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Oxygen in tree stems and possible relations to pathogens and red heart formation

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Table of contents

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Abstract

Aerobic processes in plant metabolism require a constant supply with oxygen. Whereas gas exchange of leaves and roots is a central topic in plant eco-physiology and has been studied in much detail, only few studies have dealt with gas exchange and especially the oxygen content of stems. This was probably due to the lack of a proper methodology. Recently developed micro-sensors, based on fluorescence quenching, allowed the direct measurements of oxygen in the stem of standing trees.

The research conducted in this study measured the oxygen concentration in standing spruce and beech trees, investigating how the living parts of the sapwood are supplied with enough oxygen for respiration. It was hypothesized that oxygen is either transported to the parenchyma cells by means of radial diffusion through bark, phloem and cambium or in dissolved form with the transpiration stream upwards from the roots to the crown. It was further investigated what factors can influence the oxygen content of a stem, and if and how a reduction of oxygen can affects a tree.

First, the velocity of axial and radial oxygen diffusion was measured in wood of various native trees to determine if diffusion alone can supply the living sapwood with oxygen. Results showed that oxygen diffusion is strongly dependent on wood anatomy and water content, and model calculations implied that active sapwood can be supplied with oxygen under certain conditions. However, if water content and respiration are too high to assure a sufficient supply by radial diffusion, respiration in the sapwood is reduced by oxygen deficiency.

Continuous long-term measurements in stems of spruce and beech trees showed distinct diurnal fluctuation of oxygen, and differences in oxygen between various depths and positions in the stem (sapwood versus heartwood, stem height). These results suggest that both transport pathways play a role. On the one hand, the decrease of oxygen with stem height found in spruce and the dependency of oxygen in beech on soil moisture indicated the importance of the transpiration stream as medium of oxygen transport. On the other hand the fact that oxygen replenished even in times of zero sapflow, and it did not decrease when sapflow was reduced suggests that at least some oxygen enters by radial diffusion.

Experiments with potted spruce saplings were conducted to investigate if drought stress or flooding induces oxygen deficiency in the stem. A hypothesis was tested, suggesting that the emissions of ethanol and volatile terpenes are enhanced under conditions of hypoxia or anoxia, which successively could lead to an increased attractiveness for bark-beetles that locate stressed individuals by their emission spectrum. In this study oxygen was not reduced in stressed individuals and emissions of ethanol and volatile terpenes were not increased.

Further the hypothesis was tested that oxygen, penetrating into the heartwood through injuries in beech trees, induces the formation of red heart, while bacteria and fungi are irrelevant in the process. Oxygen was measured in beech trees with and without red heart and wood samples were screened for the presence of micro-organisms. In exposure experiments beech wood was stored at various oxygen concentrations in the laboratory. Although wood discolorations were stronger in the presence of elevated oxygen concentrations in the laboratory, discolorations were also found in beech wood exposed to 0% oxygen and in already formed red heart. In the standing tree a difference in oxygen concentration between beech trees with and without red heart was found only on few occasions, and oxygen was present in both in a sufficient concentration to induce red heart. The results of this study imply that oxygen is not the only factor necessary for the formation of red heart in beech, and the involvement of micro-organisms needs to be studied in more detail.

Zusammenfassung

Alle aeroben Stoffwechselprozesse hängen von der Versorgung mit Sauerstoff ab, doch während die Untersuchung des Gaswechsels von Blättern ein zentrales Gebiet der Ökophysiologie ist, haben sich erst wenige Studien mit dem Gasaustausch und besonders dem Sauerstoffgehalt von Stämmen beschäftigt. Neu entwickelte Sauerstoffoptoden, die auf der Technik des Fluoreszenzquenching basieren, ermöglichten es Messungen direkt im Holzkörper von Bäumen durchzuführen.

Die im Rahmen dieser Studie durchgeführten Versuche sollten die Frage beantworten, wie die lebenden Zellen im Splintholz mit genügend Sauerstoff für die Atmung versorgt werden. Sauerstoff kann entweder durch radiale Diffusion über Borke, Phloem und Kambium, oder in gelöster Form mit dem aufsteigenden Saftstrom von den Wurzeln in Richtung Krone zu den Parenchymzellen transportiert werden. Weiters wurde untersucht, welche Faktoren den Sauerstoffgehalt im Stamm reduzieren und welche Bedeutung dies für den Baum haben kann.

Zunächst wurde in einem Laborversuch die Diffusionsgeschwindigkeit von Sauerstoff in axialer und radialer Richtung im Holz verschiedener heimischer Arten gemessen um festzustellen, ob die lebenden Zellen im Splint allein durch Diffusion mit Sauerstoff versorgt werden können. Die gemessenen Diffusionskoeffizienten waren stark vom anatomischen Bau und dem Wassergehalt des Holzes abhängig, und Modellrechnungen zeigten, dass aktives Splintholz unter bestimmten Bedingungen alleine durch radiale Diffusion mit Sauerstoff versorgt werden kann. Ist der Wassergehalt des Stammes zu hoch, und wird durch gesteigerte Atmung viel Sauerstoff gezehrt, kann der Sauerstoffgehalt im Splint jedoch stark absinken.

Langzeitmessungen im Holz von Buchen und Fichten zeigten deutliche Tagesschwankungen und Unterschiede zwischen verschiedenen Positionen im Stamm (Splint/Kernholz, Höhe im Baum). Eine bei Buchen beobachtete Abhängigkeit der Sauerstoffkonzentration vom Bodenwassergehalt, und die Abnahme der Sauerstoffkonzentration mit steigender Stammhöhe scheinen auf die Bedeutung des Transpirationstromes und indirekt des Sauerstoffgehaltes im Boden für die Sauerstoffversorgung des Stammes hinzuweisen. Allerdings wurde auch ein Anstieg des Sauerstoffgehalts in der Nacht und bei reduziertem Saftfluss beobachtet, sodass zumindest ein Teil des Sauerstoffs auch über radiale Diffusion eindringen dürfte.

Bei Versuchen mit eingetopften Fichten wurde getestet, ob Trockenstress oder Überflutung zu starkem Sauerstoffmangel im Stamm führt. Nach einer Hypothese sollte unter Sauerstoffmangel im Stamm gebildetes Ethanol als Signalstoff für Borkenkäfer dienen, die

damit gestresste Bäume orten könnten. Allerdings zeigten gestresste Bäume weder reduzierte Sauerstoffkonzentrationen, noch erhöhte Ethanolemissionen.

Weiters wurde die Hypothese getestet, dass Sauerstoffzufuhr im Holz der auslösende Faktor für die fakultative Ausbildung von Rotkernen in Buchen ist, während Bakterien und Pilze nicht an dem Vorgang beteiligt sind. Dazu wurden Messungen an verkernten und nicht verkernten Buchen durchgeführt und Holz auf das Vorhandensein von Bakterien und Pilzen untersucht. Bei Lagerungsversuchen im Labor zeigte sich zunächst, dass das Ausmaß der Rotfärbung von der Sauerstoffkonzentration abhängig ist. Zwar zeigten Holzstücke die bei höheren Sauerstoffkonzentrationen gelagert wurden stärkere Verfärbungen, allerdings verfärbten sich auch Holzstücke bei 0% Sauerstoff und auch bereits gebildetes Kernholz. Die Sauerstoffkonzentration in stehenden Bäumen mit und ohne Farbkern unterschied sich nur selten, aber sowohl in verkernten als auch in nicht verkernten Buchen wurde Sauerstoff im Stamm in einer genügend hohen Konzentration festgestellt um potentiell eine Farbkernbildung auszulösen. Die Ergebnisse in dieser Arbeit lassen vermuten, dass Sauerstoff zwar ein essentieller, aber nicht der einzige Faktor bei der fakultativen Rotkernbildung ist, und eine Beteiligung von Mikroorganismen noch besser untersucht werden sollte, um die Bildung des Rotkerns in Buchen zu erklären

Introduction

The present thesis is based on studies conducted within the FWF research project Nr. 16145: "Oxygen in tree stems and its role in tree-parasite interactions", running from 2003 to 2006. The principal goal of the project was to analyze how oxygen is supplied to the stem and what affects its concentration, and to test the hypothesis that the ethanol produced and emitted as a consequence of oxygen deficiency identifies stressed trees to bark beetles.

Belowground organs of plants regularly experience hypoxia under conditions of water logging, and their response and adaptations to oxygen deficiency are well-studied. Oxygen can decrease to very low levels also in above-ground parts of plants, particularly in tree stems (Eklund, 2000). To supply live cells in the sapwood, oxygen can either diffuse radially through periderm, phloem, cambium and wood, or be taken up by the roots and transported upwards with the transpiration stream. In most trees the cambium plus bark appear to be quite impermeable to gases and the transpiration stream is supposed to be the main source of oxygen for the xylem. Thus, a major proportion of the oxygen supplied to the live sapwood appears to be transported with the transpiration stream rather than diffuse through the nearly impenetrable cambium (Gansert, 2003). However, during the night and on wet and cool days with no sapflow, the only oxygen available is either diffusing radially or is present in gas-spaces or dissolved in water.

Very little is known about the short-time dynamics of oxygen concentrations in the wood. A main reason why relatively little attention has been paid to stem oxygen is that it was very difficult and cumbersome to measure with traditional methods such as the Clark electrode. An oxygen electrode based on fluorescence quenching (Klimant *et al.*, 1996) made instantaneous, prolonged and small-scale measurements of O_2 concentrations possible for the first time and was found to be an important tool for a better understanding of oxygen relations in plants. Recent experiments with this method have found a clear relationship between sapflow and stem oxygen in *Betula pendula* (Gansert *et al.*, 2001; Gansert, 2003) and *Laurus nobilis* (del Hierro *et al.*, 2002; Spicer & Holbrook, 2005) used this method to measure oxygen in *Acer rubrum*, *Fraxinus americana*, *Tsuga canadensis*, and *Quercus rubra*.

Apart from the question of how oxygen is supplied to the living sapwood, two applied questions were investigated in this study:

Norway spruce (*Picea abies* [L.] Karst.) is the economically most important conifer in Austrian forestry, and bark beetles (Coleoptera: Scolytidae) are their major pest. The susceptibility of conifers to bark beetle infestation depends on tree physiology: trees become susceptible when subjected to massive injury or unfavorable environmental conditions such as drought stress or flooding (Führer, 1996). Unhealthy or injured trees release higher

amounts and different bouquets of ethanol and volatile terpenoids than healthy trees, and these volatiles serve as attractants to some species of bark beetles (Byers *et al.*, 2000). Ethanol is an end-product of energy production under hypoxic or anoxic conditions, frequently found in oxygen depleted roots or stems (Kimmerer & Stringer M.A., 1988). It was investigated if and how the stem oxygen content of drought stressed or flooded spruce influences the emission of ethanol and volatile monoterpenes. It was assumed that stress reduces oxygen in the roots or in the stem xylem and cambium to levels where ethanol is produced and emissions of volatiles are enhanced, which would explain the causal relationship between tree stress and bark beetle attack. Analyses of correlations between water stress and stem oxygen would also reflect on the basic hypothesis: If oxygen is supplied via the sapflow, several types of stress may result in decreasing wood oxygen concentrations or even anoxia: water logging, which reduces soil oxygen content, severe drought, which reduces sapflow, and infections, which increase respiration and may decrease sapflow.

Another interesting field of plant physiology where the involvement of oxygen has been suggested but not yet clarified is the facultative formation of red heartwood in beech (*Fagus sylvatica* L.). The currently predominant theory suggests that discolorations associated with red heart formation are caused by oxidation processes, and oxygen enters the central parts of the stem through wounds in the root or crown area, while bacteria and fungi are not involved (Knoke, 2003). However, a review of the literature shows that so far there is no hard evidence that rules out micro-organisms as the cause of red heart, and oxygen was never measured in standing beech. While there is little doubt that the oxidation of phenolic substances causes the discolorations (Koch *et al.*, 2000), the possibility that oxygen disperses within the stem is still questionable, because it does not seem plausible that oxygen disperses several meters in axial direction within the stem, but not several centimeters in radial direction within the sapwood of a healthy individual. Measurements in various depths of standing trees with and without red heart could test this theory, because it would require a constantly very low oxygen concentrations in the central regions of the stem in individuals with clear heartwood and higher concentrations in specimens with red heart.

This thesis consists of three chapters that summarize different studies conducted during the project. The following section gives a brief overview:

Chapter 1 (original article) describes laboratory experiments made from 2003 to 2004 to measure oxygen diffusion against nitrogen gas in the wood of six native species to test if radial diffusion is feasible to support the living sapwood in the stem. Coniferous (*Picea abies* (L.) Karst. and *Taxus baccata* L.), ring-porous (*Quercus robur* L. and *Fraxinus excelsior* L.) and diffuse-porous (*Fagus sylvatica* L. and *Carpinus betulus* L.) trees were measured at different water and gas contents. The diffusion coefficient (D) in radial direction was mostly

between 10^{-11} and 10^{-7} $m^2 s^{-1}$ and was strongly related to the gas content. Model calculations showed that under conditions found in the living stem, the support of the respiring sapwood can be assured by radial diffusion. However at a very high water content of the stem radial diffusion can be too low to ensure the supply with sufficient oxygen and an important function of gas in living stems appeared to be the supply of oxygen through storage and diffusion.

Chapter 2 describes the experiments with spruce conducted from 2003 to 2004. This part gives also an overview of the current state of knowledge regarding oxygen in stems (Chapter 2.1.1) and its influence on host-parasite interactions (Chapter 2.1.2). Although several authors e.g. (Gansert *et al.*, 2001; del Hierro *et al.*, 2002; Gansert, 2003; Spicer & Holbrook, 2005) successfully used sensors based on fluorescence quenching, several adaptations were necessary until proper results could be obtained. That process is described in detail in the Materials and Method section (Chapter 2.3).

Oxygen was measured in a mature spruce tree in the arboretum of the BOKU (Chapter 2.4.1), where stem oxygen differed between heartwood and sapwood. Diurnal variations were measured and oxygen decreased from the bottom to the crown which pointed to towards the transpiration stream as the transport medium for oxygen. Results corresponded to (Eklund, 2000), who found that O_2 concentrations slightly declined with height in the stem of spruce trees, which may be explained by the oxygen depletion of the xylem sap.

Oxygen was also measured in two bark beetle-infested spruce trees in Lower Austria in fall 2003 (Chapter 2.4.2) where longer periods of anoxia where measured in the stem and in eleven mature trees with and without heart rot in a forest stand (2005, Chapter 2.4.3). Wood-infecting fungi may lower oxygen concentrations through increased respiration or by additionally clogging xylem vessels and tracheids and thus preventing transport via sapflow. It was assumed that oxygen is lower in species affected by heart rot, but not difference in oxygen was found between the groups.

Between 2003 and 2005 oxygen and sapflow was measured in 19 potted spruce saplings in the BOKU arboretum (Chapter 2.4.4). To find further evidence that oxygen is transported with the transpiration stream, oxygen supply to the roots was cut off by drought-stress (reduction of sapflow) or flooding of the pots. To investigate if stressed trees have increased emission rates of ethanol and volatile terpenoids, emissions were measured three times during extended periods of stress. Oxygen was measured periodically and continuously in various depths. Although oxygen decreases during flooding in *Laurus nobilis* (del Hierro *et al.*, 2002), it did not differ between flooded, desiccated and control spruces. Also emission rates of ethanol and volatile terpenes did not differ between control and stressed specimen. But although the results downplay the role of the transpiration stream as the major pathway of oxygen, the interpretation of the results is difficult because volatile resin components probably biased oxygen measurements in spruce (Chapter 2.3.7.3). Oxygen and emission

rates of ethanol and monoterpenes were also measured during desiccation in cut spruce logs and in spruce lumber used for bark beetle control (Chapter 2.4.5) where an increase in oxygen was found in the course of desiccation possibly due to the increased permeability of oxygen in dry wood (Sorz & Hietz, 2006). Emission rates did not differ between logs stored under wet conditions and bench dried logs

Chapter 3 describes experiments conducted with beech from 2004 to 2006. The current state of knowledge on red heart in beech is briefly reviewed in Chapter 3.1. Oxygen and sapflow were measured in eleven mature trees in a forest stand in Lower Austria. Oxygen was measured periodically (Chapter 3.4.1) and continuously (Chapter 3.4.2) during two successive vegetation periods in trees with and without red heart. Oxygen fluctuated widely (between 0 and 95%) and with diurnal variations similar to those measured in spruce.

In this thesis, unless stated otherwise, all oxygen values are in % / air saturation, where 100% = 20.9% [O_2].

Stem oxygen declined rapidly during and after heavy rainfall when the stem was wet with reduced permeability and soil water content increased. Also oxygen replenished even in times of zero sapflow, pointing towards radial diffusion. Regarding red heart formation, the predominant theory was falsified. Although longer periods of anoxia were measured in both peripheral and central areas of the stem, anoxia was never continuous longer than three-four weeks and oxygen exceeded 0% in all measured depths in all specimens.

To investigate the involvement of bacteria and fungi three storage experiments (Chapter 3.4.3) and microbiological examinations on cut wood disks (Chapters 3.4.4 and 3.4.5) were conducted. Cores were harvested from standing trees prior to oxygen measurements in April 2005 and additionally in November 2006. Wood blocks were cut from disks of freshly felled trees in January 2006. All cores and blocks were stored for three month under oxygen concentrations ranging from 0% to 100% air. To test the influence of endophytic bacteria and fungi, one half of the cores and blocks were sterilized in an autoclave before storage. Color intensities at wavelengths between 400 nm and 700 nm were measured before and after storage and color index values (CIELAB) were calculated. Results indicated that oxygen strongly influences the discolorations and cores exposed to higher oxygen concentrations showed stronger discolorations than cores exposed to 0%. However, discolorations were also found in cores exposed to 0% oxygen and in already formed red heart which does not consent with the predominant hypothesis that red heart is only formed by living cells on the sapwood/heartwood border. Combined with the oxygen measurements in the stem and the microbiological experiments, where bacteria and fungi were found abundantly in healthy and discolored tissue, the results indicate that oxygen is not the only initiating factor of red heart formation.

1 Gas diffusion through wood: implications for oxygen supply

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ORIGINAL ARTICLE

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Gas diffusion through wood: implications for oxygen supply

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Abstract Living tissue in tree stems has to be supplied with oxygen, which can be transported upwards with the transpiration stream; but in times of zero sapflow, the only source is the oxygen stored or diffusing radially through bark and xylem. We measured radial and axial diffusion of oxygen against nitrogen gas in wood of coniferous (*Picea abies* (L.) Karst. and *Taxus baccata* L.), ring-porous (*Quercus robur* L. and *Fraxinus excelsior* L.) and diffuse-porous (*Fagus sylvatica* L. and *Carpinus betulus* L.) trees at different water and gas contents in the laboratory. The diffusion coefficient (D) in radial direction was mostly between 10^{-11} and 10^{-7} m^2 s^{-1} and was strongly related to the gas content. At 40% gas volume, D increased 5–13-fold in *Picea*, *Taxus* and *Quercus*, 36-fold in *Fraxinus*, and about 1000-fold in *Carpinus* and *Fagus* relative to D at 15% gas volume. In the axial direction, diffusion was 1 or 2 orders of magnitude faster. Between-species differences in diffusion velocities can largely be explained by wood structure. In general, D was lowest in conifers, highest in diffuse-porous and intermediate in ring-porous hardwoods, where the large vessels were mostly blocked by tyloses. Model calculations showed that at very high water content, radial diffusion can be too low to ensure the supply of respiring sapwood with sufficient oxygen and an important function of gas in living stems appears to be the supply of oxygen through storage and diffusion.

Keywords Gas diffusion · Oxygen supply · Wood structure

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Introduction

Oxygen is required for oxidative respiration, which under most conditions provides the energy for plant cells. Plants growing in submerged or waterlogged soil often show anatomical adaptations for the transport of oxygen to below-ground parts, and gas flow can be substantially enhanced by Venturi-ventilation (Strand and Weisner 2002) and thermo-osmosis (Buchel and Grosse 1990; Grosse et al. 1992). Tree stems are normally exposed to light and air, but unless the bark is transparent, they do not produce oxygen. Stem tissue respiration in the growing season can be substantial (Stockfors and Linder 1998; Teskey and McGuire 2002; Gansert 2004) and bark, cambium and wood may pose considerable, but mostly unquantified, barriers to gas diffusion. To supply live cells in the sapwood, oxygen can either diffuse radially through periderm, phloem, cambium and wood, or be taken up by the roots and transported upwards with the transpiration stream. In some tree species adapted to waterlogged soil, the cambium has small intercellular spaces permitting oxygen supply through the bark (Hook and Brown 1972; Buchel and Grosse 1990). In other species lacking such intercellular spaces, the cambium plus bark appear to be quite impermeable to gases and the transpiration stream is supposed to be the main source of oxygen for the xylem (Hook et al. 1972). More recently, direct measurement of oxygen in the stem support this idea (Eklund 2000; del Hierro et al. 2002; Mancuso and Marras 2003; Gansert 2003), though in times of zero sapflow, such as during the night and on wet and cool days, only the oxygen either diffusing radially or present in gas spaces or dissolved in water is available.

All sapwood contains, by definition, living cells but not all sapwood conducts water (Phillips et al. 1996). Thus, whether oxygen passes through the cambium or is transported via the transpiration stream, it has to diffuse through wood to reach parts of the inner sapwood with no sapflow. Indeed, the oxygen deficiency in the innermost part of the sapwood has been implicated in heartwood formation (Eklund and Klintborg 2000). By contrast, Knoke (2003) suggested that dark heartwood in *Fagus sylvatica* forms

when oxygen enters the stem through wounds, in contrast to white heartwood, which keeps the original sapwood colour presumably under low oxygen concentrations in undamaged stems. Oxygen is also important for wood decomposition, and its diffusion through dead wood may affect the metabolic activity of wood-degrading microbes and thus the time it takes for a log to decompose (Hicks 2000; Kazemi et al. 2001).

The gas permeability of wood has been investigated for technical applications (Comstock 1967; Hansmann et al. 2002), as it affects the speed of drying (Bramhall and Wilson 1971) and the ease of chemical modification of wood (Tesoro et al. 1966; Choong et al. 1975). However, for technical applications, gas permeation is generally measured under a pressure difference in either steady or unsteady state, which is different from the diffusion at constant pressure occurring in living stems or decomposing logs (Comstock 1967; Prak 1970; Isaacs et al. 1971; Petty 1973; Sebastian et al. 1973; Siau 1976; Militz 1993). Gas flow under a pressure difference is characterized by Darcy's law and expressed in $m^3\ m^{-1}\ s^{-1}\ Pa^{-1}$ and substantially differs from diffusion as a molecular mass flow under the influence of a concentration gradient, which follows Fick's first law and is measured in $m^2\ s^{-1}$ (Comstock 1967; Siau 1984; Nobel 1991). Measurements of permeation under a pressure gradient are therefore not very relevant for analysing oxygen supply to wood in biological systems. To evaluate the effect of wood anatomy and water content on gas transport, we measured gas diffusion through the wood of two coniferous, two diffuse-porous and two ring-porous species in axial and radial direction and at different water content. We used an approach similar to Yokota (1967), who tested the diffusion of air in helium through heartwood in a set-up that provided constant gas concentrations at both sides of the wood specimen tested, but used nitrogen instead of helium and a different set-up.

Materials and methods

Plant material and experimental set-up

Trees with a diameter at breast height of 15–20 cm and no obvious physical damage or pathogen infection were cut at the BOKU Experimental Forest Garden Knödelhütte and the Lainzer Tiergarten in Vienna during late December 2003. To test the effect of wood structure on gas diffusion, we sampled three trees each of *Picea abies* (L.) Karst., *Taxus baccata* L. (conifers), *Quercus robur* L., *Fraxinus excelsior* L. (ring-porous), *Fagus sylvatica* L. and *Carpinus betulus* L. (diffuse-porous). A 10–15 cm piece of the lower half of the stem was cut and stored at 4°C submerged in water with an anti-microbial agent (Micropur forte, Katadyn, Wallisellen, Switzerland) added to prevent microbial growth.

For the diffusion experiments, branchless and undamaged parts close to, but not including, the pith were used. This was entirely heartwood in *Taxus*, *Fraxinus* and *Quercus*, which have only a few annual rings of sapwood and

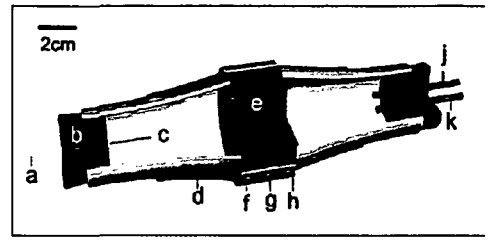

Fig. 1 Set-up used for measuring gas diffusion through wood. Syringe housing the oxygen sensor (a), silicone septum or rubber plug (b), cut-off flask (c), heat-shrinkable tubing (d), wood specimen (e), thin layers of laboratory film (f, h), polymer sealant (g), N_2 inlet and outlet (j, k)

coloured heartwood, heartwood probably with some sapwood in *Picea*, and sapwood in *Fagus* and *Carpinus*. Cylindrical pieces were cut out of the stems, avoiding the central pith, in either axial or radial direction. On a lathe, these pieces were processed to a precise size with a diameter of 45 mm and a length of 25 mm, a smooth surface and notches at both ends that fitted into the glass flasks. The samples were infiltrated under a vacuum at 25°C for several days, after which all except *Picea* wood sank in water, i.e. had a density >1. Gas diffusion was measured with the set-up shown in Fig. 1. The specimen was placed between two flasks with the bottom cut off. To avoid gas leaks, the mantle of the cylindrical wood sample was wrapped with a thin layer of laboratory film (Parafilm, SPI Supplies, West Chester, PA, USA), followed by a ca. 1.5 mm coating of polymer sealant (Teroson, Henkel, Heidelberg, Germany) and another thin layer of Parafilm. The piece plus flasks were enveloped in heat-shrinkable tubing and put into an oven for about 5 min at 150°C. Measurements with thermocouples showed that during this short exposure the temperature of the wood below the sealing layer did not exceed 70°C and rose by only a few degrees inside the wood. To avoid the desiccation during the shrinking of the tubing, the flasks were filled with wet cotton and closed with aluminium foil. The shrinkable tubing was additionally tightened with a silicone tube to ensure a perfect seal also when the wood shrank during drying. This procedure produced a gas tight sheath that also compensated for roughness of the wood surface. The wood was weighed alone and as enclosed with flasks and wrappings.

The opening of one flask was sealed with a gas-impermeable silicone septum only penetrated by the syringe housing the oxygen optode, which was connected to an oxygen meter (Microx TX3-AOT, PreSens GmbH, Regensburg, Germany; Holst et al. 1997). The temperature sensor needed for temperature compensation of the oxygen measurement was attached outside the set-up, as there was no substantial temperature gradient in the laboratory. To test for leaks, the sensor side of the set-up was flushed with N_2 externally, and this did not affect the O_2 concentration measured in the flask. The other flask was flushed with nitrogen gas flowing in and out through a rubber stopper at a rate of 5–10 l min^{-1}. The N_2 gas had to be bubbled through water to prevent the exposed end of the wood from drying too

quickly. The oxygen concentration was measured at 1 min intervals using a CR10X datalogger (Campbell Scientific, Logan, UT, USA) to trigger the oxygen measurement and to read and store the analogue output signal of the TX3-AOT.

The signal of the oxygen sensor is linearly related to oxygen concentrations and was calibrated with pure N_2 (0% O_2) and ambient air (20.95% O_2). A detailed description of the optical measurement of oxygen with an optode sensor can be found in Gansert et al. (2001).

The oxygen sensor recorded the decrease in O_2 concentrations when flushing with N_2 and an increase in O_2 after flushing ceased and the open end was exposed to ambient air. The first experiments lasted until the O_2 concentration had decreased to almost 0%. Since the whole curve could be described by one exponential function (Fig. 2), later experiments were stopped when O_2 had declined to about 70% or after 24 h if the diffusion was very slow.

After testing at maximum water content, the wood was left to dry on the laboratory bench for 1 or more days, which also served to equilibrate the gas concentration with the ambient air. Because the wood slightly shrank during drying, the shrinkable tubing had to be adjusted again. After several experiments had been conducted with each sample, the set-up was tested for leakage by allowing methyl green to percolate through the wood. When the wood was cut afterwards, the staining showed that the solution passed through the wood, and not between the wood and the sealing. In initial experiments, when the seal was not air tight, this was seen in a very fast decrease of the oxygen concentration measured, and these data were not used. Finally, the wood was dried to constant weight at 105°C.

The proportion of volume occupied by cell walls was calculated as (dry weight/wood volume/1.5), 1.5 being the density of the cell wall, which is constant across species (Niemz 1993). The proportion of water was calculated as (fresh weight – dry weight)/wood volume, and the proportion of gas as 1 – [water] – [cell wall]. The volume of the wood pieces was calculated from the dimensions of the saturated wood.

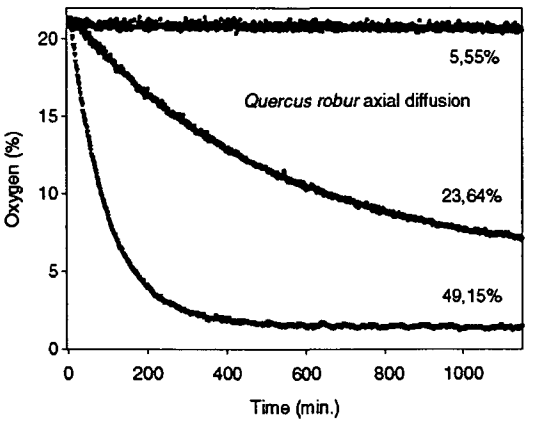

Fig. 2 Oxygen concentrations measured in a closed air volume sealed to *Quercus robur* heartwood through which oxygen diffused in exchange of nitrogen while the other side was exposed to pure N_2 gas. The three curves were measured at different proportions of gas/water (in percent volume) in the wood

No cracks were found in the wood after the diffusion measurement and prior to oven drying, but observations were only superficial. To verify this, we left wood of similar dimensions, as used in the diffusion experiment and partially wrapped in aluminium foil to delay drying, on the laboratory bench and monitored weight and possible cracks on the surface over several days.

Calculation of Fick's diffusion coefficient (D)

The oxygen concentration was measured in a gas volume of 4.466×10^{-5} m^3. At standard temperature and pressure (0°C and 101.3 kPa), 1 mol has 0.0224 m^3, and the amount of oxygen in this volume at an ambient oxygen concentration of 20.95%, the average atmospheric pressure of 101.5 kPa, and the laboratory temperature of 20°C is $4.466\times10^{-5}\times0.2095/0.0224 * 1015/1013 * 273.15/293.15=3.899\times10^{-4}$ mol. The measured oxygen concentration was used to calculate the amount of oxygen/nitrogen diffusing through the wood and the changing gradient of the two gasses. Oxygen concentration, at the other end, was assumed to be 0 when flushed with N_2 and 20.95% when exposed to ambient air.

The amount of gas diffusing depends on the concentration gradient (Δc; mol m^{-3}), the area (A; m^2) and length (l; m) of the diffusion pathway and the diffusion constant (D). D was calculated from the measured change in the concentration and absolute amount of oxygen as follows:

$$D(m^2\,s^{-1}) = \text{diffusion}\,(\text{mol}\,s^{-1}) * l/\Delta c/A$$

To smooth D, exponential curves of the form $[O_2] = a\,e^{-b*t}$, where t is the time in seconds and a and b are coefficients, were fitted to the measured oxygen concentration between 95 and 70% of ambient air.

