al., 2010).

3.3 METHODS

Plant material

We used 20 red-leaved Japanese maples (*A. palmatum* 'Bloodgood' Thunb.) with stem diameters of 3.2 ± 0.2 cm (mean ± standard deviation, n = 10) in 2010 and 4.2 ±0.7 cm (n = 10) in 2011 at ca. 30-cm stem height. The average tree height was 135 ± 15 cm in 2010 and 149 ± 19 cm in 2011. The trees had been grown at a local nursery, where they had been planted in pots in 2009, when the trees were ca. 5 years old. In March 2010, the potted trees were placed in a 2 × 2 m grid in an experimental garden in Wageningen, the Netherlands (51.98848N, 5.66448E). The trees were watered with a semi-automatic fertigation system.

Experimental setup

Ten trees were wounded by inserting a 1-mm diameter nail ca. 1 cm into the stem at ca. 30-cm stem height on 20 December 2010, ca. 2.5 months after leaf shedding. Directly after wounding, five randomly selected trees were placed in a dark climate chamber at 4 °C; the remaining five were also stored in a dark climate chamber, but at 15 °C. On 10 January 2011, a stem segment ca. 10 cm long that included the wound was cut from each of the 10 trees and stored in a 50% ethanol solution at 4 °C. One year later, between 20 December 2011 and 10 January 2012, a new batch of 10 trees was subjected to identical treatments, using identical climate chambers.

Sample preparation

All 20 stem sections were sawn transversally through the middle of the wound. Using the G.S.L.-1 sliding microtome (Gärtner et al., 2014) we then cut transverse thin sections (20 – 25 mm) through the wounded part. All cross-sections were stained with a safranin/astra-blue solution for 5 min, to colour unlignified cells blue and the lignified cells and cells filled with inhibitory compound red (Gärtner and Schweingruber, 2013). Additional samples were immersed in potassium hypochlorite (5%) for ca. 8 min, then rinsed with water and stained with (i) a safranin/astra-blue solution to stain unlignified cells blue and lignified cells red (Gärtner and Schweingruber, 2013) or (ii) Sudan III, in order to indicate suberine. Following dehydration in graded series of ethanol (50 – 95 – 100%), all samples were rinsed with xylol, mounted on microscope slides in Canada balsam and dried in an oven at 60 °C for 15 h. Photographs were taken with a digital camera (DFC 320, Leica, Cambridge, UK) mounted on a microscope (DM2500, Leica), using Leica imaging software (version 3.6.0).

Measurements and statistical analyses

We examined all the treated thin sections and recorded the presence or absence of the formation of ligno-suberized layers and wound periderms in the living bark (Fig. 3.1). All other analyses were based on thin sections that were stained with safranin/astra-blue solution and not treated with potassium hypochlorite. In the cambial zone, we measured the extent of tangential cambial dieback as the distance between the wound and the intact cambium (Fig. 3.1), using the software Leica Application Suite (version 3.6, Heerbrugg, Switzerland). In addition, the presence of callus tissue (traumatic parenchyma cells) and the locally present wound xylem were recorded after the outermost tree-ring boundary (TRB) had been located (Fig. 3.1). We used Leica Application Suite software (version 3.6) to determine the mean tangential width of xylem discolouration from measurements taken left and right of the wound (Fig. 3.1). Differences in wound response between the warm and cold treatments were tested per winter with the statistical software package SPSS version 19 (SPSS, Inc., Chicago, IL, USA), applying a significance level of 0.05. The effects of temperature on cambial dieback and xylem discolouration were analysed using the non-parametric Mann– Whitney U-test; the presence of ligno-suberized layers, callus and wound xylem was analysed using Pearson's Chi-square tests.

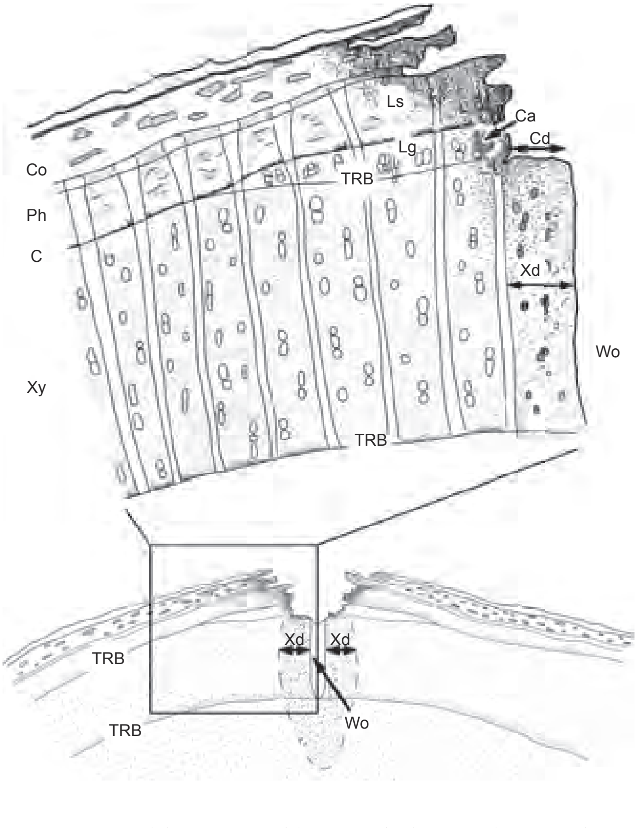

Figure 3.1. Schematic overview of the anatomical features which occur after wounding (Wo, wound) and were recorded or measured in the phloem (Ph), cortex (Co), cambial region (C) and xylem (Xy). In the living bark, i.e. phloem and cortex, we recorded the presence of ligno-suberized layers (Ls) and wound periderms. In the cambial zone, we measured the extent of tangential cambial dieback (Cd) and recorded the presence of callus (Ca) and local growth (Lg) of wound xylem, which develops after the TRB has been formed. In the xylem, we measured the extent of tangential xylem discolouration (Xd) left and right of the wound (Wo).

3.4 RESULTS

All buds remained dormant during both the 3-week experiments (winters of 2010/2011 and 2011/2012).

In the living bark, i.e. phloem and cortex, no wound periderms were observed. Nevertheless, during both the winters ($P < 0.001$), we detected the formation of a ligno-suberized layer between the wounded and intact living bark in all the trees subjected to the warm treatment (Fig. 3.2A). This layer was absent in the trees subjected to the cold treatment. Furthermore, we observed that discolouration in the phloem and cortex was minor in trees subjected to the cold treatment (Fig. 3.3A), but appreciably greater in trees from the warm treatment (Fig. 3.2A).

In both winters, the tangential extent of cambial dieback after the 3-week experiments was significantly less ($P = 0.009$) in the cold treatment than in the warm treatment (Fig. 3.4). In the warm treatment, cambial dieback extended on average 1050 ± 257 µm (mean ± SD, $n = 10$) while in the cold treatment it was virtually absent, with an average value of 84 ± 96 µm ($n = 10$); in two trees from the cold treatment there was no cambial dieback (Fig. 3.3A). Only in trees stored at 15 °C, few irregularly shaped callus cells had formed near all the wound margins ($P = 0.001$) (Fig. 3.2B). In that treatment, the cambium of all trees was locally reactivated and formed wound xylem ($P < 0.001$) over a radial distance of ca. 0.7 – 1.5 µm near the wound margin within 3 weeks of wounding. Within that period, some vessels and fibre cells lignified (Fig. 3.2C) but others did not (Fig. 3.2D). Vessels were mostly clustered and were smaller in area than the vessels that had formed during the previous growing season (Fig. 3.2D). In the 4 °C treatment no wound xylem was formed (Fig. 3.3A and B).

In the injured xylem, there was a large difference ($P < 0.001$) between the warm and cold treatments in the amount of tangential discolouration on either side of the wound that resulted from the deposition of inhibitory compounds (Figs 3.2E, 3.3C and 3.4). In trees stored under warm conditions, xylem discolouration extended laterally from the wound for an average of 1550 ± 219 µm (mean ± SD, $n = 10$), whereas in trees kept under cold conditions no discolouration occurred (Figs 3.2E, 3.3C and 3.4). Generally, discolouration of cell walls occurred close to the wound, while with increasing distance from the wound and closer to the unwounded xylem, many vessel and fibre lumens were blocked by inhibitory compounds (Fig. 3.2E and F).

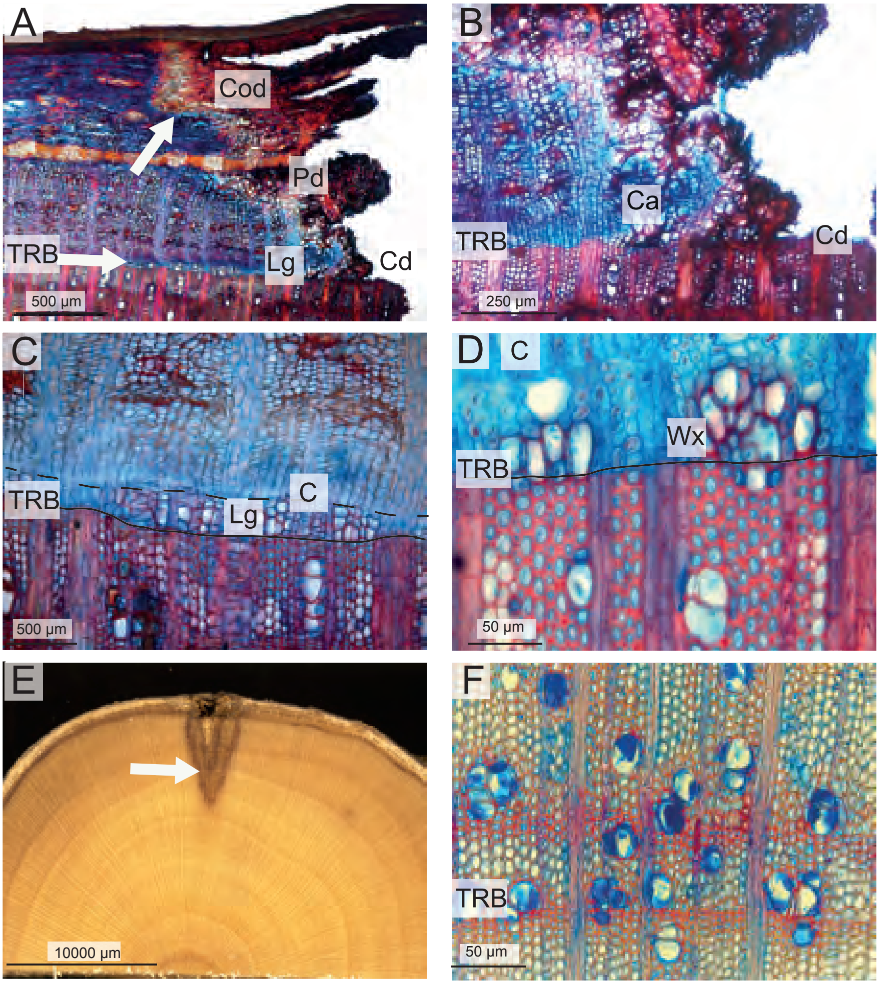

FIGURE 3.2. Wound reactions of dormant *A. palmatum* after being stored for 3 weeks in a climate chamber at a temperature of 15 °C. (A) Thin transverse section showing Cd and Lg resulting from the formation of wound xylem to the right of the lower arrow. The upper arrow indicates the ligno-suberized layer that is developing between the area with phloem discolouration (Pd), cortex discolouration (Cod) and the living phloem and cortex, to compartmentalize the wound in the phloem and cortex. (B) Thin transverse section showing callus formation (Ca) in relation to the TRB and Cd. (C) The reactivated cambium has formed wound xylem locally near the wound margin in between the TRB (solid line) and the cambium (c) above the dashed line. (D) Partly lignified and unlignified vessels. Fibre cells and ray parenchyma are still unlignified below the cambium (c). (E) Transverse section through the wood and the bark surrounding the wound; the arrow indicates xylem discolouration produced by secretion of inhibitory compounds. (F) Transverse section showing secretion of inhibitory compounds into vessels and fibre lumen close to the intact xylem, as indicated by the arrow in Fig. 3.2E.

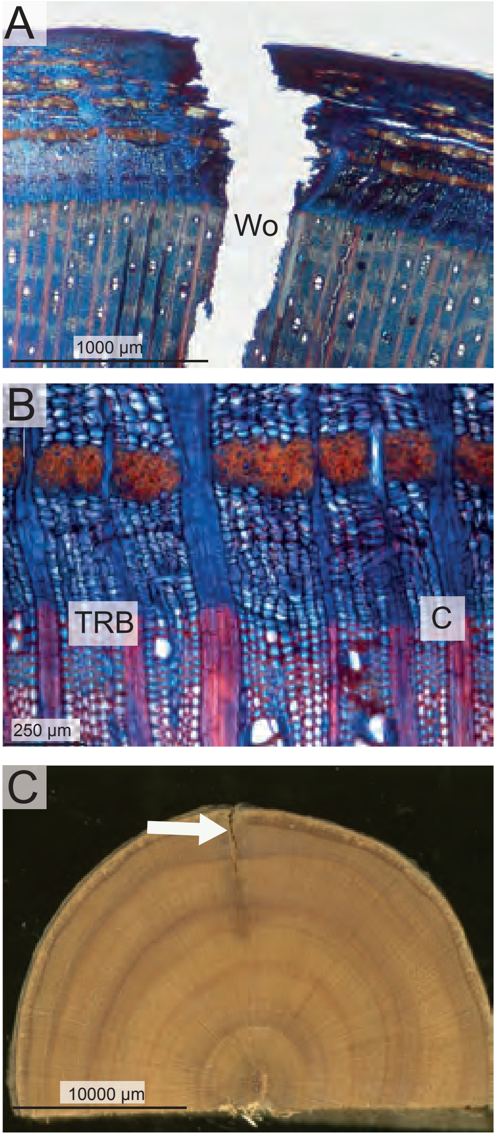

Figure 3.3. Wound reactions of dormant *A. palmatum* trees after being stored for 3 weeks in a climate chamber at a temperature of 4 °C. (A) Xylem and bark surrounding the wound (Wo). The virtual absence of Cd is characteristic for this cold treatment. (B) Details of the cambial zone near the wound margin, showing the TRB and dormant cambium (c). (C) Transverse section through the wood and bark surrounding the wound (arrowed). Compartmentalization of the wound by inhibitory compounds is virtually absent.

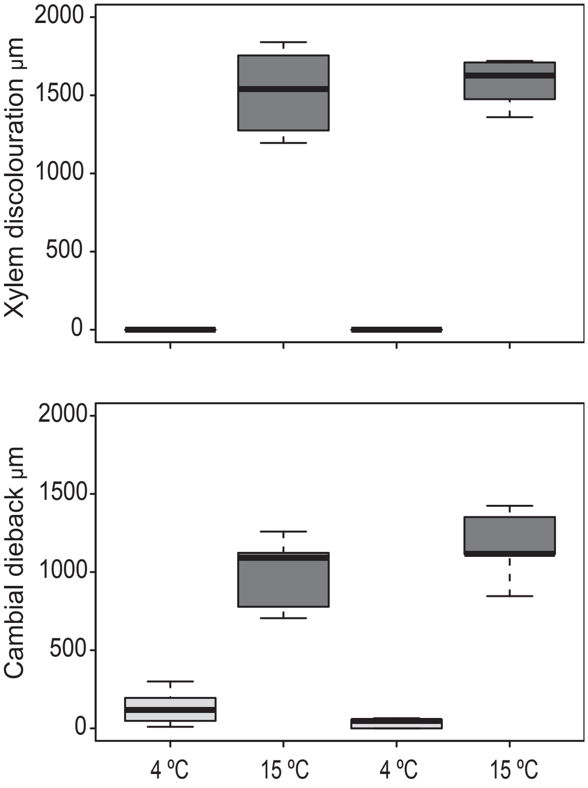

Figure 3.4. Boxplot showing the extent of tangential xylem discolouration (top) and cambial dieback (bottom) in trees (n = 5 per treatment) that were wounded and stored for 3 weeks under either a cold or a warm temperature regime in an experiment conducted during the winters of 2010/2011 and 2011/2012. During both the winters significant (Mann–Whitney U, $P < 0.01$) and consistent differences occurred in the extent of xylem discolouration and cambial dieback: at 4 °C hardly any cambial dieback or discolouration was present but at 15 °C cambial dieback and discolouration averaged 1050 and 1550 µm, respectively.

3.5 Discussion

Callus and local wood formation at 15 °C

When we examined the samples taken 3 weeks after the trees had been wounded during winter bud dormancy, we found, in contrast to our hypothesis, that the formation of callus cells and local formation of wound xylem had already occurred in trees kept at an ambient temperature of 15 °C. To our knowledge, xylem differentiation has never previously been reported in temperate deciduous trees during winter bud dormancy (Begum et al., 2013; Copini et al., 2014). Begum et al. (2007) reported that in unwounded trees kept under warm conditions (locally heated), xylem differentiation occurred only after bud break. This suggests that in our experiment the local formation of wound xylem in *A. palmatum* was triggered by a wound signal that is active only at a higher temperature. The wound signal might comprise hormones such as jasmonates and ethylene, which are important in wound closure (e.g. Seo et al., 1997; Bari and Jones, 2009; Ursache et al., 2013). In horticulture, cell proliferation following grafting has been reported in deciduous trees during dormancy, when grafted stem sections were exposed to temperatures between 24 and 27 °C while the roots and crown were kept at low temperatures (Lagerstedt, 1981; Hartmann, 2002). This indicates that our findings might be applicable to other deciduous species. In a garden experiment, Copini et al. (2014) found that *A. palmatum* trees that had been wounded in October when most leaves had abscised had formed no local wound xylem or callus 14 or 28 days later: in the 14-day period the average temperature was 7.3 °C (±2.7) and in the 28-day period it was 8.2 °C (±3.3). In the same study, *A palmatum* trees that had been wounded in March, just before the onset of bud burst and tree-ring formation, showed local wood and callus formation around wounds within 4 weeks during which the average temperature was 9.2 °C (±4.1). In addition, local formation of wound xylem was found in trees wounded at the end of August or September when the TRB had formed and the leaves were still fully developed or had begun to acquire their autumn colour (Copini et al., 2014). Why were local wound xylem and callus formation for wound closure both absent in October (Copini et al., 2014) but present in December under favourable temperature conditions (this study)? A possible explanation is that the trees are in transition from a resting stage (endodormancy) during October to a quiescent stage of dormancy (ecodormancy) in December (Perry, 1971; Lang et al., 1987; Begum et al., 2013).

Wound-periderm formation

In samples from the warm treatment we detected the formation of ligno-suberized layers between the living and wounded bark, which form before the wound periderm develops (Biggs, 1986; Trockenbrodt and Liese, 1991; Trockenbrodt, 1994; Woodward and Pocock, 1996). Other studies have shown that wound periderm formation stops in October and resumes during March of the following year (Deflorio et al., 2009; Copini et al., 2014). During the growing season, a ligno-suberized zone can form quickly—within 1 – 3 weeks—in *Populus tremula*, *Platanus × acerifolia*, *Salix caprea*, *Tilia tomentosa*, *Sorbus* *aucuparia*, *Acer pseudoplatanus* and *Betula pendula* (Trockenbrodt and Liese, 1991; Trockenbrodt, 1994; Woodward and Pocock, 1996), as happened in our study. To our knowledge, however, there have been no previous reports of ligno-suberized layers being formed during winter dormancy.

Cambial dieback is temperature dependent

We found that the extent of cambial dieback in response to wounding during dormancy depends on temperature. The virtual absence of cambial dieback in trees stored under cold conditions during dormancy is unexpected, as in an earlier study (Copini et al., 2014), cambial dieback in *A. palmatum* trees wounded between March and October, i.e. shortly before, during and after the growing season, always occurred within 2 weeks of wounding. To the best of our knowledge, the absence of cambial dieback after wounding during winter dormancy has never previously been reported. In contrast, studies on wound reactions in *A. pseudoplatanus*, *B. pendula*, *Fagus sylvatica* and *Fraxinus excelsior* have found that more cambial dieback is measured at the end of the growing season after wounding during winter dormancy (December) than after wounding in autumn and spring (Dujesiefken and Liese, 1990; Dujesiefken et al., 1991). This implies that cambial dieback in *A. palmatum* wounded during winter dormancy is delayed until the temperature rises again. As the extent of cambial dieback is highly correlated with xylem discolouration (Fig. 3.4) and with discolouration observed in the phloem and cortex, we assume that cambial dieback is a temperature-dependent physiological process associated with xylem and bark discolouration.

Compartmentalization caused by inhibitory compounds in the xylem

We observed compartmentalization of the wound by deposition of inhibitory compounds in fibre cells and vessel elements only in the trees stored at 15 °C. This is consistent with a finding reported by Schmitt and Liese (1992) for a fi experiment: that during winter dormancy fibrillar inhibitory material may be secreted into fibres and vessel elements within 4 weeks of wounding. They found that whereas *B. pendula* was able to continue secretion throughout the winter, in *Tilia americana* there was no further secretion after February or March wounding. When they performed a laboratory experiment in which they stored wood samples at 4 °C in June for 3 weeks, they found that *T. americana* was unable to secrete fibrillar material—which is in line with our results—whereas *B.*

pendula was able to secrete inhibitory compounds (Schmitt and Liese, 1992). This confirms that compartmentalization by inhibitory compounds is a temperature-dependent physiological process similar to the formation of ligno-suberized layers, callus and wound xylem, which are all delayed at low temperatures during winter dormancy. Given that compartmentalization of tree wounds restricts moisture loss and damage from pathogens (Shigo, 1984; Mireku and Wilkes 1989; Pearce, 1996; Fink, 1999), it thus seems likely that trees in areas with mild winters might cope better with the effects of wounding than trees in areas with cold winters.

3.6 Conclusions

We conclude that *A. palmatum* trees are able to start wound compartmentalization in the living bark, cambial zone and xylem within 3 weeks under mild (15 °C) ambient temperature during winter bud dormancy. Wound reactions entail phloem, cortex and xylem discolouration by the secretion of inhibitory compounds, and the formation of ligno-suberized layers, callus and local wound xylem. At low temperatures, wound reactions, including necrosis of cambial cells, are virtually absent and are likely delayed until the temperature rises again. It therefore seems likely that trees that are wounded during winter dormancy in areas with mild or warm winters will be better able to cope with wounding because, unlike trees in cold environments, they can compartmentalize wounds even during winter dormancy.

Acknowledgements

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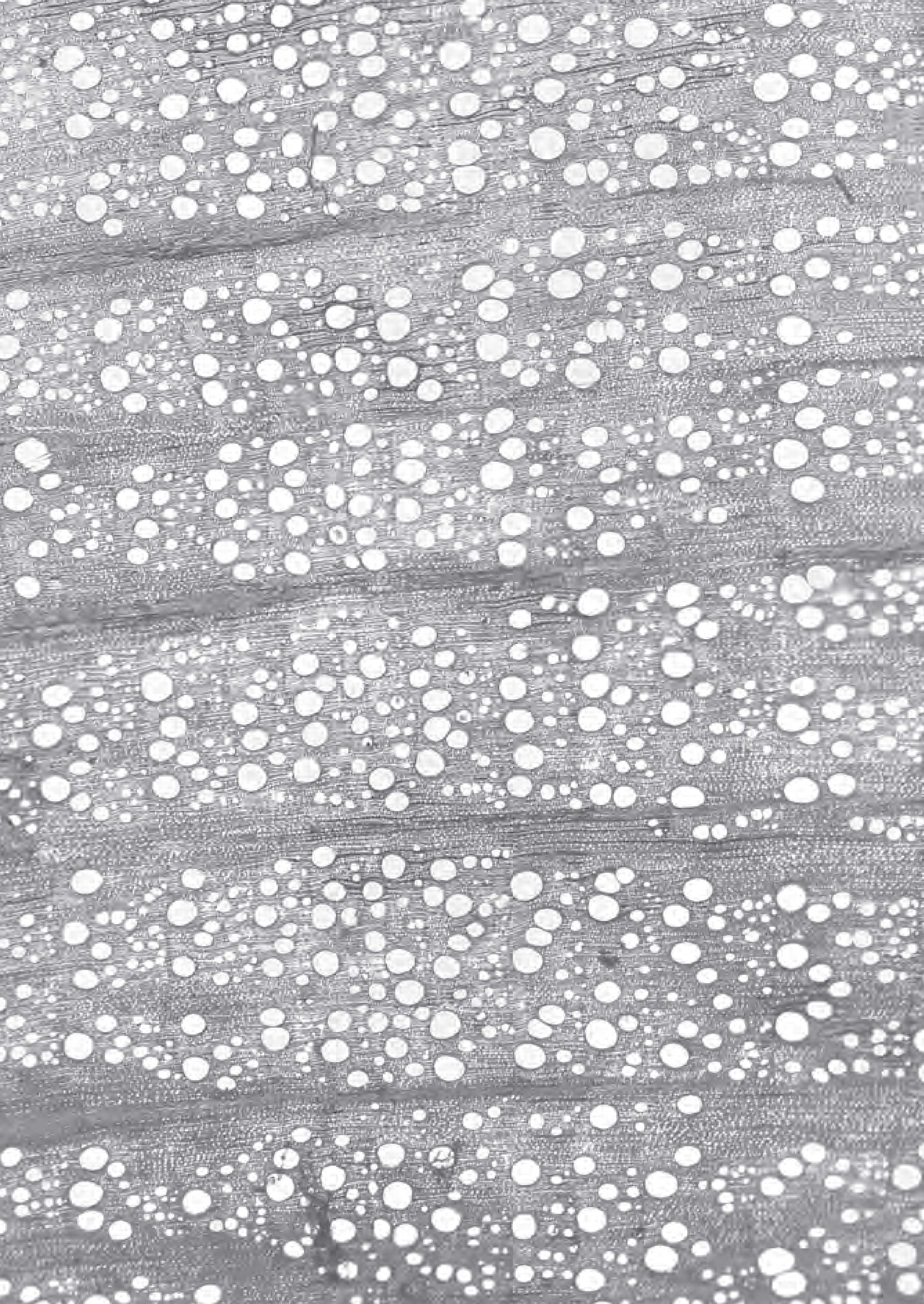

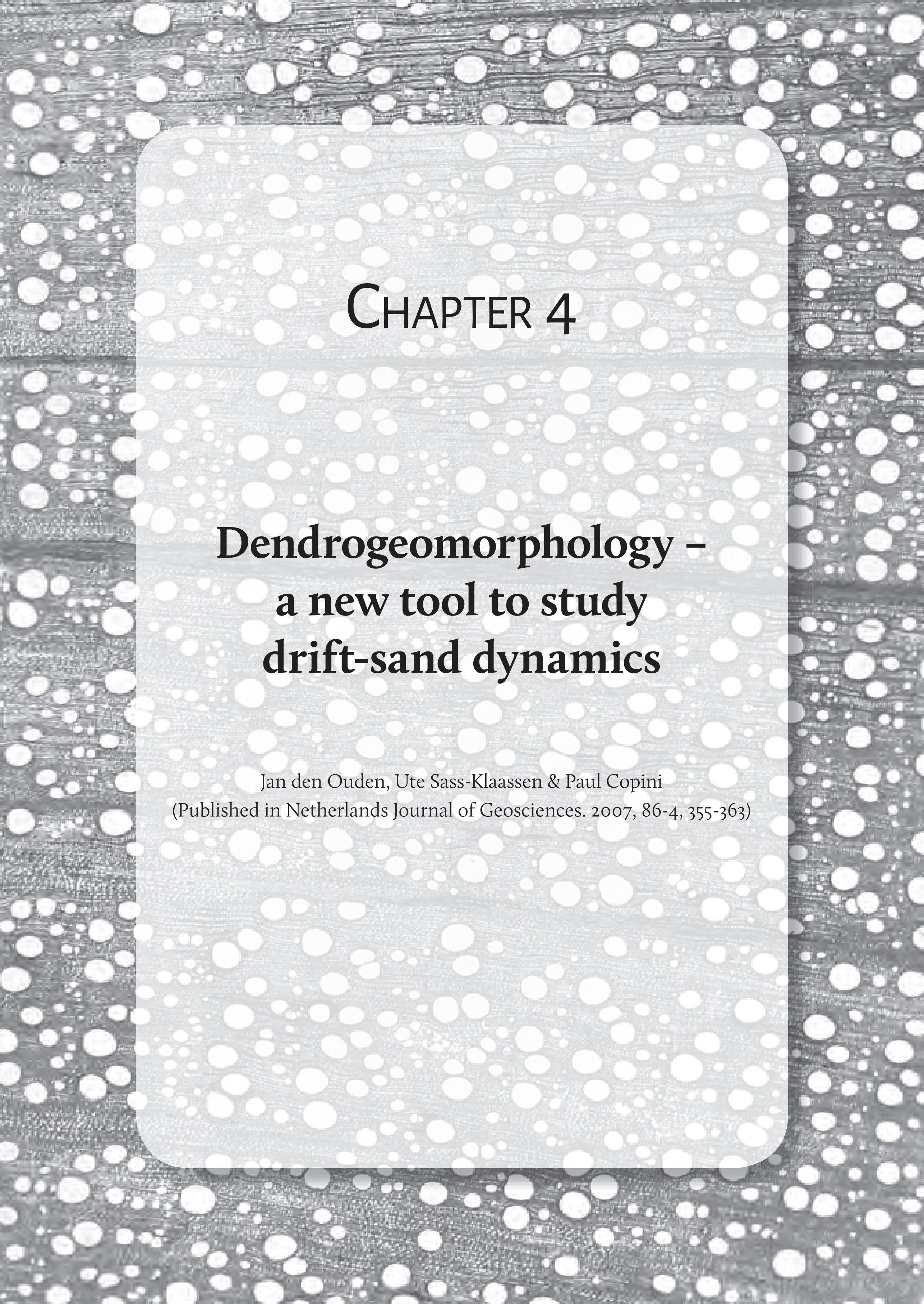

CHAPTER 4

Dendrogeomorphology – a new tool to study drift-sand dynamics

Jan den Ouden, Ute Sass-Klaassen & Paul Copini
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4.1 ABSTRACT

A dendrogeomorphological approach is presented, using wood characteristics of native oak (*Quercus robur* L.) to infer dynamics of aeolian sediment transport in drift-sand areas. Wood samples, taken from oaks in two drift-sand areas, were analysed to study changes in tree-ring pattern and wood anatomy as a consequence of burying or exposure from drift sand. In all cases, the wood of the sampled oaks showed sudden changes in anatomy and tree-ring width due to burial by drift sand or subsequent exposure after erosion of the new soil surface. After aerial stems became covered by drift sand, the wood lost its characteristic ring-porous features, and tree rings became strongly reduced in width with less distinct ring boundaries. Buried stems that became exposed after erosion showed an abrupt increase in ring width and turned distinctly ring porous again. Roots that were exposed also adopted clear ring-porous features, increased in ring width and anatomically resembled aerial stem wood. Using tree-ring analysis, it is possible to precisely date sand deposition and erosion events by detecting the concurrent changes in anatomy of woody structures. This study indicates the high potential of dendrogeomorphology as a tool to study drift-sand dynamics with a high temporal, i.e. annual, resolution for a period going back as long as the maximum age of the trees present (in this study at least 250 years). Since the signals of past deposition and erosion events are conserved in the wood, this is the only method that can be used to reconstruct drift-sand dynamics when the actual landforms are no longer present.

Keywords: drift sand • erosion • *Quercus robur* • tree-ring analysis • wood anatomy

4.2 INTRODUCTION

The Netherlands contain the largest area of active drift sands in northern Europe, but this area is rapidly declining. During the past centuries, the area of active drift sand has decreased from approximately 800 km^2 in 1850 and 40 km^2 in 1980 to only 15 km^2 in 2000 (Bakker et al., 2003; Riksen et al., 2006 Fig. 4.1). Large-scale afforestations, changes in land use, and increased nitrogen deposition have all contributed to the rapid consolidation of active drift sands. Concurrently, species related to drift sands have become rare or locally extinct (Bal et al., 2001). To curb this trend, drift sand areas have been designated a protected habitat type within the European Natura 2000 network. Many efforts are now made to conserve the last remaining drift-sand areas, or restore the consolidated drift sand by removing all vegetation and topsoil, and clearing surrounding forests to increase landscape erodibility.

The future development of the current drift-sand areas or the potential of their successful restoration depend on a better understanding of those external factors that are driving the system: climate, landscape erodibility and land use (Koster, 1978; Bakker et al., 2003; Riksen et al., 2006). There are, however, no long-term data sets available on the direct relation between these factors and aeolian activity. The reconstruction of sediment transport and erosion, based on dated events in the past, allows studying dynamics of aeolian activity in retrospect, and linking it to climatic events and changes in landscape structure.

The large Dutch drift sands originate from the medieval period (Koster, 1978). Their origin could be dated with conventional dating techniques, such as 14C, of the palaeosoils underneath the drift sand deposits. However, 14C dating of dune formation is only possible if enough carbon has accumulated in the overblown surface. This implies that between vegetated periods, gradual build-up of the dunes cannot be followed due to the lack of suitable organic material for 14C dating. Combined with the wide confidence interval, this makes 14C dating not suited to answer questions related to fine scale processes within the drift-sand system that vary on an annual to decadal scale. The recently developed technique of Optically Stimulated Luminescence (OSL) dating has made it possible to study sand-deposition phases with considerably higher accuracy (Ballarini et al., 2003; Wallinga et al., 2007).

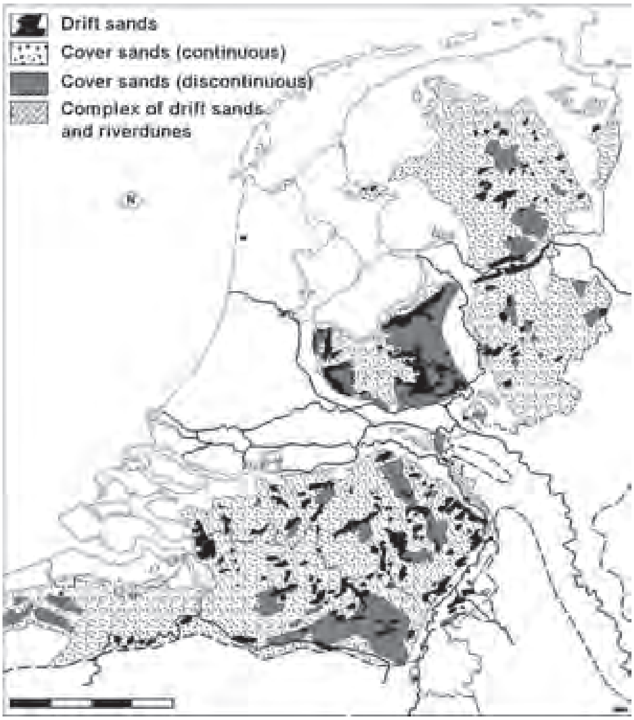

Figure 4.1. The expanses of drift sand areas in the Netherlands and northern Belgium, within the matrix of cover sands from which these drift sands originated. Most drift sands are now consolidated. The locations of the research sites are indicated by DS (Deelensche Start) and LDD (Loonse en Drunense Duinen). Figure after Koster (2005).

In this paper we propose a novel method for dating drift-sand dynamics using the anatomical changes in the wood of trees that are (partially) covered by drift sand, or have become exposed after erosion. The detection of abrupt changes in wood anatomy in combination with dendrochronology allows dating deposition and erosion events to the exact year. This will enable us to reconstruct aeolian activity with unprecedented precision. The application of dendrochronological techniques in geomorphology has led to the development of dendrogeomorphology (e.g. Alestalo, 1971; Gärtner et al., 2001; Bodoque et al., 2005). Especially in mountainous areas, studies on coniferous tree species like Norway spruce (*Picea abies* (L.) Karst.), European larch (*Larix decidua* P. Mill.) and Scots pine (*Pinus sylvestris*) have been able to exactly date and reconstruct geological events such as landslides and avalanches. This was done by using sudden changes in the wood anatomy of roots that occurred after exposure as time markers (Gärtner et al., 2001; Gärtner, 2003; Bodoque et al., 2005;

Gärtner, 2007). Also, the covering of stems and roots by sand results in distinct changes in the wood anatomy. This enables the detection of the burial time with approximate annual precision (Cournoyer and Filion, 1994).