Diffusion model

To test the effect that D had on the oxygen supply of respiring sapwood, models were designed and run with AME (Version 4.4, now called Simile, www.simulistics.com). Diffusion was calculated through 20 layers of sapwood, each 5 mm thick, and determined by the concentration gradient between two adjacent layers, layer thickness, and D. The initial oxygen concentration was that of the air at 20°C (i.e. 20.95% or 8.732 mol m^{-3}) and of saturated water at 20°C (9.16 mg l^{-1} or 0.286 mol m^{-3}) in the water phase. The water and gas phases accounted for 50 and 25% of wood volume, respectively, which is important because the amount of oxygen available for respiration strongly depends on the presence of gas. In the first model, sapwood respiration was set to 50 μmol m^{-3} s^{-1}, which is not particularly high (Edwards and Hanson 1996;

Lavigne et al. 1996), at positive oxygen concentrations and to 0 μmol m^{-3} s^{-1} when all oxygen was consumed. In the second model, sapwood respiration was also 50 μmol m^{-3} s^{-1} at O_2 concentrations above 25% (relative to air), but declined linearly to 12.5 μmol m^{-3} s^{-1} at 5% O_2. This corresponds approximately to the relationship between oxygen concentration and consumption for sapwood measured by Spicer and Holbrook (2005). In the third model, respiration was 100 μmol in the outermost 5 mm of sapwood, declined linearly to 25 μmol at a depth of 5 cm and was constant at deeper layers to a maximum depth of 10 cm. The models were run with values for D between 10^{-6} and 10^{-10} m^2 s^{-1} until the concentrations remained constant, i.e. the oxygen consumed by respiration was in equilibrium with the oxygen supplied through diffusion through the successive layers of wood. A slightly modified version, without respiration and with a closed volume of gas after the last layer, correctly simulated the oxygen measurement in the experimental set-up. The full model can be obtained from the corresponding author on request.

Results

The oxygen concentrations in the small gas volume into which N_2 diffused in exchange for O_2 declined exponentially (Fig. 2), i.e. the amount of gas diffusing decreased, as was expected when the gradient between the side exposed to pure N_2 and the side where the oxygen concentration was measured declined.

The density of wood at maximum water content ranged between 0.83 g cm^{-3} for *Taxus*, and 1.20 g cm^{-3} for *Quercus* (Table 1). With the cell wall having a density of 1.5 g cm^{-3} (Niemz 1993), it amounts to between 25% (*Picea*) and 45% (*Quercus*) of wood volume occupied by cell walls. It was not possible to completely infiltrate the wood with water and between 0.8 and 24% of volume still contained gas at the maximum water content obtained.

Since diffusion is much higher in gas than in water, D was related to the gas content, which at various stages of drying ranged between <1 and ca. 50%, rather than to the water content. In all species, the diffusion coefficient strongly increased with the volume of air in the xylem (Fig. 3). The relationship between D and gas volume was approximately exponential, but differed between species. The rate of nitrogen/oxygen diffusing through the xylem was very low at maximum saturation and low gas content. At 15% gas volume, D for axial diffusion ranged between about 9.5×10^{-10} m^2 s^{-1} for *Picea*, and 1.3×10^{-8} m^2 s^{-1} for *Quercus* (Table 1). At 40% gas volume, D increased 5–13-fold in *Picea*, *Taxus* and *Quercus*, 36-fold in *Fraxinus* and about 1000-fold in *Carpinus* and *Fagus*.

In all species except *Fraxinus*, cracks on the wood surface drying slowly on the laboratory bench were observed only at a water content <14% dry weight, which was reached in the diffusion experiments only with *Quercus*. In *Fraxinus*, the first crack was observed at a water content of 36% dry weight, which was also reached in the diffusion experiment.

The difference between radial and axial diffusion was low at low gas content, but diffusion in the axial direction was between 4 and 66 times higher than in the radial direction. In general, D was lowest in conifers, intermediate in ring-porous and highest in diffuse-porous hardwoods. The difference between species was stronger for axial D than for radial D.

The relationship between the density of dry wood (or the volume occupied by cell walls) and D in axial or radial direction was not significant, with $r^2<0.1$.

The model calculation showed that in the range of diffusion coefficients measured, D, and therefore wood gas content, had a very strong influence on the ability to supply live sapwood through radial diffusion (Fig. 4). At constant respiration and $D=10^{-8}$, oxygen reached 5.5 cm sapwood depth, and only above ca. 3×10^{-8} was at least some oxygen reaching the entire 10 cm of sapwood. At $D=10^{-10}$ m^2 s^{-1}, only the outermost mm could be supplied with oxygen to sustain a respiration rate of 50 μmol m^{-3} s^{-1}. When respiration was reduced at low oxygen concentrations, the supply of deeper layers was slightly better because the oxygen-deficient inner sapwood consumed less. When respiration declined from 100 μmol in the outermost sapwood to 25 μmol at a sapwood depth >5 cm, the supply of the sapwood was worse at all but the highest D because the high respiration of the outer layers left little oxygen for the inner layers with less demand.

Discussion

The fibre optode used was ideal for measuring changes in oxygen concentrations in a closed volume of unstirred

Table 1 Density of dry wood, of saturated wood used at the beginning of the diffusion experiments, the volume of cell walls, and diffusion coefficients (D) for axial gas transport in wood at 15% (D_{15} axial) and 40% (D_{40} axial) gas content and radial transport at 40% (D_{40} radial) gas content

	Dry density (g cm^{-3})	Saturated density (g cm^{-3})	Cell wall (vol%)	D_{15} axial (m^2 s^{-1})	D_{40} axial (m^2 s^{-1})	D_{40} radial (m^2 s^{-1})
P. abies	0.43±0.02	1.00±0.06	25.25±0.82	9.5×10^{-10}	6.2×10^{-9}	1.4×10^{-9}
T. baccata	0.79±0.18	0.83±0.21	36.82±1.64	4.5×10^{-9}	5.8×10^{-8}	1.7×10^{-9}
Q. robur	0.78±0.04	1.20±0.02	45.21±1.39	1.3×10^{-8}	6.9×10^{-8}	3.2×10^{-9}
F. excelsior	0.64±0.03	1.07±0.03	38.04±2.30	1.2×10^{-8}	4.3×10^{-7}	5.9×10^{-8}
C. betulus	0.74±0.01	0.99±0.03	39.80±1.42	1.3×10^{-9}	2.0×10^{-6}	9.3×10^{-8}
F. sylvatica	0.69±0.04	1.12±0.03	38.39±2.22	3.8×10^{-9}	2.3×10^{-6}	3.5×10^{-8}

Values of D_{15} and D_{40} are obtained by fitting exponential or linear (in the case of *Q. robur* and *C. betulus* radial) regressions curves to the data in Fig. 3

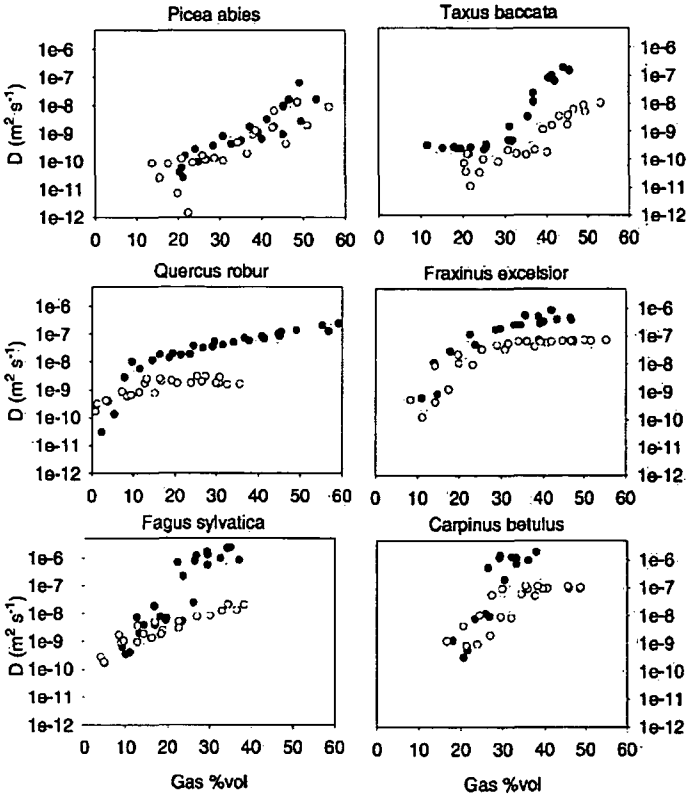

Fig. 3 Diffusion coefficient (D) of nitrogen/oxygen in wood related to heartwood air content. *Picea* and *Taxus* are conifers, *Carpinus* and *Fagus* diffuse-porous hardwoods and *Quercus* and *Fraxinus* ring-porous hardwoods. Full symbols represent diffusion in the axial direction, empty symbols in the radial direction. Each dot represents one diffusion measurement as shown in Fig. 2. For each species, three pieces from different individuals were measured at decreasing water content over several days

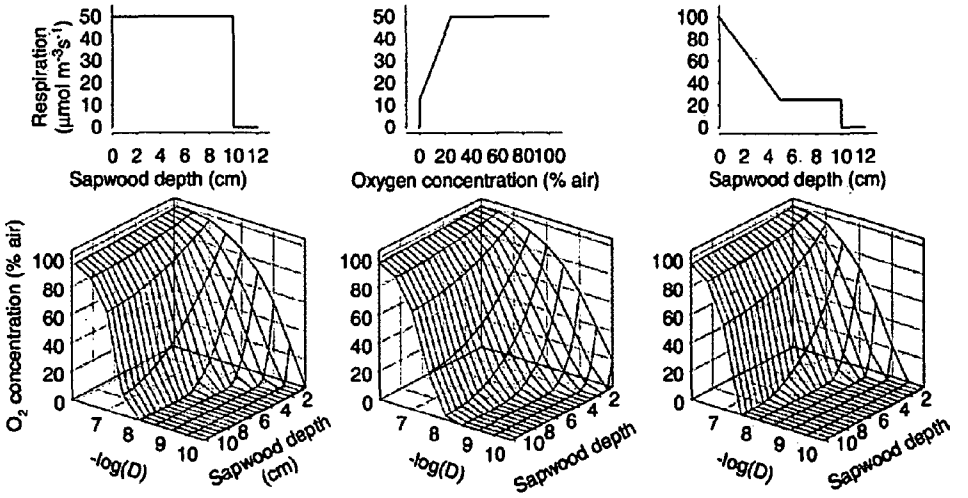

Fig. 4 Model calculations showing the effect of the diffusion coefficient (D) on the oxygen supply through sapwood of 10 cm thickness, and with the volume in gas and water phase accounting for 50 and 25% of wood volume, respectively. In Model 1 (left) respiration is constant at 50 µmol m^{-3} s^{-1} as long as any oxygen is available, in Model 2 (centre) respiration declines from 50 µmol m^{-3} s^{-1} at an oxygen concentration >25% to 12.5 µmol at O_2 >0%, and in Model 3 (right) respiration declines from a maximum of 100 µmol m^{-3} s^{-1} at the outermost sapwood to 25 µmol at a depth of >5 cm

air without any oxygen consumption, but more important is the fact that the whole set-up was as gas tight as possible.

In principle, liquids as well as gas can be transported through wood either by bulk flow along a pressure gradient or by diffusion following a concentration gradient. In live trees, liquid water is always transported along a pressure gradient, and nearly all attempts to measure the transport capacity of wood have been made by applying pressure, i.e. have quantified permeability. However, substances dissolved in water or gas will also diffuse through the wood and particularly under conditions of zero flow this diffusion can be an important factor limiting the supply of oxygen to tissues.

The diffusion coefficient (D) of oxygen is 1.95×10^{-5} m^2 s^{-1} in air and 2.0×10^{-9} m^2s^{-1} in water at 20°C and 101.3 kPa (von Willert et al. 1995). We measured D in wood between 4×10^{-11} and 2×10^{-6} m^2 s^{-1}, i.e. always lower than in air and in some cases lower than in water alone. The only other publication of gas diffusion (in contrast to permeation under pressure) in wood we could find reported air diffusion through oven-dried heartwood of various Japanese softwoods and hardwoods between 5×10^{-7} and 2.4×10^{-5} m^2 s^{-1} in longitudinal direction and between 3.5×10^{-10} and 2.8×10^{-7} m^2 s^{-1} in radial and tangential direction (Yokota 1967). Considering that we never measured completely dry wood, this agrees well with our data.

Except for a single measurement in *Fraxinus*, the minimum D measured was always lower than the diffusion in water alone in spite of the fact that no samples were completely infiltrated and minimum air content was between 0.8 and 24%. This illustrates that the cell walls present a major barrier to gas diffusion with D lower than that of water.

Given that D differs by about 4 orders of magnitude between water and air, the strong relationship between D and the proportion of air space in wood was to be expected. However, neither D at a given proportion of gas volume nor the maximal D measured at minimum water content, when diffusion should largely be limited by cell walls, was related to the volumetric proportion of cell walls which ranged between 25 and 45%. This contrasts with the inverse relationship between specific wood density and gas permeability in longitudinal and tangential direction (Yokota 1963; Isaacs et al. 1971) and underlines the difference between diffusion and permeation and the importance of anatomical features. In the case of the species measured, the arrangement of cell walls (or the size and shape of conducting elements), the structure of the cell wall matrix and/or the pit membranes must have a strong impact on D in wood. Diffusion models for water through the cell wall generally assume that the resistance of the secondary wall is extremely high and diffusion thus occurs largely through pit membranes (Siau 1984).

The very strong increase of D with air content could additionally be caused by desiccation cracks, which can also form in live sapwood (Cherubini et al. 2003). In our study, some cracks were observed only at the lowest water concentrations tested in *Quercus* and *Fraxinus* and can thus account only for the extreme D measured in some cases.

At a gas content of ca. 30%, D differed by about 4 orders of magnitude between different tree species and was lowest in the two conifers, which had the lowest wood density. The low hydraulic conductance of conifer wood is explained by the lack of large conducting elements. However, since diffusion is described by Fick's law, D through individual elements is proportional to their cross-sectional area and not to the square of that area as in water flow density through capillaries governed by Poiseuille's law. The interactions between the diffusing gas molecules and the cell wall may slow diffusion down a bit, but since the mean free path of a gas molecule, which is the average distance it travels before colliding with another molecule, is about 70 nm at normal pressure and temperature (Nobel 1991), much smaller than the diameter of tracheids, this effect should be very small. Thus, the slow diffusion through conifer wood is probably related to the length of the conducting elements, i.e. the frequency at which gas has to diffuse through cell walls, rather than the tracheid diameter. Also the bordered pits of conifers may present a higher resistance than pits in angiosperms, particularly in the heartwood where most pits are aspirated and blocked by accumulations of extraneous material and inclusions of extractives (Isaacs et al. 1971; Sebastian et al. 1973; Siau 1984; Carlquist 1988; Schweingruber 1990).

The higher diffusion in axial relative to radial direction (Table 1) is explained by the fact that the diffusing gas encounters more cell walls in the radial than in the axial direction, that gas-filled elements extend axially and that, at least in conifers, bordered pits connect cells in the axial and tangential direction, but radial cell walls generally have no pit membranes. In principle, ray tracheids and ray parenchyma can also contribute to radial permeability. In hardwoods, the contribution of rays to the overall permeability is thought to be lower than in softwoods, despite the generally higher volumetric fraction of rays in hardwoods (Siau 1984).

While we did find differences between the axial and the radial diffusion coefficients of about 1 or 2 orders of magnitude and similar to those of Yokota (1967) for dry conifer wood, radial to axial permeability measured by pressure differences differed by 3–8 orders of magnitude with the highest values measured in red oaks, which have very wide vessels (Siau 1983).

Using model calculations and data on wood anatomy of two conifers, Petty (1973) concluded that for axial diffusion through dry wood about 98% of the resistance is in the tracheid lumina, 1% in the pit apertures and 0.2% in the pit membrane pores; but for tangential diffusion, 90% of the resistance occurs in pit apertures and only 0.3% in tracheid lumina. In *Picea sitchensis*, the axial diffusion coefficient was about 40 times greater than radial D (Petty 1973), which is in the upper range of our results.

Hardwoods are less homogeneous than conifers, which makes the diffusion more complex. Similar to softwoods the pores of hardwoods are the primary path of the diffusing gas (Siau 1984). The large vessels of angiosperms typically

extend for several centimetres to decimetres (Tyree and Zimmermann 2002), thus, at least a few vessels may have extended through the prepared piece of wood without any cell wall blocking the axial diffusion. Ring-porous *Quercus* and *Fraxinus* have much larger vessels than *Carpinus* and *Fagus*, but most of their heartwood vessels are blocked by tyloses, which at least partly explains why diffusion through ring-porous heartwood was slower than through diffuse-porous wood. Also, to compensate for the low hydraulic conductivity of small vessels, those in diffuse-porous trees occupy a larger fraction of the cross-sectional area than in ring-porous species (Siau 1984). Sebastian et al. (1973) observed that the small latewood vessels of ring-porous wood can be relatively impermeable to gas, in contrast to the relatively uniform vessels of diffuse-porous and the large earlywood vessels of ring-porous wood. In ring-porous wood, the permeability of latewood vessels is probably intermediate between large vessels and fibres. The proportion of a specimen's latewood is therefore important for its overall gas permeability, which is true for both hardwoods and softwoods (Bramhall and Wilson 1971; Petty 1973) and the same may be true for diffusivity.

While the relationship between air content and D was approximately exponential for conifers, the increase of D at higher proportions of air levelled off in angiosperm wood (Fig. 3). Because large vessels have fewer cell walls in the axial direction (or none in a specimen of 2.5 cm length) they will be the first to be drained. Subsequently, as smaller and shorter vessels and fibres with thick cell walls embolize, the gain in D for an additional volume of gas in the wood is relatively small.

A recent review of gas content in wood found an average gas volume of 18% in sapwood and 50% in heartwood in 26 softwood species, of 26% in both sapwood and heartwood in 31 temperate hardwood species, and of 18% (sapwood and heartwood not distinguished) in 52 tropical hardwoods, with substantial variation between species (Gartner et al. 2004). Seasonal fluctuations in water content can also amount to over 10% of total stem volume (Pausch et al. 2000). Thus, the gas volume at which we measured diffusion is similar to that in live trees.

So far, gas in the water-conducting part of the stem has mainly been an issue because of the negative effect that gas-filled or embolized conducting elements have on water flow by reducing hydraulic conductance (Tyree and Zimmermann 2002). Recently, Gartner et al. (2004) addressed the mechanical effect of gas in stems and calculated that the normal gas content in *Pseudotsuga menziesii* stems permitted these to withstand a 26–41% higher wind force relative to stems completely saturated with water, but concluded that gas content may have evolved in response to pressures unrelated to biomechanics.

Considering the strong effect of gas content on diffusion, we believe that an even more important function of gas in stems may be to provide oxygen through storage and faster diffusion. Sapwood with 25 vol% cell wall material, 50% water, and 25% gas in equilibrium with oxygen concentration of ambient air at 20°C will store 0.14 mol O_2 m^{-3} in the aqueous phase (solubility at 20°C is 9 g O_2 m^{-3}) and 2.18 mol m^{-3} in the gas phase (the air has 8.73 mol oxygen m^{-3} at 20°C). At full saturation (75% water) only a total of 0.21 mol m^{-3} can be stored. High rates of stem respiration during the growth period are in the range of 100–200 μmol CO_2 m^{-3} sapwood s^{-1} at 15–20°C (Edwards and Hanson 1996; Lavigne et al. 1996). At 100 μmol m^{-3} s^{-1}, the oxygen stored in the sapwood example above with 25% gas will be consumed in 6.4 h. If the water content were 65%, the supply would last for 2.9 h, and at full saturation (0% gas) for only 0.6 h, assuming no oxygen is imported. At zero sapflow, the respiring sapwood will draw oxygen either through bark and cambium, if this path is open, which in many trees it appears to be not (Hook et al. 1972), or from the non-respiring heartwood. All three models tested showed that for oxygen diffusion to supply 5 cm of sapwood respiring at a rate of 50 μmol m^{-3} s^{-1}, the diffusion coefficient has to be about 10^{-8} m^2 s^{-1}, which is in the range of the values measured for radial diffusion. These models are simplifications and sapwood respiration will depend on distance from the cambium, oxygen concentration, season, temperature and other factors. But even allowing for inaccuracies in the estimates, it is clear that the gas diffusivity of wood is necessary to supply live sapwood with oxygen and that, given the strong effect of water content on D, not enough oxygen would diffuse through fully-saturated wood. This supports the notion that heartwood formation can be triggered by low oxygen concentrations in the innermost sapwood (Eklund and Klintborg 2000), but makes the idea that the dark heartwood in beech forms when oxygen enters through distant wounds on the upper stem (Knoke 2003) appear unlikely. In a recent study, oxygen concentration were found to decline from the cambium towards the heartwood boundary during times of stem respiration, but no values below 3–5% gaseous mole fraction (corresponding to approximately 15–25% of air O_2 content) were measured (Spicer and Holbrook 2005). These concentrations had only minor and reversible effects on parenchyma cells and it is concluded that low oxygen concentrations are unlikely to be the cause of heartwood formation. However, Spicer and Holbrook (2005) measured sapwood oxygen at only two times of the year, Eklund (2000) found values <1% of air in drought-stressed spruce trees, and sapwood cell death may only occur in times of stress with extremely low oxygen concentrations, which only long-term measurements are likely to detect.

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2 Oxygen in spruce stems

2.1 Current state of knowledge

2.1.1 Oxygen in tree stems

Higher plants require oxygen (O_2) for the respiratory production of energy and for various other processes where oxygen is involved, such as lignification (Halliwell, 1978; Mäder & Amberg-Fisher, 1982) or the action and turnover of various hormones (e.g. Letham & Palni, 1983; Yang & Hoffman, 1984). But aerobic respiration produces large amounts of carbon dioxide (CO_2) in return, that need to be removed from the respiring tissue, because higher levels of CO_2 inhibit aerobic respiration (Miller & Hsu, 1965; Wullschleger *et al.*, 1994). In leaves stomata and intercellulars ensure an adequate supply with O_2 and CO_2 (Jones, 1998). Respiring sapwood parenchyma cells are far from a direct contact with the atmosphere and not surrounded by sizeable intercellulars, requiring a different systems for their supply with oxygen and the removal of CO_2, which is less well understood.

Distance and high resistances exclude the possibility of oxygen diffusing from the leaves downward into the stem, thus the gas can either reach the living sapwood by radial diffusion along its partial pressure gradient through bark, phloem and cambium, or be transported upwards dissolved in the transpiration water, which is driven by the soil-plant-atmosphere water-potential gradient. Owing to their intercellulars, bark and phloem are normally quite gas-permeable and allow O_2 to supply phloem and cambium by diffusion through connected intercellular spaces (Armstrong, 1979). The cambium however has intercellulars that are important for the radial diffusion to the xylem only in trees adapted to flooding (Hook & Brown, 1972).

Environmental conditions like drought or water logging sometimes inhibit oxygen supply to all or parts of the plant body, eventually causing tissue hypoxia (oxygen deficiency resulting in stress) or anoxia (absence of oxygen) and changes in metabolic pathways. Oxygen deficiency is common in roots of wetland plants, when gaseous oxygen is expelled from soil pores by the excess of water, and the low diffusion coefficient for oxygen in water - 10^4 times lower than in gas (Atkins, 1998) - impedes further supply from the aerated zone. Several studies describe survival strategies that allow plants to cope with flooding and oxygen deficiency (Drew, 1997; Moog, 1998; Lösch & Busch, 2000; Das & Uchimiya, 2002; De Simone *et al.*, 2003; Fukao & Bailey-Serres, 2004). Adaptation includes the formation of intercellulars and lenticels, which improve the ventilation of submerged parts, a down-regulation of the metabolism to reduce the exhaustion of carbohydrate supplies, and energy production with alcoholic fermentation (Armstrong *et al.*, 1994; Kozlowski, 1997; Gibbs & Greenway, 2003; Jackson & Colmer, 2005).

So far the impact of low oxygen partial pressure (pO_2) on plant metabolism was mostly described in herbaceous species and in water-logged underground organs, but recent publications show that under certain environmental conditions oxygen depletes noticeably in tree stems. Low pO_2 is often measured during periods of high respiratory activity, when most oxygen is consumed by living parts of the stem, or when environmental conditions restrict a sufficient supply.

In Norway spruce O_2 is close to ambient levels in winter, but it decreases significantly during the vegetation period, reaching the lowest concentrations in summer (Eklund, 1990; Eklund, 1993; Eklund, 2000). In the sapwood of *Betula pendula* oxygen declines from the beginning of bud break until frondescence (Gansert *et al.*, 2001). In summer oxygen declines from the cambium toward the heartwood in *Fagus americana* and *Tsuga canadensis* (Spicer & Holbrook, 2005). It is suggested that these effects are correlated to an increase in the respiration rate (R). R of the cambium and its surrounding tissues is strongly dependent on the temperature (Lavigne, 1996; Stockfors & Linder, 1998), and it approximately doubles with a rise of 10 °C (Atkin & Tjoelker, 2003). Thus, in times of high temperatures, e.g. in summer, more oxygen is consumed by the living cells of xylem and phloem resulting in a consequent depletion of the available oxygen when sufficient supply is not assured. While oxygen consumption in the cambial zone is generally attributed to growth respiration, i.e. the production of new tissue, and is related to stem growth, oxygen demand by parenchyma cells in the sapwood and the phloem are related to maintenance respiration (Thornley, 1970). Stockfors and Linder (1998) measured respiration in Norway spruce and found that during the growing season (June to September, with a peak in mid-June) 40 - 60% of total respiration accounts for growing respiration. Maintenance respiration is highest in mid-May and it is largely related to the phloem where 75 - 80% of active cells in spruce stems exist. Phloem maintenance accounts for about 70% of overall maintenance respiration.

Active parenchyma cells account for 5 - 35 vol.% in the secondary xylem (conifers: 5 - 8%, angiosperms 10 - 35%). These cells live between two and 150 years, exist at depths of 20 cm and more within the stem (Panshin & de Zeeuw, 1980), and require oxygen for aerobic respiration. If the xylem cells are supplied with O_2 by means of radial diffusion, the O_2 diffusing through bark, cambium and wood has to pass through several layers of respiring and O_2-consuming tissues (phloem, cambium and outer sapwood layers).

Oxygen content in spruce slightly declines with height (Eklund, 2000), thus it was suggested that O_2 is transported with the sap stream, and that O_2 is successively depleted along its way as it passes respiring tissues that consume O_2 and enrich the sap water with CO_2. Sapwood pO_2 decreases when sapflow is restricted in *Laurus nobilis* (del Hierro *et al.*, 2002), *Betula pubescens* (Gansert, 2003), and *Olea europaea* (Mancuso & Marras, 2003). Continuous oxygen measurements showed diurnal variations in the sapwood in a range of 10 - 20%

(Gansert, 2003; Mancuso & Marras, 2003), which indicate a strong relationship between oxygen and transpiration stream. Gansert measured oxygen with an optode based sensor in *B. pendula* (Gansert et al., 2001), where he found highest O_2-levels during the night when sapflow was typically low - zero, and in potted *B. pubescens* (Gansert, 2003), where pO_2 was highest during daytime when sapflow was also high and when pO_2 was lowest during the night. Mancuso and Marras (2003) measured oxygen in a potted *O. europaea* tree with a tree-point electrode based sensor, and found that in during daytime almost all the oxygen present in the sapwood was supplied by the ascending sap, while during the night the diffusion of oxygen via the sap-filled lumina of tracheids and vessels (diffusion in the aqueous phase) accounted for about 87% of the supplied oxygen. The remaining 13% were transported by radial diffusion in either the aqueous or gaseous phase. During the vegetation period and during daytime when sapflow is high the transpiration stream probably contributes largely to the supply of oxygen, but during the night and when sapflow is low, e.g. in periods of heavy rainfall, radial influx from the ambient atmosphere functions as an important alternative source for the living sapwood.

2.1.2 Relationships between stress, stem oxygen, emissions rates and pathogens

When oxygen availability is limited by environmental factors and aerobic respiration is impeded by a low pO_2, plant tissues can use alcoholic fermentation, producing ethanol from carbohydrates. During anoxia, energy (ATP) and redox equivalents (e.g. NAD^+) must be generated via pathways such as lactate- and ethanol-fermentation that do not use require oxygen as an electron acceptor Fermentation is less efficient than aerobic respiration and the rate of energy production under anoxia is significantly reduced compared to the rate in air. Only two molecules of ATP per molecule of glucose fermented are generated, releasing only 5% of the ATP generated by aerobic respiration. However the capability of ATP production under anaerobic conditions is essential for adaptation to oxygen stress, since the necessary metabolitic activity can be sustained until normal levels of O_2 are restored (Gibbs & Greenway, 2003; Jackson & Colmer, 2005). In non-tolerant plants death of active tissue occurs after only a few hours of anoxia, probably caused by a shortage of ATP and cytoplasmic acidosis, the accumulation of toxic metabolites such as acetaldehyde in the cytoplasm (Roberts *et al.*, 1984). Acidosis can also arise from unregulated lactate fermentation, making it a potentially more harmful alternative to alcoholic fermentation (Armstrong *et al.*, 1994; Gibbs & Greenway, 2003; Jackson, 2006)

Under normal conditions only low levels of ethanol exist in cambium and xylem of most tree species (MacDonald & Kimmerer, 1991). Increased ethanol levels are present in the vascular cambium of water-logged trees (Kimmerer & Stringer M.A., 1988), in topped or girdled trees (Sjödin *et al.*, 1989), in tree stumps (von Sydow & Birgersson, 1997; Kelsey & Joseph,

1999a), in stems with heart rot (Gara *et al.*, 1993), and in cut logs exposed to rain (Kelsey & Joseph, 1999b).

It has been hypothesized that pO_2 in the vascular cambium is often low, as a result of low diffusion through , respiring secondary phloem and thick periderm. Ethanol levels in the cambium of non-flooded *Populus deltoides* trees were not reduced when stems were exposed to pure oxygen but exposure to low oxygen increased ethanol content substantially (MacDonald & Kimmerer, 1991). These results suggest that the cambium of non-flooded trees is not anoxic under normal conditions, despite the presence of ethanol, and that the cambium is also permeable for oxygen. Within the stem ethanol is transported with the sapflow and it shows diurnal fluctuations in *Populus deltoides* with minima during daytime and maxima during the night. It was hypnotized suggested that the reduction of sapflow is followed by a depletion of oxygen during the night, which eventually leads to conditions favoring alcoholic fermentation and the production of ethanol. The concentration of ethanol does not change with tree height in non-flooded trees, but it does decrease with height in trees with flooded roots, possibly because ethanol is produced in anoxic root tissue and consecutively transported into the stem along with the transpiration stream (MacDonald & Kimmerer, 1991).

Thus factors influencing oxygen transport (e.g. reduced sapflow or bark permeability, low soil oxygen) and/or increase O_2-consumption (high temperatures, active growth, stress respiration, respiration by pathogens) are likely to result in hypoxia or anoxia in the living sapwood and may eventually lead to an increase in ethanol synthesis.

Ethanol production is of interest because it is a main co-attractant of bark beetles. Bark beetles (Coleoptera: Scolytidae) are one of the major pests of coniferous forests in central Europe and pose an intense threat to stands of Norway spruce. They are responsible for enormous losses in forest industry and according to a report of the Austrian Federal Government (2006) 2.8 million solid cubic meters of lumber were lost in 2005 due to bark beetle infestation. The larvae feed in tunnels in the live phloem beneath the dead periderm. In most cases the eventual death of an infested tree is not the direct result of the feeding, but is often the result of infections with beetle-associated blue-stain fungi, such as the *Ophiostoma* and *Ceratocystis* species, that may dry the tissue and cause tracheid aspiration or vascular plugging (Paine *et al.*, 1997; Lieutier, 2002; Wermelinger, 2004).