Recently, the possibilities of using broadleaved species have been explored to reconstruct erosion effects on ash (*Fraxinus excelsior* L.) and beech (*Fagus sylvatica* L.). These studies were able to date the exact year of exposure (Sahling et al., 2003; Hitz et al., 2008b). To our knowledge, no research has yet been conducted to test whether broadleaved species can be used to study the time of burial due to geomorphic events. In this paper we explore the potential of dendrogeomorphology, using native oak (*Quercus robur* L.), to reconstruct aeolian sediment transport in drift-sand areas with high accuracy. Specific questions we address are (i) what are the effects of covering by sand and exposure on the wood anatomy of stems and roots of oaks, and (ii) can we exactly date the year of burial and/or exposure?

4.3 Methods

Trees in drift sand

Trees frequently establish in drift-sand areas, mainly in local areas where vegetation cover has developed in later stages of the succession. Oak (*Quercus robur*, *Q. petraea* (Matt.) Liebl.), Scots pine (*Pinus sylvestris* L.) and Birch (*Betula pendula* Roth) are the main species occurring in Dutch drift sand (Fanta, 1982). Birch has a short life span, leaving oak and pine as the most promising species to be used for a dendrogeomorphological study. Scots pine was imported on a large scale in afforestations of drift sand during the late 19th and first half of the 20th century. Older trees are usually oaks. These may be trees that have spontaneously established from seeds, or have been planted and managed to serve as sand traps (Buis, 1985). Oaks occur as single trees or clustered in groups in drift-sand areas (Fig. 4.2). The clusters of oak are characterised by having many stems growing closely together, and DNA-analysis has shown that such oak clusters consist of a single or a few different genotypes forming one or more clones (Copini, 2005). These clusters originate from oak shrubs or trees that have been partially covered by drift sand (Tesch et al., 1926; Stoutjesdijk, 1959; Copini, 2005).

FIGURE 4.2. Drift-sand area in the Loonse en Drunense Duinen (LDD) with scattered oaks. What appears as oak shrubs are the crowns of partially overblown trees.

FIGURE 4.3. Oak cluster in the Loonse en Drunense Duinen (LDD). The line follows the level where stems were thickened, indicating the former soil level. The four crosses below the line indicate sample locations at heights of 0.2, 0.4, 0.8 and 1.2 m. The sample taken at 0.4 m (cross at the right) is shown in Fig. 4.5. The crosses above the line indicate sample locations in stems that were not affected by drift-sand burial.

FIGURE 4.4. Two oak clusters growing on a dune ridge in the Deelensche Start (DS). The line follows the level where stems were thickened, indicating the former soil level. Top: The cross indicates the sample location of one of the two cores drawn from the structure, and presented figures 4.6 and 4.7B. Bottom: The arrows indicate the zones of adventitious roots (AR) that were formed after three sand deposition phases. The crosses indicate sample locations in two out of seven sampled stems that were not affected by drift-sand burial.

Study area and sampled trees

Two drift-sand areas were selected (Fig. 4.1): the Deelensche Start (DS) in the central part of the Netherlands (52°.05' N, 5°.50' E), and Loonse en Drunense Duinen (LDD) in the south (51°.40' N, 5°.05' E). In these areas, oak trees were present as clusters that appeared as having been covered by sand, and subsequently exposed again after erosion. These trees provided an excellent opportunity to study the effect of presumed burial and exposure on the wood anatomy in both stems and roots, as they could be easily accessed for sampling. In LDD we selected an oak cluster on an eroded nebkhar dune (Fig. 4.3). This cluster was 8 m in diameter and consisted of stems growing on a complex network of stem- and root-like structures. Distinctly thickened stem parts marked the transition between the stems and the lower network. Directly below these, remnants of adventitious roots were present, so we assumed that the area directly below the thickened parts represented a former soil surface (Fig. 4.3; see also Marin & Filion, 1992; Strunk, 1997; Maun, 1998). In DS, we selected oaks growing on a dune ridge (randwal). These oaks formed small clusters, consisting of several stems, and also showed a transition between aerial stems and a network of stem and root-like structures marked by a thickening of the stem, below which point adventitious roots were present at several heights. Analogous to LDD, we assumed that these trees were exposed by erosion after they had been buried by drift sand in a previous phase (Fig. 4.4).

Sample collection and processing

Samples were collected in the winter 2005/2006 using an increment corer (essentially a hollow drill). From the selected trees, a series of samples was taken from 0.4 m above the thickened stem parts (above the line in Figs 4.3 and 4.4), representing stem growth that was not influenced (unaffected) by drift-sand burial or exposure. From the oak cluster at LDD we took samples from three unaffected stems and from DS seven unaffected stems were sampled.

The second series of samples was taken from parts of the structure below the thickened stems, (below line in Figs 4.3 and 4.4), and were assumed to be affected by drift-sand covering and subsequent exposure after the soil surface eroded. In LDD, two cores were collected from opposite directions at a stem-like structure at ca. 0.4 m above the current soil surface (Fig. 4.3). Both cores extracted at this position contained all tree rings from pith to bark allowing us to date (changes in) the wood structure over the entire lifetime of this structure.

Along another stem-like structure located in the inner part of the cluster we collected samples at 0.2, 0.8 and 1.2 m above the current soil surface (see Fig. 4.3).

At DS two cores were collected from a stem-like structure approximately 0.8 m below the thickened stem part at opposite sides of the structure (Fig. 4.4).

Detecting changes in wood anatomy

The surface of all wood samples was carefully prepared with razor blades in order to make the tree rings well visible. Visual inspection of the wood anatomy was done under a Leica stereomicroscope (magnification from 20 to 120 times). The wood anatomy of oak stems (aerial parts) is characterized by very large vessels in the earlywood (produced just before the start of the growing season), making it distinctly ring porous. In the latewood, produced in the summer, smaller vessels are arranged in flame-like groups (Fig. 4.7B1). In root wood (subterraneous parts), the ring-porous feature disappears (Fig. 4.7A1). Also, the root either lacks a central pith, or the pith has a clearly different shape as compared to the stem wood (Schweingruber, 2001). However, near the root collar the difference between stem and root anatomy is often gradual. All tree rings were inspected for distinct changes in wood anatomy. The exact year in which changes in wood anatomy were detected was determined by counting back from the outermost ring, which was formed in 2005.

Tree-ring analysis

Tree-ring widths were measured to the nearest 1/100 mm using dendrochronological measuring equipment (LINTAB, TSAP; Rinn, 1996). Of the previously buried stems, only one sample per site was available that covered the entire radius of the stem, including the juvenile part in the centre. Of the remaining samples, dating was confined to the youngest, outer part of the radial as the inner part included rotten segments. The tree-ring series were visually and statistically (cross-)dated using the programs TSAP (Rinn, 1996) and COFECHA (Holmes, 1983; Grissino-Mayer, 2001). Subsequently, chronologies (= mean curves) were calculated per oak cluster from the unaffected and previously buried parts to check for differences in growth pattern and to exactly date the tree rings where changes in wood anatomy were observed.

Table 4.1. Average ring width from wood samples taken from oaks growing in two drift-sand areas. The oaks grew as multi-stemmed clusters, and showed distinctly thickened stems. Samples were taken from stems above, and from the structures below, these thickenings. In the lower samples, three distinct phases were present with different wood anatomy. They were identified as aerial stems that became buried and were later exposed again after erosion (aerial phase 2).

Sample location	Loonse en Drunense Duinen			Deelensche Start		
	N	Period represented in samples	Ring width (mm ± SD)	N	Period represented in samples	Ring width (mm ± SD)
Stems above thickening	3	1927-2005	0.90 ± 0.62	7	1919-2005	1.36 ± 0.72
Structure below thickening Total	1	1855-2005	0.62 ± 0.50	1	1755-2005	0.55 ± 0.54
Aerial phase 1		1855-1916	0.79 ± 0.60		1755-1823	1.58 ± 0.91
Buried phase		1917-1965	0.28 ± 0.05		1824-1995	0.29 ± 0.11
Aerial phase 2		1966-2005	0.79 ± 0.43		1996-2005	1.16 ± 0.57

4.4 RESULTS

For both study sites, all samples from the upper, unaffected stem parts showed the typical anatomy of aerial stem wood with a ring-porous structure. One sample from LDD contained the central pith, which enabled exact age determination of the stem to 79 years. The other samples from LDD were broken and missed the inner rings, but due to similarity in stem morphology and tree-ring pattern these stems most likely originated at the same time, i.e. around 1927 (Table 4.1). Most of the seven stems at DS also lack the pith but the maximum number of tree rings counted in one stem amounts to 87, indicating initiation of the unaffected stems around 1919 (Table 4.1). The average tree-ring width of the unaffected stems was 0.90 mm ± 0.62 mm (mean ± SD) for the three stems at LDD, and 1.36 ± 0.72 mm for the seven stems at DS (Table 4.1). The mean tree-ring series of the oaks from LDD and DS are illustrated in Figures 4.5 and 4.6 (blue line) respectively, and show that the growth level of the oaks is relatively low with the exception of the first ca. 10 years where very wide tree rings were formed. This means that the unaffected stems started to grow very fast in the beginning.

The stems below the thickened parts of the two clusters at LDD and DS differed in age. The oldest dated stem at LDD contains 150 tree rings and hence dates back to 1855 (Fig. 4.5). At DS the sampled stems were exactly 100 years older, dating back to 1755 (Fig. 4.6). Three distinct phases with clear transitions in tree-ring width and wood anatomy can be distinguished in both sites (Table 4.1, Fig. 4.5 and 4.6). First, a (juvenile) phase is present with extremely wide tree rings during the first ca. 10 years – comparable to the initial phase in the unaffected stems (blue line) – followed by the formation of somewhat smaller rings (Fig. 4.7). The wood that was formed during this first 50 to 60 years in both sites has a ring-porous structure and clearly resembles stem wood (Fig. 4.7).

The start of the second phase is marked by a transition in both tree-ring width and wood anatomy, and occurred in 1917 in LDD and in 1824 in DS. In both sites mean tree-ring width decreased considerably (below 0.3 mm, Table 4.1) with the consequence that the annual variation is clearly reduced (Fig. 4.5 and 4.6, red lines). Latewood formation is strongly reduced and mean vessel area declined (Fig. 4.5 and 4.6) which makes that the tree-ring structure ultimately became diffuse porous during this second phase (Fig. 4.7).

The third phase started in 1966 at LDD and in 1996 at DS. Again, a clear transition occurred with pronounced changes in tree-ring width and wood anatomy: tree-ring width abruptly increased and regained values similar to the period before the long-term growth depression (Table 4.1). The tree rings became clearly ring porous again and the average size of the earlywood vessels increased (Fig. 4.7).

In LDD, only single wood samples were collected at 0.2, 0.8 and 1.2 m above the current soil surface underneath the thickened stem parts (Fig. 4.5). As the inner, juvenile parts of these cores were partly broken, exact dating was impossible. However, we were able to visually discern changes in wood-anatomical features in the juvenile part. We were able to date the year in which the second transitions took place. The innermost part of the sample taken at 0.2 m above the current soil surface clearly contained root wood (Fig. 4.7A1) whereas the two samples taken at 0.8 m and 1.2 m above the current soil surface started growing as aerial stems. All three samples also showed two distinct transitions in wood anatomy and ring width. As in the earlier described sample (Fig. 4.5, red line and photographs) the first transition is characterized by a sharp decrease in ring width together with a drastic decrease in the amount of latewood. Vessel size decreased considerably during a long-lasting growth depression (Fig. 4.7A2). The second transition in all samples showed again a strong increase in ring width, with the wood becoming clearly ring porous (Fig. 4.7A3). This second transition was dated to 1978, 1948 and 1946 for the samples collected at 0.2, 0.8, and 1.2 m height respectively.

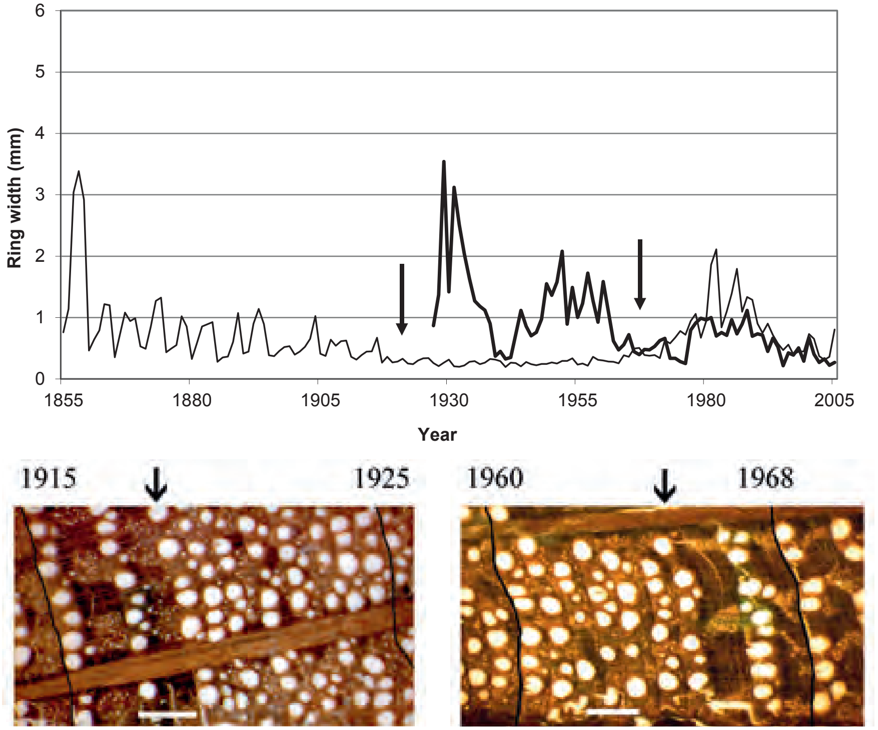

Figure 4.5. Changes in ring width and anatomy in the wood of a stem-like structure of an oak (*Quercus robur*) in the Loonse en Drunense Duinen (LDD). Top: Cluster chronology (1927 till 2005) of three stems sampled above the thickened stem parts (see Fig. 4.3), and the average ring width (1855 - 2005) from two samples drawn from a woody structure below the thickened stem parts, 0.4 m above the current soil surface. The arrows indicate the years in which transitions in wood anatomy occurred. Bottom: Photographs of the transition zones where normal stem wood changed into buried stem wood and vice versa. The numbers indicate the year in which the first and last complete visible tree ring on the photograph was formed. The arrow indicates the point where the transition occurred. Growth proceeds from left to right.

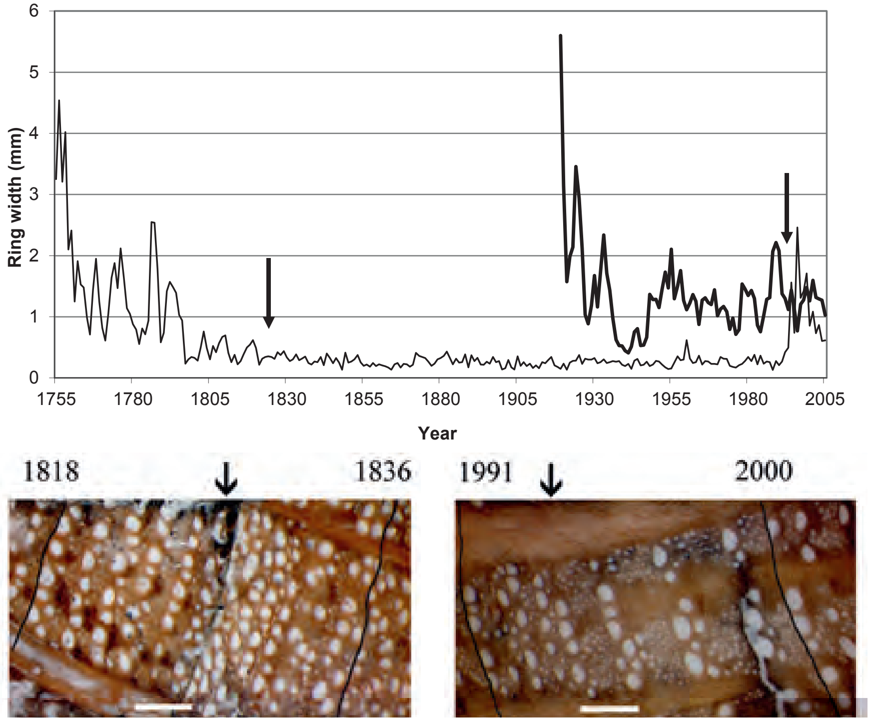

Figure 4.6. Changes in ring width and anatomy in the wood of a stem-like structure of an oak (*Quercus robur*) in the Deelensche Start (DS). Top: Cluster chronology (1919 till 2005) of seven stems sampled above the thickened stem parts (see Fig. 4.4), and the average ring width (1755 - 2005) from two samples drawn from a woody structure below the thickened stem parts. The arrows indicate the years in which transitions in wood anatomy occurred. Bottom: Photographs of the transition zones where normal stem wood changed into buried stem wood and vice versa. The numbers indicate the year in which the first and last complete visible tree ring on the photograph was formed. The arrow indicates the point where the transition occurred. Growth proceeds from left to right.

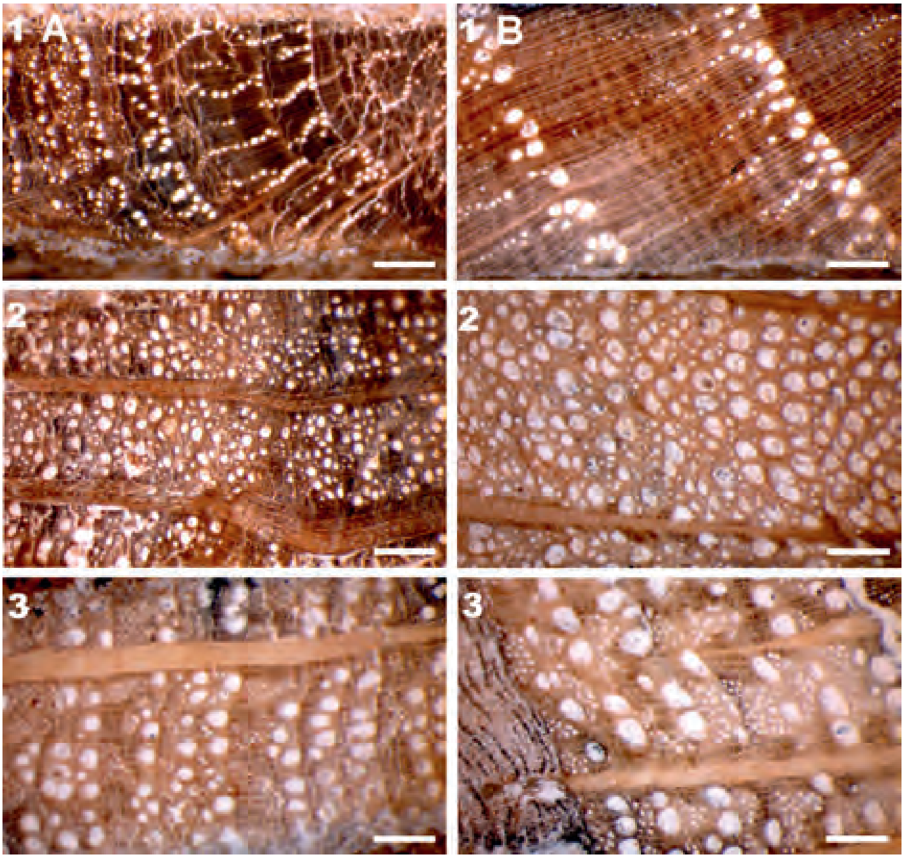

Figure 4.7. Changes in wood anatomy of oak (*Quercus robur*) as a result of burial by sand and exposure after erosion. The scale bars represent 1 mm. A. Wood anatomy of the root of an oak from the Loonse en Drunense Duinen (LDD). The sample was taken from the lowest sample position as indicated in Fig. 4.3; 1. Tree rings close to the pith with a clear root wood anatomy; 2. Long lasting growth depression (root wood) during the period in which the root was buried deeper after being covered by drift sand; 3. Distinct tree rings with a ring-porous anatomy are formed when the root became exposed after erosion. B. Wood anatomy of an oak stem from the Deelensche Start (DS). The sample was taken from the location indicated in figure 4.4 (top); 1. Tree rings around the pith with the distinct ring-porous anatomy of aerial stem wood; 2. Extremely narrow rings, resembling the anatomy of deep root wood, that were formed after the stem was covered by drift sand; 3. Distinctly ring-porous tree rings were formed again after the buried stem part was exposed again after erosion.

4.5 Discussion

Tree morphology

In both oak clusters (LDD and DS), the samples taken from below the thickened stem parts showed sudden changes in wood anatomy and ring width, while the samples above that point show no such changes and consisted of distinctly ring-porous wood across the entire radius of the stems. The aberration from normal stem or root wood in the lower stem parts indicates that these parts had experienced a change in the local environment. The narrow age range of the higher stem parts (above the line in Figs 4.3 and 4.4) suggests that these stems started growing around the same year. Together with the fact that very wide rings are formed during the first up to 10 years followed by a rapid decrease in tree-ring width (Fig. 4.5 and 4.6) this is a clear indication of simultaneous re-growth after cutting. This growth pattern is consistent with the re-growth pattern of sprouts on stumps (Copini et al., unpublished results). Based on the maximum ages, we estimate that the trees have been cut around 1927 in LDD and 1919 in DS. The thickened parts of the stems indicated the level where the former generation of stems was cut. As it can be assumed that cutting took place near the soil surface (Boer, 1857), the thickened stem part thus represents the approximate level of a former soil surface in 1927 (LDD) and 1919 (DS), respectively.

Wood-anatomical changes

The anatomy of the first-formed (juvenile) wood in the lower parts of the trees indicates that at the lowest sampled location of the oak cluster in LDD (Fig. 4.3), the structure started as a root (Fig. 4.7A1). At all other sampled locations in both sites, stems started growing as aerial shoots. The subsequent change in wood anatomy and ring width is consistent with changes that have been observed in stems that become buried by sand (Wieler, 1891; Heikkinen and Tikkanen, 1987; Filion and Marin, 1988; Marin and Filion, 1992; Strunk, 1997), or roots that are buried deeper in the soil. These changes in wood anatomy mainly comprise a decrease in the amount of latewood and distinct changes in early-wood vessel size (Fig. 4.7), reflecting a drastic change in cambial activity. It is not clear yet as to what environmental variables bring about these changes, but these may include reduced temperature and/or oxygen levels or the increased pressure by the covering substrate (Fayle, 1968; Marin and Filion, 1992; Cournoyer and Filion, 1994; Gärtner et al., 2001; Gärtner, 2003). Marin & Filion (1992) showed that when stems of *Picea glauca* were re-exposed after burial, they started producing rings that were approximately of the same width as rings produced before burial. This is consistent with the second transition found in our samples from the lower part of the trees at LDD and DS.

Potential for dating drift-sand dynamics

The changes in wood anatomy induced by burial or exposure of stems or roots occur most likely instantaneously. Changes in ring width may show a delay of several years (Cournoyer & Filion, 1994). Our data indicate that exposure of previously buried stems and roots is expressed by a simultaneous change in wood anatomy and ring width (Figs 4.5 and 4.6). This allows exact dating of erosion events and erosion rates. At LDD, the locations at 1.2 and 0.8 m above the current soil surface (Fig. 4.5) were exposed in 1946 and 1948 respectively, which yields an erosion rate of 0.2 m.y–1. The lowest sample location at 0.2 m became exposed in 1978, indicating that erosion rates decreased to 0.02 m.y–1.

A period of sand deposition, resulting in the covering of stems or deeper burial of roots did result in a simultaneous change in wood anatomy and ring width in LDD. However, in DS prior to the first transition starting at 1823, radial growth had already decreased a number of years before the anatomical change occurred in the wood (Fig. 4.6). This difference is probably induced by one or more sand deposition events prior to the covering of the stem at the sampled location in the structure. At this moment it remains unknown how changes in wood anatomy and ring width in the stem or root are related to the exact position of the new soil surface. Detailed observations and experimental research are needed to determine within-tree variation in wood anatomy and radial growth along the vertical axis of the stem and at different heights above, and depths below, the exact level of sand deposition.

Although only a limited amount of samples was taken, this pilot study has demonstrated that the history of covering and exposure of stems and roots is recorded in the wood of oaks growing in drift-sand areas. Changes in wood anatomy can be dated using dendrochronological techniques, resulting in a great potential to study the dynamics in drift-sand areas. Using this dendrogeomorphological approach, dune formation and erosion can be reconstructed in areas where trees are present and may allow for the reconstruction of aeolian activity with annual precision. An additional advantage of this method is the fact that the 'signals' of past deposition and erosion events are conserved in the wood. The records of past deposition and erosion events therefore remain present in the tree, even when the sand dunes are fully eroded. Dendrogeomorphology can thus be used to reconstruct drift-sand dynamics when the actual landforms are no longer present. No other method is able to do so. However, we still have to resolve questions as to what extent the ‘signal’ of covering or exposure is present along the vertical axis of the tree. In combination with optical dating of sediments (Wallinga et al., 2007) in locations without trees, it will become possible to build a high resolution data set about dynamics of aeolian sediment transport in drift-sand areas. Ultimately, this allows the reconstruction of the effects of changes in climate and land-use on drift-sand dynamics, providing baseline data for restoration and conservation potential of active drift sands.

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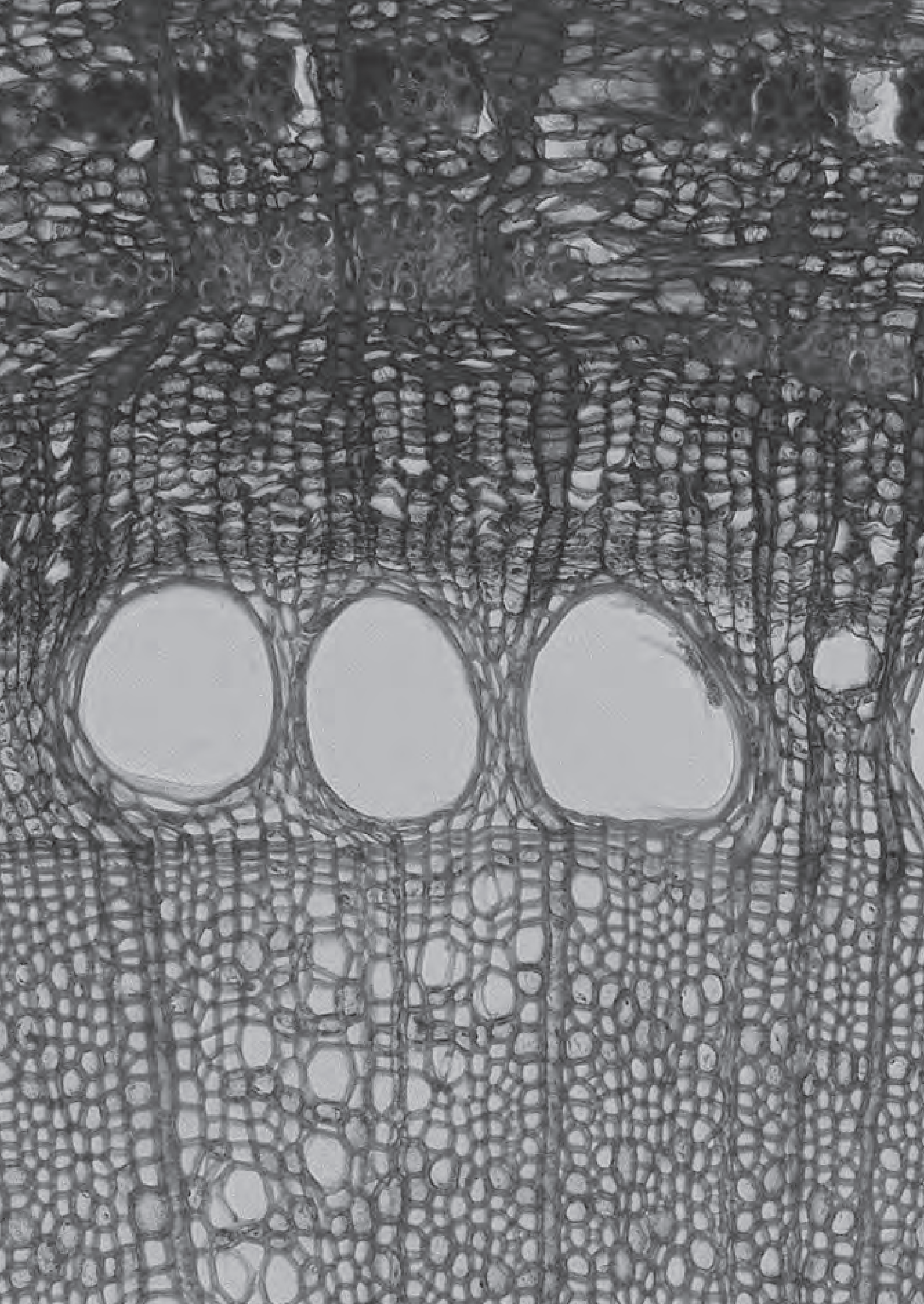

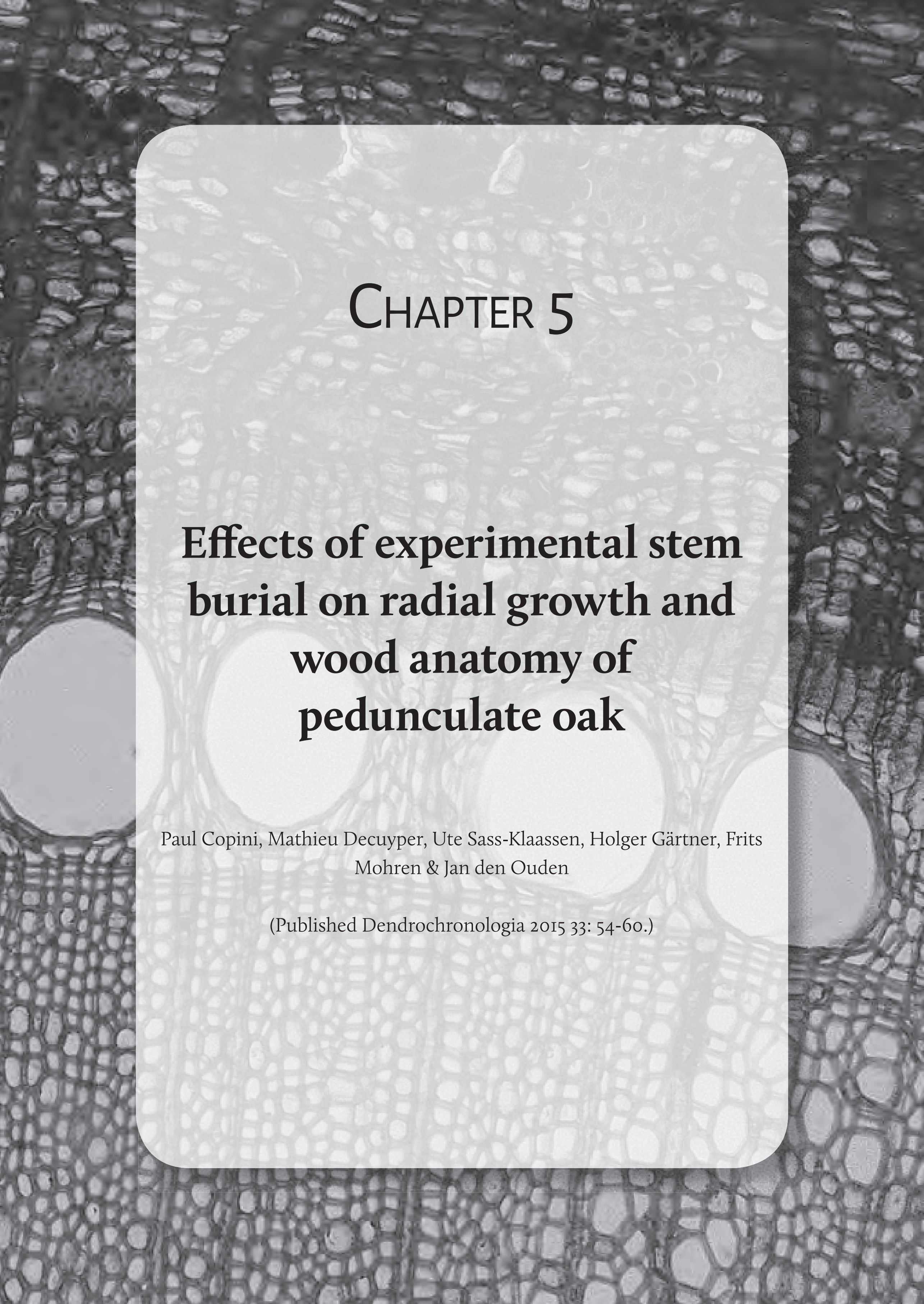

Chapter 5

Effects of experimental stem burial on radial growth and wood anatomy of pedunculate oak

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5.1 Abstract

In dendrogeomorphology, abrupt changes in wood anatomy are frequently used to date the exact year of burial and exposure events. However, few studies have addressed the precision and underlying mechanisms of these changes. In a field experiment, performed in a drift-sand area in the Netherlands, we buried the stems of mature pedunculate oak trees (*Quercus robur* L.) up to a height of 50cm and analysed the responses in ring width and vessel characteristics, while monitoring the course of temperature above and below the soil surface. After 3 years of stem burial, we found no significant differences in ring width and vessel characteristics between control and buried trees both above and below the burial level. Burial however strongly reduced temperature amplitude and the occurrence of sub-zero temperatures around the buried stems. All buried trees formed epitropic roots that grew upward into the new sediment layer, but no adventitious roots were formed on the buried stems. Irrespective of the burial treatments, we found that the mean ring width was largest at the original stem base and lowest at breast height. In contrast, vessel sizes were significantly larger at breast height compared with the stem base. Differences in vessel density barely differed between years and heights. In our field experiment on mature pedunculate oak trees, the burial of stems by 50cm of drift sand did not induce any local growth suppression or detectable changes in wood anatomy. As wood-anatomical changes in response to burial have previously been reported for trees that had formed adventitious roots, we stress the role of adventitious-root formation as a possible trigger behind the local changes in wood anatomy, reflecting a functional change of a buried stem towards a root. Based on our field experiment, it seems unlikely that years of shallow or moderate burial events (≤50 cm) can be reconstructed using the wood structure of buried stems. As epitropic roots develop quickly after burial, dating such roots may potentially yield better estimates of burial events. Further research on the relation between adventitious root and changes in stem anatomy is needed to ascertain the precision of dating sand-burial events using tree rings.

Keywords: dendrogeomorphology • drift sand • growth response • *Quercus robur* • wood anatomy

5.2 Introduction

Dendrogeomorphology is a powerful tool to reconstruct geomorphic processes with high temporal resolution (Alestalo, 1971; Stoffel et al., 2010). This method is frequently used to ascertain erosion and accumulation rates or dynamics of sediment transport in many different ecosystems (Gärtner et al., 2001; Bodoque et al., 2005; den Ouden et al., 2007; Stoffel et al., 2013). Transitions in ring width and vessel or tracheid-lumen size caused by burial or exposure have been used to date the exact year when such events occurred (Cournoyer and Bégin, 1992; Gärtner et al., 2001; Friedman et al., 2005; Stoffel et al., 2013). Normally, stems react to burial with reduced growth, whereas exposure of roots leads to the opposite (Fayle, 1968; Marin and Filion, 1992; Friedman et al., 2005; Matisons and Brūmelis, 2008). In conifers, exposed roots may develop tracheids that are reduced by 50% in the lumen area, whereas stems show a 50% increase in tracheid lumen after burial (Marin and Filion, 1992; Cournoyer and Filion, 1994; Gärtner et al., 2001). In broadleaved species, the response differs between diffuse-porous and ring-porous trees. Whereas diffuse-porous species drastically increase vessel size after stem burial (Beakbane, 1941; Sigafoos, 1964; Fayle, 1968; Friedman et al., 2005), ring-porous species exhibit decreases in earlywood-vessel size (Knowlson, 1939; Cournoyer and Bégin, 1992; Friedman et al., 2005; den Ouden et al., 2007). In addition, ring-porous species show the tendency to appear diffuse porous (Fig. 5.1), or root-like, after burial and shift back to ring-porous after exposure (Fayle, 1968; Cournoyer and Bégin, 1992; den Ouden et al., 2007; Hitz et al., 2008a)

Many dendrogeomorphic studies have assumed that the wood-anatomical changes in response to burial or exposure events were caused by sudden changes in soil pressure, aeration, moisture, light, and/or temperature along buried stem parts (Fayle, 1968; Gärtner et al., 2001; Den Ouden et al., 2007). Especially temperature has been pointed out as a triggering factor (Richardson and Dinwoodie, 1960; Fayle, 1968; Gärtner et al., 2001). While studies generally assume an immediate response of tree stems to burial, few studies have explored the abruptness at which wood-anatomical changes occur after burial or exposure events and results are often inconsistent. Some studies indicate that trees respond to burial within the growing season following the event (Wieler, 1891; Gärtner et al., 2001; Friedman et al., 2005). Findings from other studies suggest that transitions in ring width and wood anatomy only occur in particular plant parts, most likely through mechanical constraints (Bannan, 1941; Fayle, 1968; Stokes and Mattheck, 1996; Heinrich and Gartner, 2008), or could be substantially delayed (Knowlson, 1939; Strunk, 1995).

In this study, we used a field experiment to study the effects of stem burial by drift sand over a period of three years on mature pedunculate oak (*Quercus robur* L.) trees. In line with previous findings (Friedman et al., 2005; Den Ouden et al., 2007), we hypothesized that burial of stems up to 50 cm would cause an immediate and significant reduction in ring width and vessel size as well as a shift from a ring-porous towards a more diffuse-porous wood structure within the growing season following the burial event. To assess the potential role of temperature (Richardson and Dinwoodie, 1960; Gärtner et al., 2001), we recorded the course of temperature above the soil surface and at various heights in the new sediment layers.

Figure 5.1 Stem burial of pedunculate oak (*Quercus robur*) in the 'Loonse en Drunense Duinen' in the Netherlands. A: Stems of pedunculate oak are naturally covered by drift sand after a storm. B: A transverse thin section showing typical ring-porous stem wood with large earlywood vessels with diameters of up to 300 µm. C: Abnormal wood structure below adventitious roots after a stem was buried by 2 meters of drift sand. Ring width and vessel size are strongly reduced and the ring porosity is absent, making ring boundaries difficult to recognise.

5.3 Material and Methods

Study site and experimental setup

Within the extensive drift-sand area 'Loonse en Drunense Duinen' in the Netherlands, we selected a site dominated by pedunculate oak (51.6508 °N, 5.0991 °E). In an area of 100 by 100 metres, we randomly assigned 10 trees as controls and 10 trees for burial treatment. In March 2010 when the experiment was established, the 20 trees had a mean diameter at breast height (DBH, 130 cm) of 19.6 ± 3.4 cm (mean ± sd; n =20) and a mean height of 11.4 ± 1.4 m and were all dormant. Around each of the 10 trees to be buried, we constructed a squared wooden enclosure of 2 x 2 x 0.6 m (length, width, height) with the tree in the centre (Fig. 5.2). We marked the 50-cm stem height around the entire circumference of the tree and then filled the enclosure up to 50-cm stem height with drift sand (fine grained sand) from the adjacent area. On four buried trees, we installed HOBO Pro temperature data loggers (Onset Corporation, Bourne, MA, USA) to record the temperature at 0 (original soil surface), 25, and 47 cm (both near the buried stem parts) as well as above the new soil surface at 75 cm. The loggers were installed on the north side of the trees. In two control trees, HOBO Pendant loggers (Onset Corporation, Bourne, MA, USA) were installed at the same heights. All loggers were synchronised and temperature was measured on an hourly basis.

Sampling and sample preparation

In November 2012, the buried trees were excavated and the north side of each trunk was marked. The formation of adventitious roots on buried stems and negative geotropic roots or epitropic roots, i.e. roots growing from the original root system upwards into the new sediment layer (Stone and Vasey, 1968; Alestalo, 1971), was noted. Next, all trees were felled, and stem discs were taken at stem heights of 12.5, 25, and 130 cm. Per stem disc, two radii with a tangential width of 1.5 cm were extracted from the north and south side of each disc. We avoided sampling in buttress zones in the discs. The surfaces of all radii were prepared with razorblades for tree-ring measurements and scanned using a high-resolution (1600 dpi) flatbed scanner (Epson Expression 10000 XL). We then cut transverse thin sections (20 µm) of the tree rings formed between 2007 and 2012 using a G.S.L.-1 sliding microtome (Gärtner et al., 2014). All thin sections were stained with a Safranin/Astrablue solution for five minutes. Following dehydration in graded series of ethanol (50-95-100%), the samples were rinsed with Roticlear®, mounted in Rotimount® (Carl Roth, Karlsruhe, Germany), and dried under pressure for three days. Photos were taken with a digital camera (DFC 320, Leica, Cambridge, UK) mounted on a microscope (DM2500, Leica, Cambridge, UK) using Leica imaging software (version 3.6.0). The photos were stitched together using PTGui software (v. 9.1.8, New House Internet Services B.V., Rotterdam Netherlands).

Figure 5.2 Setup of the field experiment in the 'Loonse en Drunense Duinen'. Stems of mature pedunculate oak trees (*Quercus robur* L.) were covered by drift sand up to a stem height of 50 cm, before the onset of the 2010 growing season. The left arrow indicates one of the two temperature loggers that were installed to measure temperature at stem heights of 0, 25, 47, and 75 cm. The right arrow indicates one of the control trees.

Measurements and analyses

To determine the age of the trees and for internal cross-dating, we measured the tree-ring widths using the WinDENDRO tree-ring image analysis software (version 2009b, Regent Instruments, Quebec, Canada). The tree-ring series were visually and statistically cross-dated using the programs WinTSAP (Rinn, 1996) and COFECHA (Grissino-Mayer, 2001). To determine the potential effect of stem burial on tree-ring width and wood anatomy, we measured ring width (RW), earlywood width (EW), latewood width (LW) and earlywood-vessel area in the tree rings formed between 2007 and 2012 at the three sampling heights using ImageJ software (Rasband, 1997-2012). We measured all earlywood vessels larger than 30 µm in diameter. The first row of earlywood vessels was measured separately. To check the quality of vessel measurements, the generated masks belonging to individual vessels were overlaid with the original picture and adapted when necessary. From the vessel area measurements, we calculated the mean earlywood vessel area (MVA) and mean area of the first-row earlywood vessels (MVA-r1). Earlywood-vessel density (VD) was determined as the number of first-row earlywood vessels divided by the tangential width over which the vessels were measured in the sample. Maximum earlywood-vessel size (MAX-20) was calculated as the mean diameter of the 20 largest vessels in a particular tree ring at a given height.

The hourly temperature measurements were used to calculate the mean daily temperature and amplitude (max. temperature – min. temperature) at 0-, 25-, 47- and 75-cm stem height for all buried and control trees. In addition, the number of days and hours with sub-zero temperatures were calculated per height and treatment.

Statistical analyses

The effect of burial on ring-width (RW, EW, LW) and earlywood-vessel variables (MVA, MVA-r1, MAX-20, and VD) was tested using mixed factorial ANOVAs in the statistical software package SPSS, version 19 (SPSS Inc. Chicago, Il, USA) applying a significance level of 0.05. Ring width, earlywood width, and latewood width were log transformed before analyses to adhere to the assumptions of normality. As Mauchly's test indicated that the assumption of sphericity had been violated for the effect of year, the degrees of freedom in all tests were corrected using the Greenhouse-Geisser correction (Greenhouse and Geisser, 1959).

First, we performed analyses on the actual years of stem burial (2010 - 2012) per height to determine whether the burial treatment only affects the stems at 12.5 and 25 cm, i.e., below the new soil surface. For this, the burial treatment (burial or control), was used as the between-subject factor and the year was used as within-subject factor (repeated measure). Subsequently, we ran individual tests for each dependent variable (RW, EW, LW, MVA, MVA-r1, MAX-20, and VD).

Second, we performed analyses including both the pre-burial years (2007 - 2009) and the actual years of burial (2010 - 2012) and included all heights (12.5, 25, 130 cm above the original soil surface) to test whether drastic changes occur in ring-width and earlywood-vessel variables due to burial. The burial treatment (burial or control) was used as the between subject factor, and year and height were used as within subject factors. After this, we ran individual tests for each dependent variable (RW, EW, LW, MVA, MVA-r1, MAX-20, and VD) followed by post-hoc tests with Bonferroni corrections to show which heights or years were significantly different.

5.4 RESULTS

Tree characteristics

During the experiment, one control and one treatment tree died; both were excluded from the analyses. The age of the remaining trees ranged between 42 and 77 years with the control trees on average being slightly older (58 ± 12 years, mean ± SD; n = 9) than the trees of the burial treatment (53 ± 9 years, mean ± SD; n = 9). In spring 2010, all trees of both the control and burial group were moderately to heavily defoliated mainly by winter moth larvae (*Operophtera brumata* L.) just after leaf emergence. All buried trees reacted to stem burial by forming new roots from the original root system that grew upwards into the new drift-sand layer (Fig. 5.2). These epitropic roots were thin, approximately 3 mm in diameter, and were most abundant close to the buried stem (Fig. 5.3). No adventitious roots were formed along the buried stems.

Temperature along stems

The burial treatment drastically reduced the daily amplitude in temperature and the number of hours with sub-zero temperatures along the buried stem parts in the new sediment layer (Table 5.1). At 75 cm height, trees from all treatments experienced comparable overall temperatures during the run of the experiment, with temperatures ranging between -17 to >30 °C (Table 5.1). Burial leads to reduction of the temperature range, with the minimum range from 0.8 to 18.2 °C at 0 cm, beneath 50 cm of drift sand. In the buried trees, a clear gradient was observed along the stem with the smallest daily amplitudes at the original, later buried, soil surface (0 cm) followed by increasing temperature differences until the amplitude reached 7 °C in the unburied upper stem portion (75 cm), resembling the situation at the soil surface of control trees (Table 5.1). Sub-zero temperatures occurred in all control trees but to a lesser extend at the soil level. Along buried stems, sub-zero temperatures never occurred at the original soil level at 0 cm and only during 13 days at the 25-cm stem height. Just below the new surface, at 47 cm, the frost conditions (73 days in total 1261 hours) were comparable to the situation at the soil surface in control trees, with a somewhat higher average sub-zero temperature of -1.5 °C (Table 5.1).

Figure 5.3 Experimental stem burial of mature pedunculate oak trees (*Quercus robur* L.) by 50 cm of drift sand. (A) Epitropic roots (arrow) growing from the main roots into the newly deposited drift-sand layer. The asterisk indicates the original soil level whereas the dashed line shows the new soil surface. (B) A transverse thin section showing the wood structure at breast height, 70 cm above the new soil layer. The line indicate the transition between the tree rings that were formed prior (2007–2009) or during the burial experiment (2010–2012). (C) A transverse thin section showing the wood structure at a 12.5-cm stem height below 37.5 cm of drift sand. Mean ring width increased whereas earlywood vessel size decreased compared with breast height. Vessel density did not significantly differ between years and heights. The line indicates the transition between tree rings that were formed before (2007–2009) or during the burial experiment (2010–2012).

Table 5.1. Mean ambient temperatures and standard deviations (SD) measured at stem heights of 0, 25, 47 and 75 cm above the original soil surface of control and buried trees between 2010 and 2012. Note that the trees were buried by 50 cm of drift sand, such that 0, 25 and 47 cm above the original soil surface equals 50, 25 and 3 cm below the new soil surface, respectively.

Height (cm)	Temperature °C (Mean ± SD)	Temperature range 2010-2012 °C	Daily amplitude. °C (Mean ± SD)	Days <0°C	Hours <0°C	Mean temp <0°C (Mean ± SD)
Control trees						
0	10.8 ± 6.4	-10.2, 32.5	6.9 ± 4.2	79	1139	-2.2 ± 2.2
25	10.9 ± 7.3	-16.9, 34.5	9.4 ± 4.8	150	1734	-3.2 ± 3.2
47	10.9 ± 7.4	-17.3, 35.0	9.8 ± 4.9	157	1762	-3.2 ± 3.2
75	11.0 ± 7.4	-17.2, 34.6	9.6 ± 4.9	157	1752	-3.2 ± 3.2
Buried trees						
0	10.7 ± 4.3	0.8, 18.2	0.4 ± 0.2	0	0	-
25	10.6 ± 4.9	-2.0, 19.5	0.8 ± 0.5	13	296	-0.7 ± 0.6
47	10.6 ± 5.7	-6.1, 24.4	3.4 ± 2.1	73	1261	-1.5 ± 1.4
75	10.8 ± 6.8	-16.7, 31.9	7.2 ± 3.8	114	1569	-2.9 ± 3.1

Tree-ring variables

The three ring-width variables (RW, EW, LW) showed similar trends between 2007 and 2012 in the control and buried trees (Fig. 5.4). We found no effect of the burial treatment on ring-width variables at 12.5-, 25- or 130-cm stem height between 2010 and 2012 (Table 5.2). A significant interaction between year and treatment was observed at 25 cm stem height in EW (Table 5.2). However, this difference was highly variable between years, and when the year 2010 was omitted from the analyses the interaction became insignificant. We found significant differences between years, heights and in the interaction between year and height (Fig. 5.4) when all years (2007 - 2012) and all heights were examined (Table 5.3). The general trend was that RW and LW were significantly larger at 12.5 cm compared with breast height ($P < 0.05$), whereas EW was significantly wider at breast height ($P < 0.001$) (Fig. 5.4). In addition, the ring width of 2010 was significantly smaller compared with the pre-burial years 2007 to 2009 ($P < 0.001$) (Fig. 5.4). The three-way interactions between treatment, year, and height were not significant.

Table 5.2 Significant results of mixed factorial analyses per height over the experimental years (2010–2012) for the dependent variables: ring width (RW), earlywood width (EW), latewood width (LW), mean earlywood-vessel area (MVA), and mean vessel area of the first row of earlywood vessels (MVA-r1) and of the 20 largest earlywood vessels (MAX-20). F values and their significance are given as *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). The trees were covered with 50 cm of sand between 2010 and 2012, thus covering the stem sections at 12.5 and 25 cm.

Variables (height, cm)	Treatment		Year * treatment		Year	
	F	*P*	*F*	*P*	*F*	*P*
RW (130)	-	-	-	-	15.818	***
RW (25)	-	-	-	-	6.096	*
RW (12.5)	-	-	-	-	-	-
EW (130)	-	-	-	-	9.418	**
EW (25)	-	-	10.488	***	4.212	*
EW (12.5)	-	-	-	-	-	-
LW (130)	-	-	-	-	23.478	***
LW (25)	-	-	-	-	-	-
LW (12.5)	-	-	-	-	-	-
MVA (130)	-	-	-	-	-	-
MVA (25)	-	-	3.594	*	6.656	**
MVA (12.5)	-	-	-	-	3.632	*
MVA-r1 (130)	-	-	-	-	-	-
MVA-r1(25)	-	-	-	-	6.656	**
MVA-r1 (12.5)	-	-	-	-	3.632	*
MAX-20 (130)	-	-	-	-	-	-
MAX-20 (25)	-	-	-	-	4.360	*
MAX-20 (12.5)	-	-	-	-	-	-
VD (130)	-	-	-	-	-	-
VD (25)	-	-	-	-	-	-
VD (12.5)	-	-	-	-	4.445	*

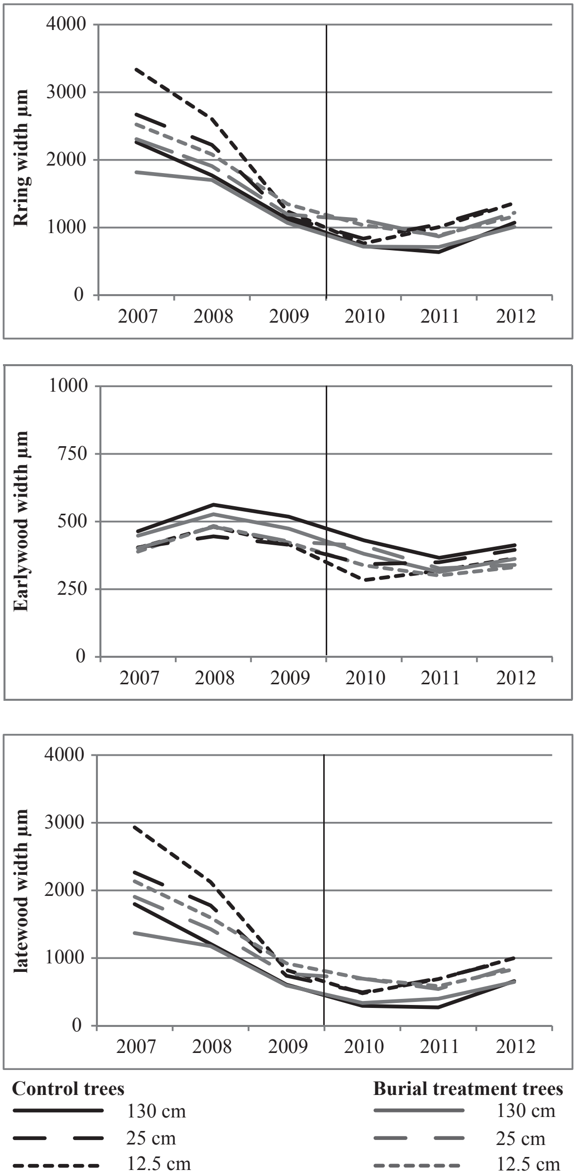

Figure 5.4. Mean ring width, earlywood width and latewood width in years before burial (2007–2009) and during burial (2010–2012). No significant differences were found between buried trees and controls. Growth in both groups is strongly reduced in 2010. Moreover, ring width was significantly smallest at breast height, whereas earlywood width was largest at breast height.

Earlywood-vessel variables

The earlywood-vessel variables (MVA, MVA-r1, MAX-20, VD) showed similar trends in both control trees as well as in buried trees between 2007 and 2012 (Fig. 5.5). No significant differences were found in earlywood-vessel variables between control trees and buried trees at 12.5-, 25- or 130-cm stem height during the run of the burial experiment (2010–2012) (Table 5.2). A significant interaction between year and treatment was found in vessel size at 25 cm (Table 5.2). However, when the year 2010 was omitted from the analyses, this interaction appeared to be insignificant. When all years between 2007 and 2012 and all heights were included (Table 5.3), we found significant differences in all vessel variables except for earlywood vessel density (VD). On average, vessel size was larger at 130 cm and significantly reduced towards 12.5 cm ($P < 0.001$) (Fig. 5.3 and 5.4). Moreover, vessel size in the tree ring of 2010 was strongly reduced compared with the pre-burial years 2007 – 2009 ($P < 0.001$) (Fig. 5.5). The three-way interactions between treatment, year, and height were insignificant.

Table 5.3 Significant results of mixed factorial analyses including all heights (12.5, 25, 130 cm) and both the pre-burial years (2007–2009) and the experimental years (2010–2012) for the dependent variables: ring width (RW), earlywood width (EW), latewood width (LW), mean earlywood-vessel area (MVA), and mean vessel area of the first row of earlywood vessels (MVA-r1) and of the 20 largest earlywood vessels (MAX-20). F values and their significance are given as *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Three-way interactions between treatment, year, and height were insignificant (not shown).

Variables	Treatment		Year * treatment		Height * treatment		Year		Height		Year * height	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
RW	-	-	-	-	-	-	57.558	***	6.448	**	5.114	**
EW	-	-	-	-	-	-	24.440	***	14.077	***	2.706	*
LW	-	-	-	-	-	-	38.425	***	-	-	-	-
MVA	-	-	-	-	-	-	28.45	***	21.443	***	2.706	*
MVA-r1	-	-	-	-	-	-	25.284	***	34.376	***	2.302	*
MAX-20	-	-	-	-	-	-	20.141	***	29.134	***	3.865	**
VD	-	-	-	-	-	-	-	-	-	-	-	-

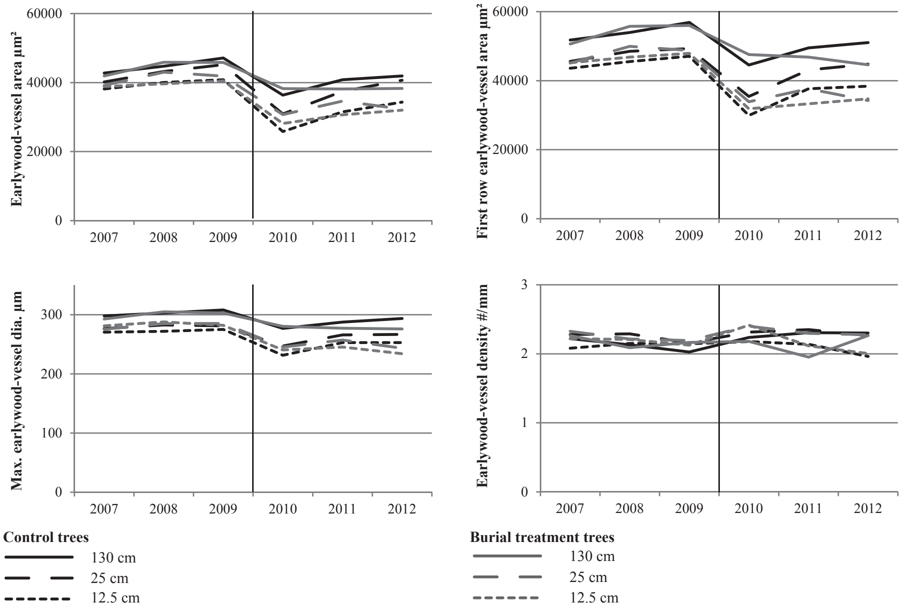

Figure 5.5 Mean earlywood-vessel area, mean first row earlywood-vessel area, max. vessel diameter and vessel density before burial (2007–2009) and during burial (2010–2012). Vessel size was largest at breast height. Earlywood vessel size was strongly reduced in 2010 compared with the years between 2007 and 2009. Vessel density barely differed between years and heights.

5.5 DISCUSSION

Effect of stem burial on wood structure

We found no effect of burial of oak stems by 50 cm of sand on ring-width variables and wood anatomy after three growing seasons of burial, whereas the temperature amplitude and the occurrence of sub-zero temperatures were strongly reduced. This is in contrast to the view that burial events, such as exposure events, instantly influence vessel area and/or ring width within the year of burial (Patel, 1965; Cournoyer and Bégin, 1992; Gärtner et al., 2001; den Ouden et al., 2007), even when trees are covered by only 50 cm of sediment (Friedman et al., 2005; Copini et al., 2007). However, our results agree with Knowlson (1939), who studied sessile oak trees (*Q. petraea* (Matt.) Liebl) that were covered with sandy soil to a stem height of 4.5 m (15 feet) for ca. 52 years and indicated that wood-anatomical changes only occurred 33 years after the burial event. Also Strunk (1995) found delays up to seven years between debris flows and the start of growth suppressions. Mechanical constraints of the stem base may limit the capacity of trees to change their anatomy in response to burial or exposure events (Bannan, 1941; Fayle, 1968; Stokes and Mattheck, 1996; Heinrich and Gartner, 2008).

The strong changes which we observed in ambient temperature along buried stem parts are in line with the view that temperature amplitude and frost occurrence decrease with increasing soil depth (Jong, 1979; Hillel, 1998; Walter et al., 2009). However, this strong gradient in temperature in combination with obvious changes in light environment did not affect the cambium in a way that changes in radial growth and vessel patterns were induced in buried stem parts during the three years when the experiment took place. The exceptionally narrow tree ring with many narrow vessels formed in 2010 was most likely the result of defoliation by winter moth larvae (Thomas et al., 2006) that were highly abundant in the Netherlands in both 2009 and 2010 (Moraal, 2011).

Adventitious roots as a possible trigger?

The formation of adventitious roots on the buried stem is one of the most important responses of trees after burial, most likely because of oxygen or moisture deprivation in the original root system (Cowles, 1899; Kurz, 1940; Dech and Maun, 2006; Maun, 2009). Many studies reporting on abrupt changes in ring width and wood anatomy in response to burial or exposure have been conducted on trees or stem cuttings that had formed adventitious roots (Wieler, 1891; Knowlson, 1939; Cournoyer and Bégin, 1992; Marin and Filion, 1992; Copini et al., 2007). Also Den Ouden et al. (2007), studying pedunculate oak trees with a history of past stem burial and recent stem exposure in the same drift-sand area as used in this study, found long-lasting growth suppressions and abrupt transitions from ring-porous stem wood towards diffuse-porous, root-like wood and vice versa along stems. This suggests that sudden changes in radial growth and wood anatomy in buried stems or branches of pedunculate oak may only occur after a functional change of the buried stem towards a root due to the formation of adventitious roots in the upper part of the buried stem segment. The absence of changes in ring width and wood anatomy, three years after the onset of the present field experiment, could therefore be attributed to the lack of functional changes of the buried stem parts in the absence of adventitious roots. Deeper burial might have had induced adventitious root formation. Also, the buried trees might just have needed more time to initiate adventitious root formation (Stone and Vasey, 1968; Strunk, 1995, 1997; Maun, 1998). Further research should address the relation between wood-anatomical changes and the occurrence of adventitious roots.

Implications for dating sand-burial events

Our results showed that, at least in the case of burial of the stem base of pedunculate oak by 50 cm of drift sand, no drastic changes in ring width and wood anatomy occurred within a burial period of three years. Although sand dynamics can be highly dynamic depending on the location within a drift-sand area (Riksen and Goossens, 2007), most sand transport occurs nowadays – in line with our experiment – after large incidental erosion events (Koster, 1978; Koster, 2009). So far wood-anatomical changes were observed in trees that were covered gradually with drift sand for more than 1.5 m (Den Ouden et al., 2007), which may indicate that more shallow burial events at least till a stem height of 50 cm (this study) might not be recorded in the wood of mature pedunculate oak trees. Consequently, the annual resolution of dating burial events, which was assumed by Den Ouden et al. (2007), might be invalid for shallow or moderate burial events. However, if transitions from stem wood toward a structure resembling root wood depend on the formation of adventitious roots, as speculated in this study, then stems may provide a minimum age of sediment deposition. Another possibility to date burial events is to determine the age of epitropic roots that grow quickly into the newly deposited sediment layers and may indicate the minimum period of burial (Alestalo, 1971). Further research encompassing a cross validation e.g. with optically stimulated luminescence dating (Ballarini et al., 2003; Vandenberghe et al., 2013), may strongly contribute to understanding the precision of dating drift-sand dynamics using tree rings.

5.6 CONCLUSIONS

In our three-year lasting field experiment on mature pedunculate oak trees, the mere burial of stems by 50 cm of drift sand did not induce any local growth suppression or detectable changes in ring width or wood anatomy. The absence of changes within buried stem parts occurred despite significant ones in temperature and light conditions. Whereas all buried trees formed epitropic roots, no adventitious roots were observed along buried stems. As wood-anatomical changes in response to burial have previously been reported for trees that had formed adventitious roots, we stress the role of adventitious-root formation as a possible trigger behind the local changes in wood anatomy, reflecting a functional change of a buried stem towards a root. Based on our field experiment, it seems unlikely that years of shallow or moderate burial events (≤ 50 cm) can be reconstructed using the wood structure of buried stems. As epitropic roots develop quickly after burial, dating these roots may provide a more precise estimate of the year of burial. Further research on the formation of adventitious root and changes in stem anatomy is needed to ascertain the precision of dating sand-burial events using tree rings.

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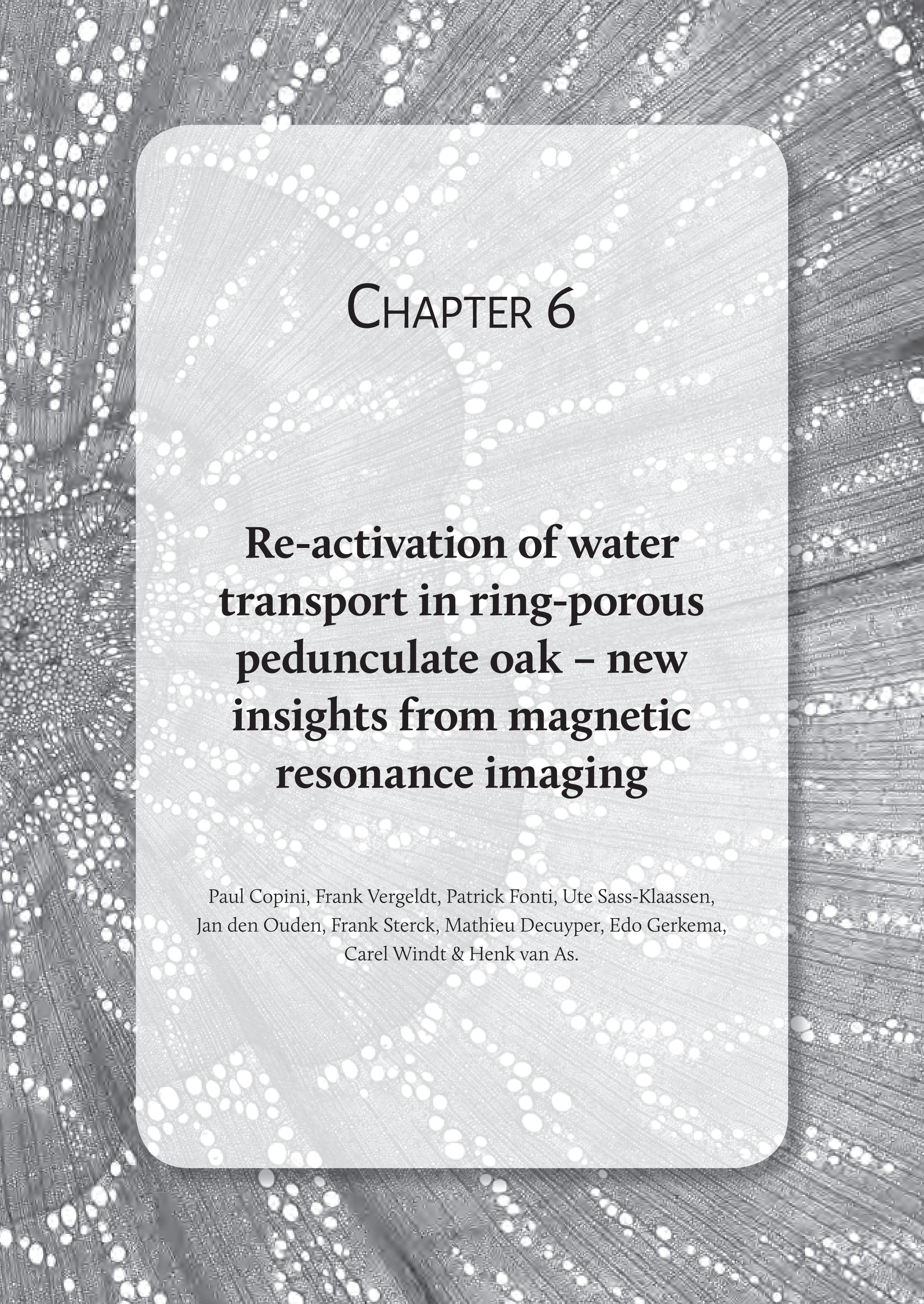

CHAPTER 6

Re-activation of water transport in ring-porous pedunculate oak – new insights from magnetic resonance imaging

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6.1 ABSTRACT

We investigated re-activation of water transport in relation to earlywood-vessel formation and sapwood-water content in three-year-old pedunculate oaks trees (*Quercus robur* L.), using a combination of non-invasive Magnetic Resonance Imaging (MRI) and wood-anatomical research. At six leaf phenophases, from bud dormancy in late winter until the second growth flush in summer, axial xylem flow and water content were measured per tree ring and for the pith and bark respectively. Wood anatomy was studied to trace connections between newly formed earlywood vessels and previous year latewood vessels. During budswell 90% of the newly formed earlywood vessels occurred at positions where previous year latewood vessels and related vasicentric tracheids were connected to the tree-ring boundary. These newly formed earlywood vessels accounted for 26% of the axial xylem flow early in the growing season, at budswell, but their contribution increased to ca. 70% at the second growth flush in summer. Previous year latewood vessels started transporting water upon budswell and remained conductive throughout the study period. No significant changes in mean water content were observed throughout different leaf phenophases in the bark, the newly formed outermost tree ring, and the pith. However, the water content of the previous tree ring dropped considerably from approx. 37% during dormancy to 15% at the second growth flush in summer; this difference was mainly attributed to a ca. 77% reduction in water content of the fibre tissue i.e. fibre cells and surrounding parenchyma cells. Our *in vivo* approach led to new insights into processes around quantification and observation of dynamics in water content and re-activation of xylem flow in ring-porous species. We found that previous year conductive latewood-vessel tissue and new developing earlywood vessels form a functional unit that conducts effectuate water transport in the beginning of the growing season. This becomes possible through connections across the tree-ring boundary. The role in xylem flow during reactivation in spring gradually shifts from conductive latewood to new earlywood, concomitant with the stage of development of earlywood vessels and leaf formation. During reactivation, water which is mainly stored in fibre tissue in the previous ring, moves to the cambium and into the developing new tissue. Our study confirms the great potential of in vivo studies using MRI for quantification and observation of dynamics in water content and sap flow.

Keywords: Cambial activity • Earlywood-vessel formation • MRI • Phenology • *Quercus robur* • Xylem flow.

6.2 Introduction

The change in deciduous hardwood trees from winter dormancy to active growth is a spectacular phenomenon in nature. Within a short period of time, trees reactivate their conductive tissues and form leaves in order to resume photosynthesis. Xylem vessels that are mostly embolised after freeze-thaw cycles in winter need to be refilled or renewed. Some species, such as diffuse-porous *Acer*, *Betula* or *Juglans*, are able to refill embolised vessels by positive root or stem pressure and may use multiple tree rings for water conductance (Sperry et al., 1988; Hacke and Sauter, 1996; Ameglio et al., 2001). Ring-porous trees such as *Quercus* or *Fraxinus* form large earlywood vessels in spring followed by small latewood vessels later on in the growing season. As the large earlywood vessels of ring-porous species become dysfunctional after frost, these species need to renewed their conductive tissues every year (Cochard and Tyree, 1990; Utsumi et al., 1996; Davis et al., 1999). This makes ring-porous species highly dependent on their newly formed earlywood vessels, which are formed synchronously with or prior to leaf development (Essiamah and Eschrich, 1986; Cochard and Tyree, 1990; Granier et al., 1994; Sass-Klaassen et al., 2011).

In ring-porous species the small latewood vessels are irrelevant for water transport compared to large earlywood vessels as, according to the Hagen-Poiseuille law one large earlywood vessel with a size of 300 µm in diameter may transport as much as 10.000 latewood vessels with diameters of 30 µm. However, small latewood vessels might become of vital importance during leaf and earlywood formation in spring once the large previous year early-wood vessels have embolised during winter (Zimmermann, 1964; Braun, 1970; Granier et al., 1994; Utsumi et al., 1999; Tyree and Zimmermann, 2002). The exact mechanisms are not known but wood-anatomical studies revealed that earlywood vessels are connected to previous year latewood vessels: in *Fraxinus lanuginosa* the connection is direct via pits (Kitin et al., 2004; Kedrov, 2012) while in *Quercus robur* the connections consist of vasicentric tracheid cells that are surrounding the latewood vessels (Braun, 1970; Sano et al., 2011). In addition, it has been suggested that previous latewood vessels may already be conductive in axial direction when swelling of the buds occurs in early spring (Essiamah and Eschrich, 1986) as water is needed e.g. for hydrolyses of starch and for cambial activity. Based on dye injection and cryo-scanning electron microscopy, Sano et al. (2011) showed that also during the growing season, the tracheids in e.g. *Quercus* *crispula* around the previous year latewood may conduct water. Yet, it was only possible to speculate about these processes, as contemporary measurement techniques such as heat-based methods (heat balance and heat dissipation) or radioisotope methods, frequently lack the required spatial resolution or are invasive and therefore may not represent the intact plant (Clearwater and Clark, 2003; Renninger and Schafer, 2012; Vandegehuchte and Steppe, 2013).