The susceptibility of conifers to bark beetle infestation depends mostly on host tree and stand characteristics. Bark anatomy and physiological status of the attacked tree are crucial and pioneer bark beetles select susceptible hosts that were weakened by previous episodes of abiotic or biotic stress (Guerard et al., 2000). Thus intense outbreaks are often associated with unfavorable environmental conditions like drought (Vité, 1961; Mattson & Haack, 1987; Croise & Lieutier, 1993) or heavy windbreak damage (Peltonen, 1999). The probability of an

attack is strongly related to stand characteristics, with outbreaks occuring more frequently on dry, south-exposed and sunlit sites (Lobinger & Skatulla, 1996; Jakus, 1998) and on stands with a higher proportion of older trees (Becker & Schröter, 2000). Further known influences are stand altitude, the availability of soil nutrients (Dutilleul *et al.*, 2000) and water supply (Lexer, 1995).

Vital trees possess several defense mechanisms to prevent attacking bark beetles from successfully establishing broods. Healthy individuals withstand isolated attacks by most species of bark-beetle and only mass attacks can result in successful broods and the eventual death of the attacked tree (Guerard et al., 2000).

The primary or constitutional defense level of an attacked tree is the release of stored resin upon attempted penetrations of the bark (Paine *et al.*, 1997). This initial defense is especially effective in individuals with sturdy bark and dense resin ducts (Baier, 1996) and in trees with normal water status (Vité, 1961; Lorio & Hodges, 1968). The secondary defense level involves changes in local metabolism around the entrance hole. Toxic chemicals such as procyanidine are synthesized to restrict the establishment of a brood (Rohde *et al.*, 1996). The tertiary defense level is a systemic change in tree metabolism that enhances the synthesis of special defense proteins which deteriorate the food quality of the substrate. The fourth and final level of defense is the new formation of periderm tissue and resin ducts (Lieutier, 2002).

Next to their contribution to plant defense liquid oleoresins play an important ecological role in host–beetle interactions. Low-molecular monoterpenes are major components of conifer resin (Führer *et al.*, 1991; Langenheim, 1994), and although they are toxic at high concentrations, they are also important for the attraction of bark beetles (Byers, 1995). Pioneer bark beetles use tree-emitted volatiles (kairomones) as signals to locate and detect susceptible host species within a population, which is crucial for successful colonization and the consequent establishment of a brood (Raffa & Berryman, 1987). The defense of weakened individuals is substantially reduced, and bark beetles preferentially attack unhealthy or injured individuals which also release higher amounts and different bouquets of ethanol and volatile terpenoids than healthy trees (Moeck, 1970; Gara *et al.*, 1993; Byers *et al.*, 2000).

Certain compositions of monoterpenes emitted from spruce trees increase the initial attack rate of *Ips typographus* and other species (Baier *et al.*, 1999) and affect their post-landing behavior (Wallin & Raffa, 2000). The insects enter the attacked host through lenticels because they are softer than the surrounding tissue, have fewer resin canals, and are the origin of volatile emissions (Rosner & Führer, 2002). Terpene content and composition are influenced by various environmental factors such as drought stress (Kainulainen *et al.*, 1992; Croise & Lieutier, 1993), air pollution (Cobb *et al.*, 1972), and by previous infections with tree

diseases e.g. heart-rot (Madziara-Borusiewiz & Strzelecka, 1977). The attraction of *Ips typographus* to affected spruce trees is reinforced synergistically when volatile monoterpenes appear in combination with ethanol (Borden, 1985).

(Kelsey & Joseph, 1999b) found substantially higher ethanol emissions and higher attack rates of ambrosia beetles on cut logs of Douglas fir exposed to rain compared with wood protected from rain. It was suggested that rain water is absorbed by the outer bark of the wet logs where it restricts the gas exchange between the living tissue and the outer atmosphere. Such conditions would facilitate the development of hypoxic conditions necessary for ethanol synthesis and its accumulation in the trunk.

2.2 Objectives

A review of the few studies done shows, that several factors influence short- and long-term fluctuations of oxygen concentrations in the sapwood. The probable effects of water stress, root oxygen supply, fungal infections and tissue temperature have been described in the previous chapter. Although several authors presented preliminary evidence that an important proportion of sapwood oxygen is transported via the transpiration stream (Eklund, 2000; Gansert *et al.*, 2001; Gansert, 2003; Mancuso & Marras, 2003), some of these results were obtained from single trees and should be confirmed under controlled conditions. Oxygen supply is most likely influenced by the external oxygen concentrations, sapflow rates, gas permeability of the stem and consumption of adjacent tissues, while O_2 demand is affected by tissue temperature, growth, biotic and abiotic stress and by the respiration of pathogens or pests themselves. It was the main objective of this thesis to quantify the combination of these effects on stem oxygen content and to find answers on the question how oxygen is transported into the stem. Initial hypotheses were:

H1 The main transport pathway of oxygen in tree stems is the transpiration stream

If oxygen is transported with the transpiration stream, dissolved in the ascending sap, xylem pO_2 should increase with sapflow rates. Furthermore it should decrease with stem height in well-aerated soils, because O_2 successively depletes when it passes through respiring tissues. If, however, oxygen enters the sapwood predominately through radial influx and penetrates the stem through phloem and cambium, pO_2 should be unaffected by changes in sapflow, assuming the root area is well-aerated and oxygen-rich water is taken up by the roots. Also the oxygen content should decline along the cross-section from bark toward pith, because the increasing distance to the atmosphere restricts gas diffusion.

H2 Various types of stress, including drought and flooding result in a decrease in sapwood oxygen

If oxygen is transported by the transpiration stream, flooding of the soil should result in a rapid decline of soil oxygen and consequently of oxygen concentrations in roots, sap water and the stem. Also severe drought stress should result in a decline of stem oxygen because when sapflow is restricted less oxygen is transported into the stem. If oxygen is predominantly transported by radial diffusion, flooding should have little effect on stem oxygen. Drought stress, however, could increase stem oxygen, if conducting elements get embolized and gas-filled (Higgs & Wood, 1995; Tyree & Cochard, 1996; Borghetti *et al.*, 1998; Irvine *et al.*, 1998), which results in an increase of stem gas permeability.

H3 Stem oxygen deficiency results in ethanol production.

Various forms of stress can result in oxygen depletion (Chapter 2.1), but where and when ethanol is synthesized and accumulated should depend on the type of stress. Drought stress restricts the sapflow and successively the O_2 transport to the sapwood, but does typically not enhance respiration. Thus sapwood pO_2 should deplete during prolonged drought, resulting in increased ethanol production in the upper stem. Anoxic conditions associated with prolonged flooding should induce ethanol synthesis primarily in the roots, from where it is transported to the stem via the sapflow.

There is isolated evidence for most of the above hypotheses. It is also known that bark beetles are attracted to terpenes in combination with ethanol (Borden, 1985; Schroeder & Lindelöw, 1989) and that they are able to locate stressed trees. If the above hypotheses can be demonstrated to be correct in stressed spruce trees, i.e., if stress results in oxygen deficiency and this in turn in ethanol production and higher emission rates, then this would offer a mechanism through which bark beetles identify weakened trees.

2.3 Material and Methods

2.3.1 Basics of oxygen measurements

All oxygen measurements in this study were conducted with oxygen meters (Microx TX3-AOT, Presens GmbH, Regensburg, Germany) with Needle-Type Housing optical micro-sensors. The Microx TX3 system is of a small, portable oxygen meter connected to a fiber-optic oxygen micro-sensor that measures on the principle of fluorescence quenching. A laser light source inside the oxygen meter illuminates an indicator dye at the tip of the micro sensor. A fluorescence signal is generated in the tip of the sensor that is transported back to a photo-detector inside the oxygen meter in a flexible optical fiber. The oxygen meter processes the optical signal and automatically calculates the correspondent oxygen value. The oxygen meter can either be connected to a PC or a datalogger for automated operation and data storage.

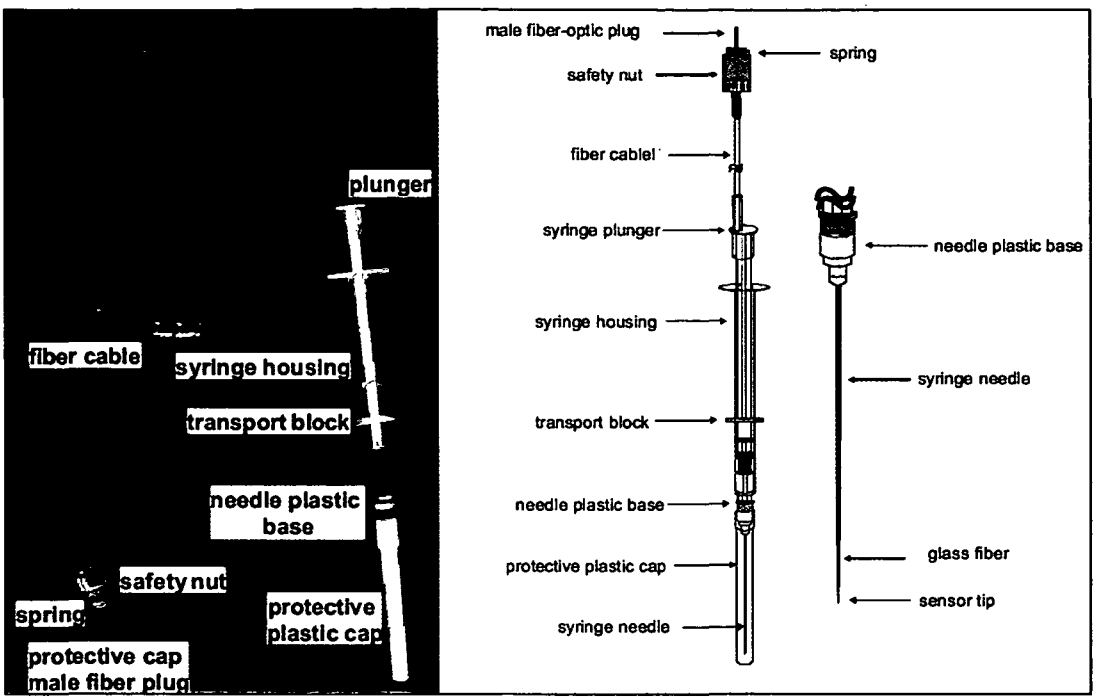

Fig. 1: The optical micro sensor in detail. The micro sensor consists of a glass fiber with a luminophore dye on its tip. The fragile fiber is housed inside a syringe and is connected to an oxygen meter (not shown). The sensor tip is sheltered inside the needle but can also be extracted for cleaning or to shorten the response time of the measurement (from Microx TX3 instruction manual).

The sensor itself consists of a 140 µm silica optical fiber with a luminophore on the tapered tip (Fig.1). The fiber is housed inside a polypropylene syringe tube (diameter Ø = 1 mm). The distal end of the fiber is sheltered inside a stainless steel needle (Ø = 1.2 mm) which protects the luminophore from mechanical damage. In a safe environment the sensor can be extended from the needle by pressing the plunger. This is important in liquid media, where oxygen diffusion is slow. Gas diffusion is fast enough through the needle, at least for the response time required, and in field experiments the sensor was not extracted to avoid mechanical damage. The sensor tip that contains the indicator dye is protected by a thin black isolation layer that functions as a screen for ambient light sources. Removal of that layer renders the sensor unserviceable. The glass fiber which connects the syringe is protected by shrinkable tubing. The oxygen meter is built into a small aluminum case, which can easily be stored in a weatherproof plastic trunk together with the datalogger for long-term measurements in the field.

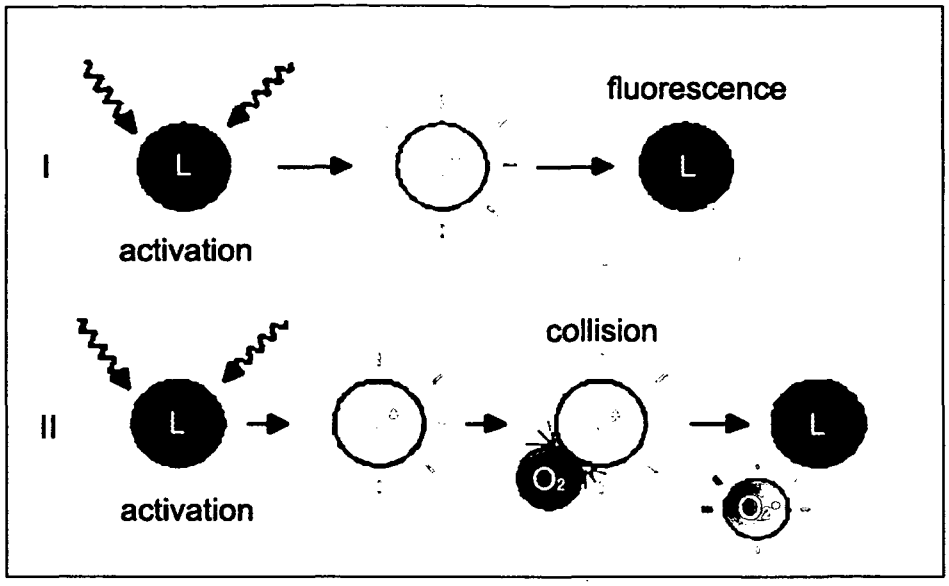

Fig. 2: The principle of dynamic quenching: I: A luminophore molecule (L) gets activated by laser light and enters an instable energized state (L*). In case no oxygen is present, the activated L* emits its absorbed energy in form of fluorescence light. II: In case molecular oxygen is present (O_2), it collides with L*, and receives its activation energy (quenching). Triplet ground state oxygen is elevated to energized singlet oxygen (O_2*) and L* is degraded to L without emitting any energy in the form of fluorescence radiation. Thus the net fluorescence signal of the luminophore is reduced by collisional or dynamic quenching of molecular oxygen (from TX3 instruction manual).

In all optical sensors an analyte interacts with an indicator which changes its optical properties in the course. This results in a change of color (absorbance or spectral distribution) or of luminescence properties like intensity, lifetime or polarization. The principle of measurement applied in the TX3 system is based on the effect of dynamic quenching of luminescence by molecular oxygen (Fig.2).

A collision between a luminophore in its excited state and oxygen results in radiationless deactivation of the excited molecule. Such a collision is followed by an energy transfer from the excited indicator molecule to the oxygen, which is transferred from its stable triplet ground state to its excited singlet state. As a result, the indicator molecule does not emit luminescence and the measurable light signal decreases. Substances usable as indicator dyes are e. g. polycyclic aromatic hydrocarbons, transition metal complexes of Ru (II), Os (II) and Rh (II), and phosphorescent porphyrins containing Pt (II) or Pd(II) as the central atom (Klimant *et al.*, 1996; Holst *et al.*, 1997).

The relation between the oxygen concentration in the sample and luminescence intensity and lifetime is described in the Stern-Voltmer equation:

$$\frac{I}{I_o} = \frac{\tau}{\tau_0} = \frac{1}{(1 + K_{sv} \cdot [O_2])}, \quad (1)$$

where I_0 and I are the luminescence intensities in the absence and presence of oxygen, τ_0 and τ are the respective luminescence lifetimes, and K_{sv} (quenching constant) quantifies the quenching efficiency and sensitivity of the sensor. The quenching effect for the indicator dye used in the TX3 is highly specific for molecular oxygen.

The phase-modulation technique is applied to evaluate the fluorescence lifetime of the indicator. When the luminophore is excited with a frequency-modulated light source, the excitation lifetime causes a time delay of the emitted signal. This delay is the phase angle between the exciting and the emitted signal. The phase angle is shifted as a function of the oxygen concentration and is inversely proportional to it.

The sensor can be calibrated at a specified temperature in oxygen-free water (0% O_2) and water-vapor saturated air (100% O_2), or, as for this study, with gaseous nitrogen (0% O_2) and ambient air. After calibration two specific phase angles (Φ_{20}, Φ_0) for a high and low oxygen content are automatically calculated and stored.

Many factors which influence the oxygen content are temperature dependent, (e. g. water vapor pressure and solubility of oxygen in water), as are decay time and intensity of the fluorescence signal. The TX3 therefore measures temperature with a Pt-1000 resistance and automatically compensates for temperature. The temperature sensor was generally inserted into a whole in the trunk along the oxygen sensor. The indicator dye is embedded into an ion-impermeable matrix, which minimizes external influences on the measurement such as pH, ions and many solutes. The matrix successfully inhibits cross-sensitivity to CO_2, H_2S, NH_3, and aggressive ion species such as S^{2-}, SO_4^{2-} or Cl^-, but the luminophore is sensitive to certain organic solvents (e. g. acetone, chloroform, methylene chloride), which swell the sensor matrix. This can be a problem for long-term measurements in stems containing resin that contains volatile organic compounds (Chapter 2.3.7.3).

Optical sensors do have advantages in comparison with traditional methods:

- Unlike steady-state electrodes, optodes consume no oxygen during the measurement. The sample does not have to be constantly stirred to provide a proper dispersal of oxygen.
- Optodes feature an exceptional high spatial (<50µm) and temporal resolution (t_{90}< 1s) and a high sensitivity (ppb-range).

- The small dimensions of the fiber (tip diameter = 30 µm) enables measurements in very confined and otherwise not accessible areas (e.g. inside the sapwood of a tree).
- Photo-decomposition of the luminophore is almost negligible when the measurements are made in minute or hour intervals, thus long time measurements are possible without the need to exchange the sensor for recalibration.

Prior to this study, optical sensors for oxygen measurements in the stems of woody plants have been successfully used by (Gansert *et al.*, 2001; del Hierro *et al.*, 2002; Gansert, 2003; Spicer & Holbrook, 2005).

2.3.2 Sapflow measurements

Water transport in the stem is linked to the transpiration rate of the entire tree, and sapflow thus provides information on a tree's physiological conditions (Smith & Allen, 1996; Köstner *et al.*, 1998)

The tissue heat balance (THB, P4.1 EMS, Brno, Czech Republic, (Cermák *et al.*, 2004) was used for sapflow measurements in the stem of one spruce during long-term oxygen measurements in 2003 (Chapter 2.4.1). Otherwise, Granier-type tissue heat dissipation (THD, (Granier, 1985)) sapflow sensors (from UMS, München, Germany and home-made) were used during oxygen measurements with potted spruce from 2003 to 2006 (Chapter 2.4.4) and with beech in a forest stand in 2005 and 2006 (Chapter 3.4.2). The following sections briefly describe design and technical specifications of the two applied methods.

2.3.2.1 Tissue heat balance (THB) method

THB is intended for trees with larger diameters (>120 mm) and basically determines the sapflow rates by examining the heat balance of heated stem tissue. Heat is applied by five stainless steel electrode plates inserted into the conducting sapwood (Fig. 3). The plates (10 mm x 25 mm) are positioned parallel to one another with a lateral separation of 20 mm. Alternating current (1 kHz) is passed through the xylem at a voltage regulated to give a constant power of 1 W. A THB sapflow meter normally features four pairs of differentially connected Cu-Cst thermocouples. Two pairs sense the difference between the heated and unheated part of the xylem at two depths, while the other two pairs are installed parallel with the first pairs, but are positioned outside the heated segment, to compensate for the natural temperature gradients in the stem. Sapflow [kg h^{-1}] is then calculated from the heat balance of the defined heated space.

The input energy has to be split between conductive heat loss and the warming of passing through water, and can be expressed by following equation:

$$P = Qdt_{cw} + dT\lambda \quad (4)$$

where P is the applied heating Power (W), Q is the rate of sapflow (Kg s^{-1}), ΔT is the measured temperature difference (K), c_w is the specific heat of water (J kg^{-1} K^{-1}), and λ is the coefficient of heat loss from the measuring spot (W K^{-1}). Heat loss at times of zero sapflow (early morning) is subtracted to compensate for all thermal losses not related to water transport (Cermák & Kucera, 1981).

THB electrodes and sensors with a newer arrangement using only two pairs of thermocouples were installed at 2 m height on opposing sides of the trunk. Voltage output was recorded in minute intervals and stored as 15-min means. Raw data was processed with EMS software for graphical zero-line subtraction and net-sapflow calculation.

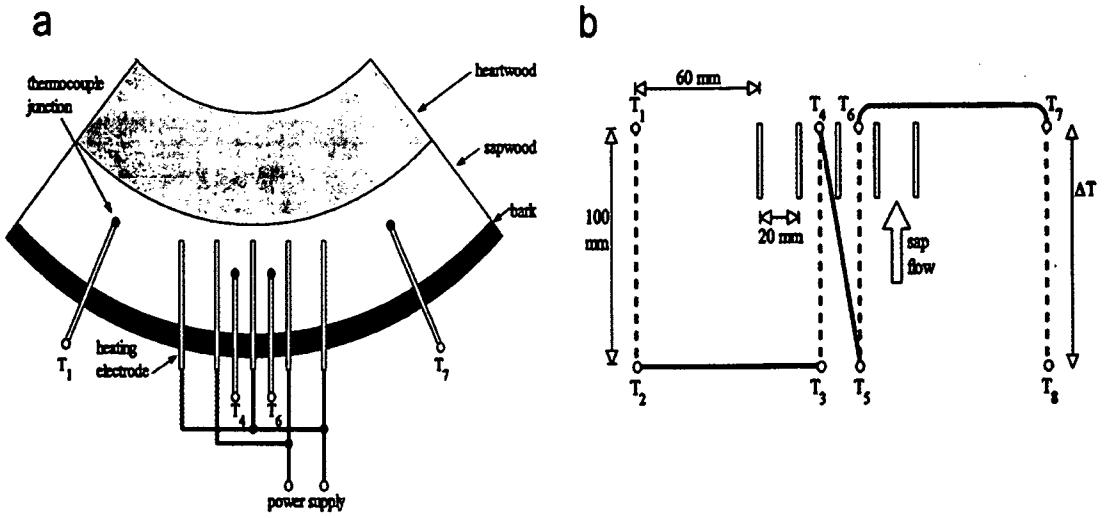

Fig. 3: Design and function of a THB-sapflow sensor. A: Horizontal view on a typical sensor setup installed in a tree stem. B: Functional scheme of the installation on the stem with relative positions of heating electrodes and thermocouple junctions (T_1-T_8). Junctions between thermocouples with copper connections are represented by solid lines, constantan wire connections are plotted as dashed lines. T_3-T_6 measure the difference in temperature between heated and unheated parts (ΔT) and T_1, T_2, T_7 and T_8 automatically subtract the natural temperature gradient (Smith & Allen, 1996).

2.3.2.2 The Thermal Dissipation (THD) method

The Granier-type thermal dissipation probe (Granier, 1985) consists of two cylindrical probes (Ø = 2 mm) inserted radially into the tree stem, with one probe placed approximately 10 cm above the other. Each probe is housed in a 2 cm long, hollow aluminum capsule. The upper probe consists of a copper-constantan thermocouple junction and is connected to a reference thermocouple in the lower probe. Additionally, the capsule of the upper probe is coiled with a constantan wire for its whole length that functions as the heater element. The two thermocouples are connected differentially and the measured voltage difference represents the actual temperature difference between the thermocouples. Similar to the THB method, heat is carried away by sapflow and the temperature difference (ΔT) between the two probes is non-linearly dependent on the rate of sapflow in the vicinity of the thermocouples. In contrast to the THB, sapflow density (Q_s) is calculated using an empirical formula that is generally applied for all tree species (Granier, 1985; Smith & Allen, 1996):

$$Q_s = 119 \cdot K^{1.231} \text{ (g m}^{-2} \text{ sapwood s}^{-1}\text{)}, \quad (5)$$

$$K = \frac{\Delta T_0 - \Delta T_i}{\Delta T_i}, \quad (6)$$

with K as the sapflow index, and ΔT_0 representing the value of ΔT_i, at times of zero sapflow. Total tree-level sapflow (Q) is further calculated as:

$$Q = Q_s \cdot A_s \text{ (m}^3 \text{ s}^{-1}\text{)}, \quad (7)$$

where A_s represents the cross-sectional area of the sapwood at heating probe (m^2). Granier-type sapflow sensors were produced by UMS (München, Germany) or home-made. For sapflow measurements in potted spruce (Chapter 2.4.4), all probes (Ø = 2 mm) were inserted into the stem at a height of ca 20 cm. The probes were inserted into drill holes (Ø = 3 mm) which were filled with thermally-conductive paste. The installed thermocouples were insulated with polyurethane foam, reflective sheets and protected from rain with plastic sheets. The sensor pairs were connected to power-regulating unit (UMS) with potentiometers regulated at 20 mA. Outgoing voltage was recorded in 5 or 10 s intervals and stored as 1 or 10 min. means with a CR10X datalogger (Campbell, Shepshed, UK) and an AM416 multiplexer (Campbell, Shepshed, UK).

(Köstner *et al.*, 1998) recommended a removal of installed sensors after a growing season to avoid damage to the surrounding wood tissue. However the removal of the fragile Granier sensors was hardly possible without destroying the sensor. Thus, sapflow sensors were not removed after initial installation except for exchange in case of defectiveness. Granier sensors were also installed in beech stems to monitor sapflow during oxygen measurements in 2005 and 2006 (Chapter 3.4.2).

2.3.3 Soil moisture measurements

Soil moisture was monitored during experiments with potted spruce trees from 2003 to 2006 (Chapter 2.4.4) with time domain reflectometry (TDR) using a TRIME FM system with a T3 tube access probe (IMKO, Ettlingen, Germany).

In May 2003 access tubes (Ø = 44 mm, length l = 30 cm) were installed permanently near the center of the 19 pots. The open tube ends were closed with plastic caps or aluminum foil to block rain water and dew. The distinct ellipsoid form of the measuring field made moisture measurements problematic, because the measuring field somewhat exceeded the pot. To minimize errors three measurements were conducted for each pot, and the probe was revolved for ca. 120 degrees after each measurement. Mean values of the measurements were calculated and used for analysis.

2.3.4 Wood moisture measurements

Wood moisture (% / dry wood mass) was measured with an electrical moisture meter measuring the electric resistance (Hydromette M4050, Gann GmbH, Gerlingen, Germany) in cut spruce logs to monitor the process of desiccation (Chapter 2.4.5). The increase in resistance is especially strong until fiber saturation point (FSP) is reached (~ 25 - 30% moisture content), above the FSP the effect attenuates and the measurement gets less exact. The needles of the Hydromette are 40 mm long, isolated along the shank, and attached on a sturdy electrode (Gann M18), which is rammed firmly into the wood prior to measurement. The instrument determines the electrical resistance at the depth of the needle tips automatically compensates for temperature and calculates water content using a wood-specific calibration curve.

Wood moisture was determined at a depth of 25 mm to accompany oxygen measurements that were conducted at the same depth. To minimize errors caused by the considerable fluctuations of moisture content in the log, a mean of three consecutive measurements made at different locations of the log, was calculated.

2.3.5 Water potential measurements

Water status of potted spruce trees (Chapter 2.4.4) was determined with a pressure bomb (Soilmoisture Equipment Corp., Santa Barbara, CA, USA).

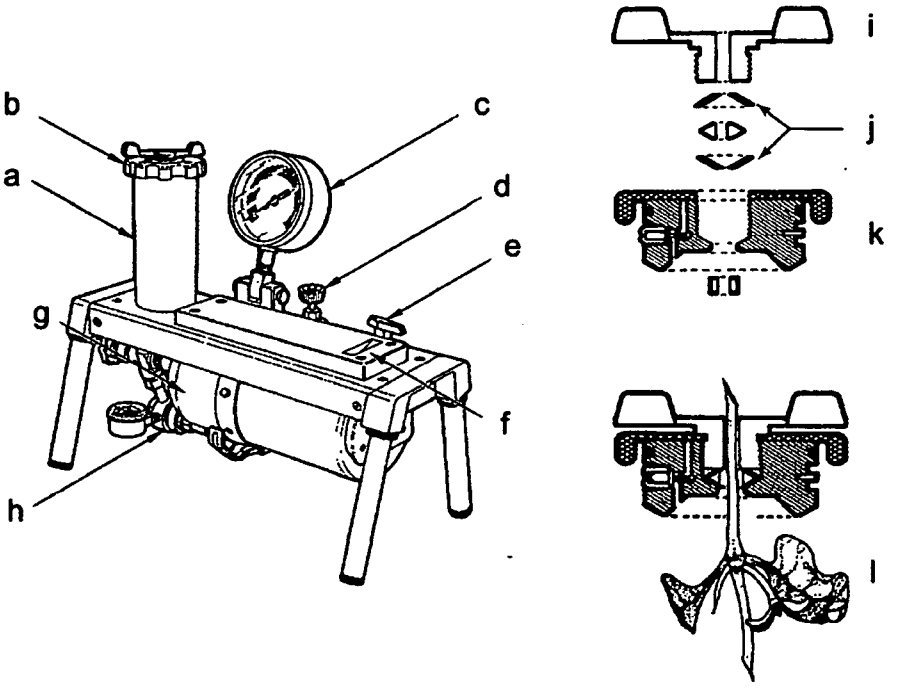

Fig. 4: Pressure bomb to measure plant water potential. Left: Overview with stainless steel measuring chamber (a) with the specimen holder on top (b), the pressure gauge (c), the metering valve (d), the three-way control valve (e), a preparation board (f), and a (g) pressurized air canister (the model used in this study featured a stand alone air canister instead of a strapped on canister), connected to the setup through a reduction valve (h). Right: Close-up of the chamber top with (i) sealing knob, (j) support washer, (k) specimen holder, (l) specimen (from Plant Water Console Series 3000 Operating Instructions, altered).

The pressure bomb (Fig.4) basically consists of a stainless steel cylinder, capable of withstanding high pressures (up to 10 MPa). This vessel is mounted on a sturdy four-legged console and is connected to a pressurized air container. The pressurized air passes a pressure-reducing valve, a metering valve and a pressure gauge before the measuring chamber. The metering valve controls the speed at which the pressure is built, and the gauge enables the exact determination of the actual pressure inside the cylinder. An additional three-way control valve is installed into the circuit to direct the flow into or out of the chamber and to seal the gas within the pressurized measuring chamber. The top of the cylinder is a detachable specimen holder with a bayonet breech for gas-tight connection to the chamber and a sealing knob located in its center. The sealing knob actuates the collet-type closure that pressure seals the specimen and sealing sleeves during the run. For operating safety a fail-save valve is located inside the bayonet breech, which prevents pressurization of the chamber when the specimen holder is not locked properly into position.

Pressure is applied until the sap returns to the cut surface of the xylem (endpoint), which gives a measure of the hydrostatic pressure in the xylem. The positive pressure applied can be read on the gauge, and is equal to the negative hydrostatic pressure in the xylem of the twig.

Healthy, unscathed 5-10 cm long twigs were cut the spruce trees and the periderm around the cut end of the twig was removed to ensure a tight sealing and to reduce the amount of resin on the cut end. Freshly cut twigs were put into the chamber as fast as possible to minimize water loss. After the specimen holder was locked tightly onto the chamber, slow pressurization was initiated until the endpoint was reached and water appeared on the cut surface.

2.3.6 Measurement of bark emissions of ethanol and volatile terpenoids

Bark emission rates of ethanol and volatile terpenoids were determined from the potted trees during stress experiments between 11:30 and 15:00 on two to four consecutive days, and from cut logs during desiccation (Chapters 2.4.4 and 2.4.5). These measurements were made by Dr. Peter Baier of the Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), Dept. of Forest and Soil Science, BOKU, Vienna.