Currently, accurate *in vivo* measurements on both water content and axial water transport are possible on actively transpiring plants due to the application of Magnetic Resonance Imaging (MRI) (Windt et al., 2006; Van As et al., 2009; Borisjuk et al., 2012; De Schepper et al., 2012; Windt and Blümler, 2015). The most important feature of MRI is the possibility to non-invasively measure the presence and mobility of protons (i.e., the nucleus of the hydrogen isotope ^{1}H) spatially over the interior of the plant. Over the last decades MRI has become an important technique to study cavitation, root development, anatomy, and xylem- and phloem-water transport (Windt et al., 2006; Helfter et al., 2007; Homan et al., 2007; Borisjuk et al., 2012; De Schepper et al., 2012; Robert et al., 2014).

We applied Magnetic Resonance Imaging (MRI) to study the reactivation of water transport in relation to leaf phenology and earlywood vessel formation in four-year old pedunculate oak trees (*Quercus robur* L.). We expected that (i) previous year latewood vessels conduct water during earlywood vessel and leaf formation (Zimmermann, 1964; Utsumi et al., 1999; Tyree and Zimmermann, 2002), whereas earlywood vessels conduct water during the remainder of the growing season (ii) that earlywood-vessel formation starts at the position where latewood vessels are connected to the tree-ring boundary (Braun, 1970; Kitin et al., 2004) and (iii) during leaf and earlywood-vessel formation the sapwood water distribution changes as more water is needed in the outermost ring for both cambial activity and axial xylem flow (Arend and Fromm, 2003; Woodward, 2004; Arend and Fromm, 2007).

6.3 Material and Methods

Plant material and selection

We used 12 four-year-old pedunculate oak trees (*Quercus robur* L.) with a mean stem diameter of 12.5 ± 1.5 mm (mean ± SD) at 50 cm stem height and a mean tree height of 171 ± 12 cm (mean ± SD). The trees were container grown in a 1 x 1 m grid in a garden experiment in Wageningen, the Netherlands (Latitude 51.9884°, Longitude 5.6644°). The trees were watered, using a semi-automatic fertigation system. We scored spring and summer leaf phenology on the upper branch of each tree every other day between April 15th and June 15th 2009. For the classification of spring and summer phenology, we used information provided by Derory et al (2006) and defined six phenophases: dormant, buds swelling, leaves visible, internodes growing, leaves fully developed and second growth flush. For the experiment, two trees were selected for analyses at every phenophase.

NMR experimental setup

All trees were scanned in a 3T MRI scanner (Bruker, Karlsruhe, Germany) consisting of an Avance console (Bruker, Karlsruhe, Germany) and a superconducting magnet with a vertical 0.5 m-diameter bore (Magnex, Oxford, UK) generating a magnetic field of 3 T (128 MHz proton frequency). (Van As, 2007). A unique openable radio frequency coil with an inner diameter of 4 cm, surrounded by an gradient coil with a maximum gradient strength of 1 T/m was mounted around the stem (Homan et al., 2007; Van As, 2007). All measurements were conducted in the centre of the coil at ca. 50-cm stem height. Light was generated by 8 projection bulbs (Philips, type 13117, 17V, 150W, GX5.3) providing photosynthetically active radiation of ca. 350 µmol $m^{-2}s^{-1}$ at a temperature between 25 and 27 °C within the upper part of the crown. The relative humidity varied between 30 and 60%.

Stem water content and T2 relaxation times mapping

Stem water content and T2 relaxation times were measured in a matrix of 256 × 256 pixels and a slice thickness of 3 mm, representing a field of view of 2.0 × 2.0 cm (1 voxel = 78.125 × 78.125 × 3000 µm) (Fig. 6.1) using a multiple spin echo imaging sequence (Edzes et al., 1998). We used the following settings: repetition time 2500 ms, 64 spin echoes with an echo time of 7.5 ms. The spin echoes were fitted to a mono-exponential curve to obtain the T_2 value. The amount of free water per voxel (%) within the tree was calibrated by comparing the signal to the intensity of the tree with reference tubes placed around the tree.

Xylem flow

We measured xylem flow in a matrix of 64 × 128 voxels representing a field of view of 2.0 × 2.0 cm (1 voxel = 312.5 × 156.25 × 3000 μm) (Fig. 6.1) using a pulsed field gradient – spin echo – turbo spin echo sequence (PFG-SE-TSE) (Scheenen et al., 2001; Windt et al., 2006). The following settings were used: turbo factor of 16, small delta of 4 ms, big delta of 50 ms and maximum gradient of 0.4 T/m. On average seven successive sap flow measurements were conducted of which the mean and standard deviation were calculated yielding, inter alia, mean xylem flow (mm^3/s) and mean linear velocity per voxel (Scheenen et al., 2001; Windt et al., 2006).

Leaf area

Immediately after NMR analyses, we scanned all leaves of the trees belonging to phenophase IV- VI on a flatbed scanner (Epson Expression 10,000 XL) with a resolution of 600 dpi. We used Image J software (version 1.44) (Rasband, 1997-2012) to measure total leaf area.

Stem anatomy

After MRI scanning, we marked the west side of all stems by making a vertical cut in the cortex with a razor blade and stored the stem segment, including the 3 mm scanned slice in a 50% ethanol solution at 4 °C. Subsequently, we made transverse thin sections (Fig. 6.1) with a thickness of 20-25 μm using a sliding microtome (Gärtner et al., 2014). All cross-sections were stained with a safranine/astrablue solution for 5 min. Following dehydration in graded series of ethanol (50–95–100%), the samples were rinsed with xylol, mounted in Canada balsam and dried at 60 °C for 15 h. Photos of the entire cross sections were taken with a digital camera (DFC 320, Leica, Cambridge, UK) mounted on a microscope (DM2500, Leica, Cambridge, UK) using Leica imaging software (version 3.6.0) (1 pixel is 2.19 μm). Subsequently the photos were stitched using PTgui software (version Pro 9.1.7).

Sapflow and water content analyses

To study water transport and water content within the stem, we made overlays of the xylem-flow data, water content data, the T2 values and the marked thin sections, using the ArcMap 10.2.1 package (ESRI Redlands, California, U.S.). Subsequently we created masks of the different tree rings (2007-2009), the pith, and the bark (Fig. 6.1). For the 2008 tree ring, additional masks were created to differentiate latewood-vessel tissue i.e. latewood vessels, vasicentric tracheid and parenchyma cells from fibre tissue i.e. libriform fibres and axial parenchyma) (Fig. 6.1). The masks were used to estimate the mean xylem flow and mean water content in the different compartments (tree rings, bark, pith).

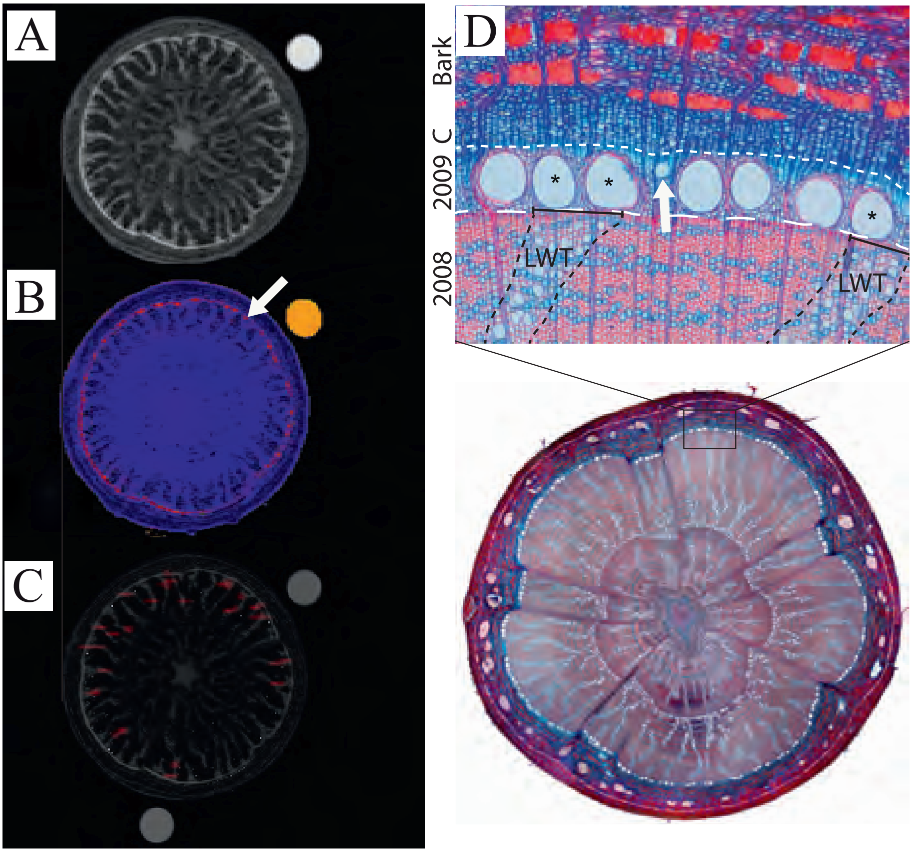

FIGURE 6.1. Measurements and overlays of MRI-derived water content, T2 relaxation values, xylem flow, and wood anatomy. A: Water content as a percentage per voxel was calculated. B: T2 relaxation times were measured and used to identify the location of newly formed earlywood vessels, i.e. the red dots representing relatively long T2 relaxation times. C: Xylem-flow characteristics were measured i.e. xylem flow (mm^3/s) and flow velocity (mm/s). D: Thin sections were prepared and masks were constructed

representing the bark, individual tree rings and the pith. In addition the 2008 tree ring was used to study the water content of latewood-vessel tissue (LWT) and the fibre tissue in between. Earlywood vessels indicated with asterisks, represent earlywood vessels that are connected to latewood-vessel tissue. The arrow indicates a blue, unlignified vessel. The dashed line at the 2008/2009 tree-ring boundary represents the circumference that was measured to calculate the earlywood-vessel density, whereas the two black lines show the tangential distance over which latewood-vessel tissue occurred at the tree-ring boundary. Note that this thin section belongs to another stem compared to A-C.

Wood-anatomical analyses

The area of developing lignified and unlignified earlywood vessels was measured for the entire circumference during each leaf-phenological stage using Image J software (version 1.44) (Rasband, 1997-2012). We also tested whether there was a significant difference between the number of earlywood vessels connected to previous year latewood-vessel tissue or to fibre tissue, taking into account the proportion of the 2008 / 2009 tree-ring boundary covered with fibre or latewood-vessel tissue using Chi-square tests.

6.4 Results

Earlywood-vessel formation in relation to leaf phenology

New earlywood-vessels were present at the phenophase budswell (I) and occurred irregularly around the circumference (Fig. 6.2). In one tree 5.7% of new earlywood vessels had been lignified while in the other 41% of the vessels were already lignified. Vessel density ranged between 2.3 and 3.0 vessels per millimetre of circumference. When the first leaves were visible (II) ca. 50% of all present earlywood vessels were lignified (Table S6.1) and in one tree a few second row earlywood vessels were already forming. In the beginning of May, when the internodes started expanding (III) between 50 and 64% of all present vessels were lignified and the vessel density increased to 5 and 6 vessels per mm respectively (Table S6.1). By then in all trees a few second row, unlignified, earlywood vessels were present. Once the leaves were fully developed (IV) more than 80% of all present earlywood vessels had been lignified. Some second row earlywood vessels were lignified, whereas unlignified first row earlywood vessels were also still present. The vessel density ranged between 7 and 9 vessels per mm (Table S6.1). With the onset of the second growth flush (V) all earlywood vessels were lignified (Fig. 6.2) and many latewood vessels had been formed. The mean earlywood-vessel density ranged between 8.0 and 9.4 vessels per millimetre.

Overall mean earlywood vessel size of lignified vessels ranged between 4050 and 9113 μm^2 respectively (Table S6.1).

Connection between latewood vessels and the onset of earlywood-vessel formation

Significant differences occurred between the number of earlywood vessels connected to latewood-vessel tissue compared to those connected to fibre tissue. At budswell, almost 90% (Chi square tests, P = < 0.001) of all new earlywood vessels were connected to previous year latewood-vessel tissue that was only present around 28% of the 2008-2009 tree-ring boundary (Fig. 6.3A). During all phenophases significantly more vessels were connected to previous year latewood-vessels tissue (Chi square tests, P = < 0.001), although the relation became weaker during earlywood-vessel formation. At the beginning of the second growth flush almost 50% of all earlywood vessels were connected to previous year latewood-vessels tissue (Fig. 6.3A), which only occupied 25% of the circumference of the tree-ring boundary.

Axial water transport in relation to leaf area

During bud dormancy, in early March, axial water transport did not yet occur (Fig. 6.2 and 6.3). Upon budswell (I), water transport started predominately (84%) in the latewood vessels, and in some lignified new earlywood vessels of the outermost tree ring in one of the two trees. Xylem flow was low with values of 0.02 and 0.05 mm^3/s (Fig. 6.3) and the average linear velocity was 0.48 mm/s. When the leaves were visible (II), axial xylem flow differed considerable between the two trees: whereas one tree only conducted 0.05 mm^3/s and a velocity of 0.47 mm/s, the other with the largest mean earlywood size transported more water with 0.27 mm^3/s and a velocity of 0.84 mm/s (Table 6.1). Once the internodes started expanding (III) and the total leaf area was ca. 883 cm^2, xylem flow was still low and ranged between 0.07 to 0.15 mm^3/s, while the total leaf area ranged between 700 and 900 cm^2 (Table S6.1). When the leaves were fully developed (IV) and the leaf area was 1760 and 2753 cm^2, a significant increase in water transport occurred with values of 0.76 and 0.90 mm^3/s and a high average velocity of 1.2 and 1.3 mm/s (Table S6.1). At the onset of the second growth flush, xylem flow was 1.2 and 1.3 mm^3/s with average flow velocities of 1.2 and 0.8 mm/s and a leaf area of 3000 and 3500 cm^2 respectively.

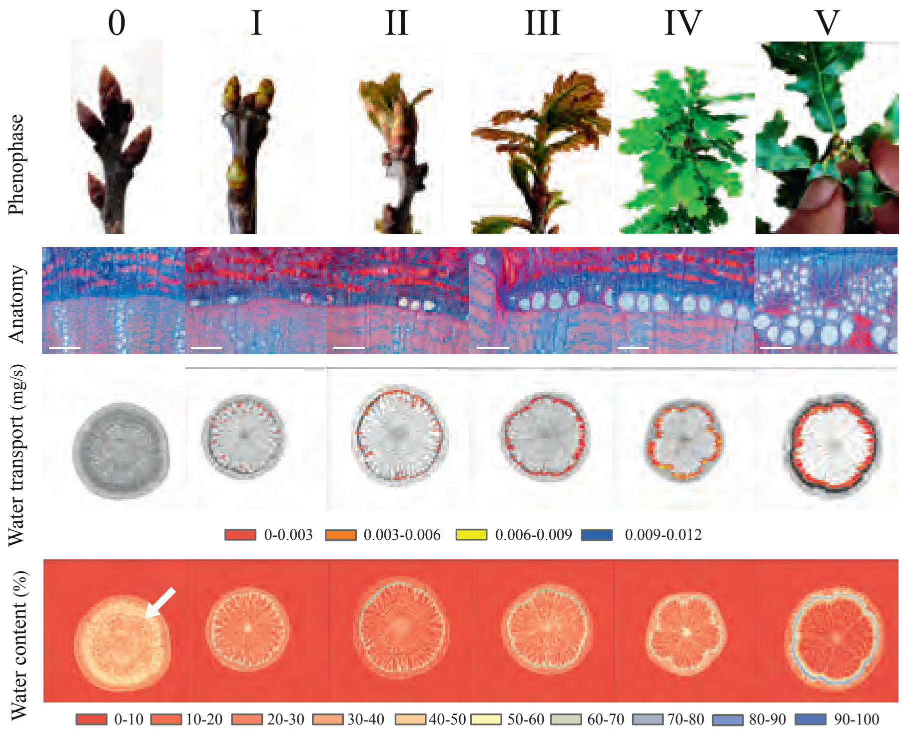

FIGURE 6.2. Earlywood-vessel formation, water content and water transport in relation to leaf phenology: 0 bud dormancy; I buds swollen; II leave visible; III internodes extending; IV leaves fully developed and phase V buds swollen upon second growth flush. At bud dormancy earlywood-vessel formation and axial water transport are absent. The highest water content can be found in the previous year tree ring. The arrow indicates previous year earlywood vessels which hardly contain any water. At budswell, earlywood-vessel formation had started in both trees, normally in the vicinity of previous year latewood vessels. Axial water transport had started in one tree mostly in previous year latewood vessels. Highest water content occurs in the developing tree ring of 2009 and in previous year latewood vessels. When the leaves were visible, more earlywood vessels had formed and axial water transport occurred both in previous year latewood vessels as in the newly formed tree ring. When the internodes were expanding many first row earlywood vessels had formed and axial water transport occurred both in previous year latewood vessels as in the newly formed tree ring. When the leaves were fully developed most earlywood vessels had lignified and a considerable increase in water transport occurred. In summer, upon the start of the second growth flush, all earlywood vessels had lignified and latewood was being formed. Highest water content occurred in the 2008 tree ring. Water content of older tree rings was low, especially in the fibre tissues. The scale bar of the anatomy photos represents 200μm.

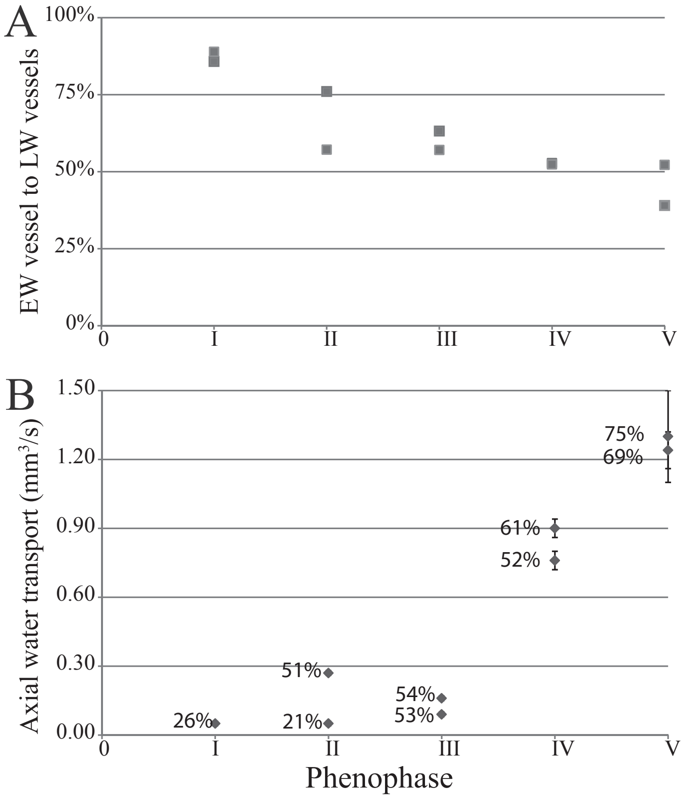

Figure 6.3. Connection between new earlywood (EW) and previous latewood (LW) vessels (A) and mean axial xylem flow (± SD) (B) per leaf phenophase for both trees: 0 bud dormancy; I buds swollen; II leave visible; III internodes extending; IV leaves fully developed and phase V buds swollen second growth flush. A: The percentage of earlywood vessels formed in 2009, that were connected to previous year latewood vessels (squared boxes). At budswell, ca. 90% of all developing earlywood vessels were connected to previous year latewood tissue. B: Xylem flow only occurred in the latewood vessels of the 2008 ring and in the newly formed ring of 2009. The percentage indicates the estimate of water transport in the 2009 ring versus the 2008 ring i.e. at budswell 26% of axial water transport occurred in the 2009 tree ring, while at the second growth flush in summer between 69 and 75% of axial water transport occurred in the outermost tree ring of 2009. Axial xylem flow significantly increased after the leaves were fully developed.

Sapwood water distribution

The average water content of trees barely differed in relation to leaf phenology and ranged between 29 and 37% (Table S6.1). However, the water distribution changed considerably between dormant trees and trees that had started with leaf and earlywood formation (Fig. 6.2). During dormancy, the water content was distributed most equally over the stem and highest water content, ca. 37%, occurred in the tree ring of 2008 (Fig. 6.4). Note that the earlywood vessels of 2008 contained hardly any water (Fig. 6.2, arrow). In the latewood of 2008, the highest water content (ca. 42%) was found in latewood-vessel tissue and 32% occurred in the fibre tissue. At budswell, when earlywood vessel formation had started, most water (ca. 50%) was found in the developing tree ring of 2009 (Fig. 6.4). Also, during the following phenophases most water was located in the outermost developing ring of 2009, whereas the water content in the previous ring of 2008 decreased until on average of 15% during the second growth flush in summer (Fig. 6.4). This water was almost exclusively located in the latewood-vessel tissue (35%); hardly any water (7%) was present in the fibre tissue.

6.5 Discussion

Axial xylem flow occurs in previous year latewood vessels and new earlywood vessels

In vivo monitoring and quantification of water content and xylem-flow resumption supported the hypothesis that previous year conducting latewood tissue and new earlywood vessels operate as a functional unit. No axial flow occurred in old earlywood vessels. This is in line with results of other studies using a combination of invasive dye injection and cryo-scanning electron microscopy that showed the presence of water in previous year's latewood vessels of *Fraxinus mandshurica* after freeze-thaw cycles (Utsumi et al., 1999) and in vasicentric tracheids around previous year's latewood vessels and around the outermost earlywood vessels in ring-porous *Quercus crispula* and *Q. robur* (Sano et al., 2011). We proved that in the initial phase slow axial water transport occurs in the latewood and is then successively taken over by new lignified earlywood vessels, which are responsible for ca. 75% of the water flow when the second leaf flush occurs in early summer. This means that even in young oaks with relatively narrow earlywood vessels (mean diameter 36 -54 µm), only the

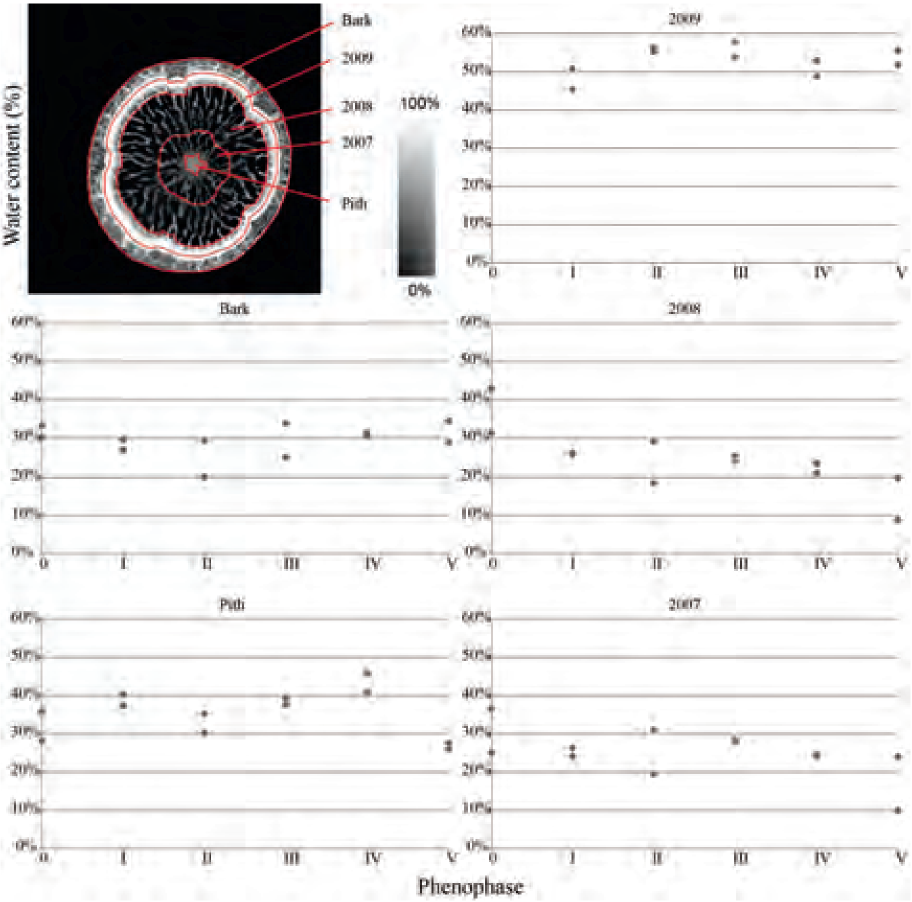

Figure 6.4. Mean water content of the bark, different tree rings and the pith per phenophase: 0 bud dormancy; I buds swollen; II leave visible; III internodes extending; IV leaves fully developed and phase V buds swollen second growth flush. The water content in the bark was on average 29 ± 4% (mean ± SD) and the mean water content of the most recent tree ring of 2009 was highest, on average 53 ± 3%. The previous year tree ring of 2008 showed a clear trend from high water content during dormancy, toward least water in summer upon the second budswell phase, whereas the 2007 tree ring contained on average 25 ± 6%, which remained fairly stable over the different phenophases. The pith contained on average 35 ± 6% of water; at the second growth flush the amount of water was lowest with values below 30%.

previous year latewood vessels and new earlywood vessels are conductive, and confirms the general view that earlywood vessels function only for one growing season (Zimmermann, 1964; Ellmore and Ewers, 1986; Cochard and Tyree, 1990; Granier et al., 1994; Hacke and Sauter, 1996). Our estimates of a 69 to 75% share in water transport by the outermost ring matches results by Granier et al. (1994), based on a combination of sapflow-measurement techniques.

They estimated that 20 to 30% of the total flow occurs outside the outermost tree ring in pedunculate oak, and suggested that this might include vessels in several older rings as well. We found that only the previous year latewood vessels in combination with actual earlywood vessels and surrounding tracheids are involved in axial transport. Differences in the percentage of water transport by the outermost earlywood vessels can be attributed to either the small size of the earlywood vessels in our young oaks and/or a possible shift in contribution once the actual latewood vessels get involved. We did not address the current year latewood in this study. Also there might be species-specific influences, as Ellmore and Ewers (1986) reported a substantial higher conductance of 90% in the outermost ring of *Ulmus americana* trees using tracer dye.

Water transport in our experimental setting was generally low and is most likely influenced by the light conditions which resemble a cloudy day. We observed a significant increase in axial xylem flow when both the leaves were fully expanded and about 80% of the earlywood vessels were lignified. Interestingly, by then most vessels in the second row of earlywood vessels were still developing. Sass-Klaassen et al. (2011) reported the same phenomenon for mature oak trees. At full leaf expansion, most water is obviously needed for transpiration, which leads to increasing xylem flow. In the preceding period, i.e. during budswell, water is also needed but in much lower quantities, e.g. for the hydrolysis of starch and for the reactivation of cambial activity (Essiamah and Eschrich, 1986).

Earlywood-vessel formation starts near previous year latewood vessels

The first new earlywood vessels were formed at positions where conductive latewood tissue bordered the cambium. To our knowledge the onset of earlywood-vessel formation has never been studied in relation to the position of latewood vessels and related vasicentric tracheids. Dynamics in radial water transport in sapwood, but also from wood to bark through the cambium has not received much attention. Rays, comprising ray parenchyma and/or ray tracheid cells, are assumed to play an important role in safeguarding water transport to the cambium (Van Bel, 1990; Fuchs et al., 2010; Spicer, 2014). However, Barnard et al. (2013) challenged the importance of radial conductance through rays and showed that tracer dye did not infiltrate ray tracheids, and hardly penetrated into ray parenchyma cells in different conifers. In line with our finding there are also several species that may conduct water radially through pits on the tangential walls of the last conduits in the latewood of conifers (Kitin et al., 2009) and angiosperms (Fujii et al., 2001; Kitin et al., 2004). According to Kitin et al. (2004) all earlywood vessels at the tree-ring boundary of *Fraxinus lanuginosa* made contact via inter-vessel pits with latewood vessels in the previous tree ring. Kitin et al. (2004) also showed that the cambium is bordered by vessels containing many bordered pits. In *Quercus* the transfer of water from vessel to vessel or from vessel to the cambium is most likely mediated by vasicentric tracheids (Braun, 1970). Together with the results in axial xylem flow, it indicates that water, which is necessary for earlywood-vessel formation (turgor) is provided by previous year latewood vessels and thus forms a functional unit with earlywood vessels across the tree-ring boundary. Future research combining high resolution X-ray computed tomography (Brodersen et al., 2011) with MRI may further support our claim of a ring-crossing conductive vessel network.

Changes in stem water content changes during xylem-flow resumption

When we compared trees at various stages of leaf formation, the overall stem-water content barely differed, however the distribution of water across the stems changed significantly in relation to leaf phenology and vessel development. During dormancy most water was located in the outermost tree ring (2008), while during leaf and earlywood-vessel formation most water occurred in the cambial zone, i.e. the physiological active cells such as the cambium, differentiating xylem and phloem cells and conductive sieve elements. This latter observation is in line with MRI studies on *Pinus thunbergii* (Umebayashi et al., 2011), *Populus* (Windt et al., 2006) and *Quercus* (Kuroda et al., 2006; De Schepper et al., 2012) and with studies using cryo-scanning electron microscopy on *Picea jezoensis*, *Larix kaempferi* and *Abies sachalinensis* (Utsumi et al., 2003). The observed continuous decrease in water content of the previous tree ring is mainly related to a significant decrease of water within fibre tissue, i.e. libriform fibres and surrounding parenchyma. This points to the relevance of fibre tissue within the sapwood for water storage, especially during winter dormancy. This finding is at odds with the general consensus that libriform fibres have a supporting function because of their thick cell walls and narrow lumina and are not part of tree water relations (Metcalfe and Chalk, 1983). Nevertheless, different studies report the function of fibres for water storage, e.g. in *Fraxinus mandshurica,* fibre lumen were normally empty but after cavitation of earlywood vessels, water was observed in fibre lumina, indicating that water might have moved from embolised vessels to fibres (Utsumi et al., 1999). Also Zimmermann and Milburn (1982) reported on water storage in xylem fibres, especially in *Acer* trees during leaf dormancy. De Schepper et al. (2012) found water to be present in fibre tissue in proximity of the cambium at the end of the growing season in young pedunculate oak trees. Potentially, fibre tissues may also play a role in diurnal patters in water movement resulting from depletion and replenishments of internal stem-water reserves caused by a delay between leaf transpiration and soil water uptake (Zweifel et al., 2000).

6.6 Conclusions

Our *in vivo* approach led to new insights into processes around quantification and observation of dynamics in water content and resumption of xylem flow in a ring-porous species. We found that previous year conductive latewood tissue and new developing earlywood vessels form a functional unit that conducts water transport in the beginning of the growing season. This most likely becomes possible through connections across the tree-ring boundary. The role in conductance during reactivation in spring gradually shifts from conductive latewood to new earlywood, concomitant with the stage of development of earlywood vessels and leaf formation. During reactivation, water which is mainly stored in fibre tissue in the previous ring, moves to the cambium and developing new tissue. Our study confirms the great potential of *in vivo* studies using MRI for quantification and observation of dynamics in water content and especially water flow.

Acknowledgements
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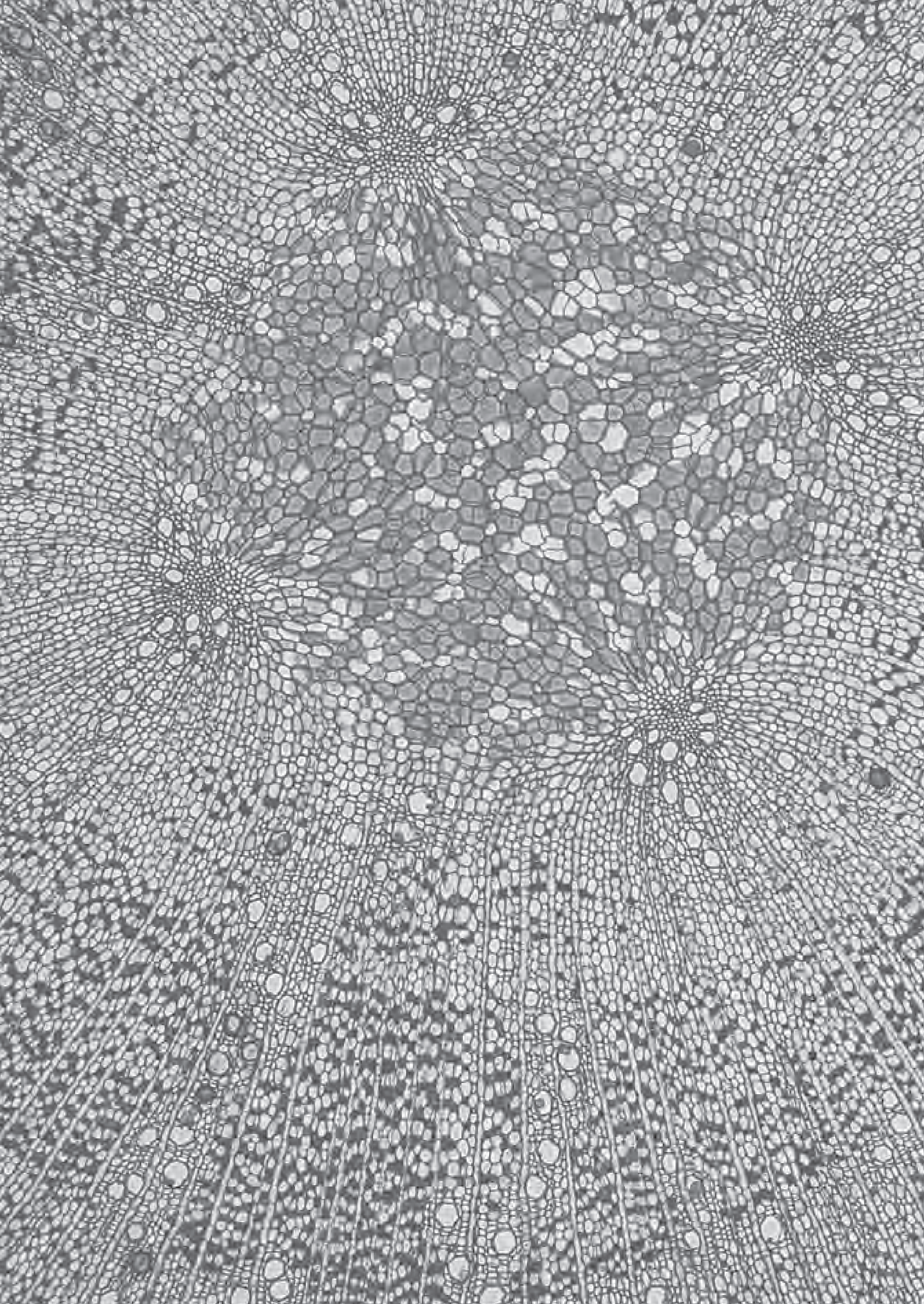
Table S6.1. Characteristics of all trees used for MRI and wood-anatomical research.

Tree	Pheno-phase	Date MRI-scanning	Tree height (cm)	Tree diameter (mm)	Mean EW vessel area ± SD (µm^2) Unlignified / Lignified	EW vessel density (#/mm) Unlignified / Lignified	# of EW vessels /% lignified	Leaf area (cm^2)	Mean. water content ± SD (%)	Flow conducting area ± SD (mm^2)	Xylem flow ± SD (mm^3/s)	Linear velocity ± SD (mm/s)	# flow measure-ments
1	0	4-Mar	188	13.1	- -	- -	0 -	-	30 ± 8	ND	ND	ND	14
2	0	5-Mar	180	13.5	- -	- -	0 -	-	37 ± 9	ND	ND	ND	12
3	I	29-Apr	169	11.3	2289 ± 1740 5193 ± 904	2.15 0.13	70 5.7	-	27 ± 11	ND	ND	ND	1
4	I	29-Apr	174	12.7	4120 ± 2817 7271 ± 2477	1.76 1.26	103 41	-	31 ± 12	0.10 ± 0.01	0.05 ± 0.01	0.48 ± 0.02	3
5	II	1-May	155	13.3	3588 ± 2469 9113 ± 3053	1.83 2.02	141 52.5	-	23 ± 15	0.32 ± 0.01	0.27 ± 0.01	0.84 ± 0.03	4
6	II	30-Apr	155	11.0	3506 ± 2552 7573 ± 2013	2.23 2.02	126 47.6	-	31 ± 15	0.11 ± 0.02	0.05 ± 0.01	0.47 ± 0.01	10
7	III	03-May	156	12.6	4337 ± 3312 8866 ± 2723	2.28 3.45	196 60.2	710	29 ± 14	0.29 ± 0.02	0.16 ± 0.01	0.51 ± 0.01	6
8	III	13-May	154	10.8	2922 ± 1812 6649 ± 1802	2.43 2.36	140 49.3	883	29 ± 12	0.15 ± 0.01	0.09 ± 0.01	0.62 ± 0.04	4
9	IV	21-May	178	12.5	1240 ± 783 8313 ± 3555	1.10 5.25	213 82.6	1760	27 ± 12	0.69 ± 0.04	0.90 ± 0.04	1.32 ± 0.02	9
10	IV	22-May	182	11.6	1605 ± 2495 6054 ± 3243	0.87 7.05	226 87.6	2753	29 ± 14	0.62 ± 0.03	0.76 ± 0.04	1.22 ± 0.05	8
11	V	30-Jun	184	13.3	- 6543 ± 4300	- 9.42	288 100	3576	33 ± 20	1.02 ± 0.08	1.24 ± 0.08	1.21 ± 0.03	3
12	V	1-Jul	176	14.0	- 4050 ± 2696	- 7.95	267 100	2983	31 ± 21	1.6 ± 0.20	1.3 ± 0.20	0.82 ± 0.03	9



CHAPTER 7

Flood-ring formation and root development in response to experimental flooding of young *Quercus robur* trees

Paul Copini, Jan den Ouden, Elisabeth M. R. Robert, Jacques C. Tardif,
Walter Loesberg Leo Goudzwaard & Ute Sass-Klaassen

7.1 Abstract

Spring flooding in riparian forests can cause significant reductions of earlywood-vessel size in submerged stem parts of ring-porous tree species. The resulting 'flood rings' can be used as a proxy to reconstruct past flooding events, potentially over millennia. Yet, the mechanism of flood-ring formation and the relation with timing and duration of flooding has never been systematically investigated. In this study, we experimentally flooded four-year-old pedunculate oak trees (*Quercus robur* L.) at three spring phenophases (late bud dormancy, budswell and internodes extending) and over different flooding durations (two, four and six weeks) to a stem height of 50 cm. At budswell an additional treatment was conducted in which only the roots were flooded. The effect of flooding on root and vessel development was assessed by sampling one set of trees immediately after the flooding treatment, and the remaining trees at the end of the growing season. Earlywood-vessel size and density, as well as ring width, were measured at 25- and 75-cm stem height. In addition, collapsed vessels were recorded. Stem flooding inhibited earlywood-vessel development in flooded stem parts. In addition, when flooding occurred upon budswell and internode expansion, it led to collapsed earlywood vessels below the water level. At the end of the growing season, mean earlywood-vessel size of flooded trees (upon budswell and internode expansion) was always reduced compared in the flooded stem parts by approximately 50% compared to non-flooded stem parts and 55% compared to control trees. This effect occurred independent from flooding duration, being present already after two weeks of flooding. Stem and root flooding were associated with significant root dieback after four and six weeks and mean radial growth was always reduced with increasing flooding duration even though after root flooding many hypertrophied lenticels were formed. We conclude that relatively short periods of flooding (two weeks) reduce earlywood-vessel size in flooded stem parts drastically, on average by 50% compared to the unflooded stem part, in juvenile trees if flooding occurs upon budswell or internode expansion. By comparing stem and root flooding, we demonstrated that flood rings only occur in trees of which the stem has been flooded. As earlywood-vessel development was hampered during flooding, a considerable number of narrow earlywood vessels present later in the season, must have been formed after the actual flooding events. Our study indicates that root dieback, together with strongly reduced hydraulic conductivity due to drastically smaller vessels in the flooded stem part, contribute to reduced radial growth after flooding events in juvenile trees.

Keywords: flooding • hypoxia • leaf phenology • pedunculate oak • *Quercus robur* • vessel development • root development

7.2 INTRODUCTION

Trees growing in riparian forests must cope with regular flooding events and may survive the anoxic conditions associated with flooding (Kozlowski, 1984; Siebel et al., 1998; Glenz et al., 2006). While flooding during dormancy may not affect trees, flooding during the growing season can severely affect development and growth (Gill, 1970; Kozlowski, 1984; Glenz et al., 2006). Species of oak (*Quercus*) and ash (*Fraxinus*) trees frequently occur along river systems in Europe (*Q. robur* L., *F. excelsior* L.), the United States of America, and Canada (e.g. *Q. macrocarpa* Michx., *Q. lyrata* Walter., *F. nigra* March., *F. pennsylvanica* March.). These species are ring porous and form large earlywood vessels in spring, followed by small latewood vessels later on in the growing season and have shown to be able to cope with 50 days of flooding as juveniles or even 100 days as adult trees (Siebel et al., 1998; Kreuzwieser et al., 2004; Glenz et al., 2006). In years with spring flooding events, these trees may alter their wood anatomy and frequently form tree rings with anomalously small earlywood vessels – such rings are known as 'flood rings' (Astrade and Bégin, 1997; St. George and Nielsen, 2003; Tardif et al., 2010; Therrell and Bialecki, In Press). These earlywood vessels may sometimes be accompanied by sickle-shaped, collapsed earlywood vessels (Land, 2014). When flooding occurs during summer, trees may defoliate and exceptionally large latewood vessels may occur (Yanosky, 1983; Yanosky and Cleaveland, 1998; Land, 2014). As flood rings are not only found in living trees but are also preserved in old timber and in subfossil trees, they can be used as a proxy to reconstruct flooding events with an annual or even intra-annual accuracy over potentially millennia and may shed light on the forcing factors between climate, human impact and flooding events (Yanosky, 1983; Friedrich et al., 2004; Wertz et al., 2013; Land, 2014). However, the application of flood rings as proxy for flooding events is hampered by our limited understanding of their formation, in the absence of experimental evidence (St. George, 2010).

The formation of flood rings is, *inter alia*, depending on the time window during which developing xylem cells are able to register the flooding signal (Fonti et al., 2010; Sass-Klaassen et al., 2011). Flooding events during winter dormancy are most likely not recorded whereas during the period of radial growth the flooding signal can be directly recorded in the earlywood (St. George and Nielsen, 2002; Wertz et al., 2013) or latewood (Yanosky, 1983; Land, 2014). In ring-porous species radial growth may either start during late bud dormancy or after budswell, while earlywood formation normally ends after the leaves are fully expanded (Zasada and Zahner, 1969; Huber, 1993; Bréda and Granier, 1996; Sass-Klaassen et al., 2011; Takahashi et al., 2013) and fine roots have developed (Ponti et al., 2004). Radial growth cessation is highly variable among trees and from year-to-year, and may end before leaf abscission (Michelot et al., 2012) or immediately after earlywood formation in spring (Land, 2014). Besides timing, the duration of a flooding event is also of importance (Astrade and Bégin, 1997; St. George and Nielsen, 2002; St. George, 2010). So far, it is known from flooding experiments that six weeks of flooding during leaf development can induce the formation of a flood ring in pedunculate oak (*Quercus robur*) (Stuijfzand et al., 2008). Flooding height is less important, as 20 cm of flooding already induced flood rings in the submerged stem parts of pedunculate oak (Stuijfzand et al., 2008).

The physiology of flood-ring formation is poorly understood. During flooding, hypoxic conditions occur as gas diffusion rates are reduced by $\sim 10^{-4}$ in water compared to air (Cannon, 1925; Kozlowski, 1986). During the growing season, this may inhibit root growth and cause decay and dieback of roots, especially in non-woody fine roots (Coutts, 1982; Yamamoto and Kozlowski, 1987b, a). The reduction of root biomass negatively influences root/leaf ratio and might be the key factor to explain reduced growth of flooded trees (Schmull and Thomas, 2000). Furthermore, reduced growth in flooded trees might occur as trees shift from aerobic respiration to anaerobic respiration which is much less efficient (Hook, 1984). Increased levels of hormones like ethylene and auxin in flooded stem parts have been related to morphological adjustments to cope with the effects of flooding, i.e. the enlargement of lenticels, formation of aerenchyma tissue and adventitious roots which deal with gas exchange and water uptake (Gomes and Kozlowski, 1980; Reid et al., 1984; Yamamoto et al., 1995; Parelle et al., 2006). These hormones could also be related to the formation of flood rings, as increased concentrations are associated with decreases in cross-sectional vessel areas and increases in vessel densities (Aloni, 1991; Tuominen et al., 1995; Junghans et al., 2004).

In this study, we experimentally investigated the formation of flood rings in pedunculate oak in relation to spring leaf-phenology and flooding durations lasting for two, four, and six weeks. We hypothesised that (i) earlywood-vessel development starts at the same moment in flooded and non-flooded trees independent of flooding duration, since ring-porous species need their current year's earlywood vessels for axial water transport (Cochard and Tyree, 1990;

Tyree and Zimmermann, 2002); (ii) spring flooding leads to anomalously narrow earlywood vessels in flooded stem parts when the timing of flooding coincides with earlywood-vessel development within six weeks of flooding (Stuijfzand et al., 2008; Sass-Klaassen et al., 2011); (iii) developing vessels collapse in response to flooding (Land, 2014); (iv) flooding leads to reduced radial growth and root dieback when flooding occurs during the growing season (Yamamoto and Kozlowski, 1987b; Astrade and Bégin, 1997; Schmull and Thomas, 2000).

7.3 Material and Methods

Plant material

We used 200 four-year-old potted pedunculate oak trees (*Quercus robur* L.) with a stem height of approximately 180 cm, that were randomly selected out of 600 available trees. All 600 trees were obtained from a tree nursery in March 2009, one year before the experiment was conducted, and immediately potted in 17 l containers (diameter 30 cm, height 24 cm) in a sand-loam mixture. The trees were then placed in a 1 × 1 m grid in an experimental garden in Wageningen, the Netherlands (51.9884°N, 5.6644°E). The trees were frequently watered using a semi-automatic fertigation system.

Experimentalset-up

The flooding experiment was conducted at the Sinderhoeve Research Station, Wageningen University, The Netherlands (51.9983°N, 5.7523°E) between March and July 2010. To simulate flooding, we used 1.4 × 1.2 × 0.7 m basins (length, width, depth) containing pumps for water re-circulation and maintaining a water level to flood a stem over a height of 50 cm (Fig. 7.1). The water came from a rain fed basin. We installed six two-channel HOBO Pro temperature data loggers (Onset Corporation, Bourne, MA, USA) to record water temperature 25 cm below the water surface (i.e. at 25-cm stem height) of every basin and air temperature 25 cm above the water surface, corresponding to 75-cm stem height of the flooded trees. During the experimental period the mean water temperature of 15.2 ± 4.6 °C (mean ± SD) was generally higher than the mean air temperature (13.1 ± 6.6 °C) while the daily temperature amplitude (max. temperature – min. temperature) in water was much lower compared to the air temperature (Fig. S7.1). Below water, dissolved oxygen concentrations (mg/L) were recorded once a week using a portable WTW Oxi 330 meter, equipped with a CellOx 325 electrode. Oxygen concentrations were on average 10.6 ± 3.9 mg/l (mean ± SD). Differences in oxygen concentrations occurred both in time and between different basins (Fig. S7.1).

Flooding treatments started at three successive leaf phenophases (Fig. 7.1) taking into account the leaf status of individual trees: late dormant trees were flooded on March 19th, trees with swelling buds were flooded between April 23rd and May 5th, and trees with expanding internodes were flooded between May 7th and May 19th 2010, approximately two weeks after budswell (Fig. 7.1). At each phenophase, 10 trees were flooded for either two, four, or six weeks by randomly placing them in one of the 10 basins (90 stem-flooded trees). For the budswell group, an additional treatment was added in which the roots of 10 trees were flooded for either two, four or six weeks by levelling the water to the soil surface (30 root flooded trees) (Fig. 7.1). Control trees, corresponding to all flooding durations were placed next to the basins (Fig. 7.1).

To assess the status of earlywood-vessel and root formation of the trees entering the experiment, five trees per phenophase were harvested (Fig. 7.1). All control trees were watered twice a week and remained – like all flooded trees – exposed to ambient weather conditions. To study the dynamics of flood-ring development, we harvested half of the trees immediately after the flooding treatment (5 treated trees, 5 control trees); the remainder was returned to the experimental garden and was harvested after the end of the growing season in November 2010. Stem sections were sampled at 25 cm (=25 cm below water level in the flooded trees) and 75-cm (=25 cm above water level) stem height. Samples were stored in 50-% ethanol at room temperature prior to further processing.

Wood sample preparation and measurements

For both stem samples collected at flooding cessation and at the end of the growing season, transverse wood sections were cut with a thickness of approximately 20 µm using a G.S.L.-1 sliding microtome (Gärtner et al., 2014) and stained with a safranine/astrablue solution for five minutes. Following dehydration in graded series of ethanol (50-95-100%), samples were rinsed with xylol, mounted in Canada balsam and dried at 60°C for 15 h. Pictures were made with a digital camera (DFC 320, Leica, Cambridge, UK) mounted on a microscope (DM 2500, Leica, Cambridge, UK) using Leica imaging software (version 3.6.0).

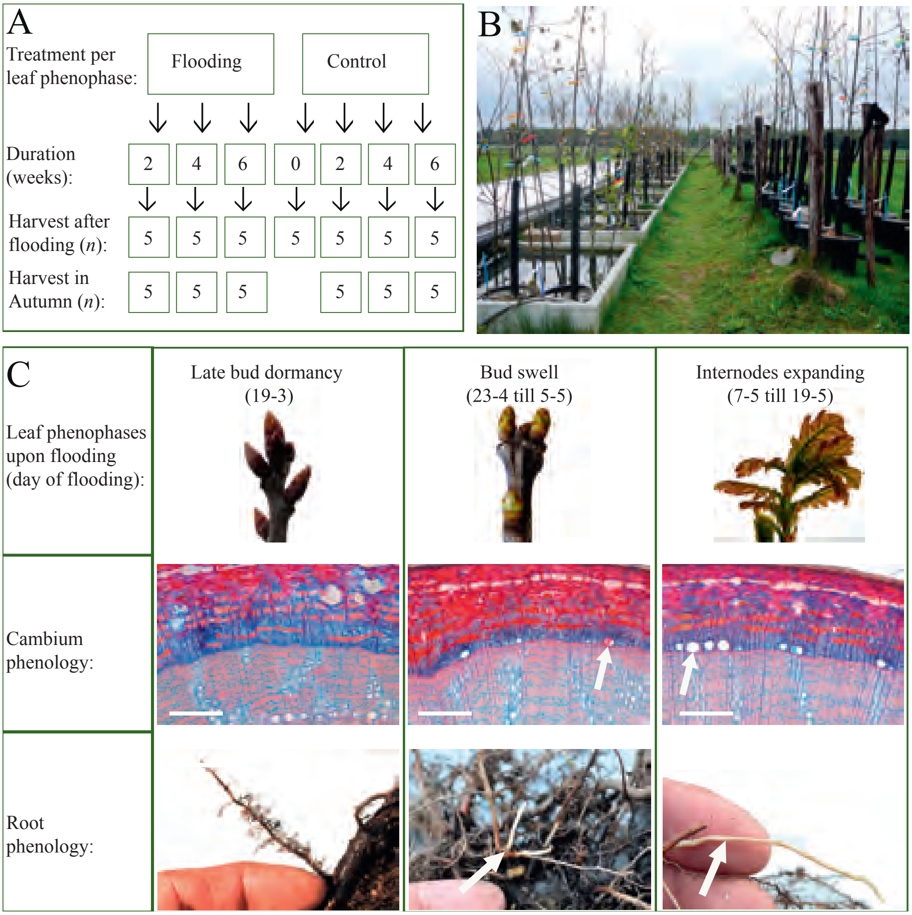


Figure 7.1. Experimental setup of the flooding experiment. (A) At three leaf phenophases, trees were flooded till a stem height of 50 cm for two, four or six weeks ($n = 10$); control trees were placed next to treated trees. Upon budswell, an additional group of trees had only roots flooded during two, four or six weeks ($n = 10$). After the flooding period in spring, 5 trees per treatment were harvested immediately while the remaining trees were returned to the experimental garden to be harvested after the end of the growing season, in autumn. (B) Experimental set up showing the concrete basins with experimentally flooded trees on the left and corresponding control trees on the right. (C) Cambium and root phenophases in relation to the three leaf phenophases. When the buds were dormant at March 19th 2010, the cambium and roots were dormant as well with no earlywood vessels or white roots present. Upon budswell, between April 23rd and May 5th, earlywood-vessel development had started irregularly around the circumference. Normally, earlywood vessels were unlignified while in some cases lignification had started (arrow). In all trees, newly formed elongating white roots were present (arrow). When the internodes started expanding 14 days after budswell, between May 7th and May 19th, earlywood-vessel development had started in all trees and both lignified and unlignified vessels were present and many new elongating white roots had formed.

For all trees harvested, we measured earlywood-vessel size (in μm^2) over a tangential width of approximately 1 cm – which equals approximately 20% of the circumference of the 2010 tree ring – using ImageJ software (ver. 1.44; http://rsb.info.nih.gov/ij; developed by W. Rasband, National Institutes of Health, Bethesda, MD, USA). In order to get an estimate of vessel development in spring, we distinguished unlignified vessels (completely blue) from lignified vessels (partly or completely red). We then determined mean earlywood-vessel area, and calculated the maximum earlywood-vessel size as the mean of the 20 largest earlywood vessels. In addition, earlywood-vessel density (vessels/mm) was calculated by dividing the number of first-row earlywood vessels formed in 2010 over the tangential width (vessels/mm). The 2010 ring width was measured at two radii at both stem heights in the trees that were harvested in November. Collapsed vessels were visually detected per stem height and rings containing three or more collapsed vessels per stem height were recorded as tree rings containing collapsed vessels.

Root development

We recorded root phenophases of all trees that were harvested immediately after the flooding experiments. To do so, we removed the basal stem part with the roots attached from the container and rinsed it with water to remove the soil. Based on studies by Hendrick and Pregitzer (1992) and Ponti et al. (2004), we defined three root phenophases: (i) dormant roots (ii) white roots elongating, and (iii) white roots maturing, forming many small lateral roots. We recorded whether root formation was affected by root dieback as visible by black discolorations or further decay. In addition, the presence of lenticels and adventitious roots was noted.

Statistical analysis

All statistical analyses were performed in the statistical software package SPSS, version 19 (SPSS Inc. Chicago, Il, USA) applying a significance level of 0.05. All post-hoc tests were performed with Bonferroni corrections.

Earlywood-vessel development - We used the trees that were harvested in spring upon the termination of the flooding treatments, to test for differences between earlywood-vessel development below and above water level as compared to control trees. First, we tested for a possible difference in vessel density at 25-cm and 75-cm stem height between all flooded and control (grouping trees with different flooding durations) using a Mann-Whitney U test (*n* = 15). Subsequently, we performed three Mann-Whitney U tests, to assess whether significant differences occurred after two, four or six weeks of flooding, compared to control trees (*n* = 5).

Earlywood-vessel area - The trees that were harvested at the end of the growing season were used to test whether spring flooding leads to anomalously narrow earlywood vessels when flooding coincides with earlywood vessel development, i.e. during budswell and to a lesser degree when the internodes start growing within six weeks of flooding. First we used mixed factorial ANOVAs to test whether mean and maximum earlywood-vessel area and vessel density within each treatment (control, flooded during late bud dormancy, budswell and upon internode expansion) was significantly different in relation to stem height (within subject factor) and flooding duration (between subject factor) (*n* = 5). Second, to test for differences between flooding treatments and their control trees, we used mixed factorial ANOVAs with mean and maximum vessel size and vessel density at 25-cm (below water) and at 75-cm stem height (above water) as within-subject factors and flooding durations and treatment as between-subject factors (*n* = 5). The analyses were followed by post hoc test.

Vessel collapse - We tested whether the occurrence of collapsed earlywood vessels was significantly different at 25-cm stem height between flooded and control trees by using the trees that were harvested after flooding and after the end of the growing season using Fisher’s Exact tests. First we tested whether there was an effect between all flooded and control trees per phenophase (*n* = 15). Subsequently, we performed three separate tests to determine whether significant differences between flooded and control trees occurred after two, four or six weeks (*n* = 5).

Radial growth - To test whether flooded trees have significantly smaller ring widths in flooded stem parts, or along the whole stem because of the flooding treatments, we used ring widths of the trees that were harvested at the end of the growing season and used mixed factorial ANOVAs to test whether the ring widths at both 25 and 75 cm (within subject factor) was affected by the flooding treatment and flooding durations (between subject factors). Post hoc tests were conducted to determine which durations affected radial growth.

Root dieback - For roots it was tested whether dieback occurred in trees that were flooded (stem or root flooded) using the trees that were harvested immediately after the end of the flooding treatments. First we used Fisher's Exact test to assess whether significant differences occurred between flooded and control trees ($n = 15$). Subsequently, we performed three separate tests to determine whether significant differences between flooded and control trees occurred after two, four or six weeks ($n = 5$).

7.4 RESULTS

Earlywood-vessel development during flooding

Late bud dormancy - at March 19th when the first flooding treatment started, none of the trees had formed earlywood vessels (Fig. 7.1A). Also, after two and four weeks of flooding none of the flooded and control trees had started earlywood vessel development (Fig. S7.2). The first earlywood vessels were present six weeks after the start of the flooding treatment in three flooded trees and in three control trees of which the buds were broken. In contrast to the control trees, that started earlywood-vessel development both at 25-cm and at 75-cm stem height, the flooded trees had started earlywood-vessel development only above the water level at 75-cm stem height (Fig. S7.2).

Budswell - upon the second phenophase, between April 23rd and May 5th 2010, earlywood-vessel development had started in all but one trees (Fig. 7.1C). Earlywood vessels were initiated irregularly around the stem circumference, mostly near latewood vessels which were bordering the tree-ring boundary. In one tree, some earlywood vessels were lignified while in the others vessels were still unlignified. The flooding treatments induced significant differences in vessel densities between 25- and 75-cm stem height compared to the control trees (Mann-Whitney U test, $U = 24$, $P < 0.001$, $n = 15$) (Table 7.1, Fig. 7.3D). Two weeks of flooding did not lead to differences in vessel densities, but after four or six weeks significant differences occurred between flooded and control trees (Mann-Whitney U test, $U = 0$, $p = 0.008$, $n = 5$ and $U = 2$, $p = 0.032$, $n = 5$ respectively). After six weeks of flooding the second row of earlywood vessels was already being completed above the water level (Fig. 7.3B, while below water vessel development was hampered (Fig. 7.3D). In the trees of which the roots were flooded upon budswell, no significant effects of the treatment or of flooding duration were observed.

Internode expansion - during the last phenophase, between May 7th and May 19th, earlywood-vessel development had started in all trees (Fig. 7.1C). While some trees had just formed small unlignified earlywood vessels, others showed few (in one tree many) lignified vessels (Fig. 7.1C). After this late flooding treatment significantly larger differences in vessel densities occurred between 25- and 75-cm stem height compared to the control trees (Mann-Whitney U test, $U = 24$, $P < 0.05$, $n = 15$) as in the flooded stem parts earlywood vessel development was slightly lower (Table 7.1). No significant differences were found in relation to flooding duration. At the end of the six-week flooding treatment, i.e. 8 weeks after budswell, all earlywood vessels of the control trees were lignified both at 25- and 75-cm stem height. In contrast, in flooded trees only the earlywood vessel above the water level were lignified whereas below the water level many vessels remained unlignified.

TABLE 7.1. Mean earlywood-vessel density and earlywood-vessel density differences and standard deviations (SD) between 75- and 25-cm stem height measured directly after the flooding treatments. The trees that were flooded for two, four and six weeks were pooled per phenophase (n = 15). The effect of flooding on the difference of earlywood-vessel density was tested using Mann-Whitney U tests. U values and their significance are given as: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NT = Not Tested.

		Mean vessel density (# / mm, ± SD)			**Treatment effect**	
Phenophase	**Treatment**	25 cm stem height	75 cm stem height	difference (75 - 25 cm)	*U*	*P*
Late bud Dormancy	Stem flooding	0 ± 0	0.25 ± 0.59	0.25 ± 0.59	NT	NT
	Control	0.19 ± 0.46	0.21 ± 0.62	0.01 ± 0.31		
Budswell	Stem flooding	1.97 ± 1.67	5.09 ± 1.32	3.12 ± 1.52	26	***
	Control	3.48 ± 1.71	4.60 ± 1.84	1.13 ± 1.62		
Budswell	Root flooding	3.45 ±1.78	5.07 ± 1.34	1.63 ± 1.39	102	-
	Control	3.48 ± 1.71	4.60 ± 184	1.13 ± 1.62		
Internodes	Stem flooding	3.51 ± 1.06	5.83 ± 1.31	2.32 ± 1.38	55	*
	Control	4.85 ± 0.84	5.80 ± 1.17	0.95 ± 1.54		

Earlywood vessel development during the growing season

Late bud dormancy - trees that were flooded upon late bud dormancy had formed slightly narrower mean earlywood vessels at 25-cm compared to 75-cm stem height by the end of the growing season (Table 7.2, Fig. 7.2A). This difference was mainly caused by the trees of the six-week flooding treatment of which two had started with leaf formation after four weeks of flooding – within the six-week flooding treatment – and showed a 38 and 66% reduction in earlywood-vessel size in flooded stem parts at the end of the growing season. Maximum earlywood-vessel size and vessel density were comparable at both heights (Table 7.2, Fig. 7.2B, 7.2C, S7.3). Compared to control trees, mean earlywood-vessel area and maximum earlywood-vessel area were significantly reduced at both heights and vessel density was slightly higher, especially in the flooded stem part (Table 7.3, Fig. 7.2). The significant interaction between duration and treatment was caused by the trees of which the buds started to develop during the flooding experiment. When the six week treatment – in which earlywood-vessel formation may have started – was omitted, there was no effect of the flooding treatments upon earlywood-vessel size or vessel density.

Budswell - one tree that was flooded upon budswell died after the four-week flooding treatment and was excluded from analyses. The remaining trees contained anomalously small earlywood vessels in the flooded stem parts at the end of the growing season; mean earlywood-vessel area and maximum earlywood-vessel area were significantly reduced on average by 47% and 32% respectively compared to 75-cm stem height, independent of flooding duration (Table 7.2, Fig. 7.2A,B, 7.3H, S7.3). Earlywood-vessel density was comparable between 25- and 75-cm stem height (Table 7.2, Fig. 7.2C). Compared to the control trees, the flooded trees contained significantly lower mean or maximum earlywood-vessel areas, and higher vessel densities (Table 7.3, Fig. 7.2C). In addition, the highly significant interactions for mean and max. earlywood-vessel size between height and treatment, indicates that earlywood-vessel size was significantly reduced in the flooded stem parts (Table 7.3, Fig. 7.2A, B).

One tree of the root flooding treatment (two weeks) died and was excluded from the analyses. The remaining trees did not show any effect of root flooding on mean or maximum earlywood-vessel size, earlywood-vessel density in relation to stem height or flooding duration (Table 7.2, Fig. 7.2, S7.3). Compared to the control trees, we found no effect of the root flooding treatment or flooding duration on mean or max. earlywood-vessel area or vessel density (Table 7.3). Only a slightly significant interaction was observed in max. vessel area between duration and treatment.

Internode expansion - at the end of the growing season all trees that were flooded upon internode expansion, contained significantly narrower earlywood vessels (mean and max. earlywood-vessel area) at 25-cm stem height compared to 75-cm stem height (Table 7.2, Fig. 7.2A,B S7.3) independent of flooding duration. On average, earlywood vessels were 50% smaller in the flooded stem parts compared to 75-cm stem height (Table 7.2). Vessel density was comparable at both heights (Table 7.2, Fig. 7.2C). Compared to the control trees, the flooded trees contained significantly lower mean and maximum earlywood-vessel areas (Table 7.3, Fig. 7.2C). In addition, we found highly significant interactions for earlywood-vessel size between height and treatment, indicating that earlywood-vessel size was significantly reduced in the flooded stem parts (Table 7.3, Fig. 7.2A, B).

Table 7.2. Effects of flooding per phenophase on mean earlywood-vessel area (Mean vessel area μm^2), mean of the 20 largest earlywood vessels (Max. vessel area, μm^2), Vessel density (# / mm) and ring width (μm), measured after the end of the growing season following the flooding experiments. The mean and standard deviations (SD) and the difference as a percentage are provided for each variable at 25- and 75-stem height. In addition, the table shows the results of mixed factorial ANOVAs with height as a within subject measure and flooding duration as a between-subject factor. *F* values and their significance are provided as: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The interactions of height and flooding duration were always insignificant (not shown).

	Stem height		Height		Duration	
Variables	25 cm	75 cm	*F*	*P*	*F*	*P*
Control non-flooded (n = 35)						
Mean vessel area	3842 ± 823	3887 ± 936	0.064	-	2.365	-
Max. vessel area	5883 ± 1346	5798 ± 1247	0.073	-	1.744	-
Vessel density	5.89 ± 1.17	6.91 ± 0.77	13.732	**	2.390	-
Ring width	425 ± 153	486 ± 130	9.551	**	1.642	-
Late bud dormancy stem flooding (n = 15)						
Mean vessel area	2467 ± 997	2847 ± 1105	4.727	*	1.832	-
Max. vessel area	4118 ± 1554	4452 ± 1601	1.894	-	1.987	-
Vessel density	7.49 ± 1.66	6.91 ± 1.08	0.056	-	1.494	-
Ring width	287 ± 125	401 ± 154	11.448	**	1.475	-
Budswell stem flooding (n = 14)						
Mean Vessel area	1797 ± 617	3422 ± 910	46.454	***	1.653	-
Max. Vessel area	3500 ± 1086	5124 ± 1180	23.886	***	2.916	-
Vessel density	7.5 ± 0.17	6.91 ± 0.37	0.063	-	0.462	-
Ring width	303 ± 148	313 ± 120	0.170	-	8.904	**
Budswell root flooding (n = 14)						
Mean vessel area	3862 ± 1295	3932 ± 1126	0.123	-	1.529	-
Max. vessel area	5486 ± 1724	5614 ± 1610	0.365	-	2.485	-
Vessel density	6.16 ± 0.87	6.50 ± 0.98	0.753	-	0.242	-
Ring width	334 ± 162	390 ± 181	0.170	-	5.765	*
Internodes stem flooding (n = 15)						
Mean vessel area	1761 ± 695	3394 ± 874	52.960	***	0.319	-
Max. vessel area	3230 ± 1393	5023 ± 1316	36.223	***	0.336	-
Vessel density	6.07 ± 1.2	6.40 ± 0.39	0.753	-	0.384	-
Ring width	269 ± 175	245 ± 114	0.581	-	0.841	-

Table 7.3. Effects of flooding measured after the end of the growing season following the flooding experiments, on mean earlywood-vessel area (mean vessel area, μm^2), mean of the 20 largest earlywood vessels (max. vessel area, μm^2), vessel density (# / mm) and ring width (μm) compared to control trees. The table shows the results of mixed factorial ANOVAs in which height was the within-subject measure and flooding treatment (treatment versus control) and flooding duration were between subject factors. *F* values and the *P* values are given as: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. * Note that the six-week group (late bud dormancy) was not analysed as these trees started with budswell and leaf formation.

	Treatment		Duration		Duration * treatment		Height		Height * duration		Height * treatment	
Variables	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Late bud dormancy stem flooding versus control*												
Mean vessel area	2.771	-	1.155	-	1.704	-	0.002	-	0.157	-	0.681	-
Max. vessel area*	0.695	-	0.606		1.576	-	0.695	-	0.173	-	0.312	-
Vessel density*	0.347	-	1.260	-	0.617	-	1.699	-	0.164	-	1.238	-
Ring width*	3.778	-	0.917	-	0.659	-	17.155	***	2.638	-	0.793	-
Budswell stem flooding versus control												
Mean vessel area	25.684	***	0.587	-	5.016	*	27.123	***	2.995	-	22.639	***
Max. vessel area	20.462	***	0.612	-	5.673	*	8.603	**	2.605	-	10.913	**
Vessel density	7.029	*	0.667	-	1.030	-	1.740	-	1.004	-	3.153	-
Ring width	15.853	**	8.755	**	1.282	-	6.165	*	1.478	-	3.287	-
Budswell roots flooding versus control												
Mean vessel area	0.145	-	1.618	-	2.917	-	0.227	-	2.101	-	0.001	-
Max. vessel area	0.096	-	1.697	-	4.099	*	0.012	-	1.183	-	0.265	-
Vessel density	0.172	-	1.591	-	1.056	-	7.658	*	0.131	-	2.385	-
Ring width	3.703	-	8.061	**	1.081	-	9.945	**	0.652	-	0.348	-
Internodes stem flooding versus control												
Mean vessel area	23.217	***	0.866	-	2.957	-	36.450	***	1.685	-	29.277	***
Max. vessel area	14.826	***	0.352	-	2.026	-	22.345	***	1.856	-	18.937	***
Vessel density	0.637	-	0.453	-	2.083	-	9.619	**	1.258	-	2.208	-
Ring width	11.366	**	0.195	-	0.866	-	1.389	-	0.864	-	6.118	*

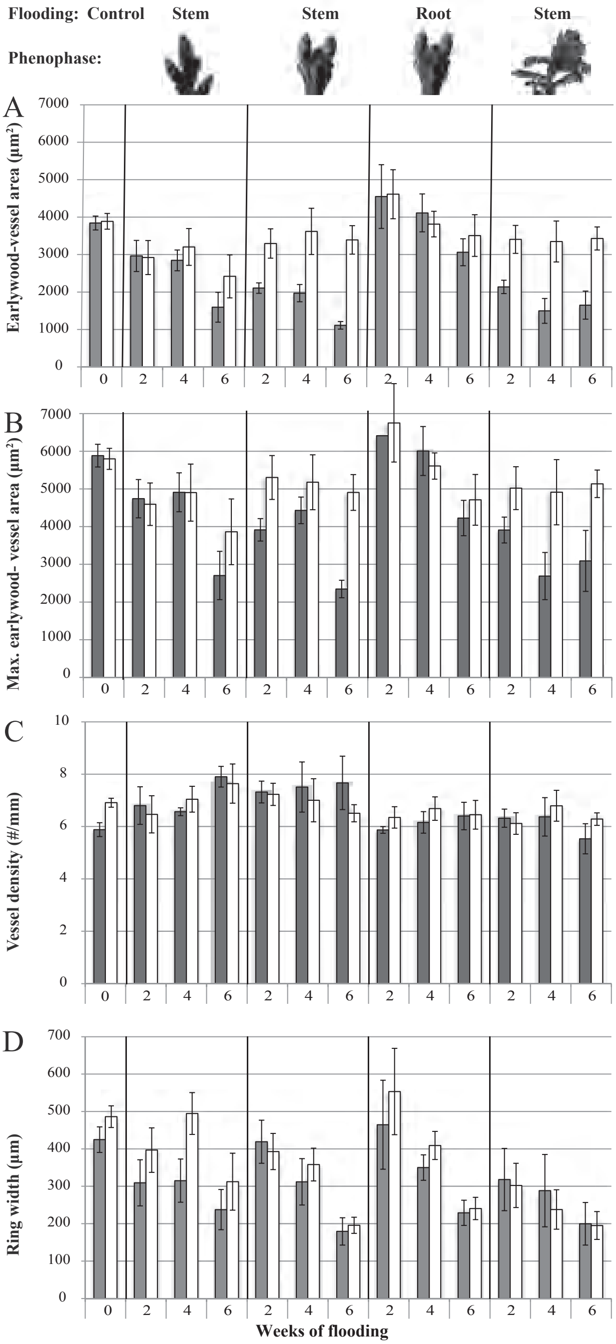


Figure 7.2. Mean earlywood-vessel area (A: µm²), means of the 20 largest earlywood vessels (B: max. earlywood-vessel area, µm²), vessel density (C: # / mm) and ring width (D: µm) after the end of the growing season following the flooding experiments for the different treatments ($n = 5$) and control trees

($n = 35$). The grey bars show the mean values and standard errors of stem sections taken at 25-cm stem height; white bar are the mean and standard errors of the stem section at 75-cm stem height. The control trees were pooled as no significant differences occurred between them.