2.3.6.1 Sampling procedures

A 16 x 4.5 x 3.2 cm plastic chamber with three boreholes (diameter Ø = 1.5 cm; two for air-inlets, one for outlet) was sealed gas-tight to the stem with solvent-free glue. To clean the ambient air before the inflow, two glass cartridges containing activated charcoal were fitted into the chamber inlets with silicone stoppers. Samples of volatile compounds were taken with a mobile vacuum pump over 19 min. sampling a total volume of ca 10 l. One glass sampling cartridge was connected to the vacuum pump with a hose, and to the outlet of the sampling chamber with a silicone stopper. The glass sampling cartridge (type B/G, Dräger AG, Lübeck, Germany) consisted of two separate charcoal layers: a control layer (300 g) and an absorption layer (750 mg). The loaded charcoal filters were sealed with polyethylene-stoppers and stored at 4°C. In the laboratory control and adsorption layers of the sampling cartridge were eluted separately with n-hexane. The eluted substances of the adsorption layer (sample volume: 0.5 ml) were analyzed in a gas chromatographer.

From cut spruce logs, wood samples (Ø = 3 cm, l = 1 cm) were taken. The samples were separated along the cambium into phloem and wood, frozen in liquid nitrogen and ground in a mortar. Monoterpenes and ethanol were extracted from 2.5 g using a closed loop stripping apparatus (CLSA) and analyzed with gas chromatography.

Air temperature and relative humidity were recorded during the measurements using a TINYTALK datalogger (Gemini Ltd., Chichester, UK). Sampling of moist logs was difficult,

since the water-based glue did not harden enough to ensure gas-tight contact between chamber and log. Moist logs thus had to be taken out of the climate chamber (rH = 75%), for two days to assure proper hardening of the glue.

2.3.6.2 Gas chromatography

All samples (sample volume: 1 - 1.5 µl for extract samples, 4 µl for emission samples) were inserted into the system through on-column injection (retention gap: apolar; methyldesactivated; length: 2.5 m; ID: 0.32 mm).

Column: Chrompack CP-Chirasil-Dex CB (Varian, Palo Alto, CA, USA), (length: 25 m, ID: 0.25 mm, film thickness: 0.25 µm; stationary phase: E-Cyclodextrin).

Operational temperature program: 50°C →4°C/min. → 70°C/2.5 min. const. / 10°C/min. → 110°C/15 min. const.

A FID-detector (detector temperature: 220°C) was used for determination of retention time. Identification of the peaks was accomplished by comparison of mass spectra with reference mass spectra and by comparison of the GC-retention time with pure reference compounds. For quantitative analysis an internal-standard method was applied with n-Nonane as internal standard (Baier, 1996).

2.3.7 Testing stem oxygen measurements

2.3.7.1 Sensor installation, tests of functionality and troubleshooting

Early experiments showed that the fragile sensor fiber was easily damaged or contaminated with resin components when the syringe was inserted directly into the tree stem. Therefore, different setups were tested that allowed the measurement of oxygen inside the stem without gas leaks and without damaging the sensor.

2.3.7.1.1 Steel jacket setup

The first setup used was an adaptation of an existing system developed previously (del Hierro *et al.*, 2002), (Fig. 5 and 7). Instead of the original home-made system consisting of several metal parts and rubber or silicone gaskets, a simplified version with three metal parts and only one seal was custom-made in larger numbers.

A galvanized steel anchor (length l = 50 mm) with a 25 mm long thread (diameter Ø = 5 - 7 mm) was screwed into a drill hole of desired depth (Ø = 6 mm). The thread attached the setup firmly to the stem and should seal the hole against ambient air. Additional sealing was achieved by hot-melt adhesive (Pattex®, Henkel, Heidelberg, Germany) that was injected between bark and anchor. The sensor needle (Ø = 1 mm) was inserted into a second thick-walled hollow needle (Ø = 2 mm) with a small ventilation hole at the side of the tip. A rubber

or silicone gasket, a brass washer and a brass union nut were tightened around the hollow second needle to prevent oxygen entering. The gasket was punctuated with a heated needle shortly before the setup was assembled. The hollow needle was then slid through the anchor jacket into the drill hole. A union nut with a second punctuated rubber or silicone gasket and a washer was tightened around the jacket.

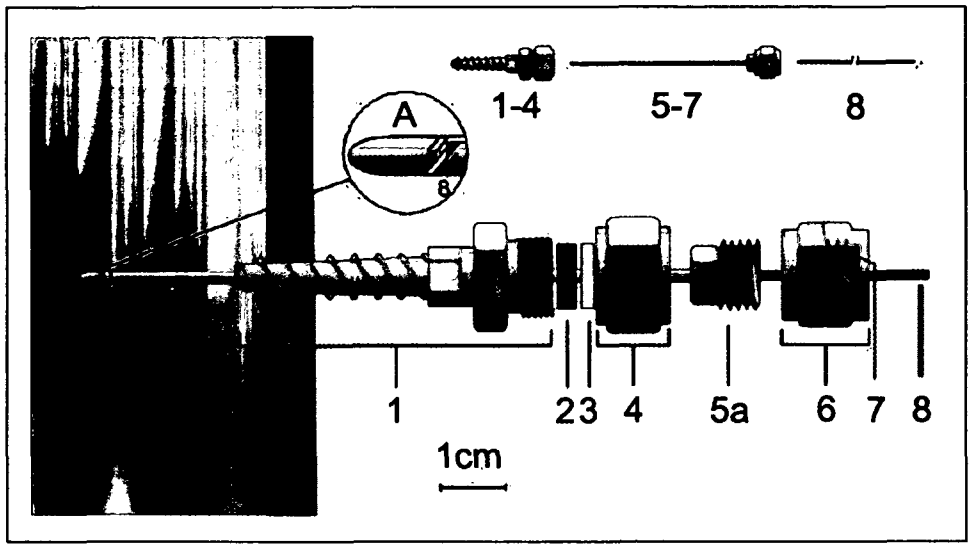

Fig. 5: Steel jacket setup used for safe insertion of the oxygen sensor into a stem. A galvanized steel anchor (1) was driven into a borehole of desired length. A rubber or silicone gasket (2), a brass washer (3), and a brass union nut (4) prevented gas leakage. A needle (5) with ventilation holes at the side of the tip (A) housed the oxygen sensor (8). The needle was sealed with a union nut (6), a rubber or silicone gasket and a brass washer (7). Potential tiny gaps between 4 and 5a and on the surfaces of 4 and 6 were additionally sealed off with Therostat® polymer sealant and silicone grease. The transition between anchor and stem was sealed off with hot-melt adhesive (del Hierro *et al.*, 2002).

The anchor jacket was closed with a rubber or silicone septum and a union nut after extraction of the sensor needle, so oxygen from the ambient atmosphere did not diffuse into the drilling hole between measurements. The greatest disadvantage of the setup was its complexity. When the sensor needle was inserted through the silicone septum small amounts of oxygen could leak. Also the headspace in the bore hole through which oxygen diffused in and out of wood was rather small in comparison to the dead space of the setup. Thus, it took ca. 20 min. to reach equilibrium in the measurement space. The many parts joined together provided several potential leaks and even when sealed with Therostat®, silicone grease or hot-melt adhesive, leak tests (Chapter 2.3.7.2) showed that the setup was not always gas-tight. Besides, the fixed length of the anchor allowed an insertion to a maximum depth of 6 cm only.

2.3.7.1.2 Metal tube setup

In 2004 a simpler method was developed that allowed a save insertion of micro sensors into the stem and turned out to be more reliable. Thick walled metal tubes, preferably of a non-oxidizing material (Ø = 8 - 10 mm) were cut into desired length. Iron tubes were additionally coated with adhesive grease to avoid oxidation. The tubes were tapered at the end driven into the tree to ensure entering without abrading wood that would clog the hole. Tubes were hammered into the hole made either with an increment corer, which had the advantage that cores gained could be studied, or an electric drill (Fig.6).

For measurements in beech in 2005 and 2006 (Fig. 8, Chapter 3.4) a second, smaller metal tube (Ø = 5 mm) was inserted into the outer tube to reduce the overall gas space. The bark around the protruding tubes was removed and sealed off with hot-melt adhesive. The outer ends of the tubes were sealed with 3 mm thick silicone septa attached with superglue and 2-component epoxy resin. The septum was punctuated with a hot needle to allow inserting the sensor needle without damaging the sensor and the needle was sealed with Therostat® and silicone. Still, the sensor, not protected by a second needle, was more exposed than in the above setup and a few sensors were damaged when penetrating the septum. This setup allowed to measure at almost any depth >1 cm in the stem without loosing either stability or gas-tightness. The head space in contact with wood was larger, and equilibrium usually established within 10 min. and within 5 min. when a second small tube was inserted to reduce the overall gas space. To be able to measure wood oxygen concentration at a defined depth requires that gas does not diffuse faster along the metal tube then within wood, which would happen if inserting the tube produced significant cracks within the wood (Chapter 3.3.1).

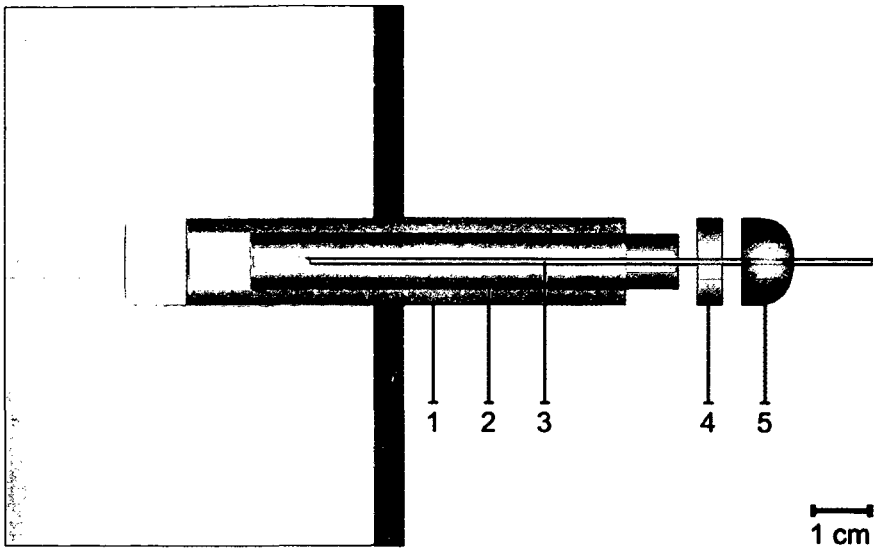

Fig. 6: Metal tube setup as used for oxygen measurements in spruce and beech. A metal tube was inserted into a bore hole (1). The innermost part of the hole (ca. 1 cm) was left uncovered to expand the actual gas exchange space between tissue and measuring chamber. A second tube was inserted into the first one to reduce the overall gas headspace in the measuring chamber (2). The syringe housing the oxygen sensor (3) was inserted through a silicone septum (4). The setup was additionally sealed with Therostat® (5) and two-component adhesive.

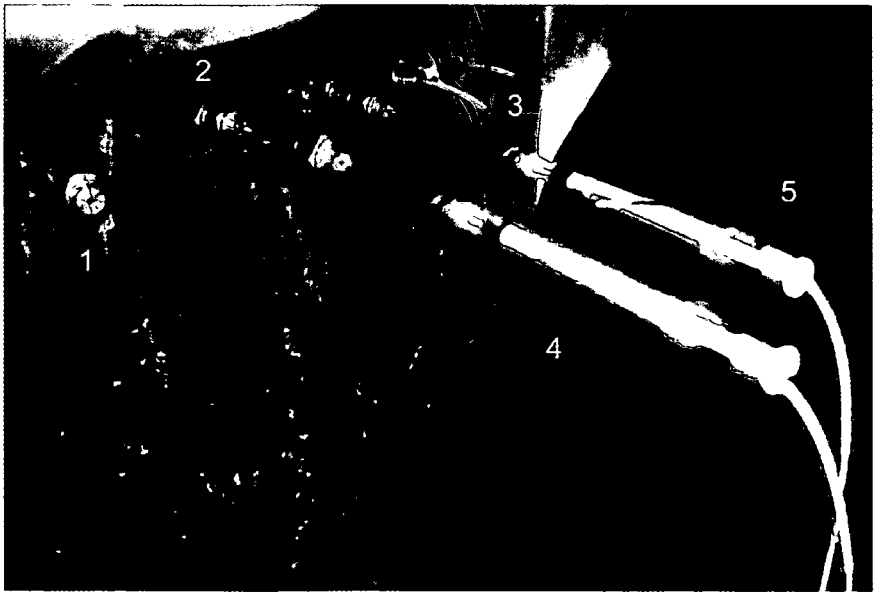

Fig. 7: Oxygen measurements in the stem of a mature spruce in 2003: Anchor jackets (1) screwed into the stem. Oxygen sensors (4 and 5) were inserted through metal jackets (2). Resin was drained through plastic tubes (3). Sensors were inserted into the sapwood at a depth (d) of 2.5 cm (4) and in the heartwood (d = 6 cm) (5). The periderm area around the setup was additionally sealed with silicone grease.

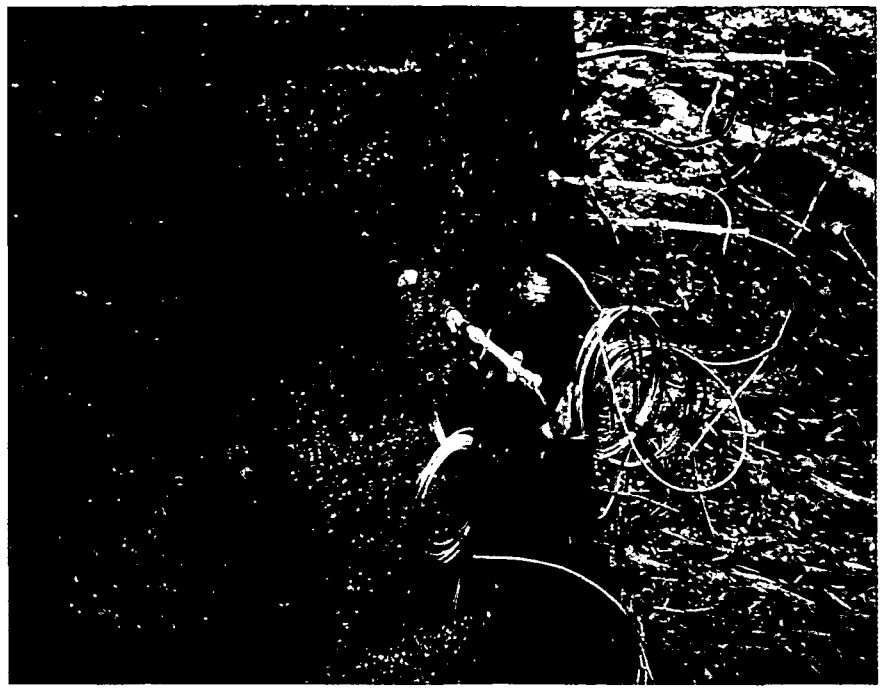

Fig. 8: Oxygen measurements in beech Nr.16 in 2006. Oxygen was measured in four depths (d = 2.5, 5, 10, 20 cm). All sensors were inserted through silicone septa glued on stainless steel tubes. The sensors were connected to TX3 units controlled by a datalogger (not in picture)

2.3.7.2 Leak testing

Strong leaks resulted in an oxygen concentration constantly around ambient and were easily detected. In the case of minor leaks the oxygen concentration at the sensor will be somewhere between ambient and the unknown concentration in wood and can only be detected by strongly changing the external oxygen concentration. When the area around the setup housing the sensor was flushed with pure oxygen or nitrogen from a pressurized container, leaks showed in slow increases or decreases, respectively, of oxygen measured in the stem (Fig.9 and 10). Usually, additional sealing with Therostat® or hot-melt adhesive was necessary to close the leaks. For leak tests, plastic bags or bottles wrapped around the setup and adjacent parts of the stem and flushed with gas from a pressurized container with a flow of 5 to 10 l / min. A second sensor measured the oxygen concentration inside this wrapping. The plastic wrapping could be constructed to either include or leave out certain parts of the setup in order to isolate leaks. Generally the anchor jacket setup was found to be less reliable than the metal tube setup.

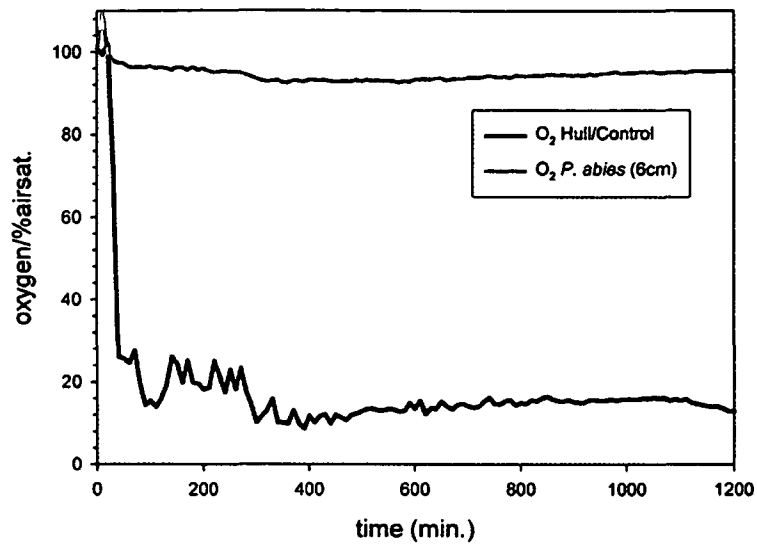

Fig. 9: Example for a leak test with negative outcome. The hull, flushed with N_2, does not need to be completely air-tight (O_2 concentrations did not decline to 0%). Oxygen remained at its high level inside the stem which indicated the gas tightness of the setup. The test was conducted during measurements with potted spruce in 2003 (Chapter 2.4.4). A steel jacket setup was used to insert the sensor into the stem (d = 6 cm)

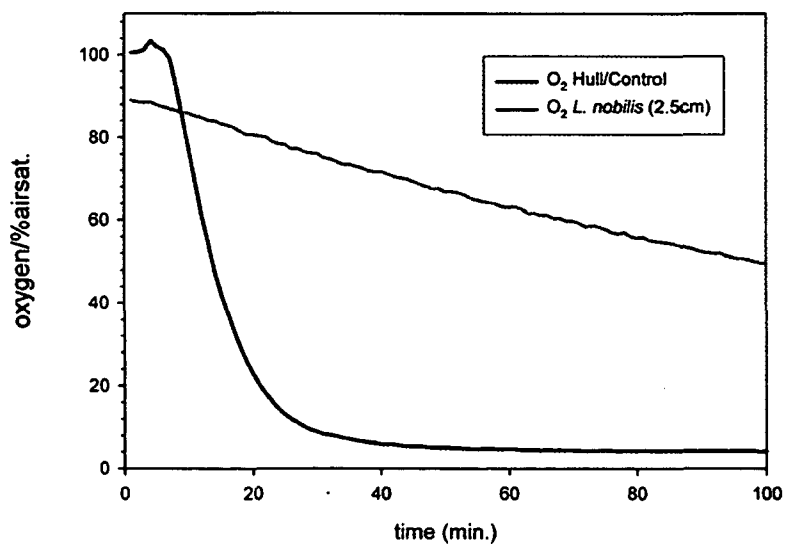

Fig. 10: Example for a leak test with positive outcome. Oxygen values declined rapidly after the setup was flooded, because nitrogen diffused through a leak into the measuring chamber and displaced the oxygen. The test was conducted with *L. nobilis* in the greenhouse in 2004. The sensor was inserted into the stem through an aluminum tube (d = 2.5 cm).

Several additional leakage tests were conducted during measurements with potted spruce (Chapter 2.4.4) because oxygen values often seemed erratic. Tests conducted in spruce Nr. 2, 3 and 5 in June and from August to September 2004 are shown in Fig.11.

After insertion into spruce Nr. 2 (anchor jacket) at a depth (d) of 5 cm, oxygen dropped to 77% and increased up to 93% where it remained longer. The surroundings of the sensor were flooded with N_2 for several hours and the setup was found gas-tight. The sensor was extracted on and inserted into spruce Nr. 3 (anchor jacket, d = 5 cm) and oxygen dropped instantly to 5%. The sensor was extracted, checked for functionality and showed 100% in the ambient air. After reinsertion values dropped again to 5% and remained low overnight. No diurnal variations in stem oxygen were noticed. The sensor was exchanged. The replacement featured no sealed-off plunger (Chapter 2.3.7.4) and elevated levels of oxygen (between 90% and 96%) and only very faint diurnal variations antipodal to air and stem temperature were measured. The elevated oxygen levels were obviously caused by a leaking sensor. The sensor was extracted and the borehole was made deeper by 1.5 cm with a small drill. After re-insertion of the non-sealed sensor oxygen dropped to 88% with faint diurnal variations antipodal to air and stem temperature.

After insertion into spruce Nr.5 (anchor jacket, d = 5 cm) oxygen decreased to 2% and remained low. To investigate the low values the surroundings of the sensor were flooded alternately with N_2 and pure oxygen from pressurized containers (Fig.11). A second sensor was installed in the hull to monitor the change of oxygen in the area around the sensor. Although the hull was flooded for several hours, none of the gases had any effect on the low stem oxygen level. No diurnal variations were noticed in spruce Nr.5. Tests were also conducted in other specimen and the area around the sensor (which was gas-tight) was either flooded with pure O_2 when stem oxygen was low, or with N_2 when stem oxygen was high. No effect of bark flooding was found during the study.

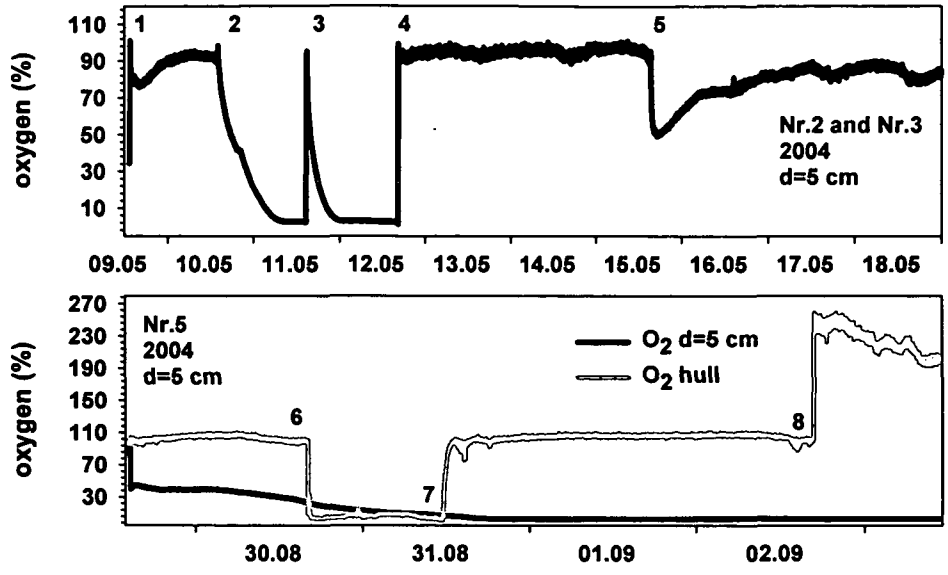

Fig. 11: Leakage tests in spruces Nr. 2, 3 and 5 conducted in June 2004 and from August to September 2004. Upper part: Insertion into spruce Nr. 2 (anchor jacket setup, d = 5 cm) followed by a leakage test with N_2 (1) that proved the setup was gas-tight. The sensor was extracted and put into spruce Nr.3 (2). The sensor was extracted, checked and reinserted (3). The sensor was extracted and exchanged for a model without a sealed off plunger (4). The sensor was extracted and the bore hole was extended (+ 1.5 cm) with a small drill. (5). Lower part: Sensor was inserted in spruce Nr.5 (anchor jacket, d = 5 cm). A second sensor was installed in the hull around the sensor to monitor leakage testing: The setup was flooded with N_2 (6 - 7), and with pure O_2 (8).

2.3.7.3 Effect of volatile resin compounds on oxygen measurements

Measurements in spruce with abundant resin in the sapwood were a problem for the measurements form the beginning on. After installing either anchor jackets or metal tubes into the fresh boreholes, the holes filled with liquid resin. This made measurements impossible and direct contact with resin damaged the sensor. Therefore, to drain the resin from the drill hole, small plastic tubes (Ø = 2 mm) were inserted into the setup (Fig.7) and exchanged with new tubes until resin flow ceased, which was usually after 7 to 10 days. According to the oxygen sensor manual, the TX-sensor is not affected by CO_2, H_2S, NH_3, and aggressive ion species such as S^{2-}, SO_4^{2-} or Cl^-, but the luminophore is sensitive to certain organic solvents (e. g. acetone, chloroform, methylene chloride), which swell the sensor matrix (Chapter 2.3.1). The effect of volatile organic substances present in conifer resin had not been tested. To investigate to what extent the presence of resin could interfere with fluorescence quenching the sensor was exposed over fresh, fluid resin harvested from potted spruces in a 100 ml Erlenmeyer flask. The entire bottom of the flask was filled with resin (Fig.12).

In two days the oxygen decreased from 100 to 52%. When the sensor was extracted, the response returned to 100% within 2 minutes, showing that the sensor was not damaged. The

results of that experiment implied that resin does interfere with the oxygen measurements, and values obtained from long-term measurements in species carrying resin (e.g. spruces) have to be discussed carefully. Several observations such as the decline of oxygen after insertion into trees Nr. 4 and 5 after insertion, and the long periods of 0% O_2 measured in several spruces were probably caused by such interferences.

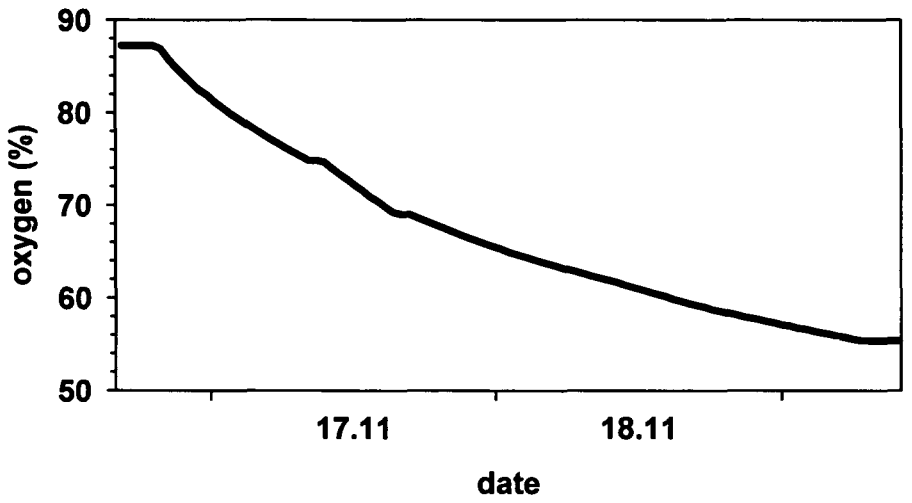

Fig. 12: Headspace oxygen concentration in an Erlenmeyer's containing fluid resin harvested from potted spruce trees. The oxygen signal was very noisy (probably due to electronic noise in the laboratory) so a running average was calculated.

2.3.7.4 Gas leaks in sensors without sealed-off plungers

Early sensor models were not completely gas-tight. During oxygen measurements oxygen entered through tiny spaces between syringe and plunger and diffused into the measuring chamber where it biased the measurements. Unfortunately this effect was first noticed during laboratory measurements in winter 2003 because in early leakage tests the sensor itself was not included and presumed gas-tight. From 2004 on new sensors with factory-made sealed off syringes were used. Older sensors were sealed of with a mixture of hot paraffin and beeswax that was inserted into the space between syringe and attached needle (Fig.13). This modification also reduced the dead gas-space within the sensor. Although 2003 measurements could have been influenced to some extent, a comparison of data from 2003 with 2004 obtained from a mature spruce in the arboretum (Chapter 2.4.1) showed that oxygen levels were in a similar range with both sensor types.

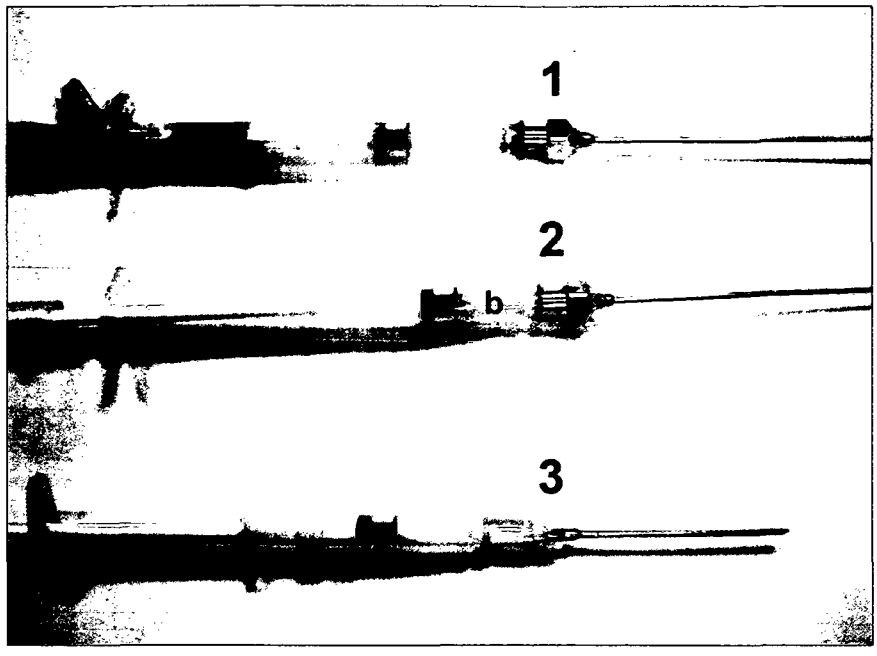

Fig. 13: Different types of oxygen sensors used in this thesis. Early sensors (1) had longer needles and were not additionally sealed. During early measurements oxygen entered the syringe through the plunger and diffused into the measuring chamber. Such sensors were modified (2) by injecting a mixture of hot beeswax and paraffin (b) into the syringe to achieve gas-tightness. Later purchased models (3) had shorter needles and were factory-made gas gas-tight.

2.3.8 Measurements of oxygen in spruce stems

Initial measurements were designed to investigate basic features of oxygen transport and distribution in tree stems and the variation of oxygen concentrations under non-manipulated conditions, i.e. without inflicting major stress. Oxygen concentrations in the stem of a mature spruce were measured in 2003 and 2004 in combination with sapflow and microclimate under natural conditions in the BOKU arboretum in Vienna (Chapter 2.4.1). Fluctuations during day-courses were monitored in the sapwood and the heartwood and from the stem base to the canopy to elucidate the pathways of oxygen. Similar experiments were also conducted with two bark beetle infested spruce trees in 2004 in Bad Vöslau, Lower Austria (Chapter 2.4.2). Problems with volatile resin compounds that biased the measurements and with the measuring setup made measurements difficult and early results erroneous (Chapter 2.3.7.3).

Experiments with 19 potted spruce saplings were conducted from 2003 to 2005 to test if, how much and how flooding and drought affects stem oxygen concentrations (Chapter 2.4.4). After measuring trees without manipulation, several randomly selected trees were either flooded or desiccated and compared with control trees. Stem oxygen was measured both continuously, and in weekly intervals with accompanying measurements of sapflow and micro climate. Experiments with *Laurus nobilis* show that flooding decreases stem oxygen

concentrations within a few hours (del Hierro *et al.*, 2002), thus a strong effect in spruce within one or a few days was expected. During three prolonged periods of applied water stress ethanol content and volatile emissions were measured by co-worker Peter Baier of the Institute of Forest Entomology, Forest Pathology and Forest Protection, Department of Forest and Soil Sciences, BOKU (Chapter 2.4.4).

Cut logs of mature spruces (length l = 50 cm, diameter Ø = 20 - 25 cm) were divided into tow groups and either stored in a climate chamber at constant temperature and moisture (T = 25°C, rH = 100%) or bench dried in the laboratory (Chapter 2.4.5). Exposure to rain results in increased ethanol concentrations in cut Douglas fir logs in the field, and to an increased attack rate of ambrosia beetles (Kelsey & Joseph, 1999a). During desiccation the dynamics of the relationship between oxygen decrease and the emissions of ethanol and other volatiles in the wood when no oxygen is transported via the sapflow was investigated. Wood oxygen concentration and wood moisture were measured in two day intervals for a month and Ethanol content and volatile emissions were measured after oxygen started to decline in the wet logs and on another occasion afterwards. Oxygen was also measured in weekly intervals from April to September 2005, in 4 and 6 cm depth, in three cut spruce logs used as bark beetle traps in Wolfsgraben, Lower Austria.

To investigate the influence of pathogens on stem oxygen content, eleven mature spruce trees, five with and six without heart rot, were measured in weekly intervals in the sapwood (depth d = 5 cm) and the heartwood (d = 13 - 20 cm) in a forest stand in Wolfsgraben, Lower Austria from April to September 2005 (Chapter 2.4.3).

2.3.8.1 Measurements in a mature spruce in the arboretum

Oxygen was measured in sapwood and heartwood of a mature spruce tree with a diameter at breast height (DBH) of 42 cm, in the arboretum of the University of Natural Resources and Applied Life Science (BOKU) between April and August 2003 and in September 2004. Oxygen was determined at 1.5 m, 7 m, and 12 m stem height and at d = 2.5 cm, 4 cm and 6 cm. Temperature sensors for compensation were inserted in 2.5 cm depth. Sapflow was recorded in 15 min. intervals using a P 4.1 (EMS, Brno, Czech Republic) with sensors installed at two opposing sides of the stem at 2 m. Air temperature and relative humidity were measured with a HMP45 sensor (Vaisala, Helsinki, Finland) in one minute intervals, means stored every 60 min. All oxygen meters and the HMP45 were connected to a CR10X datalogger (Campbell Scientific, Shepshed, UK).

2.3.8.2 Measurements in two bark beetle-infested spruce trees

In September 2004 oxygen was measured in two bark beetle infested spruces in Bad Vöslau (Lower Austria). One tree was >20 m high and >100 yrs old, the other tree about 50 years and 13 m high. Both had been heavily invested by bark beetles, probably in the dry year of 2003, and had shown massive die-back loosing most of their needles by the time of the experiment. Continuous long-term measurements were conducted in sapwood (d = 2.5 cm) and heartwood (d = 6 cm) at breast height (1.5 m) and the tree crown (10 m) of the smaller tree (Fig.17). Oxygen and stem temperature were measured and recorded in a 60s interval with a datalogger. For preliminary experiments the TX3 oxygen meter was directly linked to a notebook PC, oxygen and stem temperature values were measured and recorded in a one second interval.

2.3.8.3 Measurements in spruce trees with and without heart rot in a forest stand

Oxygen was measured in stems of eleven mature trees (DBH = 113 - 192 cm) in a forest stand in Wolfsgraben, Lower Austria. (48° 9′ 0′′ N, 16 ° 7′ 0′′E, 323 m a.s.l.). The experimental site was part of the Biosphere Reserve Wienerwald located on a south-eastward slope. Measurements were conducted between 25.05 and 12.10.2005. Oxygen was measured in the sapwood at a depth (d) of 5 cm and the heartwood (d = 13 - 20 cm). Cores were harvested before the experiments and trees were subdivided into two healthy individuals (n = 6) and individuals with heart rot in the core (n = 5). Sensors were inserted through iron tubes (diameter Ø = 8 cm) covered with adhesive grease to avoid oxidation processes.

2.3.8.4 Measurements in potted spruce saplings

Short- and Long-term measurements were conducted with 20 to 25-year-old trees (Fig.14). 19 trees (DBH = 4 – 10 cm, h = 3 – 5.5 m) growing at an experimental station near Tulln, Lower Austria, were excavated in November 2002 and cultivated in plastic pots (diameter Ø = 50 cm, height h = 50 cm) filled with a mixture of potting soil, TKS2 (pre-fertilized peat) and silica sand. The potted trees were located on an experimental plot located in the arboretum of the University of Natural Resources and Applied Life Science (BOKU), Vienna. During winter, pots were protected from frost by Styrofoam sheds filled with leaves. The trees were fertilized in April (30 g / pot) and September (60 g / pot) with mineral conifer fertilizer (NPK-fertilizer plus Mg (12 + 5 + 13 + 5) by Compo GmbH, Münster, Germany. Soil moisture was monitored periodically in the morning.

To investigate how stem oxygen was correlated to soil moisture, trees were drought-stressed (no irrigation), flooded (either by irrigating to the soil's water-holding capacity or by wrapping

the pots in plastic and completely water-logging the soil) or irrigated to a water content of 25%.

Sapflow was measured continuously during each vegetation period in a 10 min. interval at a stem height of ca. 20 cm. Oxygen was determined periodically in various depths (2003: d = 5 cm, 2004 and 2005: d = 2.5 cm) at a stem height of 10 - 15 cm. For the oxygen measurements anchor jackets were used in 2003 and in 2004. Aluminum tubes were used in 2005.

Water potential was measured in the early afternoon (around 14:00) when it tends to be minimal, and occasionally pre-dawn (maximum water potential). Air temperature and relative humidity were recorded continuously at 1.5 m height with a HMP35 or a HMP45 (Campbell, Shepshed, UK) sensor connected to a CR10X (2003 - 2004) or with a TINYTAG-logger (Gemini Data Loggers, Chichester, UK) (2005). In 2004 and 2005 emissions of ethanol and volatile terpenoids were measured during prolonged periods of desiccation or flooding (Chapter 2.4.4). From October 2004 to March 2005 two trees (Nr.14 and 15) were moved into the greenhouse where measurements of oxygen and sapflow were continued.

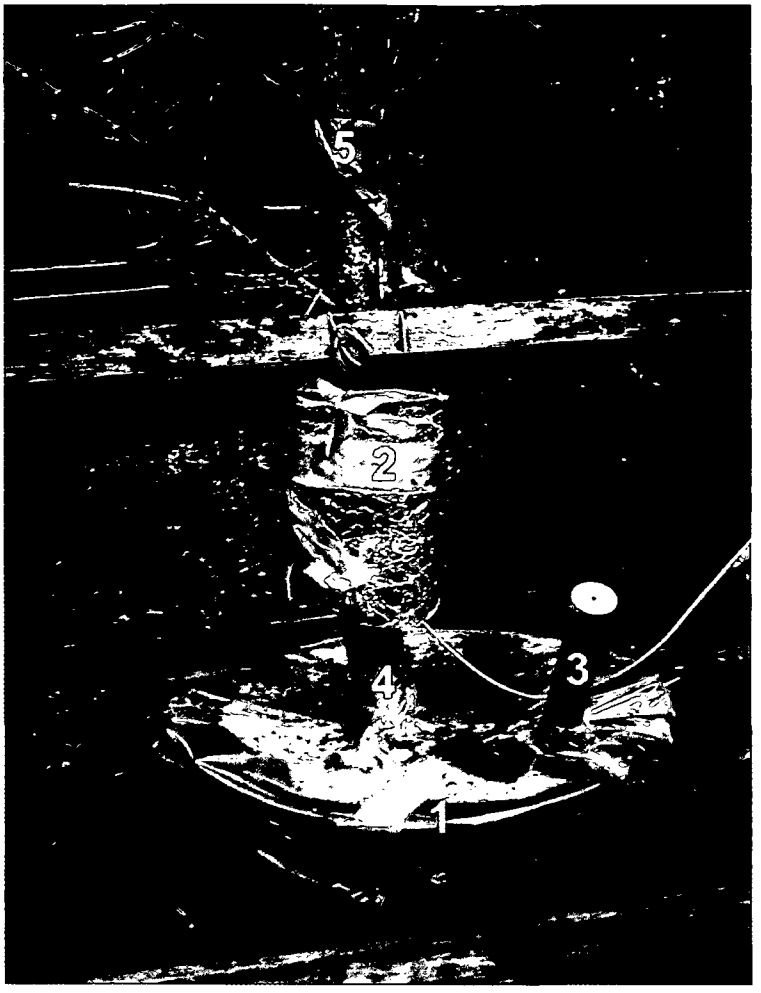

Fig. 14: Measurements with potted spruce in the arboretum. The plastic pot (1) was wrapped with foil during periods of flooding. Sapflow was monitored continuously with a pair of Granier sensors (2). Soil moisture was measured periodically through a TECAN tube (3). Oxygen was measured periodically or continuously at d = 5 cm (2003 and 2004), and at d = 2.5 cm in 2005 (4). Bark emissions were measured two times in 2004 and once in 2005 in a sealed box attached to the stem (5). Trees were secured with a wooden frame to prevent being blown over by wind. Styrofoam shielding was attached for isolation in winter (not in picture).

2.3.8.5 Measurements of oxygen and bark emissions in cut spruce logs

Oxygen and wood moisture were measured in a depth of 2.5 cm in 12 cut spruce logs in 2004. The logs (length l = 30 cm, diameter Ø = 20 - 25 cm) were cut from a freshly felled tree and divided equally into two groups. Six logs (wet storage) were stored in a climate chamber at constant temperature and moisture (T = 25°C, rH = 100%) and wrapped in wet tissue, the others were bench dried in the laboratory (dry storage). Between 05.08 and 14.09.2004 wood oxygen was measured in aluminum tubes at 2.5 cm depth and moisture with a Hydromette M4050 (Gann GmbH, Gerlingen, Germany). Bark emissions of EtOH and VTs were measured on 20.08 - 23.08 and 13.09 - 15.09.

2.4 Results

2.4.1 Oxygen in the sapwood and heartwood of a mature spruce

Long term measurements started on 23.05.2003 (Fig.15). Heartwood (depth d = 6 cm) oxygen at breast height fluctuated between 96% and 105%, maxima were reached between 10:00 and 11:00 and minima between 17:00 and 19:00. Sapwood (2.5 cm) oxygen at breast height was somewhat lower (92 - 98%) with maxima and minima a few hours earlier than heartwood (3:00 - 5:00 and 15:00 - 17:00, respectively). Sapwood oxygen decreased with stem height to 85 – 90% at 7 m and 69 – 81% at 12 m. At greater stem height, extremes in sapwood oxygen were recorded about 1 – 3 hours later than at breast height. Stem temperature ranged between 15°C and 30°C. Maximum sapflow was 12-14 kg h^{-1} in May and 14-20 kg h^{-1} in June. Sapflow maxima were recorded between 11:00 and 13:00.

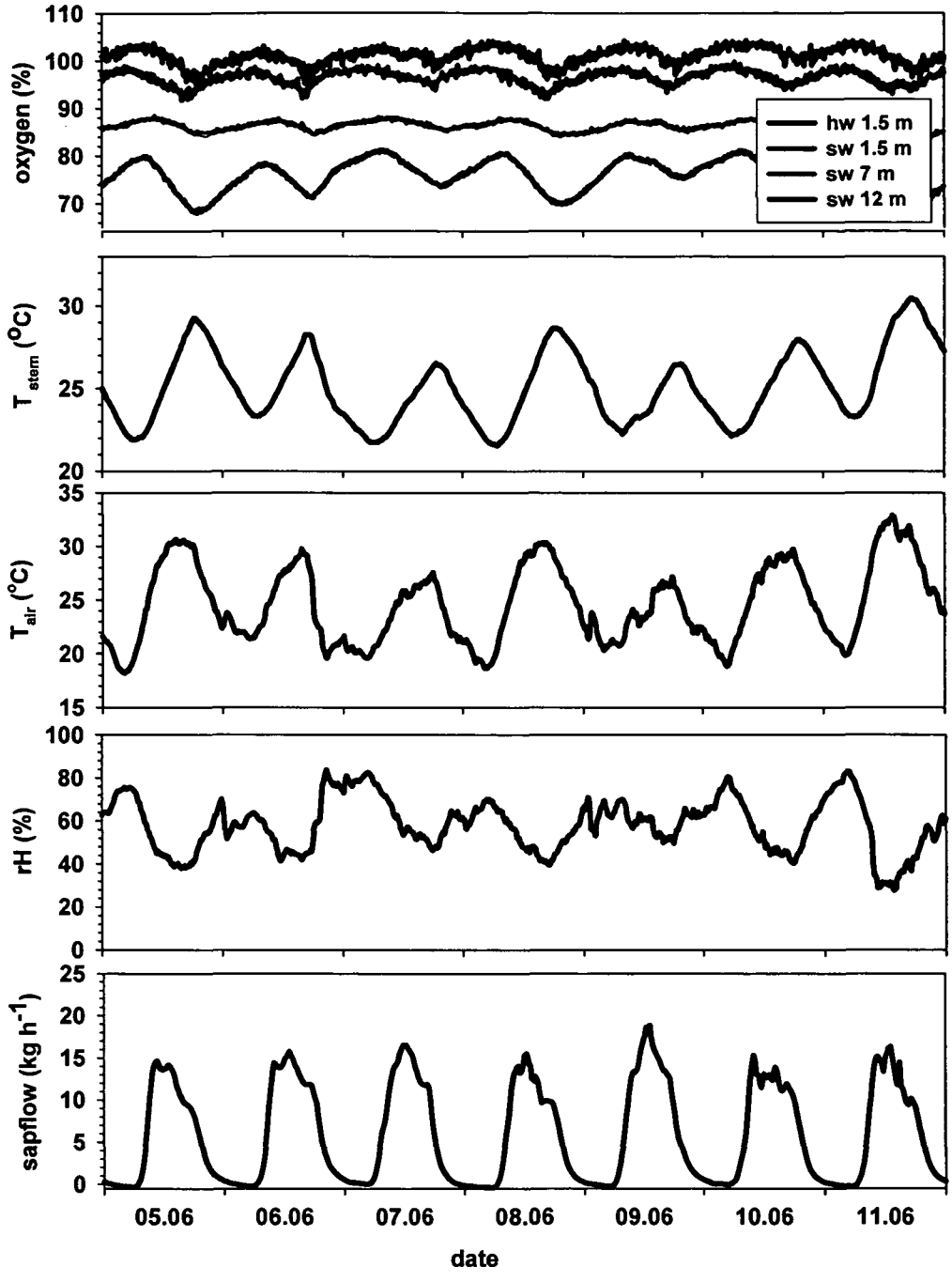

Fig. 15: Oxygen in the stem of a mature spruce tree. Hw 1.5 m = heartwood oxygen (d = 6 cm, h = 1.5 m), sp 1.5 m = sapwood oxygen (d = 2.5 cm, h = 1.5 m), sp 7m = sapwood oxygen (d = 2.5 cm, h = 7 m), sw 12m = sapwood oxygen (d = 2.5 cm, h = 12 m).

Oxygen measurements were continued in the same stem in September 2004 to test if an improved measuring setup with metal tubes and the use of sensors with shorter needles and sealed off plungers yield different values (Fig.16).

Between 09.11 and 15.11 oxygen was determined in a 60 s interval at breast height (sapwood: depth d = 2.5 cm, heartwood: d = 8 cm). A metal tube setup was used. All areas susceptible to gas-leaking were additionally sealed off with silicone grease and Therostat®. The periderm area around the tube was sealed off with heat-melt adhesive. Sapflow was not recorded. After inserting the sensors, oxygen values dropped instantly from 100% to 90% (heartwood) and 87% (sapwood). Maxima (heartwood: 101%, sapwood 98%) and minima (heartwood: 88%, sapwood 87%) were similar to measurements in spring 2003, but occurred somewhat later in the day (between 12:00 and 14:00 and between 22:00 and 01:00, respectively). Consequently, peaks were only a little earlier than the peaks in stem temperature. During the experiment the oxygen signals were noisy, probably due to imperfect shielding of the oxygen meters.

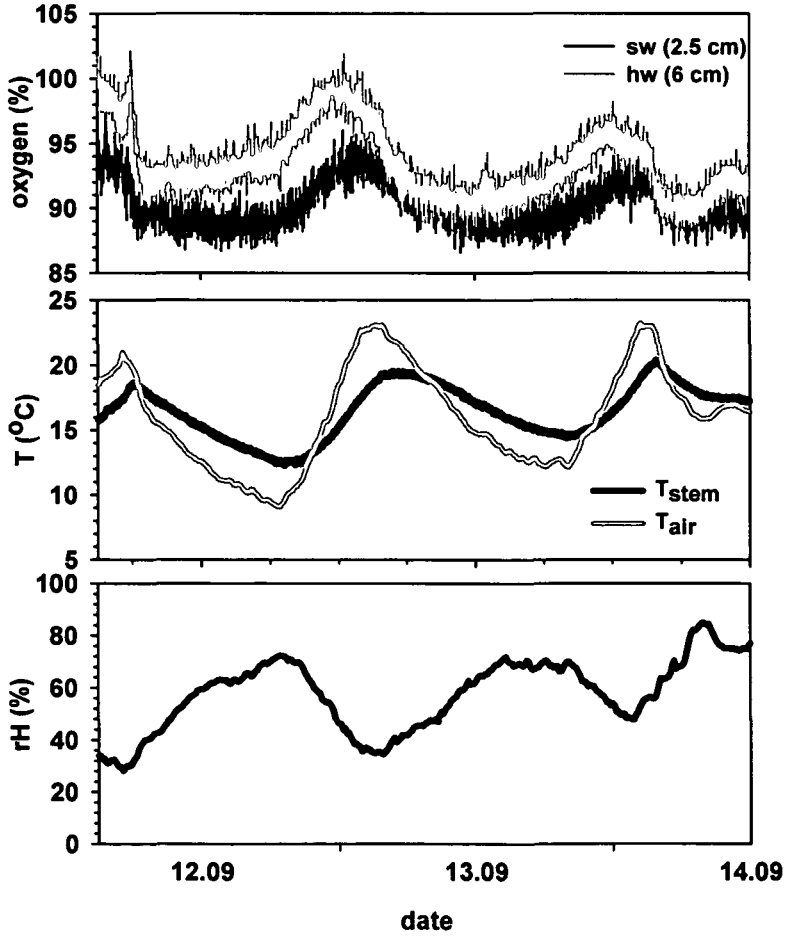

Fig. 16: Oxygen in sapwood (d = 2.5 cm) and heartwood (d = 8 cm) of a mature spruce from 11.09 to 15.09.2004.

2.4.2 Oxygen in stems of bark beetle-infested spruce trees

2.4.2.1 Short time measurements

Short time measurements: Following a sudden increase up to 120% after insertion in 2.5 cm, which was probably the result of mechanical stress, oxygen values decreased to 51% within two minutes. A second measurement conducted an hour later produced similar results: After the sudden increase to 120%, oxygen dropped down to 45% within five minutes. In the second (smaller) spruce oxygen dropped to 33% within four minutes in both sapwood (d = 2.5 cm) and heartwood (d = 6 cm).

2.4.2.2 Continuous long-term measurements

Continuous measurements were made in the smaller tree from 24.09 to 10.10 (Fig.17). Immediately after insertion the oxygen values started to drop in all three locations. Until 07.10 the oxygen values remained very low in all three locations and never exceeded 20%. While heartwood (height h = 1.5 m) and softwood (h = 1.5 m) showed no noticeable diurnal fluctuations, and a slow and steady decrease in oxygen, the concentration in the crown (depth d = 2.5 cm, h = 10m) fluctuated almost synchronous to the stem temperature between 0% and 6%. As values were extremely low, on 01.10 the sensors were extracted, checked for their response to air oxygen, re-calibrated and re-inserted. After reinsertion the sapwood oxygen in both breast height and in 10 m dropped rapidly to around 0%, but declined much slower in heartwood. Only small variations were noticed in any location. Oxygen in heartwood (h = 1.5 m) started to rise again on 07.10 and fluctuated between 70 and 110% until the 10.10. Oxygen in sapwood (h = 1.5 m, h = 10 m) remained low after their initial drop and never exceeded 5%. The slow decline and later increase in heartwood oxygen probably resulted from imperfect sealing of the metal tube.

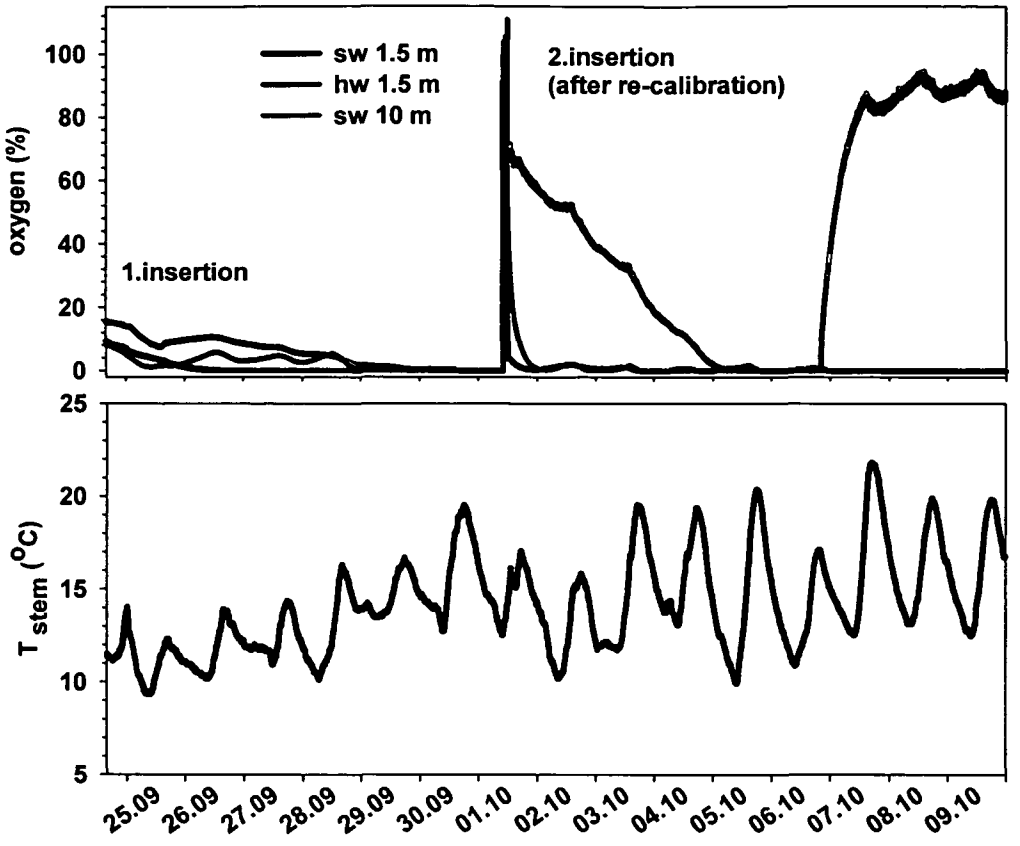

Fig. 17: Oxygen in a bark beetle infested spruce from 24.09 to 10.10. 2004. Oxygen was measured in the sapwood (sw, d = 2.5 cm) and the heartwood (hw, d = 6 cm) at h = 1.5 m and in the tree crown (d = 2.5 cm) at h = 10 m. On 01.10 all sensors were extracted, checked and re-inserted.

2.4.3 Oxygen in spruce trees with and without heart rot in a forest stand

Oxygen in a depth (d) of 5 cm ranged from 0 ± 0% (22.06) to 26.5 ± 27,6% (25.05) and in 13-20 cm from 2 ± 2.1% (15.06) to 29.4 ± 35.7% (15.07). Single measurements between trees and between measurement dates varied substantially, and oxygen content in healthy trees generally did not differ from those in trees affected by heart rot, nor did concentrations in the sapwood differ from the heartwood (Fig.18). Oxygen values were in a similar range compared to the measurements conducted with a similar setup in potted trees in the arboretum in 2005.

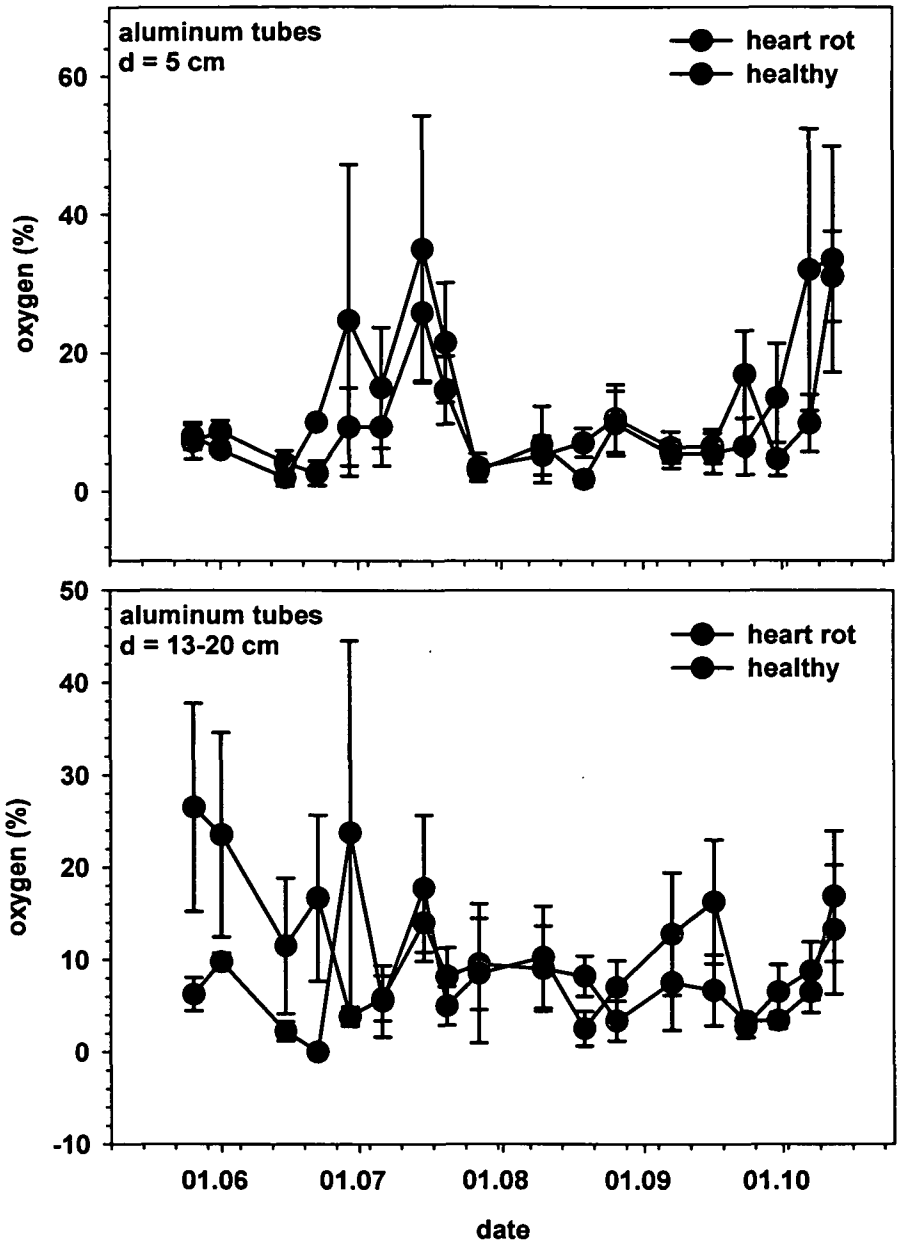

Fig. 18: Stem oxygen (d = 5 cm and d = 13-20 cm) in spruces with and without heart rot. Spruces were divided into groups of healthy specimen (n = 6) and specimen with heart rot (n = 5). Dots are group mean values, error bars are calculated from SE.

2.4.4 Oxygen, soil moisture and emission rates of potted spruce saplings

2.4.4.1 Periodical point measurements

Values obtained in 2003 (d = 5 cm) were relatively high in comparison to the results of 2004 and 2005 (Fig.19). Stem oxygen ranged between 42 and 97% and values scattered widely during the experiment. Values that obviously resulted from leakage (≥ 100%) were not included in the results. Soil moisture ranged between 9 and 48%. After their installation in September 2003 all anchor jackets remained inside the stem until 2006.

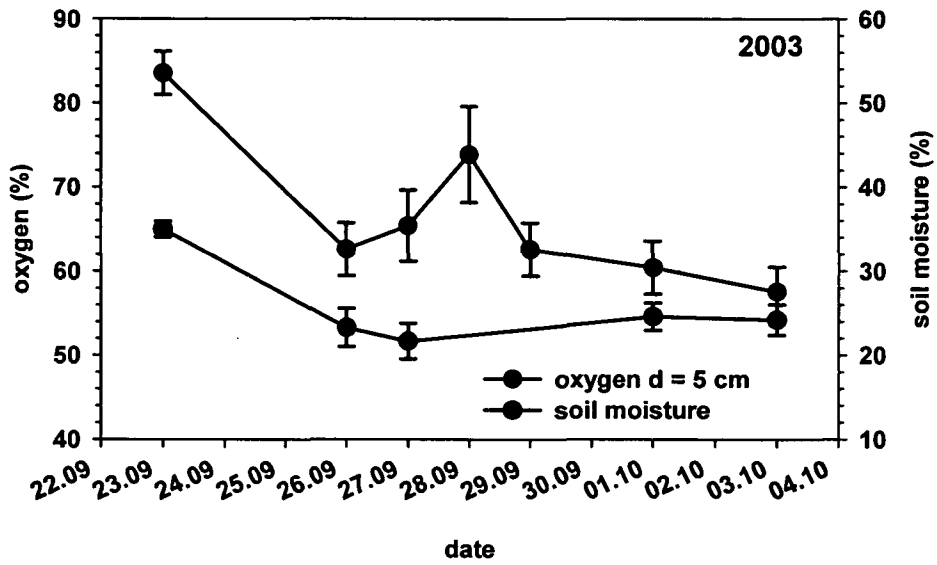

Fig. 19: Oxygen (d = 5 cm) in 19 potted spruces (2003). None of the specimens was exposed to water stress or desiccation during that period. The sensor was inserted through permanently installed anchor jackets into the stem. Dots are means (n = 19) ± SE.

In 2004 seven trees were non-stressed (C, trees Nr.: 1, 3, 4, 5, 8, 13 and 18) irrigated to a constant level of soil moisture level of 20 - 25%, six trees were drought-stressed (D, Nr.: 2, 7, 11, 12, 14, 16) to a soil moisture between 12 and 18%, and six trees were flooded (F, Nr.: 6, 9, 10, 15, 17, 19). In F soil moisture was held at water-holding capacity around 35% until the pots were completely wrapped in plastic foil, when soil moisture reached 89 - 100% (19.07 - 21.07). Afternoon water potential (mean ± standard deviation, SD) ranged between -0.65 ± 0.1 and -1.26 ± 0.3 MPa in the control and flooded trees, and was significantly lower (-0.76 ± 0.2 to -1.73 ± 0.3 MPa) during most of the experiment in drought stressed trees (Fig. 20).