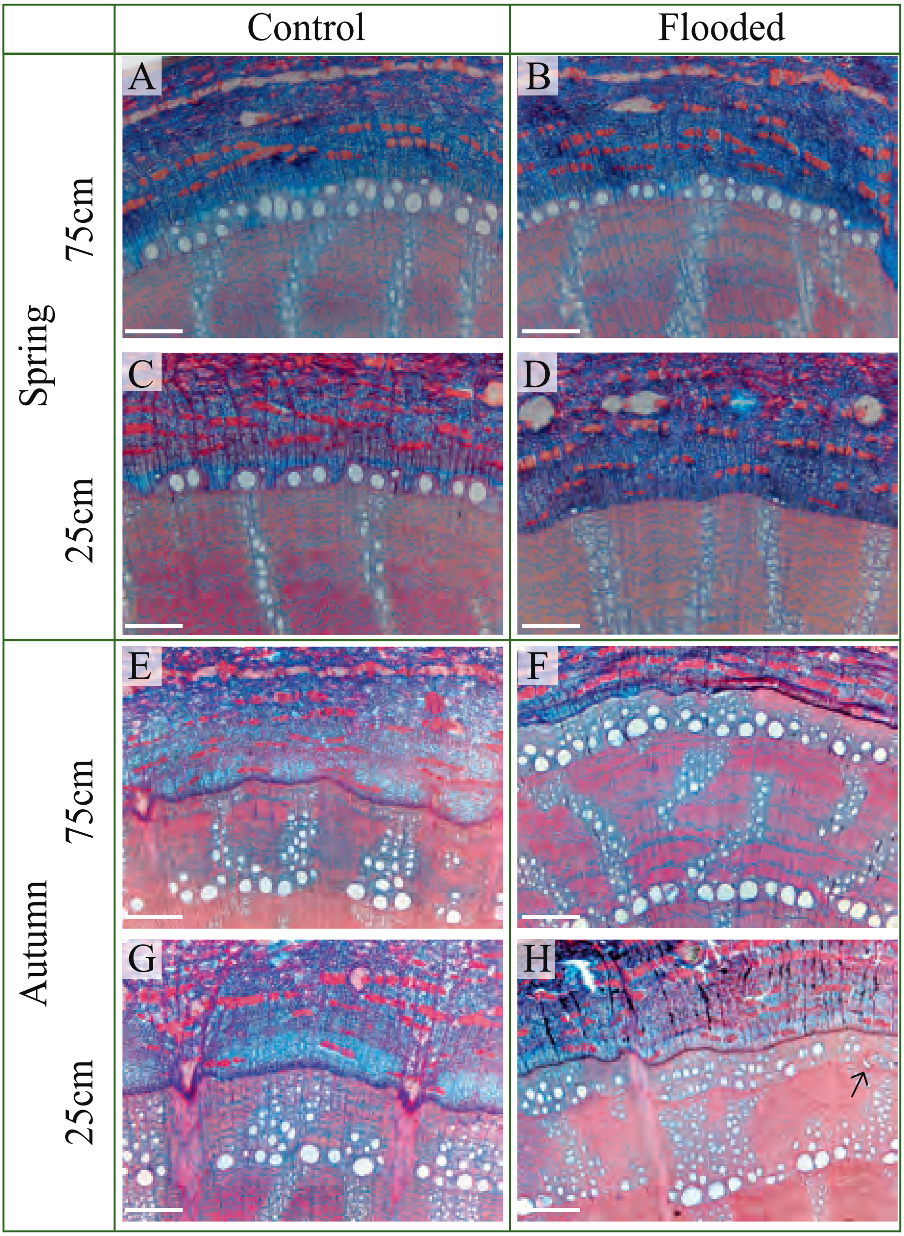


FIGURE 7.3. Effects of six weeks of flooding after budswell on vessel development and radial growth in pedunculate oak. The white scale bar represents 300 µm. The cambial zone of control (left) and flooded trees (right) at 25 cm (flooded) and 75 cm (non-flooded) immediately after the flooding treatment in

Spring 2010 (A - D) or after the growing season in Autumn 2010 (E - H). A and B show the cambial zone at 75-cm stem height. In both trees many lignified earlywood vessels are present. C and D show the cambial zone at 25-cm stem height of the same trees as in A and B. Whereas in the control tree vessel development started and many earlywood vessels are lignified in the flooded tree, 25 cm below water hardly any vessels have been formed and most are not yet lignified. E and F show the cambial zone at 75-stem height (not submerged). In both the control and flooded tree the earlywood vessels are relatively large, while ring width in the flooded tree is strongly reduced. G and H show the cambial zone at 25-cm stem height (submerged stem parts in flooding treatments). While the trees of the control group showed relatively large earlywood vessels and wide ring widths, the flooded trees formed on average 70% smaller vessels and 59% smaller tree rings.

Collapsed vessels

We found collapsed, sickle shaped earlywood vessels (Fig. 7.4) in flooded stem parts (25-cm stem height) that were flooded upon the phenophases budswell and internode expansion. The number of trees containing collapsed earlywood vessels significantly differed between control and flooded trees after budswell, both immediately after flooding (Fisher's Exact test, $p = 0.006$, $n = 15$) as well as after the growing season (Fisher's Exact test, $p = 0.017$, 15 control trees, 14 flooded trees), Immediately after the flooding experiments, six out of 15 flooded trees contained collapsed vessels, whereas after the end of the growing season seven out of 15 trees were affected by vessel collapse. In the trees that were flooded after internode expansion, vessel collapse occurred in 11 (out of 15) trees immediately after the flooding experiments and was absent in control trees (Fisher's Exact test, $p < 0.001$, $n = 15$) and in 14 (out of 15) after the end of the growing season and did not occur in control trees (Fisher's Exact test, $p < 0.001$ $n = 15$). Post hoc tests showed that the effect of flooding duration was insignificant on the number of flooded trees containing collapsed vessels.

Root development

Late bud dormancy - When the flooding treatment on dormant trees started, root formation had not yet started. The first white, elongating roots were present in four control trees belonging to the six-week treatment, whereas in the flooded trees new root formation was absent (Fig. 7.1C). One tree formed hypertrophied lenticels just below the water level during bud dormancy.

Budswell - at budswell the formation of white roots in the elongation phase had started in all sampled trees (Fig. 7.1C). We found significant differences in root dieback between all control and flooded trees (Fisher's Exact test, $p < 0.001$). During the course of the experiment, root formation of control trees

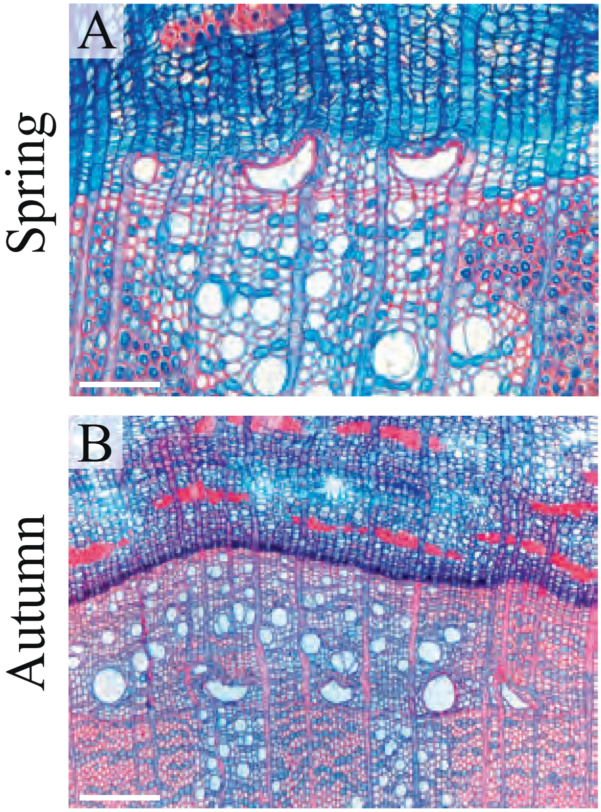


Figure 7.4. Collapsed earlywood vessels in flooded stem part at 25-cm stem height. Below the water level many irregularly shaped vessels are present both immediately after the flooding event (A) and after the end of the growing season (B).

progressed from root elongation (after two and four weeks), to roots maturing after six weeks (Fig. 7.5) and no root dieback occurred. In contrast, in the flooded trees white roots in the elongation phase were unaffected by dieback after two weeks of flooding. However, after four weeks all white roots in the elongation phase were dying back (Fisher's Exact test, $p = 0.011$) and after six weeks under water, roots were mostly decayed and easily detached from the main root system (Fisher's Exact test, $p = 0.008$) (Fig. 7.5).

Trees of which the roots were flooded, showed a similar pattern with significant root dieback occurring only in flooded trees (Fisher's Exact test, $p < 0.002$). Significant differences between flooded trees and control trees occurred after four weeks (Fisher's Exact test, $p = 0.008$) and six weeks (Fisher's Exact test, $p = 0.008$). After six weeks most roots of flooded tress were decayed (Fig. 7.5). In contrast to the trees of which the stem was flooded, nine (out of 15) of the root flooded trees formed hypertrophied lenticels (Fig. 7.5).

Internode extension- trees that were flooded upon the phenophase internode extension, showed a similar pattern in root dieback (Fisher's Exact test, $p < 0.001$). The roots of control trees progressed from roots in the elongation phase, after two weeks, to roots maturing after four and six weeks whereas their flooded counterparts were all affected by root dieback after four (Fisher's Exact test, $p = 0.008$) and six weeks (Fisher's Exact test, $p = 0.008$).

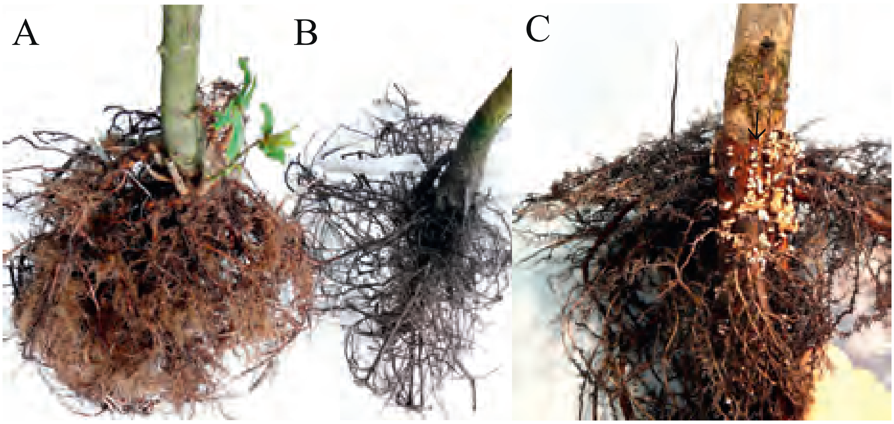


Figure 7.5. The effect of the six-week flooding treatment on root development of pedunculate oak or waterlogging treatment that started at the phenophase budswell. (A) A control tree with many maturing roots. (B) The root system of a tree that was flooded till a stem height of 50 cm; elongating or maturing roots are absent. (C) The root system of a tree of which only the roots were flooded; elongating or maturing white roots are absent but many hypertrophied lenticels have developed just below the water surface.

Radial growth

The mean ring widths were always significantly reduced in flooded trees as compared to control trees, when flooding occurred upon budswell or internode expansion (Table 7.3, Fig. 7.2D). The trees that were flooded upon budswell showed a significant reduction in ring width in relation to flooding duration between the two and six-week treatment (Bonferroni Post-hoc test, $p = 0.005$) corresponding to mean ring widths of 4.06 and 1.88 mm respectively (Fig. 7.3D). Root flooding showed a similar patterns with reduced growth with increasing flooding duration; mean ring width was significantly smaller after six weeks of flooding compared to the two week treatment (Bonferroni Post-hoc test, $p =$

0.018) (Table 7.2, Fig. 7.2D). In control trees and trees that were flooded during late bud dormancy, ring width was normally larger at 75-cm compared to 25-cm stem height. In flooded trees upon budswell or internode extension the effect of height was insignificant (Table 7.2, Fig. 7.2D).

7.5 DISCUSSION

Earlywood-vessel development is suppressed in flooded stem parts

In four-year-old pedunculate oak trees harvested immediately after the flooding treatments earlywood-vessel development was suppressed in submerged stem parts if flooding occurred at budswell or internode expansion. In the two trees that were flooded during leaf dormancy but had started leaf development while flooded, vessel development was totally absent in flooded stem parts. This local impediment of earlywood-vessel development in flooded stem parts has, to the best of our knowledge, never previously been reported and is most likely caused by the hypoxic conditions accompanied with flooding (Kozlowski, 1984). As small earlywood vessels were frequently found lignified directly after the end of the flooding treatments, it seems likely that lignification occurs under anoxic conditions during flooding. The fact that earlywood-vessel development virtually stops in flooded stem parts is remarkable as ring-porous species need to form new earlywood vessel to replace the dysfunctional earlywood in the previous tree ring before the leaves are fully expanded (Cochard and Tyree, 1990; Davis et al., 1999; Tyree and Zimmermann, 2002). In case when no new earlywood vessels are formed in flooded stem parts, water transport most likely occurs in previous-year latewood vessels that are also thought to be of importance during spring reactivation (Zimmermann, 1964; Utsumi et al., 1999; Tyree and Zimmermann, 2002; Chapter 7).

Flooding reduces earlywood-vessel size

We found that stem flooding significantly reduces mean and maximum earlywood-vessel area – on average by 50% – in flooded stem parts, when flooding occurs after budswell or internodes extending. This is independent of flooding duration (two, four or six weeks). The finding that already a two-week flooding period strongly reduces earlywood-vessel size is in line with Therrell & Bialecki (In Press) who studied flood rings in *Q. lyrata* and *Q. macrocarpa* along the Lower

Mississippi River and linked flood rings to streamflow data. They reported that flooding events of more than 10 days during spring most likely induce a flood ring. Our results are moreover in accordance with many studies that show that earlywood formation normally only lasts between four and eighth weeks during spring (Sass-Klaassen et al., 2011; Gonzalez-Gonzalez et al., 2013).

By comparing stem with root flooding, we showed that stems need to be actually flooded to induce changes in the anatomy of tree rings. This is in line with results gained from a flooding experiment in which mature pedunculate oak trees were flooded till a stem height of 20 cm with stagnant water, resulting in a flood ring in the flooded stem part (Stuijfzand et al., 2008). In addition it may explain why St George *et al.*, (2002) found that flood rings were present in bur oak (*Q. macrocarpa* Michx) at 45 cm, but were absent sometimes above 1.1 meter and in general above 3 meters. Also Land (2014) found that flood rings in *Q. robur* were present in flooded basal stem parts but absent at 4-m stem height.

Based on differences found between earlywood-vessel density recorded directly after flooding and after the end of the growing season (Table 7.1 and 7.2), our study showed that a substantial number of earlywood vessels were formed after the flooding events. These earlywood vessels either did not enlarge to normal size (as in the control trees) even though they were not directly affected by anoxic conditions. We can only speculate on the processes behind the reduction of earlywood-vessel sizes after flooding has ceased. Possibly, earlywood-vessel enlargement after flooding was affected by high concentrations of auxin and ethylene which are known to increase during flooding events (Gomes and Kozlowski, 1980; Tang and Kozlowski, 1984; Aloni, 2013).

We expected that juvenile trees flooded after their internodes were expanding, would already contain many enlarged and lignified earlywood vessels, and that consequently mean earlywood-vessel area would be less reduced compared to flooding after budswell. This was not the case in our experiment. A possible reason could be that temperatures during the first 19 days of May 2010 were far below average and among the coldest measured since weather records of the Royal Netherlands Meteorological Institute (KNMI) began. We suspect that these adverse temperatures during the experiment had strongly slowed down earlywood-vessel development, while having less effect on the ongoing leaf development.

Collapsed vessels may pinpoint flooding events

We frequently observed vessel collapse in flooded stem sections of many trees flooded upon the phenophase budswell or internode expansion. Earlywood vessel collapse in response to flooding has been reported in pedunculate oak trees growing along the river Main in Germany as well as in a experimentally flooded juvenile trees (Land, 2014). Collapsed vessels found in our experiment resemble to collapsed earlywood vessels in frost rings that are induced when temperature drops below -5 °C (Stahle, 1990; Leuschner and Schweingruber, 1996). In contrast to collapsed vessels in frost rings, our vessels were not surrounded by callus tissue (Land, 2014). As collapsed vessels were absent in control trees, and no callus tissue occurred, we can exclude frost as a triggering factor. However, as most trees in the budswell or internode-expansion phase had already started vessel formation prior to the flooding treatments, we assume that collapsed vessels are the result of vessel-development interruption during the expansion phase (Zasada and Zahner, 1969; Leuschner and Schweingruber, 1996) when the flooding events occurred. As this phenomenon only occurred in submerged stem sections after budswell and internode expansion, the presence of collapsed vessels in a tree ring can be used as a characteristic feature to pinpoint flooding events to the restricted period in the season when earlywood vessels develop.

Radial growth and root dieback

We found that flooding up to 50-cm stem height as well as root flooding reduces mean ring width when flooding occurred after budswell or internode expansion. This is in line with many studies on juvenile trees that showed that radial growth can be seriously hampered by spring or summer flooding (Coutts, 1982; Yamamoto and Kozlowski, 1987b, a). The presence of many hypertrophied lenticels upon root flooding, which are permeable to water (Groh et al., 2002) and might play an important role in water supply during flooding events (Parelle et al., 2006), did not affect the tendency of trees to grow less with increasing flooding duration.

In our experiment, root dieback occurred in all trees that were flooded (stem or roots) for four or six weeks after budswell or internode expansion. Reduced growth after flooding is most likely related to the inhibited root development. This is in line with the general view that low oxygen concentrations may inhibit root initiation and seriously affect root development so that new roots need to be developed after flooding events (Coutts, 1982; Tang and Kozlowski, 1984;

Kozlowski, 1986; Siebel et al., 1998). In addition, reduced radial growth in flooded trees could be an effect of flooding-induced local reductions in earlywood-vessel size in basal stem parts, which creates a hydraulic bottleneck. Other studies explain reduced radial growth by reduced leaf area, stomata closure and early-leaf senescence occurring during flooding (Kozlowski and Pallardy, 1984; Schmull and Thomas, 2000). Whereas stomata closure may have influenced growth in our experiment, leaf senescence did not occur in our study. Mature trees can tolerate flooding events better than juvenile trees (Kozlowski, 1984), which may partly explain why tree-ring width in mature riparian trees is not reduced in years with flooding events (Astrade and Bégin, 1997; Stuijfzand et al., 2008; Tardif et al., 2010; Land, 2014).

Implications for flood reconstructions

In this study, we synchronised the timing of the flooding for all trees by initiating the flooding treatment at a specific leaf-phenological stage. Under natural flooding conditions, trees in forests can be in many different stages of leaf and xylem development. Consequently, a flood might be recorded in a particular tree, while the flooding signal is absent in other trees. This is in line with observations by (St. George and Nielsen, 2000) who found flood rings in between 6 and 24% of the sampled *Q. macrocarpa* trees. However, note that *Q. macrocarpa* is not a true riparian species, it normally grows on the upper floodplain terraces where trees are less frequently flooded. As flood rings only occur in flooded stem parts, sampling of trees for flood signals should include the stem base. By sampling different heights, an estimation of the flooding height can be retrieved.

We found that relatively short floods, lasting for two weeks, induce the formation of narrow earlywood vessels in flooded stem parts when flooding occurs during earlywood formation. Since the earlywood vessels of ring-porous species are formed within a short time window – in our case first-row earlywood vessels were mostly formed six weeks after budswell – flood rings encode for flooding events that occur during a narrow time window: while the start and end of a flooding event does not seem relevant, the event must take place, or start in, the narrow period of earlywood formation. Collapsed vessels can be used to pinpoint flooding events that had started at the moment of earlywood-vessel development.

7.6 Conclusions

We conclude that relatively short periods of flooding (two, four and six weeks) reduces earlywood-vessel size drastically, on average by 50%, in flooded stem parts of juvenile pedunculate oak trees. This flood marker occurs in the growing season when the flooding event takes place, but only if flooding occurs after budswell or internode expansion when earlywood vessels are developing. As during flooding, earlywood-vessel development is hampered, the narrow earlywood vessels in flood rings consist of cells that are mainly formed after the actual flooding events. By comparing stem and root flooding, we demonstrated that flood rings only occur in trees of which the stem is flooded. Our study indicates that root dieback, together with strongly reduced hydraulic conductivity due to extremely narrow earlywood vessels in flooded stem parts, most likely contributes to reduced radial growth along the whole stem after flooding of juvenile trees.

Acknowledgments
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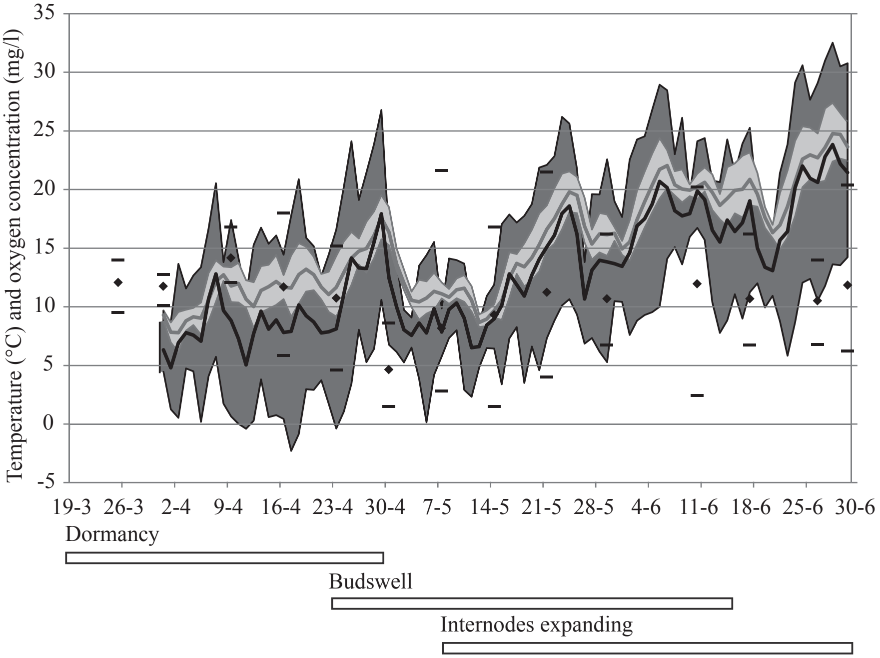


Figure S7.1. Air and water temperature measured 25 cm above and below the water level respectively in combination with oxygen concentrations during the flooding experiments. Air temperature is indicated by the black line while the dark grey area represents the daily range. Water temperature is indicated by the grey line and the light grey area indicates the daily range in water temperatures. The mean oxygen levels (mg/l) are indicated with the diamond and the range is indicated with the horizontal lines. During the flooding treatments that started at the phenophase dormancy at March 19th and lasted till April 30th, the water and air temperature were 12.0 ± 2.6 °C and 9.6 ± 5.7 °C (mean ± SD), respectively. Note that we were unable to measure temperature during the first 11 days of the experiment. During the treatments that started with budswell and were conducted between April 23rd and June 28th, the mean water and air temperature were 16.4 ± 4.2 °C and 14.5 ± 6.2 °C, respectively. In the last series of treatments that started after internodes expansion, between May 7th and June 30th, the mean water and air temperature were 17.4 ± 4.4 °C and 15.5 ± 6.2°C respectively. Oxygen concentrations were highest in the period in which trees were flooded after dormancy, with mean values of 12.4 ± 2.0 mg/l (mean ± SD). During the period when trees were flooded upon budswell or internode expansion the values were lower, the variation was higher, and the differences between different ponds increased with 9.8 ± 4.4 and 10.5 ± 4.1 mg/l respectively.

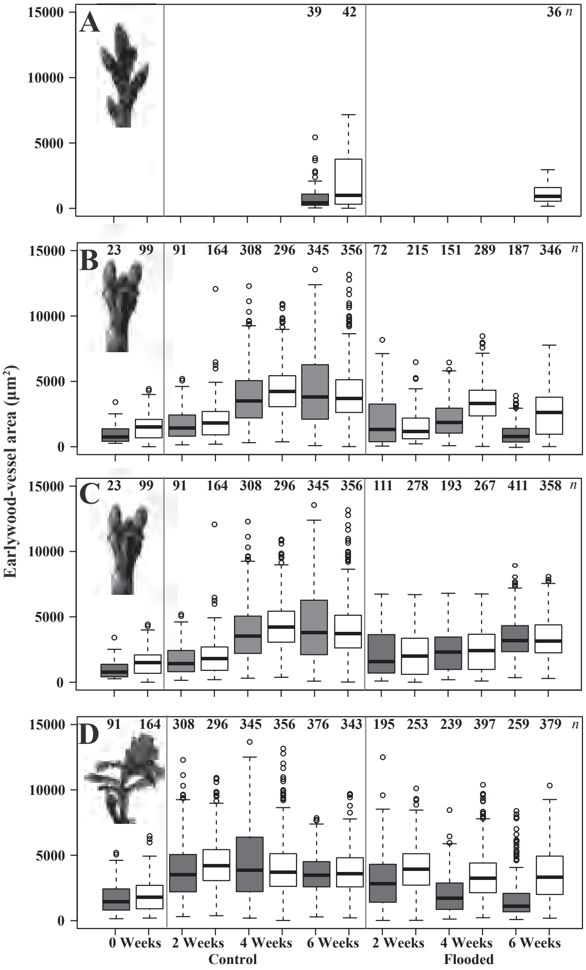


Figure S7.2. Boxplots showing the vessel areas (μm²) of all measured earlywood vessels at 25-cm (gray boxes, flooded stem part) and at 75-cm (white boxes, non-flooded stem part) stem height in pedunculate oak. Above the boxplot the number of vessels is indicated. Left from the vertical lines the control trees measured at time of treatment inititation or immediately after the flooding treatment. Right of the vertical line all treated trees. A: Stem flooding during late bud dormancy. After six weeks three trees had started earlywood vessel development of which one had started with leaf formation. While the flooded trees only formed vessel above the water level the control trees formed vessels both at 25 and at 75cm. B: Stem flooding started on budswell. Earlywood-vessel expansion and formation occured above the water level at 75cm while the vessels remained small in the submerged stem part. C: Root flooding started on budswell. Earlywood-vessel expansion and formation occured at both 25-cm and at 75-cm stem height. Note that the same control trees were used as for the stem flooding treatment upon budswell D:Stem flooding started after the internodes of the trees started extending approximately two weeks after budswell. Earlywood-vessel development above water was comparable to control trees. However below water vessel formation was hampered and vessels remained smaller compared to above water.

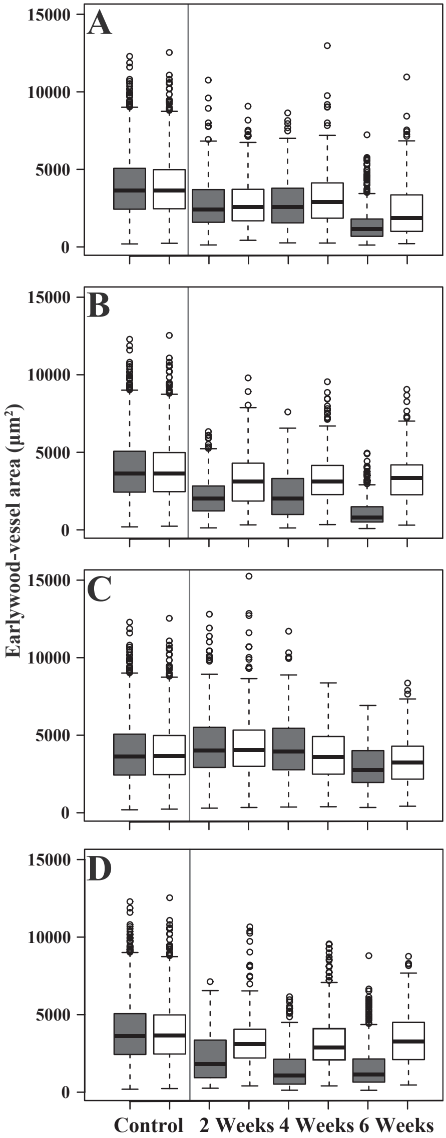
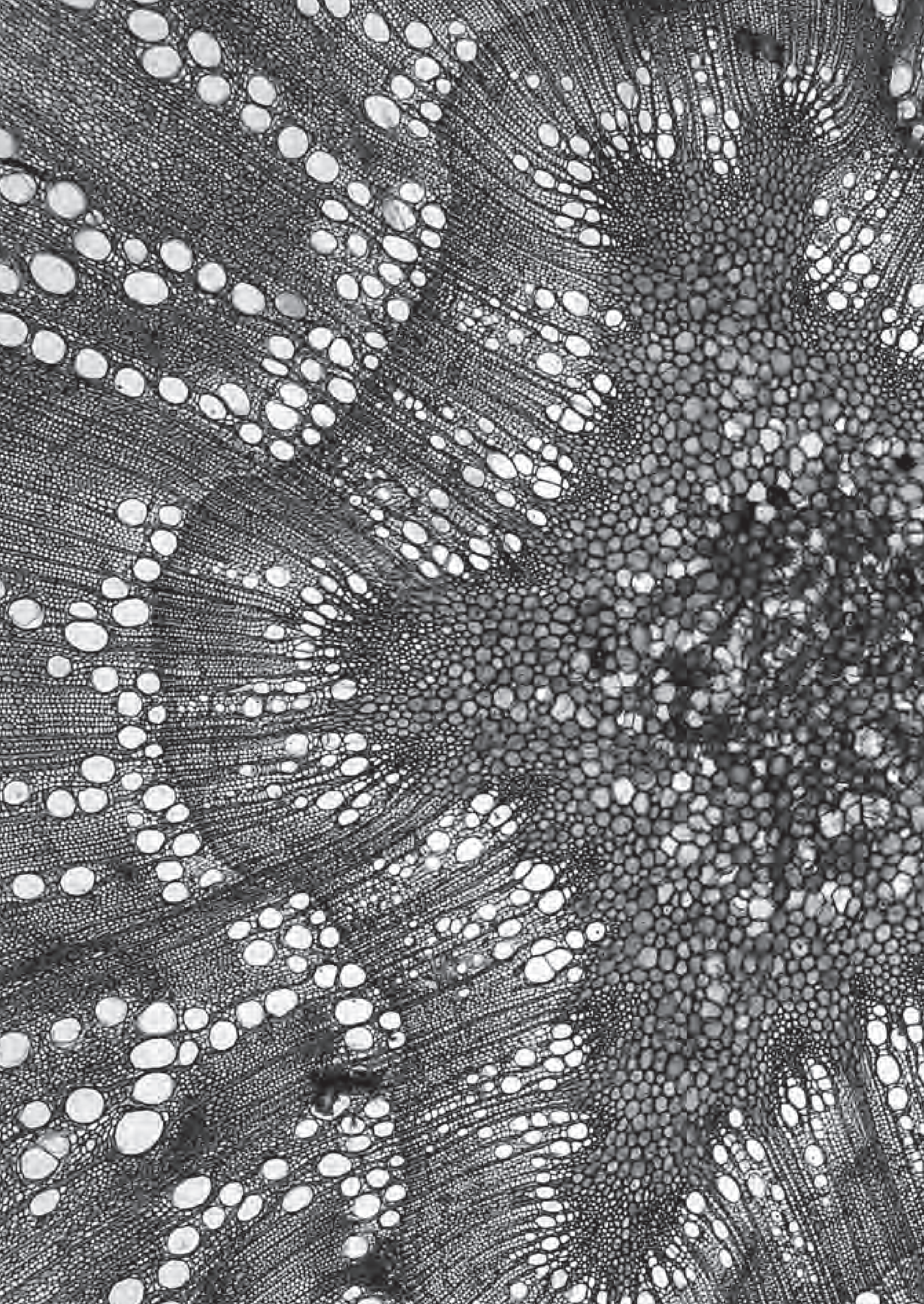
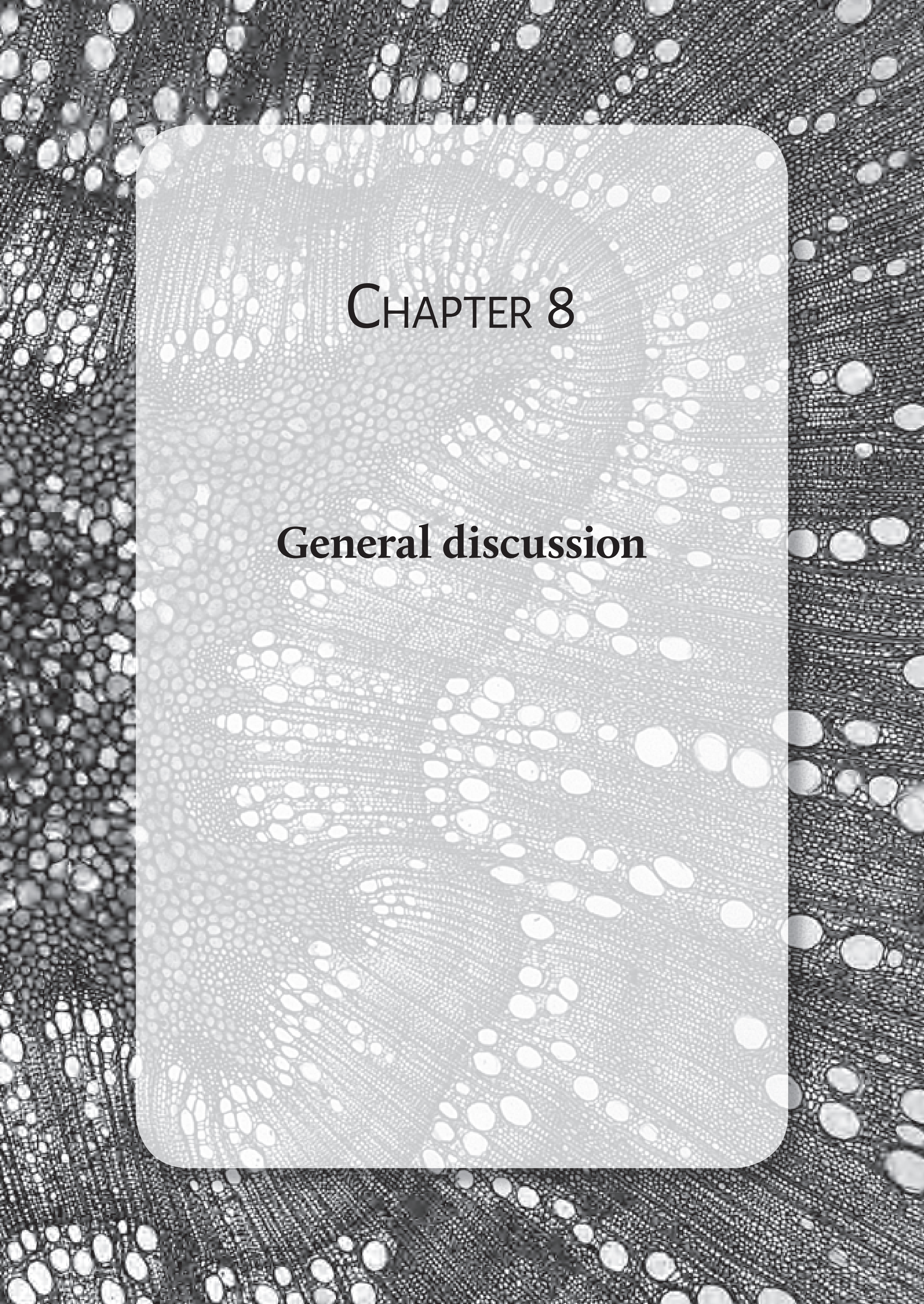


Figure S7.3. Boxplot showing the vessel area (µm²) of all measured earlywood vessels at 25-cm (gray boxes) and at 75-cm (white boxes) stem height in pedunculate oak after the end of the growing season. Per tree on average 69 ± 18 vessels (mean ± SD) were measured. As the trees were flooded till a stem height of 50 cm, a stem height of 25 cm equals 25 cm below the water and at 75-cm stem height 25 cm above the water level respectively. The control trees were grouped as no significant differences occured between them (*n* = 35). The other treatments represent five trees but note that after four weeks of stem flooding upon budswell one tree died just as after two weeks of root flooding. (A) Earlywood-vessel areas of trees where flooding started during late bud dormancy. On average the vessels were slightly smaller compared to the controls. After six weeks of flooding earlywood-vessel size decreases below water level mainly because two trees had started leaf formation while flooded. (B) Flooding started on budswell. Earlywood-vessel size was strongly decreasing in all flooding durations. (C) Root flooding started upon budswell. Earlywood-vessel size was not significantly different compared to control trees. (D) Flooding started after the internodes of the trees started extending approximately two weeks after budswell. Earlywood-vessel sizes were always smaller below water level.





Chapter 8

General discussion

8.1 INTRODUCTION

To understand changes in biodiversity, landscape or climate, there is a need to date natural phenomena that occurred in the past. One of the most accurate tools for dating such phenomena is dendrochronology: the science that uses tree rings – dated to their exact year of formation – for analysing temporal and spatial patterns of processes in physical and cultural sciences (Speer, 2010). Whereas tree-ring widths represent the aggregate influence on tree growth by factors such as climate and site condition, wood-anatomical markers in dated tree rings are proxies that may encode for specific environmental events. Moreover, by relating the position of a marker within a tree ring, an estimate can be given of the timing within the year (Ortloff et al., 1995; Stoffel et al., 2005; Kaczka et al., 2010). Such intra-annual resolution is unrivalled among dating techniques. The accurate application of wood-anatomical markers as a dating tool requires knowledge of the mechanisms underlying their formation. Yet, little experimental evidence is available for the formation of different types of markers (St. George, 2010; Stoffel et al., 2010). In this thesis a combination of field studies and experiments was used to study radial growth and the formation of wood-anatomical markers that differed in their origin: those originating by wounds and those originating from abrupt changes in the stem or root environment. This final chapter presents a discussion on the results and their implications for dating outbreaks of invasive *Anoplophora* insects, drift-sand dynamics, and flooding events.

8.2 THE TEMPORAL RESOLUTION OF WOOD-ANATOMICAL MARKERS IS LARGELY DETERMINED BY THEIR ORIGIN

Markers induced by wounding (Chapter 2) or flooding (Chapter 7) show a high intra-annual temporal resolution, whereas burial might not be recorded even three years after the triggering event (Chapter 5). Wounding and flooding both have an instant effect on tree functioning, locally around the wound or in the flooded tree. Due to wounding, cells are destroyed and vascular transport in the phloem and/or xylem is disrupted (Pearce, 1996; De Schepper et al., 2013). Flooding drastically and immediately reduces gas exchange and oxygen levels and stops radial growth (Chapter 7; Kozlowski, 1984). Such dramatic events induced the formation of wood-anatomical markers almost instantly (wounding), or during the growing period (flooding) and thus possess a potentially high temporal resolution. Burial of basal stem parts may also induce the formation of wood-anatomical markers (Chapter 4), but this may be delayed by many years or might not occur at all (Chapter 5; Knowlson, 1939). Therefore, characteristic shifts in wood structure from stem wood to root wood after burial only provides a minimum burial age. Clearly, tree functioning needs to be drastically and instantly affected to induce a wood-anatomical marker that contains an intra-annual resolution similarly to e.g. frost rings (Glerum and Farrar, 1966) and wounds caused by e.g. fire or ice (Swetnam and Lynch, 1993; Tardif et al., 2010).

Wound markers - The temporal resolution of wound dating was determined by studying the dynamics of wound reactions together with radial growth. Wounds can be assigned to three different phases related to tree-ring development (Fig. 8.1). The first two are straightforward: (1) wounds that originated during dormancy are located at the tree-ring boundary; (2) wounds that originated during the growing season are located within the tree ring (Fig. 8.1). If wound-xylem formation was observed locally around the wound (3), it could either imply that the wound originated at the very end of the growing season or just before the onset of radial growth (Chapter 2) or during dormancy when temperature was high (Fig. 8.1, Chapter 3). It is yet unknown whether local growth formed during winter dormancy will remain visible as a marker during the growing season following the triggering events, as the cambium might simply continue with the formation of the ring. This temporal resolution of wound markers is much less accurate compared to the two to four weeks that has been claimed for dating the time of wounding (Ortloff et al., 1995; Kaczka et al., 2010). The most important reason is the highly variable tree-ring growth that limits the temporal resolution. Integration of studies on intra-annual tree-ring growth using dendrometers, pinning experiments and micro-coring may allow for a better intra-annual resolution.

Flood rings - The temporal resolution of flood rings, characterised by anomalous small earlywood vessels in submerged stem parts, is different compared to wound dating as floods that occur during dormancy are not recorded (Fig. 8.2). Flooding events starting during dormancy must prevail into the growing season, or start during the period of radial growth, to leave a flood marker. The window of opportunity to record a flooding signal in the earlywood vessels may only last for

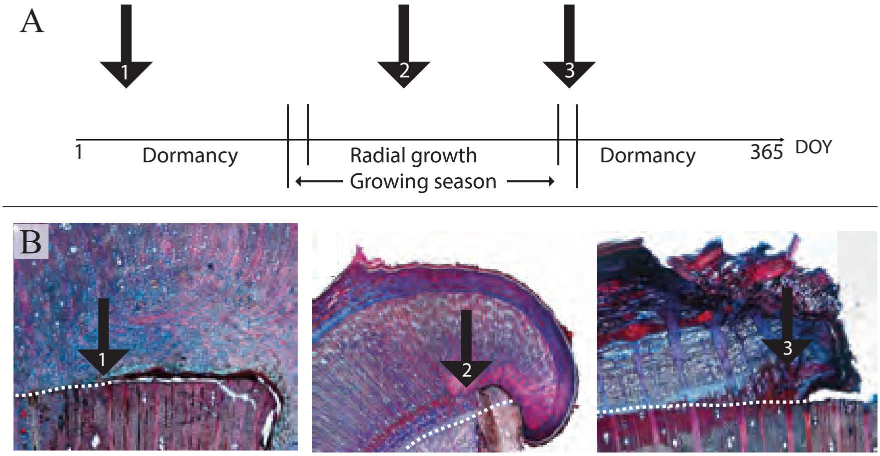


Figure 8.1. Overview of the temporal resolution of wound dating. A: Three distinct phases can be distinguished: (1) wounds that originated during dormancy are located at the tree-ring boundary; (2) wounds that originated during the growing season are located within the tree ring; and (3) at the very end of the growing season local growth occurs which remains stored in the tree-ring structure. B: Thin sections representing the three phases that can be distinguished.

ca. one month depending on actual climatic conditions, as vessel enlargement is completed in this period of time (Sass-Klaassen et al., 2011; Gonzalez-Gonzalez et al., 2013). The implication for the information captured by flood markers in earlywood is, that they could be induced by a flooding event of two weeks taking place during the growing season (Chapter 7), but the flooding could well have lasted for months before it is recorded during the formation of earlywood vessels (Fig. 8.2). On the other hand, the presence of collapsed earlywood vessels within flood rings may pinpoint floods that started when earlywood vessels were enlarging (Fig. 8.2).

This thesis focussed on formation of flood rings in response to spring flooding only. Recent research by Land (2014) indicates that summer flooding for 4, 8 or 12 weeks induces the formation of a flood ring in the late wood of young pedunculate oak trees, which indicates a similar temporal resolution for summer floods. So far, it has not been studied what will happen when trees are flooded during summer and remain flooded till dormancy has started. As flooding inhibits stem growth below the water level (Chapter 7), it seems likely that the flooding event is not stored as a flood ring in submerged stem parts (Fig. 8.2). As ring-porous species along rivers frequently contain growth suppressions in which only earlywood is formed, flooding events that occur during summer might not always be detected.

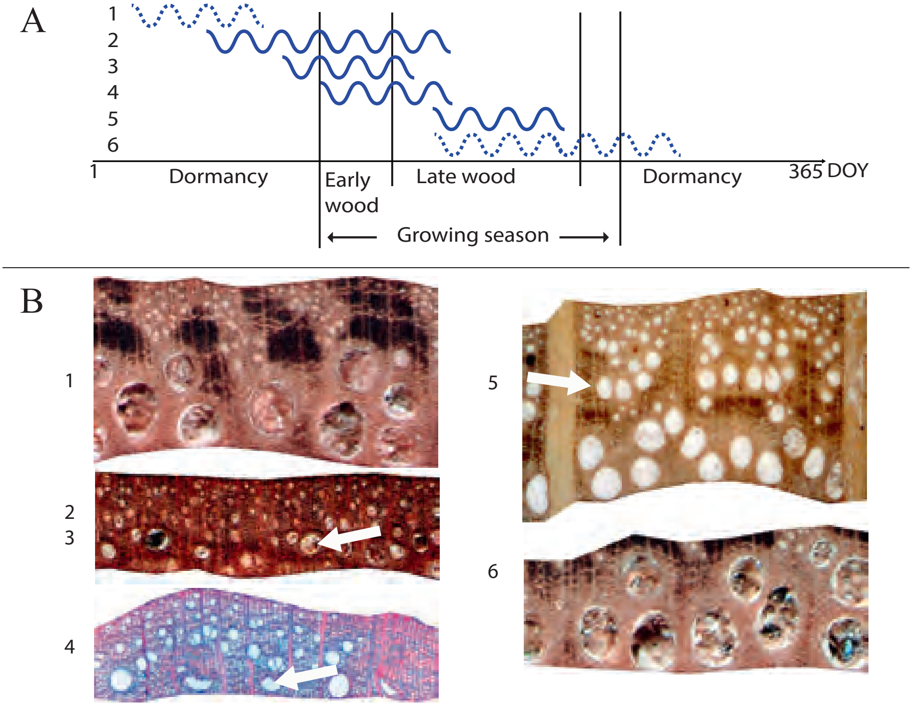


FIGURE 8.2. Schematic overview of the temporal resolution of flood rings (A) and concomitant changes in wood anatomy (B). Floods that occur before the growing season (1) are not recorded by the cambium, whereas floods that extend into the period of earlywood-vessel formation (2, 3) result in a flood ring with anomalous small earlywood vessels. Floods that start during earlywood vessel formation (4) contain collapsed earlywood vessels in addition to many anomalously small vessels. Floods that start and end during latewood formation (5) result in a flood ring with anomalous large latewood vessels. It is hypothesised that floods starting during latewood formation but lasting until winter dormancy (6) may result in a tree ring with hardly any latewood.

Burial markers - Pedunculate oak stems that are partly covered by drift sand did not show any response to a three-year lasting burial treatment. Most likely, burial only leads to a change in wood anatomy after adventitious roots have been formed (Chapter 5) and depending on the species – adventitious roots may form years after burial or might never develop (Strunk, 1995, 1997; Koprowski et al., 2010; Kogelnig-Mayer et al., 2013). Naturally buried stems of pedunculate oak in the Loonse en Drunense Duinen showed that a stem buried by ca. 60 cm of sand had not induced adventitious roots, whereas a stem covered by 2 m of drift sand in the same dune, had formed large adventitious roots (Fig. 8.3) (Beerens, 2011; Bos, 2012). This indicates that there is a threshold after which adventitious roots are formed, which could be related to e.g. water availability, nutrient availability, and oxygen levels. In contrast, young branches of pedunculate oak may form adventitious roots only when covered by a 5-10 cm organic layer (Fig. 8.3C, Copini et al., 2007). Thus tree age, bark thickness and also the presence of wounds may influence adventitious root formation (De Klerk et al., 1999; Hartmann, 2002; Dech and Maun, 2006; Maun, 2009). Consequently, the temporal resolution of transition from stem wood to root wood is inter-annual rather than intra-annual.

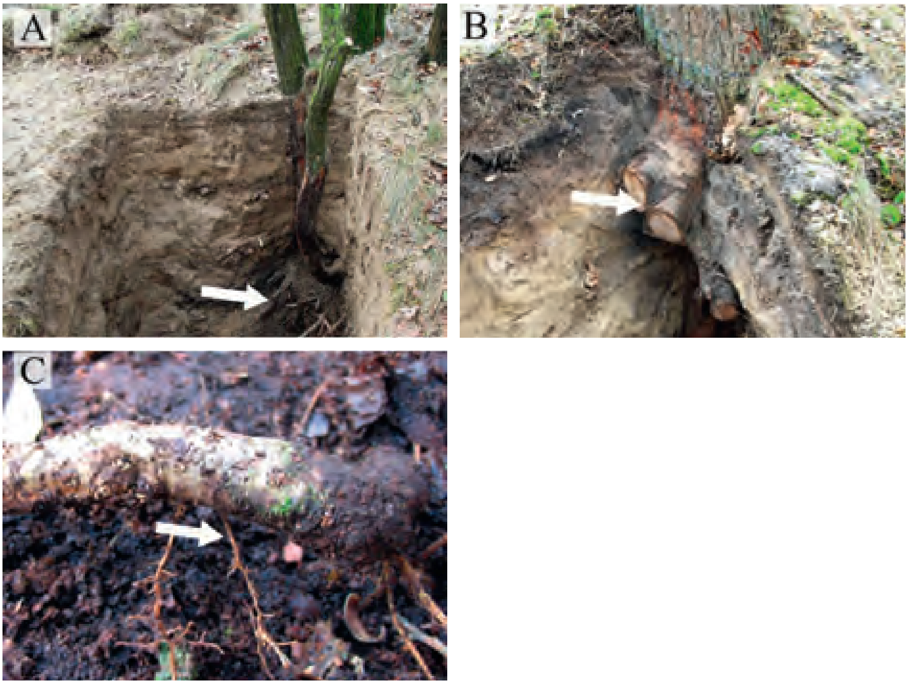


Figure 8.3. Adventitious root formation in pedunculate oak (*Quercus robur*). A: Adventitious root formation was absent in trees that were covered by ca. 60 cm of drift sand. (B) Large adventitious roots were formed in a tree covered by 2 m of drift sand. (C) Young horizontal branch that formed adventitious roots in a heath area.

8.3 Dating wounds to fight invasive Anoplophora outbreaks

The specific wounds caused by the creation of exit holes or oviposition pits (Fig. 2.1) by xylobiont insects are used to date the emergence and population dynamics of invasive insects such as the Citrus longhorned beetle (*Anoplophora chinensis* Forster) or the Asian longhorned beetle (*A. glabripennis* Motschulsky) (Sawyer, 2007; Sass-Klaassen and Copini, 2008; Copini et al., 2012). These two species, that naturally occur in Asia, are among the most destructive invasive insects found worldwide. In this thesis the accuracy of dating exit holes was studied by simulating exit-hole formation by coring holes in Japanese maple (Chapter 2). It was assumed that the origin of wounding did not affect wound closure, in line with wounding and inoculation experiments (Mullick, 1977; Dujesiefken et al., 1991; Pearce, 1996; Grünwald et al., 2002; Deflorio et al., 2009). As *A. glabripennis* contains endosymbiont microbes to digest lignocellulose (Schloss et al., 2006; Geib et al., 2008), it is conceivable that such microbes may alter the formation of wound markers in the host tree. However, no significant delays in wound closure have been reported and dating *Anoplophora* wounds caused by maturation feeding under laboratory conditions in three juvenile Japanese maples showed that these were partly overgrown during the same growing season of wounding and thus did not limit the temporal resolution (Copini, unpublished data).

Dating exit holes has proved to be of significant importance in fighting invasive insect outbreaks in the Netherlands (Copini and Sass-Klaassen, 2009, 2010; Copini et al., 2012), Italy (Sabbatini Peverieri et al., 2012) and the USA (Sawyer, 2007). When exit holes of *Anoplophora* beetles are found in imported trees, it is crucial to know when and where adult longhorn beetles have emerged: in the country of origin, at the location of import, in the nursery, or in a private garden. By pinpointing the exact year and season when *Anoplophora* beetles emerged, it was proven that almost all exit holes in imported trees were formed during the growing season at the location of import, i.e. in the Netherlands. This knowledge was subsequently used to draw up customised eradication measures and allocate proper surveillance plans (Van der Gaag et al., 2010; Loomans et al., 2013). Annual tree-ring formation is clearly a prerequisite for exact dating of wounds. After the trees had been imported, they were grown in a nursery under the temperate climatic conditions of the Netherlands, which induced the formation of annual growth rings that enabled accurate dating (Chapter 2). However, as the formation of annual tree rings is dependent on local climatic conditions (Cherubini et al., 2003; Wils et al., 2009), exit-hole dating could not be applied to exactly date the year and season of exit holes that were formed prior to import, i.e. in the country of origin. For this, first the seasonality of tree-ring formation has to be studied in the country of origin.

Dating of exit holes can also be applied to reconstruct the population dynamics of *Anoplophora* outbreaks. Studies from Italy showed that *Anoplophora chinensis* had been present for at least seven years before eradication measures were implemented (Sabbatini Peverieri et al., 2012). In the Netherlands and southern England, it was shown that *A. glabripennis* beetles are able to survive and reproduce (Sass-Klaassen and Copini, 2008; Straw et al., In Press). This confirmed the relevance of efficient eradication measures to prevent the large invasions by this insect that occurred in Italy and the USA (Haack, 2006; Haack et al., 2010). However, preliminary results also showed that an *A. glabripennis* population discovered in Almere in November 2010, the Netherlands, was declining (Copini et al., 2012). As the beetles emerged at the very end of the growing season, it seems likely that the reduced breeding success and restricted growth of this population were the result of limited food availability and unfavourable temperatures (Copini et al. 2012). Also, the outbreak of Asian longhorn beetles in Paddock Wood in southern England showed that adults emerged from late August onwards, when lower temperatures most likely caused most eggs to delay hatching until the next year (Straw et al., In Press).

From the experiments performed in this thesis, combined with insights from dating exit holes and oviposition pits, it can be concluded that with detailed knowledge on tree-ring formation, exit-hole dating is possible in all host species that form annual tree rings, and that this technique is applicable to many other xylobiont (quarantine) species with a comparable life cycle, such as *Saperda candida* Fabricius and *Apriona germari* Hope.

8.4 Implications for reconstructing drift-sand dynamics

Various dating techniques can be applied for dating drift-sand deposits, such as radio carbon, Optically Stimulated Luminescence (OSL) and dendrochronology (Alestalo, 1971; Heikkinen and Tikkanen, 1987; Marin and Filion, 1992; Koprowski et al., 2010). Using OSL dating to date quartz grains yields direct deposition ages, while radiocarbon dating may yield dates for soil organic matter extracted from buried humic horizons (Sevink et al., 2013; Vandenberghe et al., 2013). Such studies have revealed that aeolian activity had already started before ca. 5,000 BC, while other massive drift-sand phases started in the 14th to 15th century (van Mourik et al., 2010; Sevink et al., 2013). As wood is not conserved in dry sand, reconstructions based on tree rings only provide information during the lifetime of a tree, which limits dating to about the last 250 years (Chapter 4). This period is of great interest as it encompasses both the period before and after major reforestations (Tesch et al., 1926; Riksen et al., 2006). Moreover, in contrast to radio carbon and to OSL dating of drift-sand layers, trees contain the burial or exposure signal, even if the sand under which they were buried has long since disappeared (Chapter 4). The age of a tree provides a minimum date for the sand deposited below the tree, while the age of adventitious roots, growing out into the deposits that have accumulated around the tree, provides a minimum burial age (Strunk, 1997). In the Loonse en Drunense Duinen and Deelensche Start many multi-stemmed oak trees occur (Fig. 4.3, 4.4) whose stems are frequently genetically identical and that have undergone burial and subsequent exposure (Copini et al., 2005; den Ouden et al., 2008). It was described how systematically sampling both below and above adventitious roots revealed changes from root wood to ring-porous stem wood and *vice versa*, including long-lasting growth suppressions (Chapter 4). Based on earlier observations, it was hypothesised that such changes in wood anatomy most likely occur in the year of burial or exposure. However, based on our three-year field-burial experiment conducted in the same drift-sand area (Chapter 5), it should be concluded that the burial and exposure periods mentioned in Chapter 4 are in fact “minimum periods”, i.e. dependent on the time interval between burial and adventitious root formation. These estimates do not necessarily provide an annual resolution of accumulation of deposits or erosion events (Chapter 5).

In a pilot study conducted in the Loonse en Drunense Duinen on a pedunculate oak tree that was successively buried to a stem height of 2 m, dendrochronological and OSL dating were compared (Fig. 8.4) (Beerens, 2011; Wallinga et al., 2011; Bos, 2012). As 10 different organic layers were present indicating different deposit phases, of every layer an OSL sample was taken and compared with a disk from the same height (Fig. 8.4). The first tree ring around the pith of every disk provided a maximum age of the burial events, whereas the date of the uppermost adventitious roots provided an indication of the minimum period. It was found that the Bayesian OSL dating (Oxcal) provided dates which largely corresponded to these dendro-constraints, and thus indicates that OSL dating of burial events in drift sand might be as accurate as tree-ring research (Wallinga et al., 2012) and in the absence of adventitious roots, OSL dating might even be more accurate. In addition, wood anatomy of roots and buried stems was highly variable and common phenomena are striking shifts in vessel patterns (ring versus diffuse porous) in combination with drastically reduced ring widths and even missing rings (Fig. 8.4). Discontinuous rings rarely occur in ring-porous trees (Haneca et al., 2009), but were present below 2 metres of drift sand. Moreover, by cross-dating samples taken below and above the current ground level, it was found that the tree had stopped forming tree rings below adventitious roots: up to 20 years were missing below 2 metres of drift sand (Fig. 8.4C) and hardly any heartwood had been formed. Similar results were reported for below-ground connections between genetically identical oak clusters in which the last 11 tree rings had not been formed (Copini et al., 2007). In diffuse-porous species and conifers growing under stress conditions, missing outer rings are frequently found (Wilmking et al., 2012). As the cambium may remain functional during inactivity lasting up to 18 years (Wilmking et al., 2012), this may seriously hamper cross-dating when buried trees with a quiescent cambium suddenly resume tree-ring formation upon exposure. In the worst case, this may lead to the period of burial being drastically underestimated. Future research should focus on the relation between adventitious root formation and a cross validation between dendrochronology and other dating techniques such as OSL dating, as this may greatly improve our understanding of the accuracy of dating drift-sand dynamics.

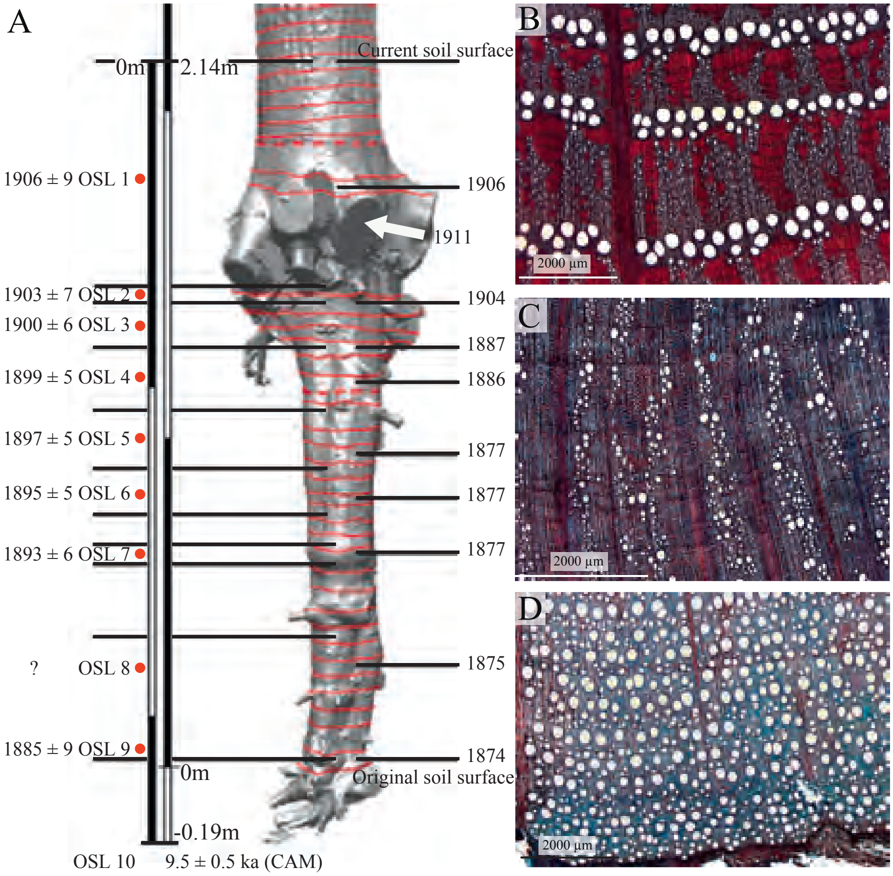


Figure 8.4. Stem morphology and wood anatomy of a buried oak stem, established in 1874, that was covered by 2 meters of drift sand in the Loonse en Drunense Duinen and formed adventitious roots (After Beerens, 2011 and Bos, 2012). A: 3D-scan of a buried oak stem in the Loonse en Drunense Duinen showing that below the uppermost adventitious roots of which one was dated to have originated in 1911 (arrow), growth was drastically hampered. On the left side of the stem the OSL dates of soil samples are given (Oxcal), whereas on the right side the date of the first tree ring is given. B: transverse thin section showing typical ring-porous stem wood with large earlywood vessels with diameters of up to 300 µm as present at 130 cm above the soil surface. C: Wood structure found two meters below the adventitious roots at the height of OSL sample 9. The ring-porous structure had disappeared and vessels are oriented in radial profiles, making ring boundaries difficult to recognise. D: Wood from the same stem section as in C, but located immediately beneath the quiescent cambium. Compared to the ring-porous aerial part, tree-ring boundaries are almost absent and radial growth is suppressed. Based on cross dating it was found that the last 20 tree rings were missing.

8.5 Flood-ring formation and implications for the reconstruction of river dynamics

In response to flooding, riparian ring-porous tree species such as *Fraxinus* or *Quercus* may also alter their wood anatomy and form "flood rings" (Fig. 8.2, Chapter 7) (Yanosky, 1983; Astrade and Bégin, 1997; St. George et al., 2002; Therrell and Bialecki, In Press). Many studies have shown that flooding drastically affects tree functioning (Gill, 1970; Kozlowski, 1984; Tang and Kozlowski, 1984; Schmull and Thomas, 2000). In line with this, in Chapter 7, it was found that all newly formed roots died within four weeks of being submerged in water and that, on average, growth was inversely related to increasing duration of flooding. However, the most striking finding is the virtual absence of earlywood-vessel formation during spring-flooding events. As shown in Chapter 6, in ring-porous trees earlywood vessels must be formed to replace the dysfunctional earlywood vessels from the previous year and thus ensure axial water transport (Cochard and Tyree, 1990; Tyree and Zimmermann, 2002). It seems likely that stomata remain closed until new roots (Kozlowski, 1984) and vessels have developed in the part of the stem submerged by flooding. The narrow earlywood vessels that mostly form after the flooding event, are connected to normal-sized earlywood vessels in the part of the stem above water. Thus, according to the Hagen-Poiseuille law, the submerged part of the stem should act as a hydraulic bottleneck for water conductance. The presence of small vessels in submerged stems is also at odds with the general hydraulic architecture of trees, in which it has been shown that vessels progressively increase in size from the leaves toward the root tips (McElrone et al., 2004; Anfodillo et al., 2006; Gebauer and Volařík, 2013). To understand the physiological consequences of flood markers in a whole tree context, further studies should focus on axial sapflow measurements in submerged and above-water parts of the stem, in relation to whole-tree functioning. To understand the actual impact of flooding events on the viability of trees in flood-plain forests and specifically the link between flooding, anatomy and tree functioning it is important to link spatial and temporal scales to actual flooding events. This can be done by studying the wood anatomy of trees growing at different locations along river systems in combination with remote sensing focussing on years with extreme flooding events. Advanced remote-sensing techniques can be used to study the extent of flooding events with a potential two-day resolution (Carroll et al., 2009). In addition, increased stress due to flooding might be derived from satellite imaging Normalized Difference Vegetation Index (NDVI) (Sims and Colloff, 2012).

Several studies have used flood rings as a flood proxy, and flood chronologies extend back several hundreds of years, e.g. to 1645 for the Red River (St. George and Nielsen, 2002) and to 1700 for the Mississippi river (Therrell and Bialecki, In Press). Flood rings chronologies have shown to document nearly all significant floods of the 20th century and severe floods documented in prior centuries as well (St. George and Nielsen, 2002; Therrell and Bialecki, In Press). As – in contrast to the wood of trees in drift-sand areas – the wood of riparian tree species is often preserved in fluvial sediments, flood reconstructions based on tree rings are possible for large parts of the Holocene (Friedrich et al., 2004; Land, 2014). As mentioned earlier, these flood markers either indicate spring-flooding events of ca. two weeks (Chapter 7) or prolonged winter flooding events. The presence of collapsed earlywood vessels within flood rings may pinpoint floods that started in spring, when earlywood vessels were enlarging (Fig. 8.2). It is important to note that collapsed vessels and also narrow earlywood vessels resembling a flood ring may occasionally form in response to defoliation, extremely cold winters, and extremely dry spring months (Garcia-Gonzalez and Eckstein, 2003; Thomas et al., 2006; Khasanov, 2013). However, in contrast to flood markers, which are restricted to submerged stem parts, these anomalies are normally present in the entire tree. This implies that in order to unambiguously reconstruct spring flooding events, it is essential to sample trees at different stem heights. In addition, during flooding, trees in riparian forests can be in many different stages of leaf and xylem development. Consequently, a flood might be recorded in a particular tree, while the flooding signal is absent in other trees. Although this thesis indicates that flood markers contain restricted information on past flooding events, this information is highly valuable in the debate whether the number of flooding events is increasing and to understand the driving factors behind floods (Knox, 2000; St. George and Nielsen, 2002; Mudelsee et al., 2003; Hall et al., 2014; Blöschl et al., 2015).

8.6 Conclusions

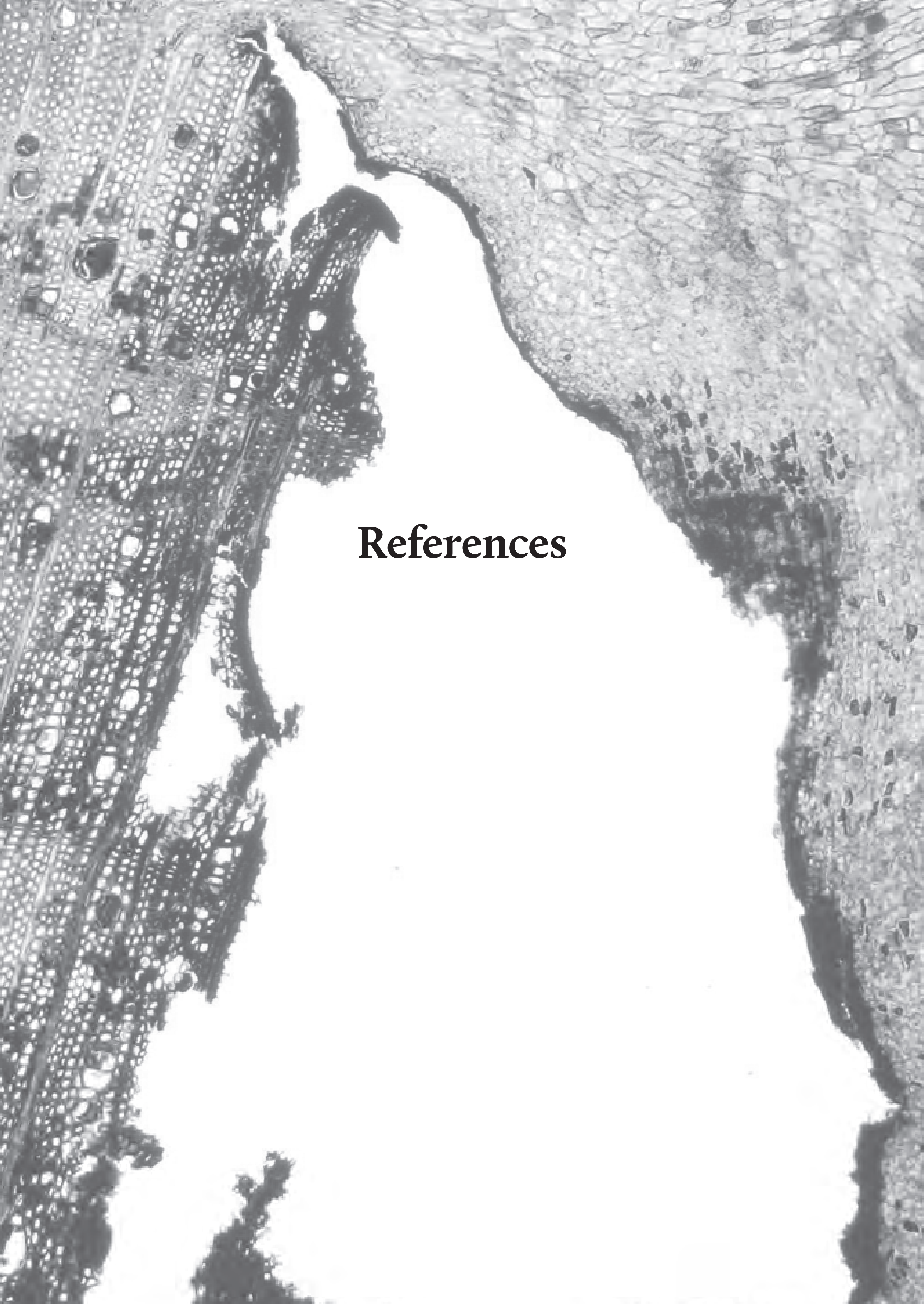
The origin of wood-anatomical markers largely determines their temporal resolution. Markers induced by wounding or flooding show a high intra-annual temporal resolution and an immediate effect on tree functioning, whereas burial might not even be recorded several years after the triggering event.

Wound dating is a precise tool that can be used to data invasive *Anoplophora* outbreaks to the exact year and season when *Anoplophora* beetles emerged. This knowledge is important to draw up customised eradication measures and to study population dynamics of these invasive insects.

Flood rings containing anomalously small earlywood vessels are formed in response to spring flooding within a narrow time window related to cambial phenology. They can be induced if a flooding event has occurred for at least two weeks during spring. The extreme reduction in vessel size is a consequence of a delay in vessel formation due to anoxic conditions related to flooding. Flood rings however, provide only limited information on the duration of flooding, as the flooding may have lasted for months (during winter) before it is recorded.

Unlike in wounded and flooded trees, anatomical changes in oak trees due to burial are not a result of physical changes in the stem environment that directly affect the cambium. Instead, they are most likely the result of adventitious root formation that transforms lower parts of the stem into root, and concomitantly induces a change from stem to root anatomy. As the formation of a wood-anatomical marker caused by burial can be delayed by many years or might be entirely absent, depending on the formation of adventitious root formation, its presence only allows for an estimate of minimum burial age.

The different wood-anatomical markers addressed in this thesis illustrate the relevance of studying the mechanism behind the formation of markers for correct interpretation of the specific triggering factor as well as their temporal resolution. Only with this knowledge it becomes possible to reliably use wood-anatomical markers as archives to understand and reconstruct dynamics in geomorphic, entomologic, pathogenic or climatic factors.