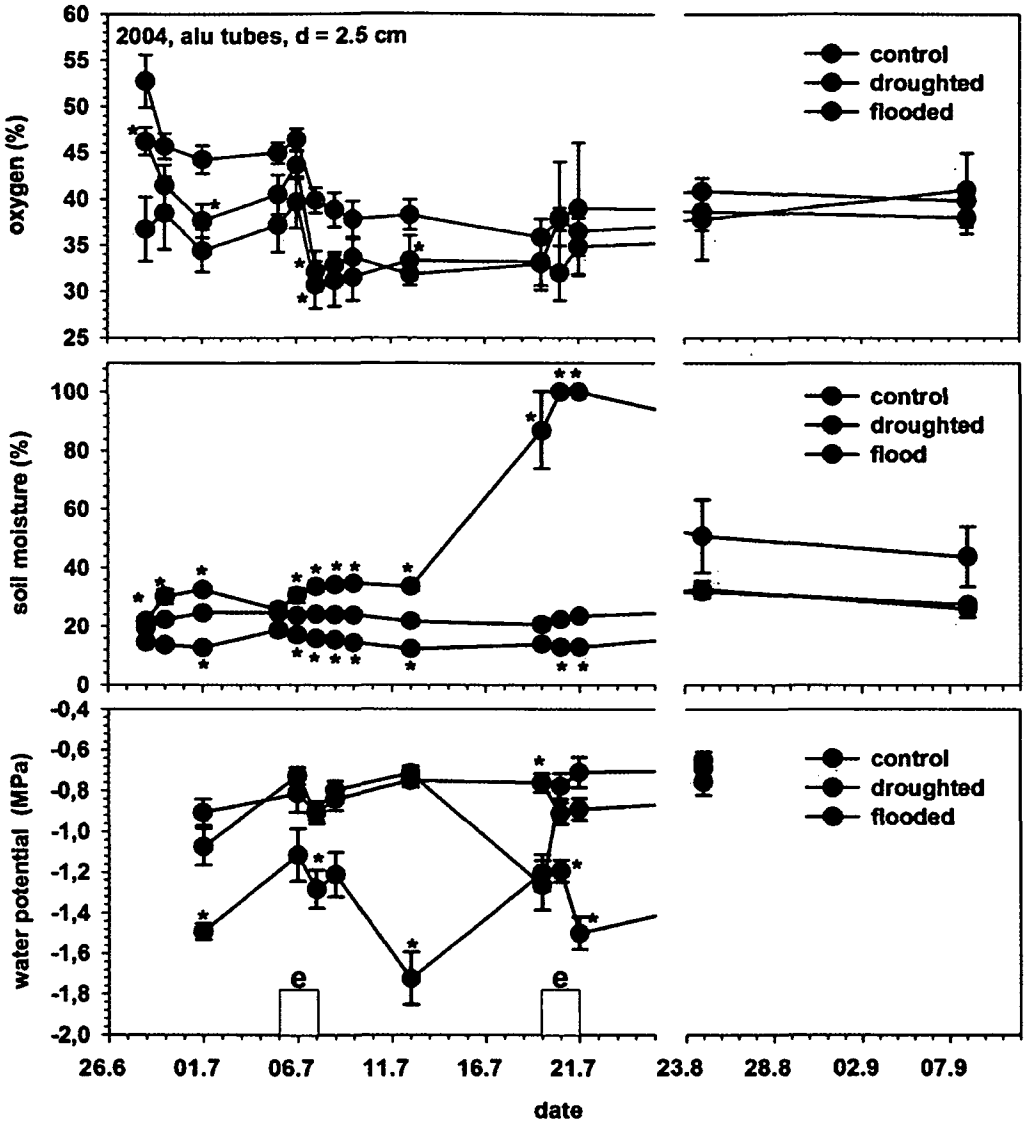

Fig. 20: Oxygen concentration in the stem of potted spruces subjected to water supply in 2004. C: control (soil water held at ca. 25% vol.), F: flooded, D: desiccated (to 12 – 18% vol.). The sensor was inserted in d = 5 cm through anchor jackets. Symbols are means (C: n = 7, F and D: n = 6, F: n = 6) ± SE. F was irrigated daily but soil moisture never exceeded 40% until the pots were completely wrapped in plastic foil (19.07 to 21.07). Dots marked with stars (*) showed a significant difference to C in a one-way ANOVA with Tamhane's T2 post hoc test (p<0.05). Grey bars with letters e mark emission sample dates.

Stem oxygen values were lower than those measured in 2003, probably due to more experience with the sensor equipment and better sealing of the anchor jackets (Fig.20). Measurements in 2003 were also conducted later in the season than in 2004. Mean ± SD ranged between 37.8 ± 3% and 52.8 ± 6% (C), 30.7 ± 6% and 41 ± 10% (D) and 31.8 ± 1% and 46.3 ± 4% (F). Generally, oxygen values scattered widely and no general correlation between oxygen and soil moisture or water potential was found. One-way ANOVA with a Tamhane's T2 post hoc test (0.95% significance) showed that oxygen in flooded and drought

stressed trees was significantly lower than in control trees on several dates (Fig. 20). The water potential of D was significantly lower on all dates except 06.07, 19.07 and 24.08. On 19.07 water potential dropped and was significantly lower than F. On several days soil moisture and water potential differed significantly between the groups, without having much effect on the oxygen content.

In 2005 trees were re-grouped (treatment C: no. 1, 3, 4, 5, 6, 8, 13, 18, 19; D: no. 7, 11, 12, 14, 15; F: no. 2, 9, 10, 17) to avoid that the treatment in 2004 affects the comparison between groups, and oxygen sensors were inserted through aluminum tubes into more peripheral areas of the stems (d = 2.5 cm). The oxygen values measured were still lower than 2004 (Fig. 21), ranging from 2.3 ± 2% to 10.8 ± 5% in control, from 0.5 ± 1% to 6 ± 4% in droughted and from 2 ± 3% to 9.5 ± 0% in flooded trees. A one-way ANOVA with Tamhane's T2 post hoc test gave no significant effect of treatment, although F had significantly higher soil moisture than C and D on 06.06 ($p<0.05$) and 12.06 ($p<0.01$).

To compare with 2004 in 2005 oxygen was also measured with the anchor jacket setup on 03.06 and 14.06 (d = 5 cm) (Fig. 23) Oxygen was noticeably when the anchor jacket setup was used with mean values between 39.8 ± 12% and 44.5 ± 15%, but measurements were also conducted in a greater depth.

Soil moisture (mean values ± standard deviation) ranged between 15.6 ± 2% and 25.1 ± 8% (C), 13.2 ± 4% and 20.1 ± 6% (D) and 15.2 ± 1 and 100 ± 0% (F). Water potential ranged between 0.73 ± 0.3 and 1.43 ± 0.3 MPa (C), 1.25 ± 0.5 and 1.69 ± 0.2 MPa (D) and 0.63 ± 0.2 and 1.56 ± 0.2 MPa (F).

Generally sapflow measurements in 2004 and 2005 showed that under strong drought stress sapflow rates were significantly reduced relative to control and flooded trees (Fig.22).

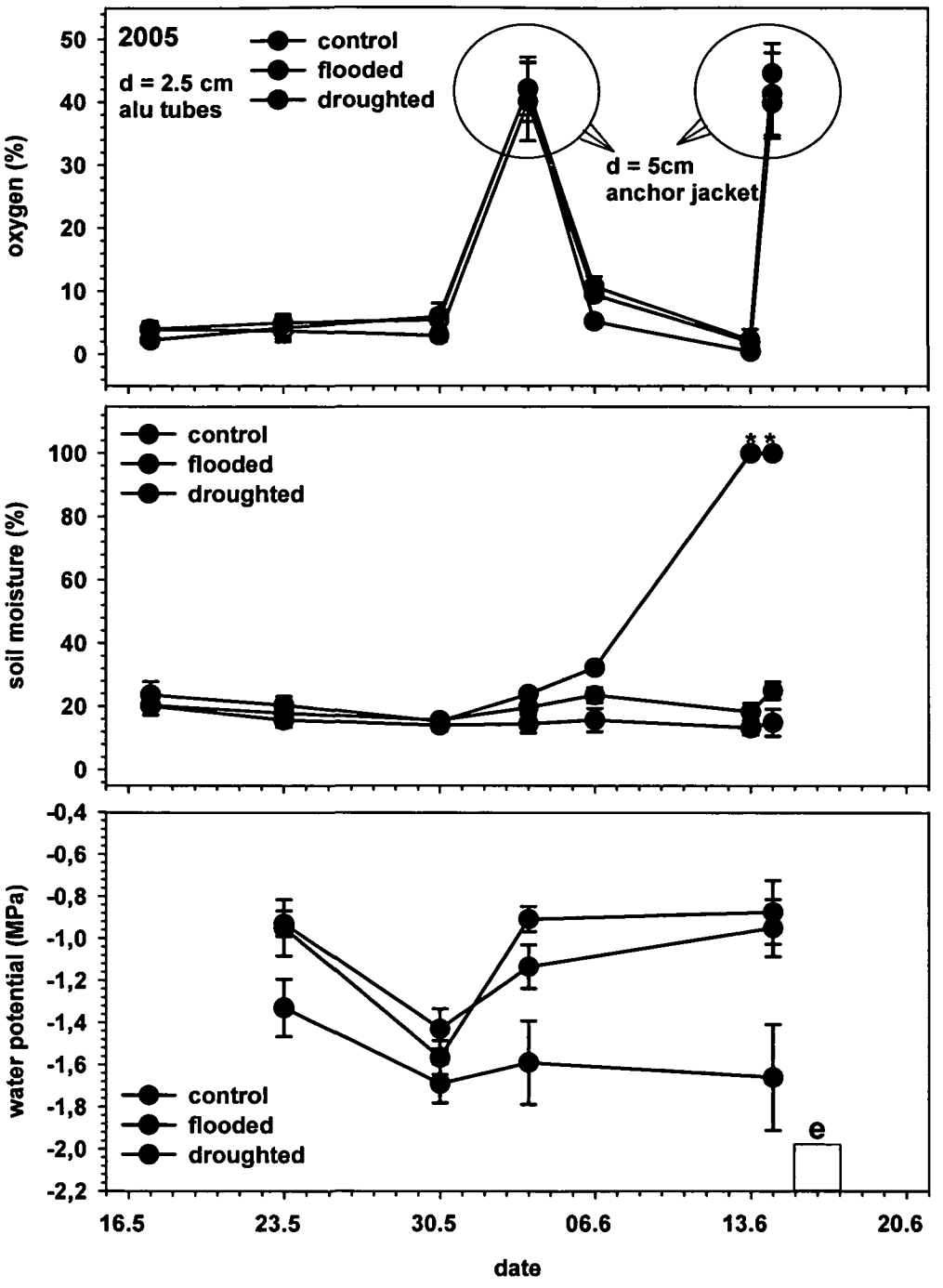

Fig. 21: Stem oxygen concentration in 2.5 and 5 cm depth in potted spruce trees subjected to different watering regimes in 2005. Each dot represents a mean (control: n = 9, flooded: n = 4, droughted: n = 6). Symbols are means ± SE. On 03.06 and 14.06 oxygen was measured with anchor jackets installed (d = 5cm, circle). Significant variations (ANOVA with Tamhane's T2 post hoc test, $p<0.05$) are marked (*). The grey bar and letter e mark the emission sample date.

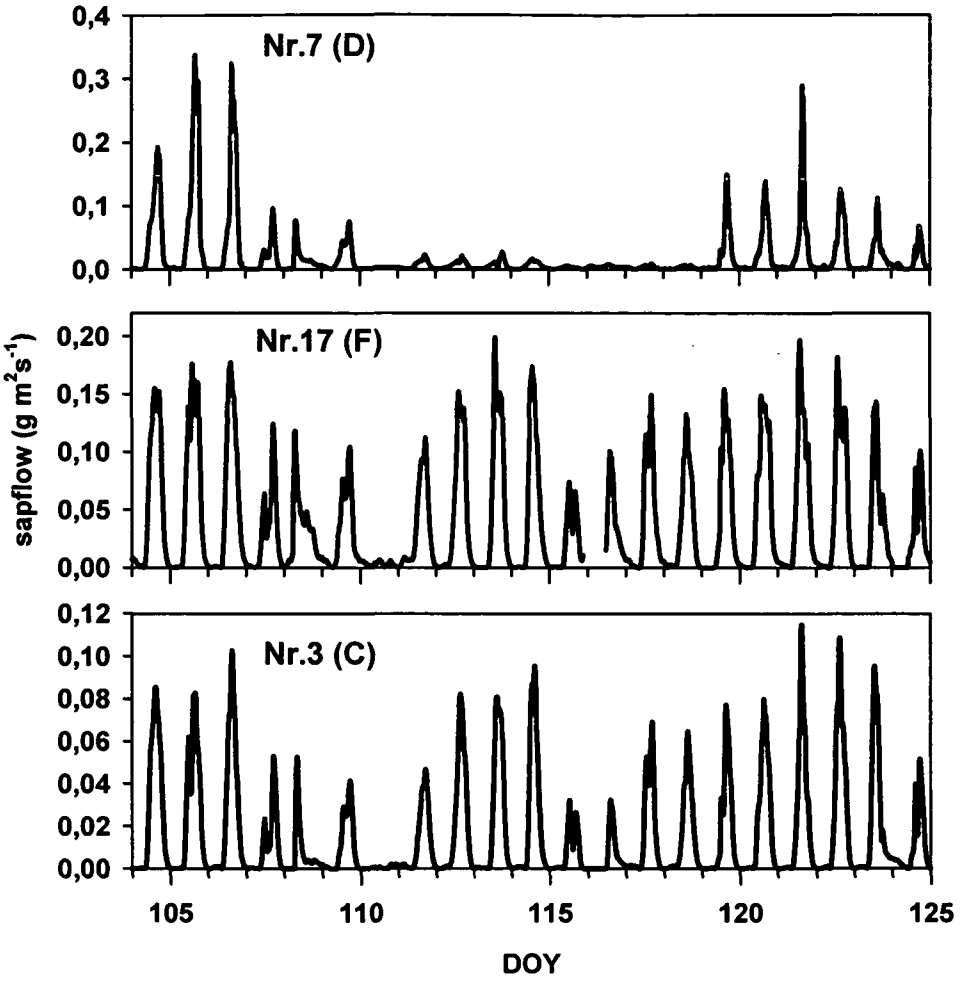

Fig. 22: Example of a decrease in sapflow in potted spruce during desiccation. Sapflow of spruce Nr.7 (D) is noticeably decreased during a period of desiccation in April 2005. Sapflow of F and C remain on a higher level. DOY = day of year, 105 = 15.06.2005.

The emission of ethanol (EtOH) and volatile terpenoids (VT) was measured twice in 2004 (05.07-08.07 and 19.07-21.07) and once in 2005 (15.06-17.06) during drought and flooding stress (Tab.1). VTs measured were (-)-α-Thujen, (-)-β-Myrcen, (-)-α-Pinen, (+)-α-Pinen, (+)-Sabinen, (-)-Sabinen, Tricyclen, (-)-Champhen, (+)-Champhen/(-)- α -Phellandrene, α-Terpinen, δ-3-Caren, p-Cymol, (+)-β-Pinen, (-)-β-Pinen, (-)-Limonen, (+)-Limonen, (-)-β-Phellandren, (+)-β-Phellandren, γ-Terpinen, Terpineol and 1.8-Cineol. With the exception of a few outliers, caused by exceptional high values of (-)-α-Pinen and (-)-β-Pinen in D and F, emission rates of EtOH and VTs were surprisingly low (Peter Baier, personnel comment) and differences were not significant (t-test, 0.95% significance).

	05.07-08.04.04			19.07-21.07.04			15.06-17.06.05		
	C	F	D	C	F	D	C	F	D
E	0 ± 0	0 ± 0	0.1 ± 0.2	0 ± 0	0.4 ± 0.6	0.2 ± 0.4	0 ± 0.1	0 ± 0	0.5 ± 1.2
-αTh	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
-βM	0.7 ± 0.4	1.3 ± 1.8	1.0 ± 0.8	0.2 ± 0.3	0.3 ± 0.4	0.2 ± 0.1	0 ± 0	0.1 ± 0.1	0.1 ± 0.1
-αP	4.2 ± 3.6	12.4 ± 19.9	14.2 ± 16.9	1.6 ± 1.4	3.9 ± 6.2	2.4 ± 1.2	0.5 ± 0.5	1.7 ± 1.9	0.9 ± 0.8
+αP	1.3 ± 0.9	2.3 ± 2.0	2.1 ± 1.5	0.9 ± 0.7	1.8 ± 2.6	1.0 ± 0.6	0.3 ± 0.1	1.3 ± 1.5	0.3 ± 0.2
+S	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
-S	0 ± 0	0.1 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Tri	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
-Ch	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0	0.1 ± 0.1	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
+Ch	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0 ± 0	0.1 ± 0.2	0 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
αTe	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
δ3Ca	0.5 ± 0.2	0.7 ± 0.4	0.4 ± 0.3	0.2 ± 0.1	0.7 ± 0.7	0.3 ± 0.2	0.1 ± 0.1	0.5 ± 0.5	0.1 ± 0.1
pCy	0.2 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0	0.2 ± 0.2	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0
+βP	0 ± 0.1	0 ± 0.1	0.1 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
-βP	8.5 ± 8.0	26.3 ± 49.2	26.2 ± 31.3	2.7 ± 2.7	3.1 ± 2.7	4.2 ± 2.8	1.2 ± 1.4	2.4 ± 2.9	1.2 ± 0.9
-L	1.3 ± 1.1	4.4 ± 8.2	2.5 ± 2.7	0.4 ± 0.3	1.0 ± 1.8	0.6 ± 0.4	0.1 ± 0	0.2 ± 0.2	0.1 ± 0.1
+L	0.5 ± 0.2	0.8 ± 0.5	0.6 ± 0.4	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
-βPh	0.7 ± 0.8	1.6 ± 2.9	1.4 ± 1.4	0.5 ± 0.7	0.3 ± 0.4	0.3 ± 0.3	0.1 ± 0	0.3 ± 0.5	0.1 ± 0
+βPh	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
γT	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ter	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1.8C	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0	0.1 ± 0	0.1 ± 0.1	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
VT	18.6 ± 13.7	50.9 ± 84.6	49.2 ± 54.0	7.0 ± 5.9	12.0 ± 15.0	9.3 ± 5.2	2.7 ± 1.9	7.1 ± 7.7	3.3 ± 1.9
VT+E	18.6 ± 13.7	50.9 ± 84.6	49.2 ± 54.0	7.0 ± 5.9	12.4 ± 14.9	9.5 ± 5.3	2.7 ± 1.9	7.1 ± 7.7	3.8 ± 3.0

Tab. 1: Emission rates of ethanol (EtOH) and volatile terpenes (VT) of potted spruce trees in 2004 and 2005. Emission rates are in µg/dm²/h. Mean values ± SD are given for each measurement and each group (n = 6). E = Ethanol, -αTh = (-)-α-Thujen, -βM = (-)-β-Myrcen, -αP = (-)-α-Pinen, +αP = (+)-α-Pinen, +S = (+)-Sabinen, -S = (-)-Sabinen, Tri = Tricyclen, -Ch = (-)-Champhen, +Ch = (+)-Champhen/(-)- α -Phellandrene, αTe = α-Terpinen, δ3Ca = δ-3-Caren, pCy = p-Cymol, +βP = (+)-β-Pinen, -βP = (-)-β-Pinen, -L = (-)-Limonen, +L = (+)-Limonen, -βPh = (-)-β-Phellandren, +βPh = (+)-β-Phellandren, γT = γ-Terpinen, Ter = Terpineol and 1.8C = 1.8-Cineol. VT = sum of all terpenes, VT + E = sum of all VTs + Ethanol. No significant difference was found between treatments (C = control, D = desiccated, F = flooded, ANOVA)

2.4.4.2 Continuous long-term measurements

Parallel to the point measurements (2003 - 2005), continuous long-term measurements were conducted in seven trees (No. 1, 2, 4, 5, 6, 14 and 15) to investigate if stem oxygen shows diurnal variations, if the fluctuations are correlated to changes in sapflow, and if stem oxygen responds to soil flooding or to desiccation (Fig.23 - 26).

During the measurements the sensors were permanently installed into the stem and only extracted for functionality test and recalibration. Stem oxygen levels were often erratic and sometimes surprisingly high (probably due to gas leaks), or dropped to zero instantly after insertion. Various leakage tests, e.g. flooding of the sensor surroundings with N_2 and pure O_2, were conducted (Chapter 2.3.7.2).

To investigate if stem oxygen changes when the oxygen supply to the roots is cut of, the trees Nr. 1, 2 and 4 were flooded in the arboretum and between October 2004 and March 2005 trees Nr. 14 and 15 in the greenhouse.

Diurnal variations were only recorded in trees Nr.1, 2 and 6. In most cases oxygen either remained on a constant near ambient level (Nr.1, 2, 14 and 15), or dropped down to values around 0% instantly after insertion and remained low (Nr.4, 5, 14, and 15). The diurnal variations recorded were either positively (Nr.1) or negatively (Nr.2 and 6) related to changes in temperature, or there was a time-lag between the maxima of oxygen and temperature (Nr.6) similar to the measurements in mature spruce and beech. Oxygen peaks also shifted during the measurements or between measurements in the same tree. Periods with distinct diurnal variations were sometimes followed by periods when oxygen remained on a constant high or low level, although sapflow remained unaffected. Also measurements were often interrupted by technical problems and the obtained data was not sufficient to describe circadian changes in oxygen. Maximum sapflow was normally measured around noon and maximum temperature in the afternoon between 14:00 and 18:00. Similar to point measurements (Chapter 2.4.4.1), an effect of flooding was not found during long-term continuous measurements of oxygen (Chapter 2.4.4.3).

During measurements in tree Nr.6 in 2003 (anchor jacket setup, d = 5 cm) oxygen fluctuated between 91 and 99%. Highest values were measured between 22:00 and 01:00, lowest between 13:00 and 15:00. In June 2004 oxygen (d = 5 cm) fluctuated between 73 and 88% in a course similar to 2003. Oxygen content was highest between 03:00 and 07:00, lowest between 14:00 and 18:00 (Fig.23).

Oxygen measured in trees Nr. 2 and 3 in June 2004 (anchor jackets, d = 5 cm) dropped to 77% after insertion and remained around 93% in Nr.2 without any diurnal variations for several days (Fig.24). In September 2004 in aluminum tubes (d = 2.5 cm) installed into freshly drilled holes oxygen ranged between 92% and 84% in a course antipodal to temperature. In tree Nr. 3 (anchor jacket, d = 5 cm) oxygen values dropped instantly to 5% and remained low for several days without diurnal variations. Oxygen ranged between 83 and 67% in spruce Nr.1 (aluminum tube, d = 2.5 cm) in September 2004 (Fig.25) varied synchronous temperature and sapflow. Oxygen measured (anchor jacket, d = 5 cm) in July 2004 in Nr.4 and 5 (Fig.26) declined continuously after insertion to 2%, remained low and without diurnal variations, although neither temperature nor sapflow were reduced during that period and showed normal diurnal variations. Oxygen measured in Nr. 14 and 15 in November 2004 in the greenhouse (aluminum tubes, d = 2.5 cm) dropped to 0% within hours and remained low for several days. In new holes were drilled to 1.5 cm oxygen remained on constant high levels (Nr.14: 91%, Nr.15: 93%) in both specimen. Detailed flooding experiments are described in the next Chapter.

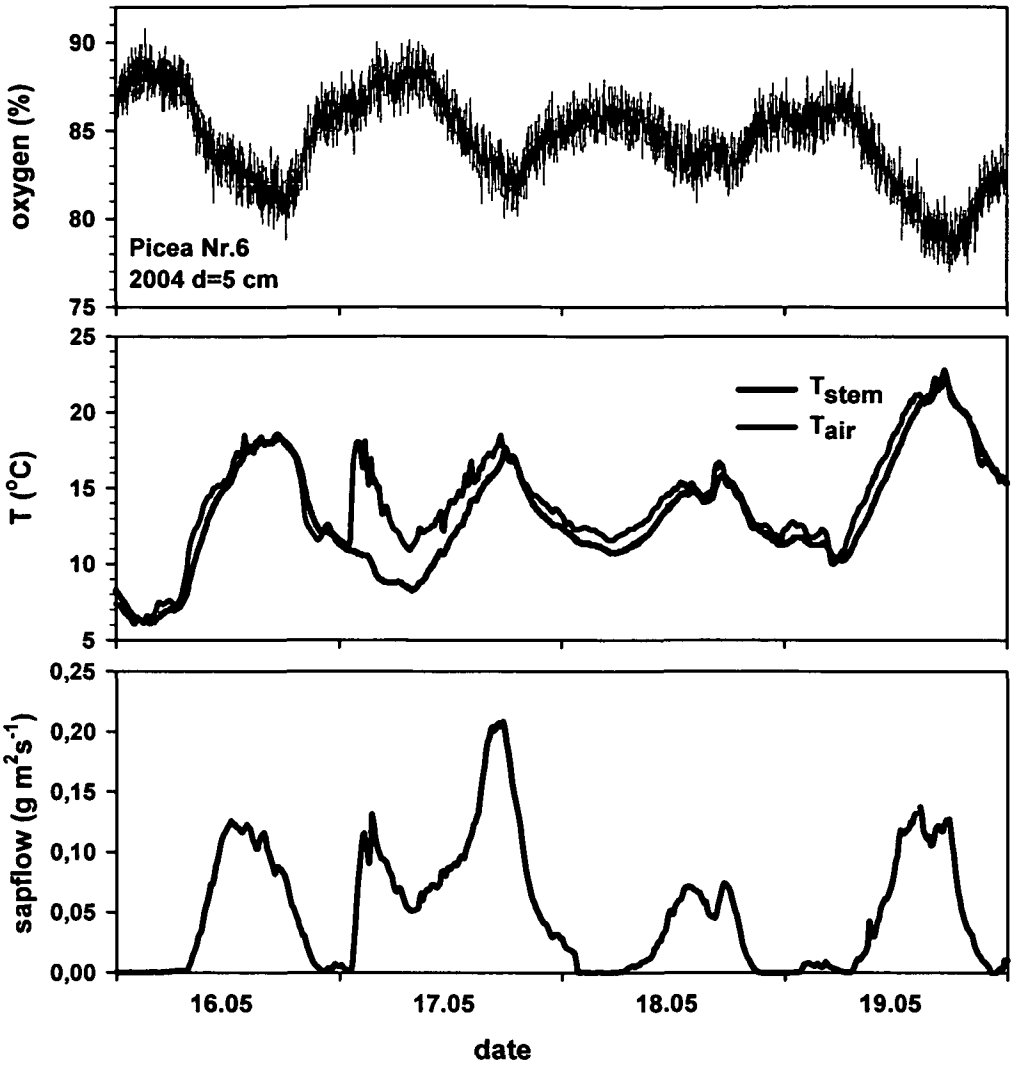

Fig. 23: Example of diurnal variations in oxygen recorded in tree Nr.6 in May 2004. During the measurements the course of oxygen was antipodal to the course of stem temperature. The apparent sapflow in the early hours of 17.05 were probably caused by inadequate temperature isolation of the setup.

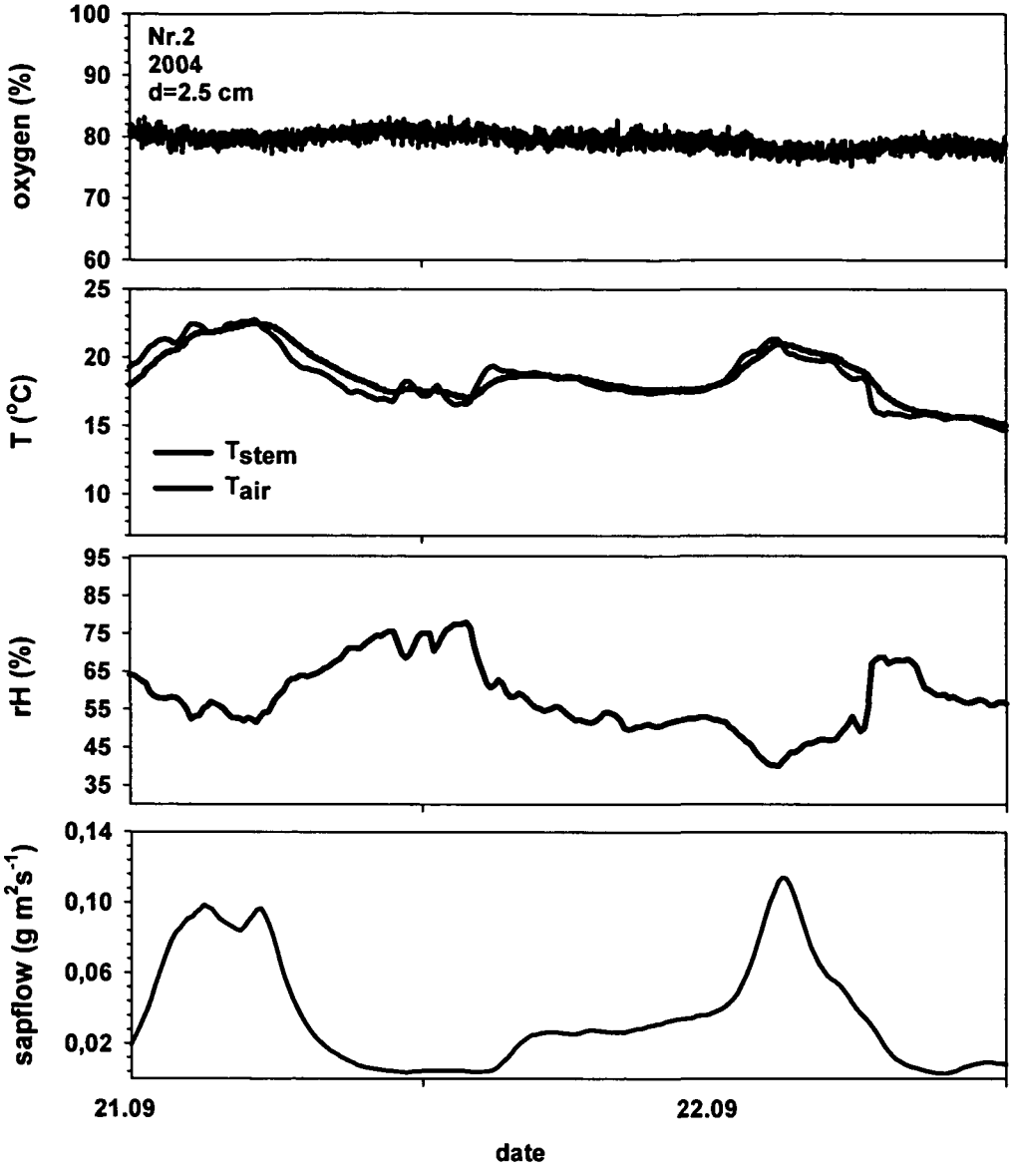

Fig. 24: Oxygen measured in tree Nr.2 in September 2004. The apparent sapflow in the early hours of 22.09 were probably caused by inadequate temperature isolation of the setup.