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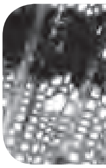

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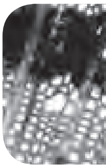

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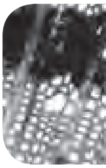

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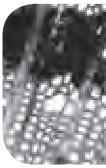

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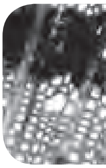

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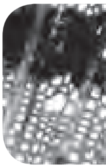

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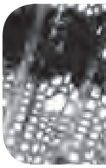
Summary

Trees are long-living organisms that record ecologically relevant information in their xylem, which can be accessed by dendrochronology, the study of tree rings. Wood-anatomical markers, also known as wood imprints or signatures, are anomalies in tree rings that can be used for studying specific environmental events as e.g. forest fires, flooding events or erosion events. By dendrochronological dating of tree rings containing wood-anatomical markers, not only the exact year of the triggering events can be reconstructed, but also the season of formation can be estimated by using the position of the marker within the tree ring. Although dendrochronological dating is amongst the most accurate dating tools, the application of wood-anatomical markers is hindered by a lack of fundamental studies that test their temporal accuracy by linking their occurrence to tree-ring growth. In this thesis, the accuracy of wood-anatomical markers that differed in their origin was investigated by a combination of field studies and experiments. First, the temporal resolution of wound dating was studied in relation to dating invasive *Anoplophora* outbreaks in Japanese maple (*Acer palmatum*), an ornamental tree occasionally containing larvae of *A. chinensis*. These xylobiont insects, that eventually kill trees, induce wounds in living trees by forming e.g. exit holes. Second, the formation of wood-anatomical markers that occur in regard to burial and erosion events in drift-sand areas was studied in pedunculate oak (*Quercus robur* L.). Last, the effect of flooding was studied in young pedunculate oak trees. It was expected that wood-anatomical markers both related to wounding and to environmental changes can be used with an intra-annual resolution.

In **chapter 2** a study is described in which the precision of wound dating with dendrochronological techniques was investigated in an experimental setting. The development of wound reactions of *Acer palmatum* was studied at the beginning, during and after the growing season, both in relation to leaf phenology and intra-annual tree-ring growth. It was found that the precision of dating exit holes is limited due to the highly variable intra-annual tree-ring growth, whereby only an accurate distinction can be made between wounds originated during, or after tree-ring formation. The resolution was improved using local growth—the local reactivation of xylem growth around the wound—as a marker for wounds that occurred at the end of the growing season. It was concluded that the intra-annual precision of dating *Anoplophora* outbreaks in *Acer palmatum* in the temperate North-western European climate is limited to three distinct phases: (i) The period of dormancy and leaf emergence when the wounds are located at the tree-ring boundary (ii) The period of tree-ring growth in which wounds are located within the tree ring, (iii) the end of the growing season in which local growth occurs.

In **chapter 3** the effect of two contrasting temperature regimes on early reactions of *Acer palmatum* trees to wounding during winter bud dormancy was investigated. By storing wounded stems of *A. palmatum* trees under an ambient temperature of 4 or 15 °C for 3 weeks during winter bud dormancy, it was tested whether trees may start compartmentalization, e.g. by producing inhibitory compounds, but it was thought that processes involving cell proliferation, such as the formation of callus and wound xylem, are delayed until the next growing season. In the 4 °C treatment, wound reactions were virtually absent. In the 15 °C treatment, however, trees reacted to wounding by dieback of the cortex and phloem and by the formation of ligno-suberized layers. In the cambial zone, cambial dieback occurred and callus tissue and wound xylem were formed locally, close to the wound margins. In the xylem, compartmentalization took place by deposition of inhibitory compounds in fibre cells and vessel elements. It was concluded that temperature is an important factor in wound reactions during winter dormancy, and may even induce proliferation of callus and wound xylem within a 3-week period of augmented temperatures. It therefore seems likely that trees that have been wounded during dormancy in areas with mild or warm winters might cope better with wounding, as unlike trees in cold environments, they may compartmentalize wounds even during winter dormancy.

A dendrogeomorphological approach is presented in **chapter 4**, that uses wood characteristics of native oak (*Quercus robur* L.) to infer dynamics of aeolian sediment transport in drift-sand areas. Wood samples, taken from oaks in two drift-sand areas, were analysed to study changes in tree-ring pattern and wood anatomy as a consequence of burying or exposure from drift sand. In all cases, the wood of the sampled oaks showed sudden changes in anatomy and tree-ring width due to burial by drift sand or subsequent exposure after erosion of the new soil surface. After aerial stems became covered by drift sand, the wood lost its characteristic ring-porous features, and tree rings became strongly reduced in width with less distinct ring boundaries. Buried stems that became exposed after erosion showed an abrupt increase in ring width and turned distinctly ring

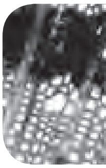


porous again. Roots that were exposed also adopted clear ring-porous features, increased in ring width and anatomically resembled aerial stem wood. Using tree-ring analysis, it is possible to precisely date sand deposition and erosion events by detecting the concurrent changes in anatomy of woody structures. This chapter indicates the high potential of dendrogeomorphology as a tool to study drift-sand dynamics with a high temporal resolution for a period going back as long as the maximum age of the trees present (in this study at least 250 years). Since the signals of past deposition and erosion events are conserved in the wood, this is the only method that can be used to reconstruct drift-sand dynamics when previously deposited sand is no longer present.

Then, in **chapter 5,** the question is asked whether mature pedunculate oak trees (*Quercus robur* L.) that were buried up to a stem height of 50 cm, change their wood anatomical structure within the growing season following the burial event. The responses in ring width and vessel characteristics were analysed, while monitoring the course of temperature above and below the soil surface. After 3 years of stem burial, no significant differences in ring width and vessel characteristics between control and buried trees both above and below the burial level were found. Burial, however, strongly reduced temperature amplitude and the occurrence of sub-zero temperatures around the buried stems. All buried trees formed epitropic roots that grew upward into the new sediment layer, but no adventitious roots were formed on the buried stems. Irrespective of the burial treatments, it was found that mean ring width was largest at the original stem base and lowest at breast height. In contrast, vessel sizes were significantly larger at breast height compared with the stem base. Differences in vessel density barely differed between years and heights. As wood-anatomical changes in response to burial have previously been reported for trees that had formed adventitious roots, the role of adventitious-root formation is stressed as a possible trigger behind the local changes in wood anatomy, reflecting a functional change of a buried stem towards a root. Based on our field experiment, it seems unlikely that years of shallow or moderate burial events (≤50 cm) can be reconstructed using the wood structure of buried stems.

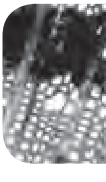
In **chapter 6**, re-activation of water transport in relation to earlywood-vessel formation, sapwood-water content, and leaf phenology in three-year-old pedunculate oaks trees (*Quercus robur* L.) was studied using spatially resolved non-invasive Magnetic Resonance Imaging (MRI) and wood-anatomical research. At six leaf phenophases, from bud dormancy in late winter until the second growth flush in summer, axial water conductance and water content were measured. Axial water conductance and water content were studied per tree ring and for the pith and bark respectively. Previous-year latewood vessels started transporting water upon budswell and remained conductive at all phenophases. Whereas at budswell 26% of the axial water conductance occurred in the newly formed tree ring, it increased to ca. 70% at the second growth flush in summer. At budswell 90% of all present earlywood vessels were formed at positions where latewood vessels were connected to the tree-ring boundary. The mean water content of the bark, the newly-formed outermost tree ring, and pith, did not show significant changes in relation to leaf phenology. In contrast, the mean water content of the previous tree ring reduced considerably from approx. 37% during dormancy to 15% at the second growth flush in summer; this difference mainly occurred due to a ca. 77% reduction in the water content of fibre tissue (fibre cells and surrounding parenchyma cells). It was shown that water conductance only occurred in the outermost tree ring and in the latewood vessels of the previous tree ring. Previous-year latewood vessels are not only important for axial sapflow but are also connected to the onset of earlywood-vessel formation in spring, most likely water in radial transport to provide water for earlywood-vessel formation. Moreover our results indicate that fibre tissue formed in the previous-year tree ring may have a crucial function of water storage during spring reactivation.

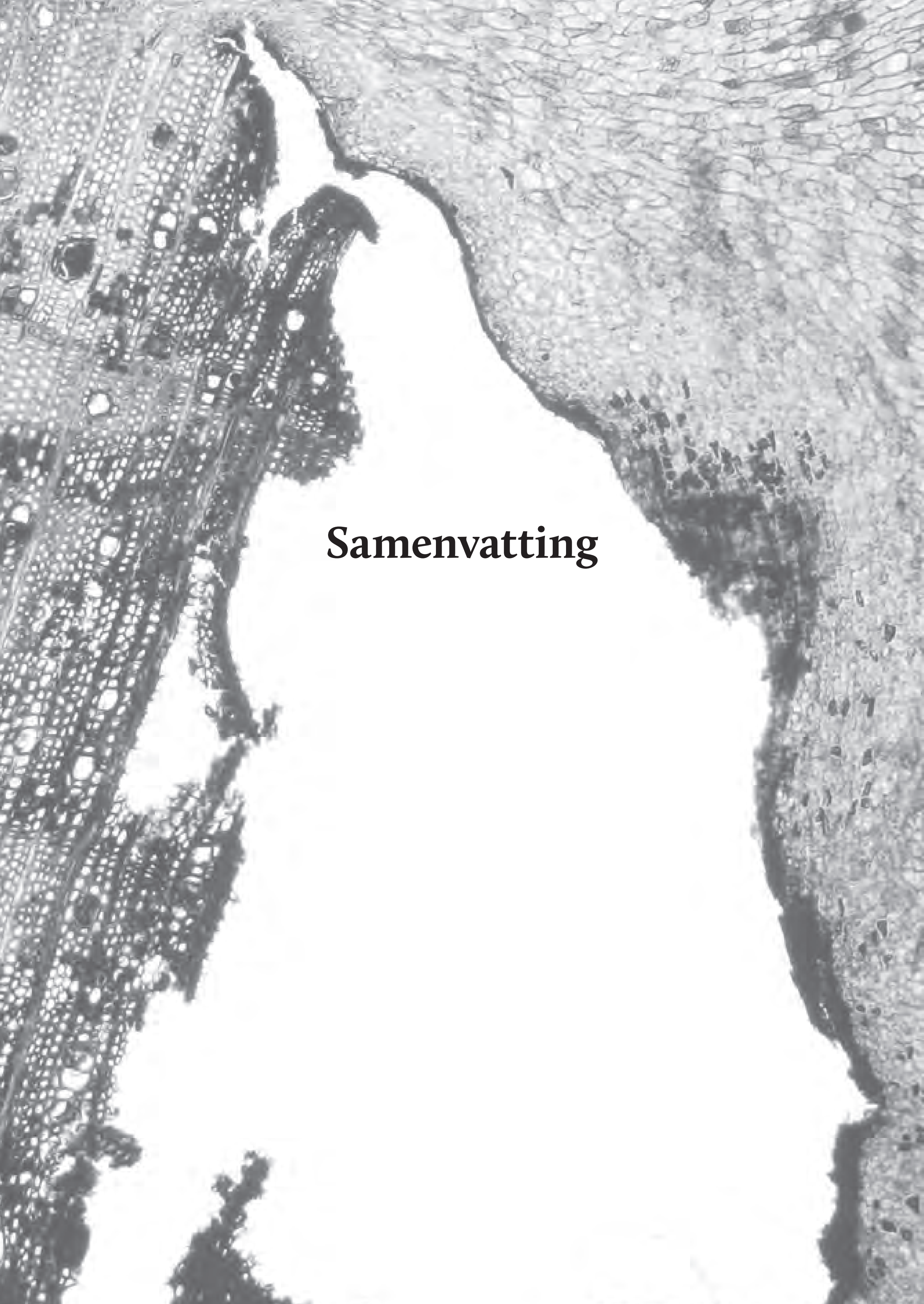
In **chapter 7** the formation of 'flood rings' that can be used as a proxy to reconstruct past flooding events is studied to understand the relation between timing and duration of floods. Four-year-old pedunculate oak trees (*Quercus robur* L.) were experimentally flooded at three spring phenophases (late bud dormancy – i.e. the buds still dormant but the cambium may become active, budswell and internodes extending) and over different flooding durations (two, four and six weeks) to a stem height of 50 cm. At budswell, an additional treatment was conducted in which only the roots were flooded. The effect of flooding on root and vessel development was assessed by sampling one set of trees immediately after the flooding treatment, and the remaining trees at the end of the growing season. Stem flooding inhibited earlywood-vessel development in flooded stem parts. In addition, when flooding occurred upon budswell and internode expansion,



it led to collapsed earlywood vessels below the water level. At the end of the growing season, mean earlywood-vessel size of flooded trees (upon budswell and internode expansion) was always reduced compared in the flooded stem parts by approximately 50% compared to non-flooded stem parts and 55% compared to control trees. This effect occurred independent from flooding duration, i.e. already after two weeks of flooding. Stem and root flooding were associated with significant root dieback after four and six weeks and mean radial growth was always reduced with increasing flooding duration. It was concluded that relatively short periods of flooding (two weeks) reduce earlywood-vessel size in flooded stem parts drastically, on average by 50% compared to the unflooded stem part, in juvenile trees if flooding occurs upon budswell or internode expansion. It was demonstrated that flood rings only occur in trees of which the stem has been flooded. As earlywood-vessel development was hampered during flooding, a considerable number of narrow earlywood vessels present later in the season, must have been formed after the actual flooding events. This study indicated that root dieback, together with strongly reduced hydraulic conductivity due to drastically smaller vessels in the flooded stem part, contribute to reduced radial growth after flooding events in juvenile trees.

Chapter 8 presents a general discussion and synthesises the main conclusions that can be drawn from the preceding chapters and shows the importance for dating invasive *Anoplophora* outbreaks, reconstructing drift-sand dynamics and flooding events. In summary, this thesis shows that the origin of wood-anatomical markers largely determines the temporal resolution. Markers induced by wounding or flooding show a high intra-annual temporal resolution and immediate effect on tree functioning, whereas burial might not be recorded three years after the triggering events. Dating wounds caused by the formation of exit holes has proved to be very significant in fighting invasive insect outbreaks. By pinpointing the exact year and season when *Anoplophora* beetles emerged, it can be substantiated whether exit holes in imported trees were formed at the location of import. This knowledge can subsequently be used to draw up customised eradication measures and allocate proper surveillance plans and population dynamics can be reconstructed. Living trees in drift-sand areas may contain burial or exposure signals even if the sand under which they were buried has long since disappeared. Anatomical changes in oak trees due to burial are not a result of physical changes in the stem environment that directly affect the cambium. Instead they are most likely the result of adventitious root formation that transforms lower parts of the stem into root, and concomitantly induces a change from stem to root anatomy. As the formation of a wood-anatomical marker caused by burial can be delayed by many years or might be entirely absent, depending on the formation of adventitious root formation, its presence only allows for an estimate on minimum burial age. Flood rings containing anomalously small earlywood vessels are formed within a narrow time window related to cambial phenology, but provide limited information on the duration of flooding, as the flooding may have lasted for months before it is recorded. Flood rings can be induced within a spring-flooding event of two weeks. The small vessels present in flood rings are mostly formed after flooding events, as flooding delays earlywood-vessel formation. The different wood-anatomical markers addressed in this thesis illustrate the relevance of studying the mechanism behind the formation of markers for correct interpretation of the specific triggering factor as well as their temporal resolution. Only with this knowledge it becomes possible to reliably use wood-anatomical markers as archives to understand and reconstruct dynamics in geomorphic, entomologic, pathogenic or climatic factors.



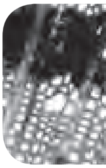


Samenvatting

Bomen zijn langlevende organismen die ecologisch relevante informatie in hun jaarringen herbergen. Deze informatie kan met behulp van dendrochronologie oftewel jaarringonderzoek ontsloten worden. Jaarringen bevatten soms houtanatomische merkers; dit zijn anomalieën in de houtstructuur die gebruikt kunnen worden om specifieke gebeurtenissen zoals bosbranden, overstromingen of erosie te dateren. Door jaarringen met houtanatomische merkers met behulp van jaarringonderzoek te dateren, kan niet alleen het exacte jaar waarin de merker werd gevormd gedateerd worden, maar er kan ook een schatting van het seizoen waarin dit gebeurde gegeven worden. Dit laatste gebeurt aan de hand van de positie van de merker in de jaarring. Alhoewel dendrochronologische dateringen erg nauwkeurig zijn, wordt het gebruik van houtanatomische merkers bemoeilijkt door een gebrek aan fundamentele studies waarin de precisie van deze dateringen onderzocht wordt.

In dit proefschrift is de precisie van verschillende houtanatomische merkers onderzocht door middel van veldonderzoek en experimenten. Ten eerste werd de temporele resolutie van het dateren van verwondingen onderzocht in Japanse esdoorns (*Acer palmatum*), een sierboom die soms larven bevat van de Oost-Aziatische boktor (*Anoplophora chinensis*). Deze houtborende insecten vormen verwondingen, onder andere door het vormen van uitvlieggaten waardoor de boom uiteindelijk dood kan gaan. Ten tweede werd de vorming van houtanatomische merkers onderzocht in zomereik (*Quercus robur*) in relatie tot begraving en vrijstelling als gevolg van eolisch sedimenttransport in stuifzandgebieden. Ten slotte werd het effect van overstroming op jonge zomereiken onderzocht. In het algemeen werd verwacht dat houtanatomische merkers gebruikt konden worden met een intra-annuele resolutie. Met andere woorden, dat er een schatting gegeven kon worden van het seizoen waarin de merker werd gevormd, bijvoorbeeld als gevolg van een overstroming of zandstorm.

Hoofdstuk 2 beschrijft de resultaten van een experiment waarin de precisie geëvalueerd werd waarmee verwondingen in bomen, veroorzaakt door de schadelijke Oost-Aziatische boktor (*A. chinensis*), kunnen worden gedateerd. Dit werd gedaan door de ontwikkeling van wondreacties in de Japanse esdoorn te onderzoeken rondom verwondingen die in het begin, tijdens en aan het einde van het groeiseizoen waren veroorzaakt. Ook werd rekening gehouden met de bladfenologie en groei van de nieuwe jaarring. De bevindingen waren

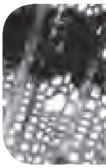


dat de precisie van het dateren van verwonding gelimiteerd was door de grote variabiliteit in jaarringgroei bij bomen onderling. Hierdoor kan alleen een verschil worden aangetoond tussen verwondingen die voor of tijdens het groeiseizoen zijn ontstaan. Aangezien bomen aan het einde van het groeiseizoen lokaal groeiden rondom de verwondingen, kon ook dit gebruikt worden om verwonding in dat seizoen te dateren. Dit hoofdstuk laat zien dat het dateren van verwondingen veroorzaakt door de schadelijke Oost-Aziatische boktor (*A. chinensis*), in de gematigde streken van Noordwest-Europa gelimiteerd is tot drie verschillende periodes: (i) de periode van winterrust en bladformatie wanneer de verwondingen op de oude jaarringgrens liggen; (ii); de periode van jaarringgroei waarin de verwondingen binnen de jaarringgrenzen liggen, en (iii) het einde van het groeiseizoen wanneer lokale groei optreedt.

In **hoofdstuk 3** wordt het effect van twee contrasterende omgevingstemperaturen op beginnende verwondingsreacties in Japanse esdoorn tijdens de winterrust onderzocht. Door bomen te verwonden en daarna gedurende een periode van drie weken tijdens de winterrust op te slaan bij een omgevingstemperatuur van 4 of 15°C, werd getest of bomen in staat zijn om in de winter verwondingen te compartimentaliseren. Verwacht werd dat er tijdens de winterrust geen callusweefsel of wondxyleem zou ontstaan en er enkel inhoudstoffen zouden kunnen worden afgezet. Na drie weken bleek dat bij de 4°C- behandeling er eigenlijk geen wondreacties optraden. Bij de 15°C-behandeling daarentegen reageerde de boom met het afsterven van de bast en met de vorming van een afscheidingslaag tussen het dode en levende weefsel. In de cambiale zone stierf het cambium rondom de verwonding en tevens werd er lokaal dicht bij de wond callus en wondxyleem gevormd. In het xyleem werden inhoudstoffen afgezet in vezels en in vatelementen. Er kan dus gesteld worden dat temperatuur een belangrijke factor is voor wondreacties die zelfs callus en wondxyleem wel degelijk kan doen laten ontstaan binnen een periode van drie weken in de winter. Hierdoor lijkt het waarschijnlijk dat bomen die verwond zijn tijdens de winter, maar die in gebieden met milde of warme winters staan, beter bestand zijn tegen deze verwondingen dan bomen in een koude omgeving. De Eerstgenoemden kunnen namelijk tijdens de winter al beginnen met het afsluiten van de verwonding.

In **hoofdstuk 4** is een dendrogeomorfologische methode gepresenteerd waarin houtkenmerken van de zomereik worden gebruikt om de dynamiek van eolisch sedimenttransport in stuifzandgebieden te reconstrueren. Houtmonsters verzameld in twee stuifzandgebieden werden geanalyseerd op veranderingen in het jaarringpatroon en in de anatomie als gevolg van begraving of erosie. In alle gevallen bevatte het hout plotselinge veranderingen in anatomie en jaarringbreedtes in relatie tot begravingen en daaropvolgende vrijstelling van stuifzand als gevolg van erosie. Na begraving van stamdelen verdween de kenmerkende ringporige structuur van het hout en de jaarringbreedte nam drastisch af. Ook waren de jaarringgrenzen moeilijk waarneembaar. Begraven stammen die vrij kwamen na erosie lieten een abrupte toename in jaarringbreedte zien en werden weer ringporig. Ook de wortels die door erosie vrij kwamen, vertoonden de kenmerken van stamhout. Dit hoofdstuk laat dus de potentie van dendrogeomorfologie zien, als een methode om de dynamiek van stuifzand te bestuderen met een hoge, tijdelijke resolutie over een periode waarin de bomen in leven zijn (in deze studie tenminste 250 jaar). Aangezien signalen van zanddepositie en erosie in het hout geconserveerd worden, is dit de enige methode die gebruikt kan worden om stuifzanddynamiek te bestuderen wanneer het afgezette stuifzand door erosie verdwenen is.

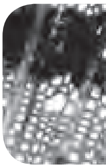
Hierna is in **hoofdstuk 5** de onderzoeksvraag gesteld of volwassen zomereiken die begraven werden tot een stamhoogte van 50cm ook hun houtanatomische structuur veranderden binnen één groeiseizoen na begraving. Hiervoor werden de veranderingen in jaarringgroei en houtanatomie geanalyseerd en het verloop in temperatuur werd gemonitord boven en in de nieuwe zandlaag. Na drie jaar van begraving werden geen significante verschillen gevonden tussen de controlebomen en de begraven bomen, zowel boven als onder de nieuwe zandlaag. Alle stammen vormden epitropische wortels die omhoog groeiden in het nieuwe sediment. Daarentegen werden geen adventiefwortels gevormd op de begraven stammen. Onafhankelijk van de begravingsbehandeling waren de jaarringbreedtes onderin de stam significant breder dan op borsthoogte (130 cm). Daarentegen waren de vroeghoutvaten significant groter op borsthoogte vergeleken met de vaten in de stamvoet. De dichtheid van vaten verschilde nauwelijks tussen jaren of op verschillende hoogtes. Aangezien in het verleden veranderingen in houtanatomie in het verleden zijn gerapporteerd in bomen die adventiefwortels hadden gevormd, is gesteld dat de formatie van



deze adventiefwortels mogelijk de oorzaak is van lokale veranderingen in de houtanatomie, die een functionele verandering reflecteert van een stam naar een wortel. Gebaseerd op dit experiment lijkt het voor de hand te liggen dat geringe of middelmatige (<50 cm) zanddeposities niet gereconstrueerd kunnen worden met behulp van de houtstructuur van begraven stammen.

In **hoofdstuk 6** is de reactivatie van watertransport in relatie tot vroeghoutformatie, spinthoutwatergehaltes, en bladfenologie bestudeerd in drie jaar oude zomereiken (*Quercus robur* L.) met behulp van Magnetic Rosonance Imaging (MRI) en houtanatomisch onderzoek. Op zes bladfenologische fasen, variërend van slapende knoppen tot gezwollen knoppen in de zomer ten tijde van de tweede bladuitloop (Sint-Janslot), werd het axiaal watertransport en de stamwatergehaltes gemeten. Deze metingen werden uitgevoerd per jaarring, voor het merg en de bast. De kleine laathoutvaten die in het voorgaande jaar waren gevormd, begonnen met het transporteren van water tijdens het opzwellen van de knoppen en bleven actief gedurende alle volgende fenofasen. Tijdens het zwellen van de knoppen vond 26% van het axiaal water transport plaats in de nieuwe jaarring. Dit liep op naar ca. 70% tijdens de tweede uitloop in de zomer. Tijdens het zwellen van de knoppen in het voorjaar was 90% van de gevormde vroeghoutvaten verbonden met laathoutvaten. De gemiddelde watergehaltes van de bast, de nieuw gevormde jaarring en het merg vertoonden geen significante verschillen in relatie tot de bladontwikkeling. Daarentegen werd het gemiddelde watergehalte van de jaarring die in het voorgaande jaar was gevormd, gereduceerd van gemiddeld 37% tijdens de winterrust, naar 15% tijdens de aanvang van het Sint-Janslot. Dit verschil werd grotendeels veroorzaakt door een afname van ca. 77% in watergehalte van het vezelweefsel (vezelcellen en omliggende parenchymcellen). Dit hoofdstuk laat zien dat watertransport alleen gebeurde in de buitenste jaarring en in de laathoutvaten van de voorgaande jaarring. Laathoutvaten gevormd in het voorgaande jaar zijn niet alleen van belang tijdens het begin van axiaal watertransport, maar zijn ook verbonden met de vorming van vroeghoutvaten in het voorjaar. Waarschijnlijk is water in radiale richting getransporteerd voor de vorming van vroeghoutvaten. Daarnaast laten de resultaten zien dat vezelweefsel gevormd in het voorgaande jaar een belangrijke wateropslagsfunctie heeft tijdens het uitlopen van de bomen in het voorjaar.

In **hoofdstuk 7** is de vorming van ‘overstromingsjaarringen’ bestudeerd, die gebruikt kunnen worden als een proxy om overstromingen in het verleden te kunnen reconstrueren. Met name werd gekeken naar de relatie tussen het moment en de duur van overstromingen. Hiervoor werden vier jaar oude zomereiken experimenteel overstroomd tot een stamhoogte van 50cm. Dit werd gedaan bij drie verschillende fenologische stadia (late winterrust; de periode waarin de knoppen nog in rust zijn maar de eerste vroeghoutvaten gevormd kunnen worden; het zwellen van de knoppen; het strekken van de internodiën) en met een overstromingsduur van twee, vier of zes weken. Tijdens het zwellen van de knoppen werd nog een extra behandeling toegevoegd waarin bomen slechts werden overstroomd tot aan de wortels. Het effect van overstroming op wortel- en vatformatie werd vastgesteld door één groep bomen direct te bemonsteren en de andere groep te laten staan tot aan het einde van het groeiseizoen. Overstroming van de stam remde de formatie van vroeghoutvaten in het overstroomde stamgedeelte. Daarnaast leidde overstroming tijdens het groeiseizoen tot misvormde vroeghoutvaten. Aan het einde van het groeiseisoen was de gemiddelde vatgrootte van overstroomde bomen in het overstroomde stamgedeelte altijd gereduceerd met ongeveer 50% ten opzichte van het niet-overstroomde stamgedeelte en met 55% ten opzichten van de controlebomen. Dit effect trad op onafhankelijk van de overstromingsduur en binnen twee weken. De overstroming van wortels en stammen ging gepaard met het afsterven van de wortels na vier en zes weken van overstroming en ook de gemiddelde jaarringbreedte was altijd gereduceerd bij een toenemende overstromingsduur. Op basis van dit onderzoek wordt geconcludeerd dat relatief korte periodes van overstroming al leidde tot een reductie in vroeghoutvatgrootte in de overstroomde stamgedeelten van juveniele eiken. Er werd aangetoond dat deze ‘overstromingsjaarringen’ alleen onstaan wanneer de stam daadwerkelijk is overstroomd. Aangezien de vorming van vroeghoutvaten sterk geremd was tijdens overstromingen houdt dit in dat een substantieel aandeel van de kleine vaten gevormd werd na afloop van de eigenlijke overstroming. Deze studie toont ook aan dat het afsterven van de wortels, samen met de sterk gereduceerde hydrologische conductiviteit veroorzaakt door de kleine vaten, bijdragen aan gereduceerde radiale groei na overstroming.



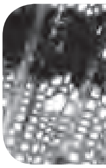
Hoofstuk 8 is een algeme discussie en synthese van de belangrijkste conclusies die kunnen worden afgeleid uit de vorige hoofdstukken. Ook het belang voor het dateren van invasieve Anoplophora-uitbraken, de reconstructie van stuifzanddynamieken en overstromingen wordt in dit hoofdstuk besproken. Samenvattend kan gesteld worden dat de oorsprong van houtanatomische merkers grotendeels de temporele resolutie bepaalt. Merkers veroorzaakt door verwondingen of overstromingen hebben een hoge temporele resolutie en een direct effect op het functioneren van de boom. Daarentegen was het effect van begraving zelfs na drie jaar niet zichtbaar.

Het dateren van uitvlieggaten bleek een belangrijke bijdrage te leveren in de strijd tegen invasieve Aziatische boktorren. Door het precieze jaar en seizoen waarin insecten uit bomen vlogen te bepalen, kan achterhaald worden of de boktor in het land van export of import is uitgevlogen. Deze informatie kan vervolgens gebruikt worden voor het opstellen van bestrijdingsmaatregelen en inspectieplannen en ook om de populatiedynamiek van deze insecten te achterhalen.

In levende bomen in stuifzandgebieden kan het effect van overstuiving en erosie aangetroffen worden zelfs als het zand alweer verdwenen is na erosie. Anatomische veranderingen veroorzaakt door begraving zijn niet het resultaat van fysische veranderingen in de omgeving van de stam die het cambium direct beïnvloeden. Waarschijnlijk worden de veranderingen veroorzaakt door de vorming van adventiefwortels, waardoor de functie van de stam verandert in een wortel en ook de houtanatomie verandert naar die van wortelhout. De vorming van houtanatomische merkers veroorzaakt door begraving kan soms pas na vele jaren optreden of helemaal achterwege blijven. Hierdoor kan deze merker alleen gebruikt worden om een schatting van de minmale periode van begraving te geven.

De ‘overstromingsjaarringen’ die gekenmerkt worden door exceptioneel kleine vaten, worden gevormd na korte overstromingen van twee weken. Deze merkers worden gevormd tijdens een korte periode gerelateerd aan de cambiale fenologie en leveren weinig informatie over de overstromingsduur, aangezien overstromingen al maanden hebben kunnen duren tijdens de winter voordat ze worden opgenomen in het hout in het voorjaar. De kenmerkende kleine vaten worden voornamelijk na afloop van de overstroming gevormd.

De verschillende houtanatomische merkers die onderzocht zijn in dit proefschrift illustreren hoe belangrijk het is om de mechanismen achter de vorming van een merker te begrijpen. Zonder deze achtergrond is het moeilijk om een correcte interpretatie te kunnen geven of om de precisie (tijdelijke resolutie) te schatten. Alleen met deze kennis is het mogelijk om houtanatomische merkers als archief te gebruiken om de dynamiek van geomorfologische, entomologische, pathologische of klimatologische factoren te bestuderen.



Acknowledgements

It is done! It has been a long interesting journey on a bumpy road with unexpected potholes, heading through stunning landscapes, beautiful places, and leading to new friendships. The support along the way was greatly appreciated! I thank you all!

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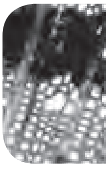

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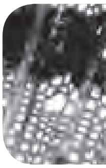
SHORT BIOGRAPHY

Paul Copini was born on March 17th, 1980 in Leeuwarden, the Netherlands. In the same place he graduated at MAVO Nylan in 1996 and at OSG Piter Jelles for higher and pre-university education in 1999. He studied Forest and Nature Management between 1999 and 2005 at Wageningen University and Research centre (WUR) in the Netherlands and European Forestry at Joensuu University in Finland between 2003 and 2006. During his studies he was actively involved in the Student Athletics Association Tartlétos. For his first thesis he worked on a molecular phylogenetic reconstruction of the Brassicaceae family at the Biosystematics Group of Wageningen University / Nationaal Herbarium Nederland. In a second thesis, he studied the genetics of oak clusters at the Veluwe in the Netherlands at both the Centre for Genetic Resources, the Netherlands (CGN) and the Forest Ecology and Forest Management group (WUR). As there was still time left, he decided to include dendrochronology to study the age and origin of these oak clusters together with Ute Sass-Klaassen at the dendro lab of Wageningen University. The combination of dendrochonology and wood science in general, genetics and historical ecology appealed to him very much. Luckily, after graduation, he was able to continue with this kind of research at the Forest Ecology and Forest Management group of Wageningen University, doing different assignments for Dutch forest management organisations. He started writing a PhD-proposal together with Ute Sass-Klaassen and Jan den Ouden, which was, after 10 months of patiently waiting, rejected. Together they decided to give it a second try. In the meantime, he had started working for the RING Foundation – The Netherlands Centre for Dendrochronology – at the Cultural Heritage Agency of the Netherlands, on a project dealing with digital data infrastructure for tree-ring research. Finally, his PhD-proposal was accepted and he was able to start his PhD in 2009, of which this thesis is the final result. Currently, he is employed by Alterra and is working for the Centre for Genetic Resources, the Netherlands (CGN). Within the cluster Forest Genetic Resources he is involved in projects on the conservation and promotion of sustainable use of genetic diversity of both trees and shrubs.



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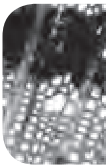

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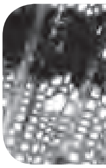

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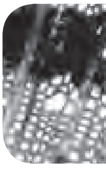


PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)
- Dendrogeomorphology: a new tool to study drift-sand dynamics
- Dendrochronologische datering van uitvlieggaten van de Aziatische boktor *Anoplophora chinensis* (Forster): verkennend onderzoek

Writing of project proposal (6 ECTS)
- Floods and sand storms: precision of dendrogeomorphic markers in trees (2008)
- Jaarringonderzoek aan uitvlieggaten in geïmporteerde en inheemse esdoorns (2009)

Post-graduate courses (4.5 ECTS)
- International winter school on wood anatomy of tree rings: wood anatomical preparation techniques and application in ecology; Swiss Federal Institute for Forest, Snow and Landscape Research WSL (2008)
- Geochronology summer school; Swiss Federal Institute for Forest, Snow and Landscape Research WSL (2009)
- Modern techniques in beech ecophysiology; Freie Universität, Berlin (2009)

Laboratory training and working visits (3 ECTS)
- Dendrogeomorphology; Swiss Federal Institute for Forest, Snow and Landscape Research (2009)

Invited review of (unpublished) journal manuscript (2 ECTS)
- Tree Physiology: ecophysiology (2014)
- Dendrochronologia: quantitative wood anatomy (2015)

Competence strengthening / skills courses (2.4 ECTS)

- Monitoring seasonal dynamics of wood formation; Slovenian Forestry Institute (2009)
- The Cambridge certificate in advanced English; British Council (2010)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.5 ECTS)

- PE&RC PhD Weekend (2009)
- PE&RC Day (2010)
- PE&RC Day (2011)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)

- Ecology theory and application (2009-2013)
- Digital Collaboratory for Cultural Dendrochronology in the Low Countries (DCCD) (2009-2010)
- ESG Science symposia (2010)
- Cost action STReESS-Studying Tree Responses to extreme Events: a SynthesiS; Italy, Bosnia, Finland, Portugal & Czech Republic (2012-2015)

International symposia, workshops and conferences (14.9 ECTS)

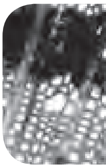
- TRACE; Slovenia (2009)
- Eurodendro; Mallorca (2010)
- Worddendro; Finland (2010)
- Eurodendro; Switzerland (2011)
- Anoplophora symposium; Milan, Italy (2012)
- Wood Structure in Plant Biology and Ecology (WSE); Naples, Italy (2013)

Lecturing / supervision of practical's / tutorials (3.6 ECTS)

- Forest resources (2009-2013)
- Ecology of forests (2009-2013)
- Habitat analyse (2010)

Supervision of MSc students

- Drift sands
- Flooding
- Anoplophora outbreaks
- Coppice systems
- Phloem-xylem interactions



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