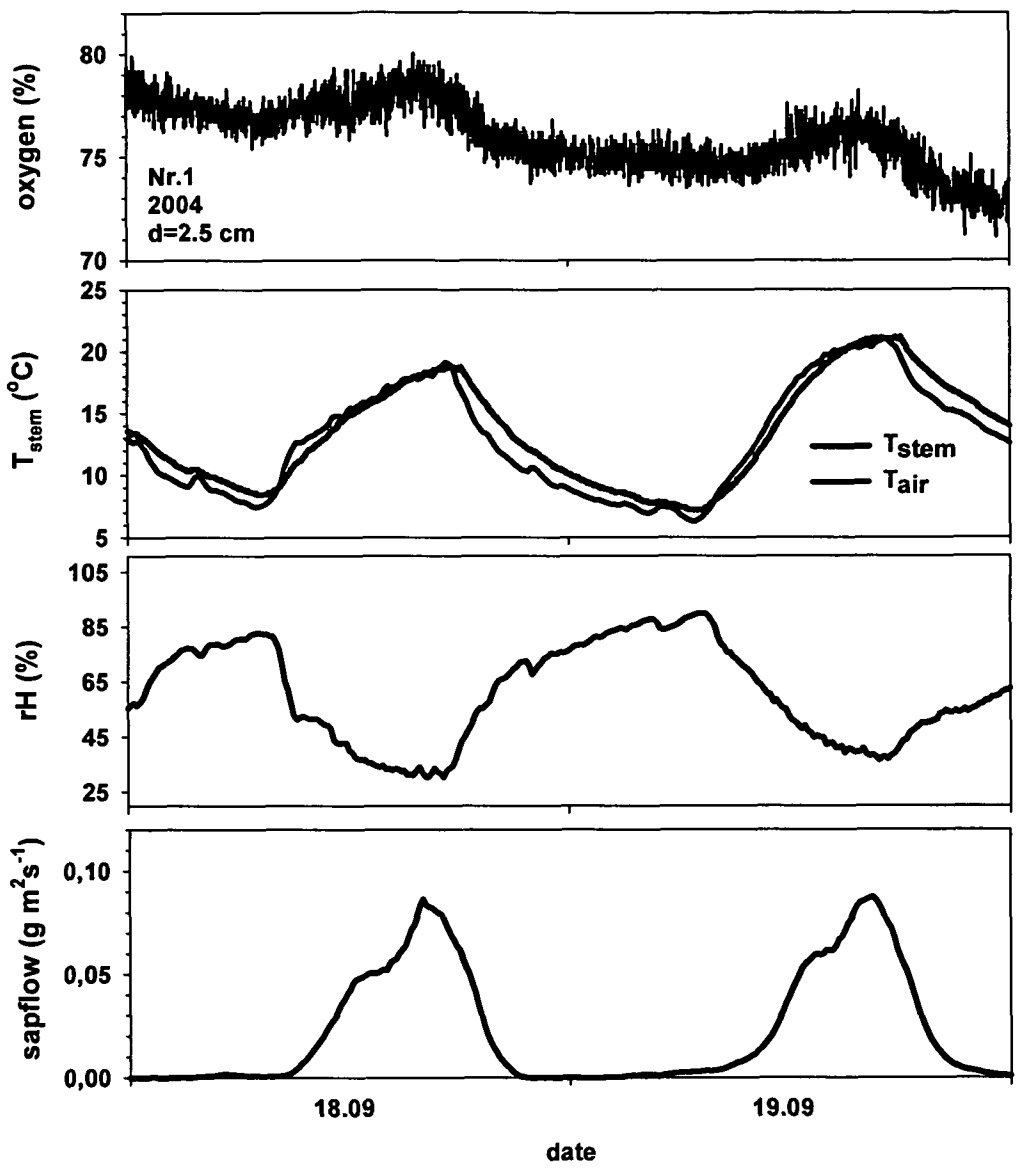

Fig. 25: Oxygen measured in potted tree Nr.1 in September 2004. Oxygen was measured through metal tubes in d = 2.5 cm.

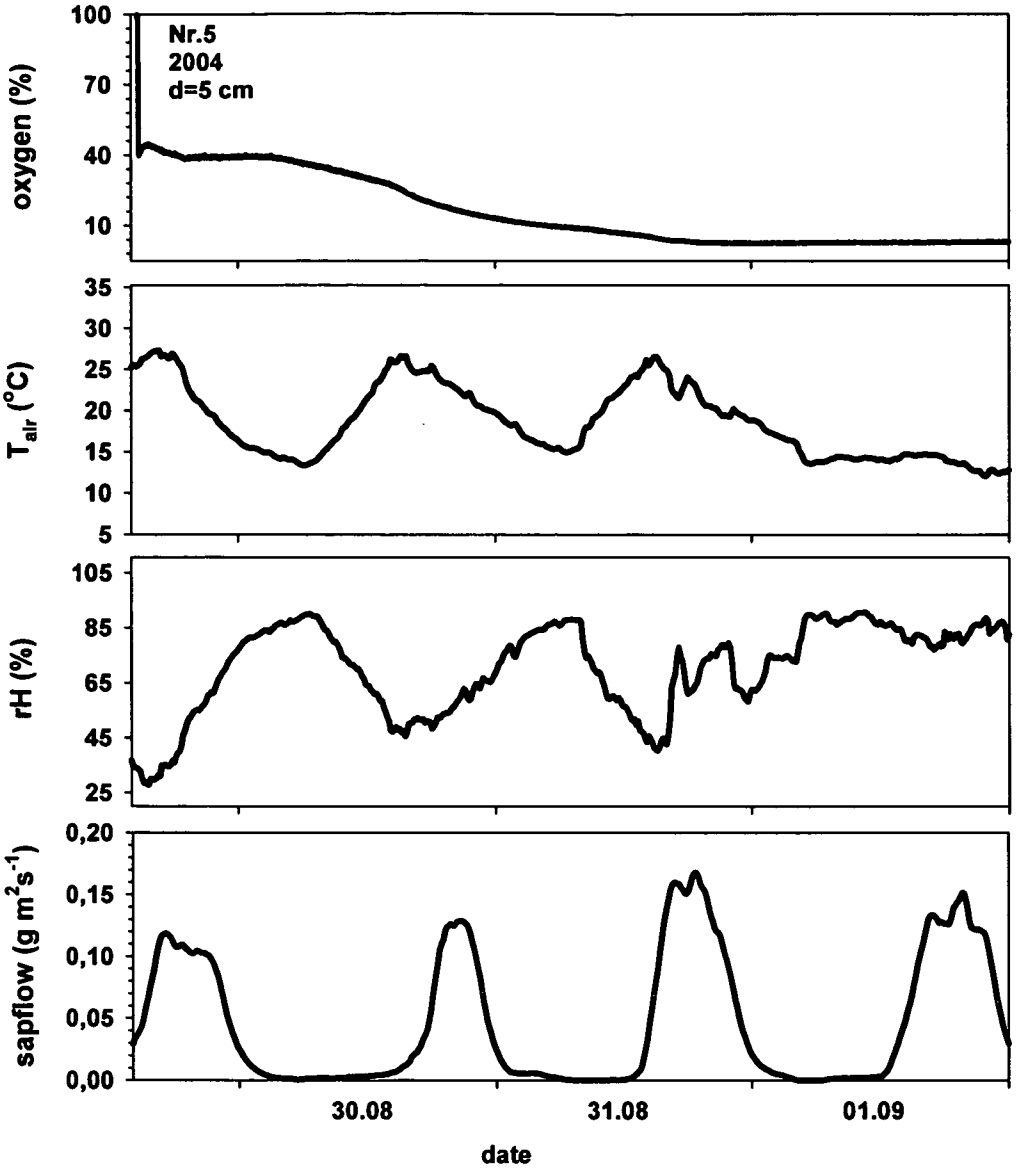

Fig. 26: Oxygen in spruce Nr. 5 measured in August - September 2004.

2.4.4.3 Effect of flooding on stem oxygen content

Pots of Nr.1, 2, 14 and 15 were flooded for several days during continuous oxygen measurements in 2004 and 2005 to investigate if stem oxygen changes with a reduced availability of oxygen in the root zone. Pots were wrapped in plastic foil to avoid drainage. In September 2004 aluminum tubes (diameter Ø = 9 mm, depth d = 2.5 cm) were installed into spruce Nr. 1 and 2. The pot of Nr.2 was flooded for several days after installation of the sensors. Oxygen was measured between 17.09 and 21.09 (Fig.27). Oxygen remained at its initial high level in both trees and no drastic decrease was noticed in either the control (C) or

the flooded specimen (F). Oxygen ranged between 83 and 67% in C and resumed a course synchronous to air and stem temperature, while it ranged from 92 to 84% in F antipodal to air and stem temperature. Sapflow was noticeably reduced during flooding in F. Sapflow peaks ranged from 0.03 to 0.05 g m^{-2} h^{-1} in F and from 0.04 to 0.12 g m^{-2} h^{-1} in C. On 21.09 the sensor was extracted from Nr.1 and inserted into the pot of Nr. 2 (F) (Fig.27). A sensor was installed in the pot that monitored the depletion of oxygen in the soil during several days of flooding. Stem oxygen in F remained around 85% during the flooding and no diurnal variations were noticed. Soil oxygen depleted within a day and remained around 0% until the pot was drained. Approximately 18 hours after drainage soil oxygen returned to 100%. During flooding sapflow peaks of F were reduced from 0.12 g m^{-2} h^{-1} to 0.04 g m^{-2} h^{-1}. Experiments in trees Nr.14 and 15 brought similar results where stem oxygen remained either on its initial high or low level, but never changed during flooding, although sapflow was noticeably reduced in both cases.

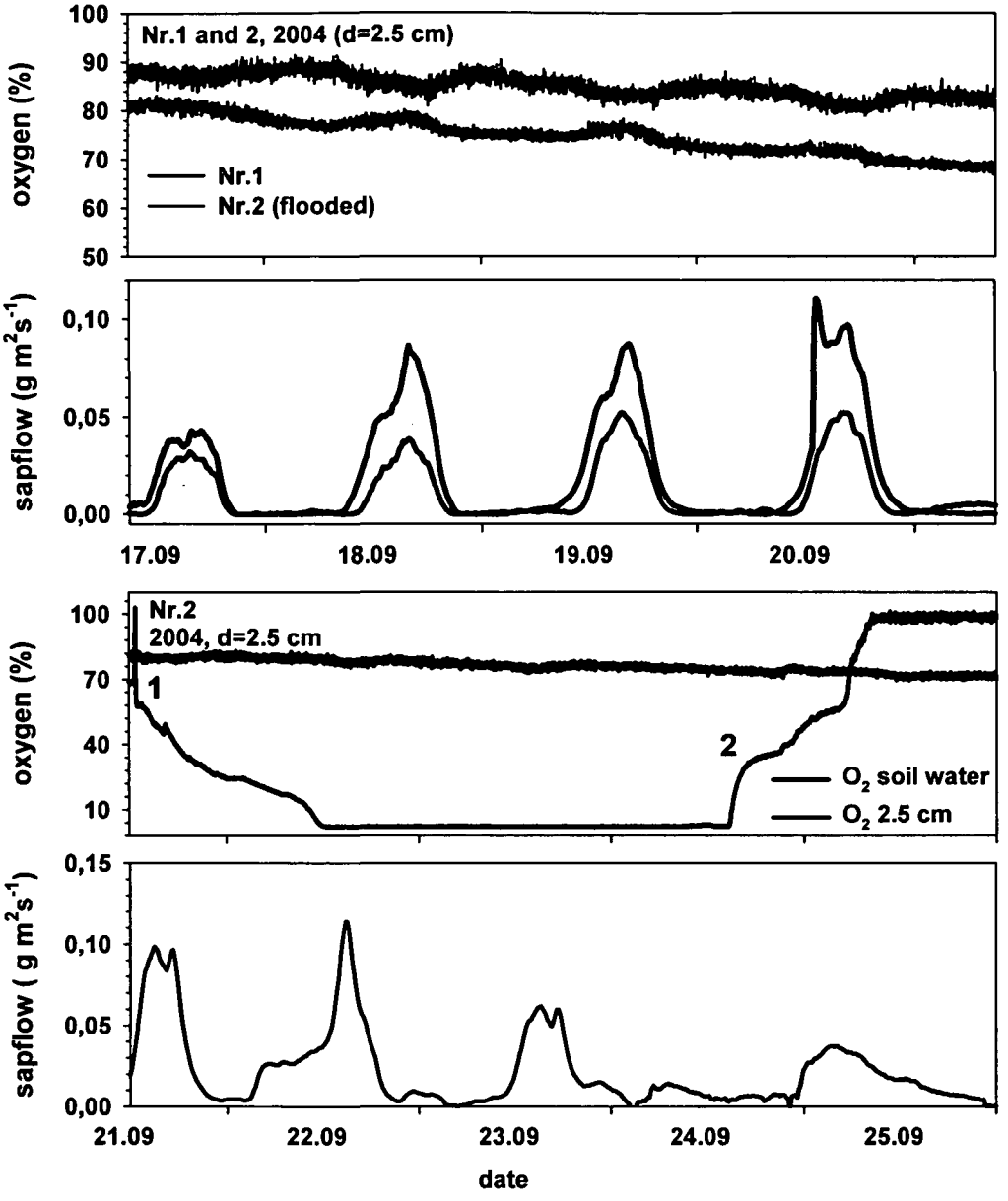

Fig. 27: Flooding experiments conducted with potted spruces Nr.1 and 2. Upper part: Nr.1 was control (C), while Nr.2 was flooded (F). Sapflow continued in F and oxygen remained uninfluenced by the flooding. Lower part: On 21.09 oxygen was continuously measured in spruce Nr.2 (F), while an additional sensor measured the depletion of oxygen in the flooded soil. Within one day of flooding oxygen decreased to 2% (1) and remained low until the pot was drained on 24.09. Sapflow was reduced during flooding while stem oxygen remained on its initial levels.

2.4.5 Oxygen and bark emissions of cut spruce logs

Stem oxygen in wet logs dropped from 26.2 ± 14% to about 10 % within eleven days, and remained between 10 and 20% for the rest of the experiment. Oxygen in desiccating wood increased from 33.2 ± 17% to 73.17 ± 19.5% and was significantly higher than in wet logs after the first two weeks (Fig.28). Wood moisture was kept constant at 100% throughout the experiment in the wet logs and ranged between 79.02 ± 8% and 87.99 ± 2.7% in desiccating wood.

Bark emissions of EtOH and VTs were generally very low (Peter Baier, personnel comment), with exceptions of a few outliners caused by a single log (log 2, wet storage). Significant difference between wet and dry logs was found in the emission rates of Ethanol (1.measurement, wet<dry) and of (-)-α-Pinen, (+)-α-Pinen and 1.8-Cineol (2.measurement, wet<dry) (Tab.2).

Stem oxygen content was also measured at two depths (d = 4 and 6 cm) in three cut spruce logs used as traps for bark beetles between March and September 2005 (Fig.29). Stem oxygen ranged from 1 ± 2% (14.09) to 40 ± 36% (15.06) in 4 cm, and from 1±1% (10.08 and 16.09) to in 45 ± 39% (15.06 and 29.06) 6 cm. Oxygen values fluctuated substantially in the first months and declined to constantly low levels at both depths by the end of August. No significant differences were found between the two depths. The logs were positioned in the opening of the forest stand and in contrast to the logs in the laboratory were exposed to changing environmental conditions including strong fluctuations in temperature, weathering and mechanical damage. While oxygen concentrations increased in desiccating logs in the laboratory, they decreased in logs exposed in the field where environmental conditions were variable and stem water content difficult to predict.

	20.08-23.08.04		13.09-15.09.04	
	wet	dry	wet	dry
E	**0.4±0.3**	**0.9±0.4**	0.5±1.1	0.0±0.1
-αTh	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
-βM	1.0±1.8	0.2±0.1	0.2±0.1	0.3±0.1
-αP	36.8±88.5	2.0±1.4	**2.6±2.5**	**9.2±6.5**
+αP	9.7±23.1	0.6±0.2	**0.6±0.4**	**2.2±1.5**
+S	0.7±1.6	0.0±0.0	0.0±0.0	0.0±0.0
-S	0.4±1.1	0.0±0.0	0.0±0.0	0.0±0.0
Tri	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
-Ch	0.3±0.8	0.0±0.0	0.1±0.0	0.1±0.1
+Ch	0.1±0.1	0.1±0.0	0.1±0.0	0.1±0.0
αTe	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
δ3Ca	3.1±6.7	0.5±0.1	0.2±0.2	0.4±0.2
pCy	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0
+βP	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
-βP	72.9±176.5	3.3±2.3	3.8±3.7	12.9±10.8
-L	2.0±3.9	0.4±0.6	0.2±0.1	0.5±0.5
+L	0.9±1.8	0.1±0.1	0.1±0.0	0.1±0.0
-βPh	4.7±11.2	0.3±0.2	0.5±0.5	0.3±0.2
+βPh	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
γT	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Ter	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1.8C	0.1±0.1	0.1±0.0	**0.0±0.0**	**0.1±0.0**
VT	132.8±317.0	7.7±4.7	8.5±7.5	26.3±19.3
VT+E	133.2±317.0	8.6±4.9	8.9±7.9	26.3±19.4

Tab. 2: Emission rates of EtOH and VT from cut spruce logs. Emission rates are in µg/dm²/h. High VT values were caused by Log 2, wet storage. Mean values (n = 6) ± SD are given for each measurement and each group. E = Ethanol, -αTh = (-)-α-Thujen, -βM = (-)-β-Myrcen, -αP = (-)-α-Pinen, +αP = (+)-α-Pinen, +S = (+)-Sabinen, -S = (-)-Sabinen, Tri = Tricyclen, -Ch = (-)-Champhen, +Ch = (+)-Champhen/(-)- α -Phellandrene, αTe = α-Terpinen, δ3Ca = δ-3-Caren, pCy = p-Cymol, +βP = (+)-β-Pinen, -βP = (-)-β-Pinen, -L = (-)-Limonen, +L = (+)-Limonen, -βPh = (-)-β-Phellandren, +βPh = (+)-β-Phellandren, γT = γ-Terpinen, Ter = Terpineol and 1.8C = 1.8-Cineol. VT = sum of all terpenes, VT + E = sum of all VTs + Ethanol. Bold numbers mark a significant difference between wet and dry storage (ANOVA).

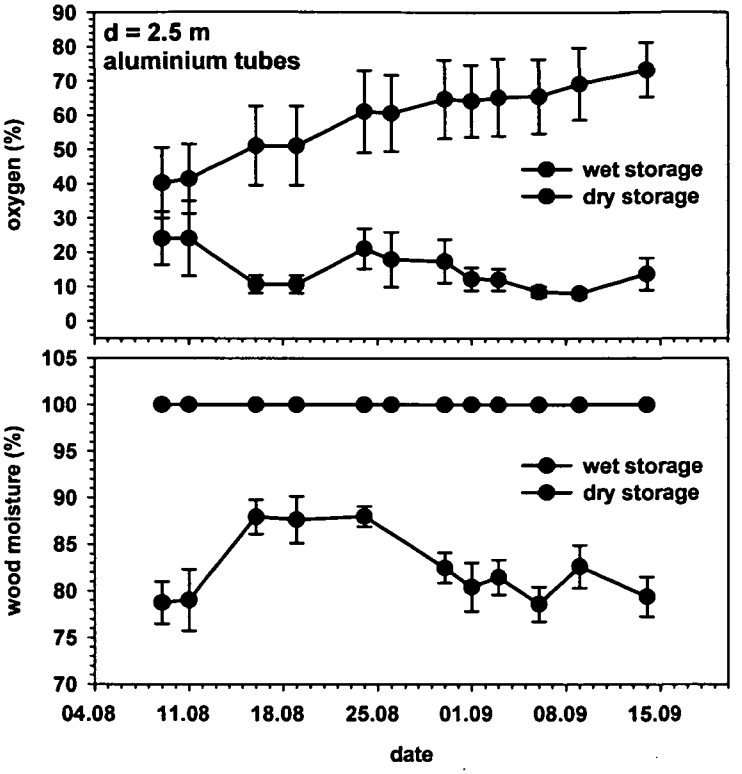

Fig. 28: Stem oxygen (d = 2.5 cm) and wood moisture of cut spruce logs. Six logs were bench dried (dry storage), and six were stored wet at (T = 25°C, rH = 100%). Dots are mean values (n = 6), error bars are calculated from SE.

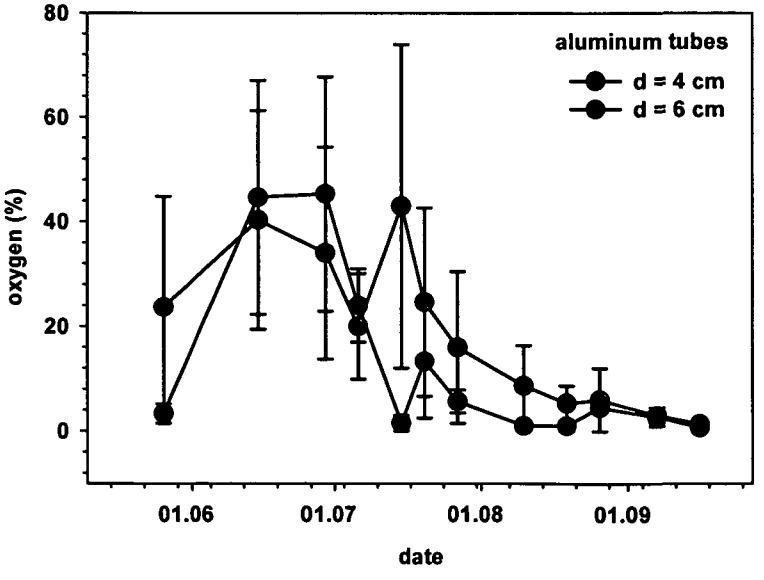

Fig. 29: Stem oxygen in three cut spruce logs used for bark beetle control in the field (Wolfsgraben, Lower Austria). Oxygen was measured on 12 consecutive dates from 25.05 and 16.09.2004 in two different depths (d = 4 and 6 cm). Dots are mean values from each depth (n = 3), error bars are calculated from SE.

2.5 Discussion

2.5.1 Uncertainties in oxygen measurements and different approaches

Oxygen measurement in standing trees is new and so far few data are available and many uncertainties exist. Various methods have been used that all have their pros and cons and none may be the optimal method under all circumstances. Although measurements with an optode sensor, as conducted in this study, are rapid and easy compared to previous methods and allow the registration of very small short-term variations and long-term continuous changes in the living stem (Chapter 2.3.1), the results obtained in this thesis show that there are still several major issues that require improvement.

The gas-tightness of the setup was of great importance to assure valid results, and although the setup can be tested for leaks (Chapter 2.3.7.2) and suspicious oxygen values (i.e., above, or at 100%) were rejected, a constant monitoring of gas-tightness during an experiment was not always possible. During periodical short-term measurements in potted spruce (Chapter 2.4.4.1) and beech trees (Chapter 3.4.1), where frequent insertions and extractions of the sensor needles caused substantial mechanical strain on the setup, the occurrence of undetected gas-leaks could have contributed to the broad distribution of oxygen values that was noticed in both experiments (Fig.19-21, Fig.35). Problems with gas-tightness were especially frequent in early stages of this study when anchor jacket setups were used (Chapter 2.3.7.1.1). Although del Hierro et al. (2001) developed and successfully applied this setup for measurements in *Laurus nobilis*, gas-tightness was never checked in that work, and comparison between data from potted spruce (Chapter 2.4.4.1) obtained in 2003 (Fig.19) with data from the two consecutive years (Fig.20 and Fig.21) suggest that anchor jackets are prone to leaks, and were less suitable for oxygen measurements in the standing tree.

The use of metal tubes (Chapter 2.3.7.1.2) resulted in more reliable data and fewer suspiciously high values. Spicer and Holbrook (2005) used a similar setup to measure oxygen in various depths in *Acer rubrum*, *Fagus americana*, *Tsuga canadensis* and *Quercus rubra*, where stainless steel cylinders were installed into drilled holes in the stem and a special construction reduced the overall gas space in the tube to a significant extent. Their tube was sealed by a silicone septum pressed against an aluminum spacer and the gas space was periodically accessed through the silicone septum with an optode-based oxygen sensor housed in a needle. A reduction of dead gas space in the measurement chamber will reduce the time needed to achieve an equilibrium between the headspace in the chamber and the xylem. If the gas-space in the headspace becomes very small, however, the minute amount of air introduced when inserting the sensor may result in a significant error, in which case it will take longer to obtain stable readings until the gas in the headspace again

equilibrates with the wood. In this study the headspace differed somewhat between measurements – depending on the length and diameter of the tubes and the hole at the end of the tube – and was in the range of 0.5 – 2 cm^3 and the area of xylem exposed was in the range of a few cm^2. With this arrangement equilibrium, i.e., stable sensor readings, was usually obtained within 5 – 10 minutes (Chapter 2.3.7).

Gansert et al. (2001) completely avoided problems associated with gas leakage by measuring oxygen of the xylem sap of *B. pendula* in the aqueous phase. He accessed the conducting sapwood using the custom-made L*i*Pax device, where a hole was drilled into the stem under water while pressure was applied to compensate for the negative capillary tension in tracheids and vessels. After the drill was removed the chamber was rinsed and the optode sensor was installed into the water filled chamber under pressure. The water in the chamber was gradually substituted by xylem sap and its oxygen content could be measured continuously. The chamber was flushed with sterilized, degassed water to replace the losses of water that was absorbed by the transpiration stream. This rather complicated method should completely avoid gas leaks and produced plausible data, however it remains unclear if the water in the chamber is completely in equilibrium with the conducting xylem, in which case the negative water potential in the xylem should rapidly absorb the water in the chamber. If the transpiration stream does not pass through the chamber, gas exchange between the chamber and the ascending sap in the xylem was probably much slower than when measurements are made in the gas phase.

During measurements in spruce trees the influence of volatile resin components on the oxygen measurements, indicated by a long-term decline in sensor output, became apparent and were also confirmed in a laboratory experiment (Chapter 2.3.7.3). Point measurements should not have been affected, but long-term measurements in spruce (or any resin containing species) are problematic, although in many cases these measurements did produce plausible readings with diel variation and no long-term decline. Possibly the use of different coatings for the sensor or different luminophores could reduce the interference of volatile organic compounds.

(Eklund, 1990; Eklund, 2000) successfully conducted periodical point-measurements of oxygen in spruce using gas-chromatography. He installed cuvettes in drilled holes and periodically extracted oxygen from the headspace of a container in equilibrium with the cuvette with a syringe. O_2 was then quantified with GC-MS. This method is not influenced by any volatiles and produces reliably data, and further allows the quantification of other gases (e.g. CO_2 or ethylene) but it is rather expensive, time-consuming and does not allow continuous measurements.

Continuous measurements using electrode-based sensors were conducted by Mancuso and Marras (2005), who inserted three micro scale electrodes in various positions of the stem of

Olea europaea. To quantify the account of radial diffusion a measuring chamber was attached to the stem, where the only access to oxygen was by radial diffusion all the way through a sector of the stem. It was suggested that the miniaturization of the Clark-type electrodes minimized oxygen consumption or can even be used to simulate consumption of respiring tissue, however, oxygen consumption still substantially biases all measurements conducted with a Clark-type electrode to some extent, which is the greatest drawback of this method.

2.5.2 Oxygen fluctuations and implications for possible transport mechanisms

A literature review shows that oxygen concentrations measured in the sapwood of trees, differing in wood anatomy and metabolic activity, vary over a broad range from pronounced hypoxia to near saturation, depending on differences in season, species, age, and position within the stem (unless stated otherwise all values are % / air saturation).

Eklund (1990, 2000) found that in winter sapwood oxygen levels in spruce were close to ambient levels (~100%), but after the onset of cambial growth they decreased to values between and 0 and 30% with exceptionally low values (<5%) in summer. (Gansert *et al.*, 2001) measured oxygen in the aqueous phase in the xylem sap of *Betula pendula* and found oxygen fluctuating in a diurnal rhythm between 40% and 75%. Gansert (2003) measured diurnal fluctuations between 90-100% (% / air saturation) in the gaseous headspace of cuvettes in contact with the sapwood in *B. pubescens*, and 30-80% (% / water saturation) in the aqueous phase. (Mancuso & Marras, 2003) measured oxygen values between 65 and 90% in the sapwood of *O. europaea*. (del Hierro *et al.*, 2002) measured sapwood oxygen between 57 and 79% in potted *Laurus nobilis* with a different optode sensor. (Spicer & Holbrook, 2005) conducted periodical measurements in various depths in *A. rubrum*, *F. americana*, *T. canadensis* and *Q. rubra*, and found an average sapwood content of 47% and lowest values in the innermost sapwood between 14 and 23%. In the present study oxygen concentrations between and 0% and 105% were measured in spruce.

The diurnal variation found in mature spruce, with the highest values shortly before noon and the lowest values in late afternoon (Fig.15) are similar to daily ranges between 10 and 20% found in various other species (Gansert *et al.*, 2001; del Hierro *et al.*, 2002; Mancuso & Marras, 2003). In mature spruce maximum oxygen concentrations were observed shortly before noon, when sapflow was increasing, pointing to transportation with the ascending sap in the daytime. Since the respiration rate (R) increases with temperature, approximately doubling with a temperature rise of 10 °C (Atkin & Tjoelker, 2003), R should be highest in the early afternoon and oxygen declined from noon onwards when sapflow started to decrease. As oxygen replenished during the night, when sapflow was near zero but respiration also lowest, radial diffusion is the likely source of oxygen in times with little sapflow.

Also the fact that in the mature spruce tree oxygen decreased and the diel variations increased with stem height points to oxygen transport by sap water (Fig.15). A similar dependency of oxygen on stem height in spruce was described by (Eklund, 2000), who suggested that the ascending sap was successively depleted of oxygen by respiring sapwood on its way from the roots to the crown.

The fact that oxygen was higher in the heartwood than in the peripheral sapwood of mature spruce (Fig.15, 16) can probably be explained by respiration that depleted oxygen in the living sapwood. Uninfected wood without living cells does not consume oxygen. The fact that oxygen extremes were measured one to three hours earlier in the sapwood than the heartwood shows that sapwood to some extent draws oxygen from the heartwood. Radial diffusion coefficients (D) in spruce are sufficiently high to support radial exchange between heartwood and sapwood, particularly with the typically low water content in the heartwood (Sorz & Hietz, 2006).

Diurnal extremes of various magnitudes were also measured in potted spruce saplings (Chapter 2.4.2.2) and in beech (Chapter 3.4.2). In potted spruce daily fluctuations were often smaller and maximal oxygen concentration often did not coincide with sapflow or was even observed during night. Oxygen in spruce saplings either remained on a constant near ambient level (trees Nr.1, 2, 14 and 15), or dropped to values around 0% instantly after insertion and remained low (trees Nr.4, 5, 14, and 15). Diurnal variations were only recorded in three of seven measured individuals, and not at all times. Maxima in oxygen coincided either with maxima (tree Nr.1, Fig.25) or minima of temperature (trees Nr.2, 6, Fig.23, 24), or the course of oxygen and temperature were shifted (tree Nr.6), and diurnal variations were similar to those of mature spruce (Fig.15, 16) and beech (Fig.38). Extremes could shift during the measurements or between measurements in the same specimen. Periods with distinct diurnal variations were sometimes followed by periods were oxygen remained on a constant high or low level, although sapflow was unaffected (Fig.37 - 42).

It is likely that sapflow contributes relatively more to the oxygen supply in large trees because the distance between living sapwood and the atmosphere is larger and the surface/volume ratio smaller. Also, the fact that in flooded trees (Fig.27), where sapflow is unlikely to contribute to oxygen supply, maxima were recorded at night, i.e., when respiration is small, and in non-flooded trees, where oxygen is present in the soil and hence in the xylem water transported upwards, during times of maximum sapflow gives support to both pathways of oxygen transport. However that particular effect was only noticed in a few specimens and can not be generalized. Assuming that radial diffusion is of increased importance in trees with a smaller diameter, it is most puzzling that the flooding of the surrounding stem with gaseous N_2 or pure O_2 induced no alteration in stem oxygen. If oxygen is transported with radial diffusion, flushing the stem with N_2 or O_2 should have shown an effect.

2.5.3 Effect of infection in large trees

Data obtained from bark-beetle infested spruce (Chapter 2.4.2) show that oxygen can decrease to very low levels (Fig.17). In September 2004 only values below 20% were measured at all depths at breast height and in 10 m. (Eklund, 1990) also measured values below 20% in spruce trees from April until late September, though measurements in different spruce trees and in beech conducted in 2005 and 2006 gave different results. The beetle-infested tree, where continuous measurements were conducted, was old and dying with heavy needle loss. Sapflow must have been very low and oxygen supply via sapflow probably negligible, and the results appear to support the importance of sapflow in large trees. In addition, as long as sapwood had not died, the stress resulting from the attack could have resulted in increased respiration. Generally infections with pathogens often alter a stems microenvironment. CO_2 levels increase and O_2 is reduced as a result of increased respiration, while the degradation of the cell walls often increases porosity, both influencing gas permeability (Boddy, 1992).

This study found no significant effect of heart rot (Chapter 2.4.3), which should have no or little effect on sapflow (Butin, 1995) and thus on oxygen concentrations in sapwood or heartwood.

2.5.4 Effects of water stress on stem oxygen and volatile emissions

Several authors report a decline of stem oxygen associated with a restriction of sapflow or root oxygen deficiency. (Gansert, 2003) found a noticeable decrease in oxygen following a restriction of sapflow in potted *B. pubescens,* (del Hierro *et al.*, 2002) found a reduction in oxygen after flooding potted *L. nobilis,* and (Mancuso & Marras, 2003), observed a decrease in oxygen after flooding the roots of *O. europaea* with gaseous N_2.

Results from potted spruce saplings were ambiguous. Data from periodical short-term measurements (Chapter 2.4.4.1), where no difference in stem oxygen was found between flooded and desiccated trees, imply that the transpiration stream contributes little to oxygen supply. Oxygen was found significantly lower in flooded specimens only on 4 out of 14 dates, but not during periods when soil moisture was highest (100%), and was lower in drought-stressed trees on a single occasion only, even though sapflow was significantly reduced in flooded (Fig.27) and drought-stressed trees (Fig.22), and water potential was significantly reduced by drought. Also no effect of flooding was found during continuous measurements (Chapter 2.4.4.2)

Apart from the size of the trees, which may have been too small for sapflow to be of much importance relative to radially diffusing oxygen, maximum soil water content and drought stress that resulted in very strong decline of sapflow was reached during a few days only, reducing the chance of detecting significant differences. As mentioned before short-term

measurements were probably susceptible to gas-leaking and data obtained from such measurements must be discussed with caution. In spruce, also resin appears to affect the output of the oxygen sensor and may have resulted in too low readings, at least in long-term measurements. Under which circumstances the abundance or composition of resin affects the oxygen sensor is difficult to conclude, but could be the reason for the slow decrease to 0% measured in November in the greenhouse and also in the arboretum (Fig.26).

Although sapflow was significantly reduced and water potential showed significant differences between the groups during times of water stress, the emission rates of ethanol and volatile monoterpenes (VT) were generally very low (Tab.1) and no significant difference was found between stressed saplings and control (Chapter 2.4.4.2). The initial hypothesis that water stressed spruce saplings show a reduction in stem oxygen and a successive increase in bark emissions of ethanol and VTs was not confirmed. Given that differences in oxygen concentrations were minor and the sample size was only 3 x 6, a larger sample with larger trees may have produced more significant differences, but the relationship in any case would most likely be weak.

Low emission rates were also found with cut spruce logs (Chapter 2.4.5), and few differences between wet stored and bench dried logs were found (Tab.2): Ethanol emissions were significantly higher in dry logs in the first measurement, (-)-α-Pinen, (+)-α-Pinen and 1.8 Cineol was significantly higher in dry logs in the second measurement ($p<0.05$), which does not correspond to (Kelsey & Joseph, 1999b), who found that cut logs exposed to rain showed a higher ethanol concentration and subsequent attack by ambrosia beetles than wood protected from rain. Again sample size was low in this experiment and outliers of VTs were found only in wet stored logs. During desiccation oxygen increased in bench dried logs while it remained at low levels in wet logs, as can be expected if the high water content reduces gas diffusion through the wood. Diffusion of gases is 10^4 times slower in water than in air (Armstrong, 1979), and, as described in Chapter 1, oxygen diffuses much faster through dry spruce wood compared to wet wood.

3 Oxygen in beech stems

3.1 Current state of knowledge

3.1.1 The formation of red heartwood in beech

Beech (*Fagus sylvatica* L.) finds its ecological optimum in Central Europe and its high shade tolerance enables the species to dominate European natural forests in most of its physiological tolerance range. Beech covers most of the natural deciduous-forest areas in Central Europe. Apart from its important role in protective forests and natural reserves, it is a very valuable species in sustainable forestry, because its high adaptability allows a broad spectrum of cultivation. Strong recruitment, resistance against insect attack, root penetration of profound soils, combined with exceptional regeneration after injuries from wind or snow break and a diameter increment until the age of 150 years, makes it the most important broad-leafed tree in Austria. The advancement of ecological considerations in forest management led to an increase of semi-natural forest stands with a high proportion of beech in recent years, and beech is likely to become even more important in the future (Kucera, 1991; Peters, 1997; Ruhm, 2004).

Beech is a diffuse-porous species with yellowish to light reddish wood that lightens during drying. Beech wood has excellent mechanical properties with an exceptionally high module of elasticity and a high bending strength considering its only moderate density of 0.68 g/cm^3. The high rate of shrinkage, which tends to influence its dimension stability, is compensated for by good machinability and good drying and sorption properties. The surfaces of light beech wood are easy to impregnate and to dye, enhancing its value for the wood industry. (Kucera, 1991; Kucera & Pohler, 1998). Beech wood was mainly used for energy generation until the middle of the 19th century, and production was focused on maximizing quantity not quality. The International Association of Wood Anatomists (IAWA) classifies beech as a tree species with facultative colored heartwood, and while the wood of younger beech trees normally features a light tone, the timber of older beech may sometimes darken significantly. For its use as energy wood the formation of red heartwood could be neglected, but with the utilization of beech wood for railroad ties, the abundance of red heartwood started to be noticed as a major defect in wood quality, since it decreased the impregnability with creosote (Kucera, 1991). Red heartwood also proved to be a disadvantage in fiber production (Dietrich, 1959).

Since the 1980s a large volume of high-quality beech wood is processed to high-value products like veneer and furniture, where red heartwood is undesired due to its inhomogeneous color. High-quality beech timber is a valuable commodity in national and international markets and the amount of red heartwood together with the knot area ratio and

tension cracks are the main features decreasing the prize (Seeling, 1998; Seeling & Becker, 2002), and today's low value of red heartwood is mainly a result of aesthetic preferences of the end users market (Kucera, 1991). Whether this is justified or not, the economic relevance of red heartwood probably explains the great interest that both foresters and scientists have in investigating beech heartwood formation.

3.1.1.1 Nomenclature of discolored wood

In spite of the importance of red heart there appears to be no consistent terminology. While some scientists took a more phenomenological approach by classifying the various types of discolorations by their shape in cross-section or their color tone, others tended to emphasize a typology based on the ontogenesis of the phenomenon (e.g. mechanical damage, fungus infection, or frost). This led to redundancy and ambiguity in the classification, where many authors gave different names to similar forms of colored heartwood formations, and differentiation was not always accurate (Stuber *et al.*, 2002). Several authors have tried to standardize the nomenclature of colored heartwood formation which led to two similar systems of classification. (Mahler & Höwecke, 1991) and (Walter & Kucera, 1991) organized the different types of colored cores by their appearance (one-zonal rotund red core, multi-zonal irregular heartwood, splash core), while (Sachsse, 1991) emphasized on the possible causes of red heartwood formation, and came up with a new classification in four categories:

- **Red heart**
- **Splash heart**
- **Wound heart**
- **Abnormal (irregular) heart**

To this typology (Klemmt, 1996) added the cracked heart, a small, elongated core, which is formed in the center of the cross-section after formation of cracks originating in the pith area. Different types of heart can be found in trees growing in one stand under similar conditions and even in a single individual (Krempl & Mark, 1962).

3.1.1.2 Red heart

The "typical" red heart of beech usually features round borders, reddish to brownish tone, and is usually not limited in its expansion by the annual growth rings (Sachsse, 1991). This irregular expansion can sometimes lead to a "cloudy" appearance on the cross-section, and therefore this type of discolored core is often referred to as cloud core (Rieder, 1997). The red heart features a spindle-like form and its largest diameter is usually found between a

third and the half of the shank height (Racz, 1961). It was assumed for a long time that the formation of red core in beech was caused by an enzymatic defense reaction triggered by a fungal invasion. (Zycha, 1948) invalidated this long-lived theory by proving that living hyphae are not necessarily abundant in the red heart, and that the formation of heartwood in beech can occur without any existence of fungi in the stem.

The most important requirement for red heartwood formation is a decrease in stem water content. A threshold for red core formation was found at 60% wood moisture (wood moisture is in % / wood dry mass, or stated otherwise) a point where much of the water is supposed to be drained out of conductive elements and fibers, and mainly the water stored in the cell walls remains. At a wood moisture < 60% living parenchyma cells die (Zycha, 1948). In a healthy young beech tree the wood moisture decreases continuously from sapwood to the pith (Sachsse, 1967). With the ageing process the water supply in the stem is reduced and the center of the stem starts to desiccate below 60%. Nuclei and mitochondriae of the parenchyma cells degenerate as a result of the water stress, while hydrolysis of starch and synthesis of tannin agents are elevated. As a defensive reaction the water stressed cells initiate the formation of tyloses at the heartwood margin, to clog the drained vessels in order to avoid further water loss and embolism. In contrast to species with obligatory heartwood formation (e. g. *Pinus sylvestris*, *Quercus robur*), the parenchyma cells located at the sapwood/heartwood boundary of beech stems are particularly vital and produce large amount of tyloses and phenolic core substances e. g. tannins prior to death. These tyloses are responsible for the inferior impregnatability, inferior sorption properties and slower drying of red hearted beech logs (Ohnacker, 1889). After the water in the stem is replaced by gas, oxygen may enter the stem through dead branches, wounds and other possible entry ports, and diffuses into the gas space driven by the gradient in partial pressure. In the presence of oxygen phenolic compounds stored in the protoplasts of the living parenchyma cells are oxidized to pigmented phenolic substances, causing the visible discoloration of the beech heartwood (Zycha, 1948; Paclt, 1953; Bosshard, 1965; Necesany, 1966; Bosshard, 1967; Ziegler, 1967; Necesany, 1969; Bosshard, 1974). Catechine- and Epicatchine-derivatives are the most frequent phenolic compounds identified in the process of discoloration. They accumulate in the living parenchyma cells before the actual heartwood formation begins, and polymerize in the presence of oxygen and under the influence of peroxidases and phenolases in the lumen. The highly condensed and pigmented end products of these polymerizations, high-molecular flavanoids and chinoids of reddish to brownish color (e. g. 2,6-dimethoxy benzochinone), can not pass through the fine molecular filters of the cell wall and remain within the protoplast (Koch *et al.*, 2000; Koch *et al.*, 2001). In species with obligatory heart formation the aging process in the parenchyma cells is much slower (Necesany, 1966) and phenolic compounds are transported into the cell walls before their

polymerization. This difference is of great significance, because, unlike species with obligatory heartwood formation, the pigmented phenolic compounds of the red heart do not enhance mechanical properties and fungal-resistance of the discolored beech heart (Ziegler, 1967; Bosshard, 1967)

While some authors believe that the formation of red heart is a pathological reaction to exogenous factors (Necesany, 1966; Schute, 1986), others state that it is merely a physiological reaction, taking place in healthy and uninjured/uninfected individuals, and its initiation and extend are mostly influenced by natural ageing processes in a persistent long-running process normally starting at the age of 90-140 years (Zycha, 1948). This theory could be confirmed by the induction of red heart under laboratory conditions by slowly desiccating beech wood in ambient air (Torelli, 1984). While the vitality of the parenchyma seems to be one the most important parameters in red heart formation, exogenous factors can have an effect as they decrease the vitality, initiating or accelerating red heart formation (Necesany, 1966). A decrease of cell vitality that goes alongside heartwood formation was described by several authors. The starch content of living parenchyma cells is a good indicator for vitality, and a decrease in cellular starch levels is also found in stem areas where red heart was formed (Ziegler, 1967). Starch content decreases exponentially from the sapwood to the heartwood, where only traces of starch can be detected, alongside a decrease in water content and soluble sugars. Thus cell vitality decreases from bark to pith. However, an opposing gradient was found for mineral nutrients such as potassium, which increase from bark to pith, with particularly high concentrations in the sapwood/heartwood boundary (Koch *et al.*, 2000), a zone of elevated physiological activity, which is described by many authors as the probable location of red heart formation (Bosshard, 1967). The cloudy structure of the red heart is caused by successive phases of several formation zones on the cross-section of the stem (Sachsse, 1991). However, the existence of mature beech trees with high DBH that exhibit no red heart, and discolorations found in zones of dead uncolored heart make that theory doubtable since a gradual decrease in water content probably eliminates the existence of living cells in the inner parts of thick stems. So far no beeches were found featuring light, not discolored central stem areas surrounded by rings of red heart. If living parenchyma is a prerequisite for red heart formation, red heart or dead uncolored heart should show no signs of further discoloration unless there are other factors (e.g. colonization by bacteria or fungi) involved in the process.

3.1.1.3 Splash heart

Splash heart, sometimes described in the literature as "butterfly core" or "flame core", features irregular jagged borders, which make it easy to differentiate from the classical red heart found in beech. Splash heart is less common (only 5% of all trees with colored

heartwood), and, in contrast to the formation of red heart, reaches its maximum diameter at the bottom of the tree. It usually extends through the whole stem length and decreases in diameter from bottom to crown. It is suggested that the formation of splash heart often originates from wounds in the central part of the root system and successively heart formation extends up to the branches (Racz, 1961; Schnell, 1986; Sachsse & Ferchland, 1988).

3.1.1.4 Abnormal (irregular) heart

Abnormal or irregular heart is often used synonymous with the splash heart, which makes analysis of literature data difficult. The most distinguished traits of the abnormal heart are its black margins found on the cross-section. Some authors also found a characteristically smell of butyric acid emerging from freshly cut lumber, accompanied by exceptional high water content in the discolored parts. The abnormal heart is similar in appearance to the wet heart of other tree species, which suggests a formation induced by infection and an ontogenesis different from the classical red core. The abnormal heart can surround an existing red heart and can extend quite rapidly along the cross-section of the tree (Sachsse & Ferchland, 1988; Walter & Kucera, 1991; Seeling & Sachsse, 1992). Abnormal hearts can extend up to half of the shank height and feature spindle or cone-shaped form. They can take up to 50% of stem volume and can impair the water transport system significantly. Beech trees with irregular heartwood can sometimes be found to have broader year rings to compensate for the loss in conducting cross-section (Seeling, 1998). An elevated abundance and higher diversity of endophytic bacteria (78 different species) was found in stems with abnormal heart (Schmidt & Mehringer, 1989). Also the numbers of bacteria colonies were elevated, and dispersed all over the cross-section, and not only in the colored parts. Lower numbers and diversity of endophytic bacteria were also found in trees without heartwood. Since high numbers of bacteria can also be detected in wet cores of other tree species (Butin, 1995), it is not yet clear if bacteria are the cause of the discoloration or if their abundance is just a secondary phenomenon accompanying heartwood formation or injury. Bacterial metabolism in the margin of abnormal heart can cause drastic change in pH of the capillary water in the infected regions, with pH either decreasing or increasing depending on the metabolism the colonizing bacteria. A decreasing pH can result from a degradation of sugars to acids such as malic-, 2-oxypropion-, acetic-, α-ketoglutaric-, lactic-, butyric- and propionic acid, while the pH can increase up to 9 by aerobic production of ammonia from nitrogen-compounds (Schmidt & Mehringer, 1989). An alkalinization of the sap above a pH of 7.3 can result in a darkening of the capillary fluids by a reversible phenolic oxidation followed by an irreversible condensation to high-polymeric substances. Discoloration caused by bacterial activity can be

far more intense than discolorations caused by oxidation processes and are, according to several authors a characteristic trait of abnormal heart (Seeling & Sachsse, 1992).

3.1.1.5 Wound heart

As the name implies, the formation of wound core is normally initiated by injuries of the stem (Sachsse & Ferchland, 1988). Wound tissue in stems of *Taxus baccata* is cytologically and biochemically very similar to wood on the sapwood/heartwood-boundary (Kucera, 1973). In beech the wound core is generally of very limited extension and not located at the center of the stem, but forms a protective layer around injuries. Wound hearts provide protection by initiating the formation of tyloses, to stop infections from entering the water transport system, and due to their relatively small area do not impair water transportation in the stem. The ascending sap stream is simply diverted around the affected parts (Sachsse & Simonsen, 1981; Seeling, 1998). tried to trigger formation of red and splash hearts by deliberately injuring beech stems, but only noticed the formation of small wound hearts in the vicinity of the injuries. These wound cores were not located at the center of the stem, were faint in color, and of limited spatial extend. They only expanded approximately 25 cm above the wound and were characterized by strong formation of tyloses, isles of living parenchymous cells, and no significant fungal infections. Rapidly progressing brown discolorations (up to 10 cm above and below the entry wound) were found in the vicinity of bullet channels after firing at beech trees with rifles of different caliber (Schute, 1986). The wound heart area increased with the size of the debris and the age of the wound.

3.1.1.6 Factors influencing facultative heartwood formation

According to empirical studies, multiple factors can influence probability and magnitude of discolored heartwood in beech. Numerous studies dealt with influences of tree, stand and forest properties on abundance or magnitude of facultative heartwood, but because of the sometimes ambiguous nomenclature, not all results could be assigned to the four distinct types of heartwood mentioned above. In most studies various types of colored hearts were classified as red heart.

A positive correlation between DBH (diameter at breast height) and abundance and diameter of red heart has been found by many authors, e.g. by (Racz, 1961; Mahler & Höwecke, 1991; Frank, 1996; Klemmt, 1996; Denstorf, 2004). Beginning with a DBH of 40 cm, the general tendency towards red heart rises significantly, and beech trees without visible signs of heartwood formation can only be found up to a DBH of 80 (Frank, 1996).

A similar dependency was found between tree age and red heart, e.g. by (Zycha, 1948; Mahler & Höwecke, 1991; Walter & Kucera, 1991; Seeling & Sachsse, 1992; Frank, 1996; Klemmt, 1996; Frommhold, 2001; Denstorf, 2004). Older trees often show a higher ratio of

colored heart than young trees of the same DBH (Frank, 1996; Klemmt, 1996). According to other studies, the red heart formation normally starts between 50 and 80 years (Necesany, 1969), and forest managers can expect a constant reduction in wood quality from an age of 100 years on, which is caused by a constantly increasing diameter of red heart (Walter & Kucera, 1991). The increase does not stagnate until an age of 140 years. Ecological modeling confirms that the tree age poses as the most influencing factor on heart formation (Knoke, 2002; Knoke, 2003). Although some authors e. g. (Racz, 1961), found only a weak correlation between heart formation and tree age, the predominant theory today states that the formation of red heart is mostly an effect of cellular ageing with loss of parenchyma vitality and exposure of stored phenolic substances to oxygen being minimum requirements. With age not only the vitality of the parenchyma decreases, but the number of potential entry ports for oxygen rises, resulting in a higher probability and larger extent of red heart (Racz, 1961).

Possible entry ports for oxygen are root injuries, stem injuries like hauling damage, falling damage, frost cracks, sunscald, rock fall, hail damage, browsing and fraying damage, insect damage, gunshot wounds, crown damage caused by snow and wind, and the severance of dead and rotten branches (Frommhold, 2001). Beech bark is generally thin and brittle and provides rather little protection to the stem, as a consequence of which small bark fissures caused by temperature fluctuations, frost or mechanical stress are very likely to occur in any individual's lifetime (Kucera & Pohler, 1998). A clear relationship was found between wounds and the shape of red heart (Kucera, 1991), which can also be influenced by the existence of knots in the stem (Wernsdörfer *et al.*, 2005).

Probabilities of red heart formation are higher in bifurcating trees (Klemmt, 1996) and red heart was marginally larger in circumference in bifurcated trees (Frommhold, 2001). Typical entry ports for oxygen (e. g. dead branches, broken branches or injuries) are not significantly correlated with the abundance of red heart (Hupfeld *et al.*, 1997), though (Krempl & Mark, 1962) found trees with a higher ratio of dead and rotten branches to have a higher abundance of red heart and also larger red heart diameters close to rotten branches. The red heart diameters also expanded in direction of the entry port. Thus, forest engineers could probably restrict the expansion of red heart by continuous thinning and removal of dead and rotten branches (Rieder, 1997).

Crown structure influences heartwood formation, and trees with flag-formed crowns are less vulnerable to red heartwood formation, than trees with broom-formed crowns (Necesany, 1969). A healthy, well-lit crown can maintain a high rate of assimilation and is better protected against natural injuries from broken branches. It was also suggested that the susceptibility to heartwood formation could be genetically determined (Necesany, 1969; Lampson, 1992), but no evidence has been produced yet. Trees with a high crown onset are

predetermined for red heartwood formation (Seeling & Sachsse, 1992), while other factors that promote red heart formation were identified as great tree height, large stem diameter and short crowns (Torelli, 1979). Trees with big and healthy crowns exhibit a higher probability of red heart, although the diameter is small (Seeling & Becker, 2002).

Stand influences on heartwood formation are heavily discussed. While some authors e. g. (Klemmt, 1996) claim that the abundance and magnitude of red heart increase on inferior stands, Krempl & Mark 1962 found the opposite, (Torelli, 1984) found good stands and high population density to be positively correlated with red heart and Klein (1992) reports that trees on exposed stands show a higher frequency and magnitude of red heart formation. Water stress was found to be of promoting both red heart (Walter & Kucera, 1991), and irregular heart (Mahler & Höwecke, 1991). An increased abundance of red heart was detected in beeches growing on alkaline stands (Walter & Kucera, 1991; Denstorf, 2004). (Knoke, 2003) however, claims that site factors do not influence the formation of heartwood at all.

An influence of environmental factors (e. g., pollution or acid-rain) on the formation of red heartwood could not be confirmed, but a higher pH in the capillary water, accompanied by reduced activity of parenchyma cells and disturbance in the water supply were found in affected individuals (Rademacher, 1986). A reduced ability of the cells to store starch and a higher abundance of tannins in the parenchyma cells was also found in affected trees. Since these factors may be prime prerequisites for heart formation, an effect of pollutants is not implausible and warrants further research.

3.1.1.7 Physical properties of red heartwood

Although beech lumber is not very enduring, and even small injuries of the standing tree can lead to infection with white rot (Kucera & Pohler, 1998), red heart exhibits an enhanced endurability towards wood decaying fungi (Molnar *et al.*, 2001). It is suggested that the formation of tyloses blocks the vessels and possible pathways for micro-organisms, which stops the spread of infection but also causes a reduction in impregnatability and sorption properties, alongside an increase in drying time (Walter & Kucera, 1991). Light sapwood and red heartwood do not differ significantly in dry density, radial and tangential shrinkage and compressive resistance (Torelli, 1979). The dry density of irregular heart decreases towards the cambium, while the dry density of red heart increases from the center of the sapwood to the cambium. The dry density of irregular heart is generally lower than the dry density of red heart (Seeling & Sachsse, 1992).

3.1.1.8 Detection of colored heartwood in standing trees

An early recognition of red heart formation in beech trees would help forest management to increase revenues by silvicultural measures and the selection of trees to be felled. Unfortunately, there is presently no way to accurately predict the presence or extent of red heart in the standing tree and the correlation between the visual assessment of its health (e. g. abundance of possible entry ports like dead branches or bark wounds) and red heart is weak (Richter, 2001). Several recent publications deal with the methodology of nondestructive assessment of wood quality e.g. (Gruber, 2001; Hanskötter, 2003), but up to now no method is sufficiently accurate and versatile to use in the field enough (Seeling *et al.*, 1998).

3.1.1.9 Discoloration effects during storage of beech lumber

Discoloration during improper storage is a common phenomenon in modern forestry and can lead to enormous devaluation of beech lumber (Trübswetter, 1995). The distinct discolorations of beech wood, which usually emerge during longer storage, are similar in appearance to those occurring in the standing tree. They are also believed to have similar influencing factors, which can either be biotic e. g. infection with fungi or bacteria, or abiotic e. g. oxidation of tannins and other phenolic substances in the parenchyma cells (Schmidt, 1994; Koch *et al.*, 2000; Koch *et al.*, 2002).

Oxygen seems to be crucial for the distinct discolorations in beech. Exposed to ambient oxygen during storage, the color of fresh beech lumber is altered from its natural light and yellowish color to a darker tone, which often exhibits reddish and grayish, lamellar patterns of discoloration. This particular effect is often referred to as "Einlauf" or "Suffocation" by forest professionals and it is believed to be caused by stress reactions of dying parenchyma cells, a process similar to facultative red heart formation in the standing tree. After cutting the deceasing parenchyma cells form tyloses and increase the synthesis of phenolic substances from starch and soluble sugars. The subsequent oxidation of the phenolic substances to highly polymerized colored substances causes the pronounced discolorations found in stored beech wood, whose intensity is correlated to the abundance and activity of living parenchyma cells in the stem. Peripheral areas of the stem can still be physiological very active at the time of the cut, where large amounts of starch are often stored in the parenchyma cells. This allows the cells to remain vital for weeks after the cut and makes stored beech lumber susceptible to discoloration for a long time (Bauch, 1984; Koch *et al.*, 2000; Koch *et al.*, 2002; Koch, 2004).

Apart from the presence of oxygen, the wood moisture content is another important factor. The formation of tyloses only occurs between wood moisture of 50% and 80% (% / dry wood mass) (Liese, 1968). However, the moisture of the log also regulates the influx of oxygen

itself because the gas diffuses at a significantly reduced rate through water and can penetrate water filled vessels only at a very slow rate (Chapter 1). During the drying of wood the water content decreases and oxygen diffuses into the log, hence the area of potential discoloration expands (Bonsen, 1991). Storage in an oxygen-free environment proved to be very effective in preventing discolorations of beech lumber, which can either be achieved by storage under water (Höster, 1974) or by storage under an atmosphere of inert gases such as nitrogen or carbon-dioxide (Mahler, 1991; Maier *et al.*, 1999).

In addition to oxidative processes, colonization of the lumber by micro organisms occurs during longer storage. This invasion of bacteria and wood decaying ascomycota (moulds) leads to intense and widespread discolorations ("Verstockung", "Stockflecken"), which is often accompanied by a degradation of wood components that eventually results in a deterioration of the mechanical properties of the affected lumber. Infestation with white rot (*Trametes versicolor*) often occurs during long-term storage and leads to massive degradation and intensive discolorations. The rate of infection is regulated by wood moisture content of the substrate, with an optimum between 40% and 120%. Hence, a reduction of the wood moisture below 40% by drying, or an increase of water content above 120%, achieved by irrigation of the freshly cut stems in the field or by storage under water, helps to prevent potential infection processes (Bauch, 1984; Schmidt, 1994; Koch *et al.*, 2000; Koch *et al.*, 2002).

A third possible source for discolorations in beech wood can be chemical reactions between tannins or other phenolic substances (e.g. catechine, taxifolin) with metal ions, resulting in grey to blue-black, intensely colored complex compounds (Di- and Tri-Brenzcatechineferric acetate). The intensity of these discolorations is influenced by the concentration of iron ions, stem water content and the pH of the wood (Bauch, 1984; Koch *et al.*, 2000; Koch *et al.*, 2002). Discolorations of that type can be prevented by avoiding contact between lumber and ferruginous metal.

Generally, all discolorations in beech wood, whether they are caused by infection with microorganisms, or by oxidation processes, are (bio)-chemical processes that are strongly influenced by water content and temperature of the substrate. The practice of felling and subsequently storing trees during winter, when temperature and wood moisture are low, helps to prevent discolorations of beech lumber to a great extent (Aufsess, 1974).

3.2 Objectives

The currently predominant theory suggests that discolorations associated with red heart formation are caused by oxidation processes when oxygen enters the central parts of the stem through wounds in the root or crown area, while bacteria and fungi are not involved (Knoke, 2003). However, a literature review shows that so far there is no hard evidence ruling out micro-organisms as a cause of red heart formation.

While there is little doubt that the oxidation of phenolic substances causes the discolorations, the possibility that oxygen disperses within the stem is still questionable. The living parts of the sapwood have to be supplied with oxygen, which is either transported horizontally through the bark or vertically with the transpiration stream. So far only few oxygen measurements in standing trees were conducted (Eklund, 1990; Eklund, 1993; Eklund *et al.*, 1998; Eklund, 2000; Gansert *et al.*, 2001; del Hierro *et al.*, 2002; Gansert, 2003) and only a single publication describes a radial profile in stem oxygen levels, where a constant decrease in oxygen from the sapwood towards the pith exists. However oxygen values never fall below 14 - 23% (Spicer & Holbrook, 2005), and only (Eklund, 2000) found oxygen concentrations around 5% in drought-stressed spruce (all oxygen values are % / air saturation, or stated otherwise). Only at such low oxygen levels the vital functions of cells are noticeably reduced (Spicer & Holbrook, 2005). Since there is no barrier for oxygen diffusion between sapwood and heartwood and only the living sapwood consumes O_2, it is not very plausible that under normal conditions the heartwood is in a state of near anoxia. The hypothesis that oxygen, entering the stem through singular injuries, initiates red heart formation further implies that O_2 diffuses several meters in axial direction within the stem, without diffusing several centimeters in radial direction to the sapwood, where it would be consumed.

According to several authors e.g. (Bosshard, 1967; Ziegler, 1967; Koch, 2004) red heart is solely formed by living cells at the sapwood/heartwood margin. However, the existence of not discolored dead uncolored heart makes that theory not very plausible. Phenolic compounds are enclosed by cell membranes after their polymerization and they cannot disperse radially along the cross-section of the stem. But so far no beeches were found featuring light, not discolored central stem areas surrounded by rings of red heart. If living parenchyma is a prerequisite for red heart formation, red heart or dead uncolored heart should show no signs of further discoloration unless there are other factors (e.g. colonization by bacteria or fungi) involved in the process.

The main objective of this study was to scrutinize the predominant theory of red heart formation, particularly the importance oxygen. Although much research was conducted in the past to pinpoint potential influencing factors, so far no oxygen measurements were conducted to confirm the basic hypothesis. Oxygen determination based on fluorescence

quenching (Chapter 2.3.1) allowed the measurement of oxygen in the stem of standing beeches for the first time.

An important prerequisite for measuring oxygen in stems was to assure that oxygen does not diffuse along the sensor into the stem reducing the gradient in intact wood. Since oxygen diffuses rather slowly through wood in radial direction (Chapter 1), even a tiny crack would suffice to eliminate an existing gradient. Thus, preliminary experiments were conducted to prove that a radial oxygen gradient can be measured in beech wood (Chapter 3.2.1).

If the penetration of oxygen through injuries in the crown or root area causes red heart formation in the central stem, unscathed, healthy individuals should show a decrease in oxygen along the radial gradient from bark to pith and the inner wood should experience more or less constant anoxia (~0% oxygen) where no oxidation processes occur. By contrast, mature trees with red heart should show a higher oxygen concentration in the central stem area.

Since holes are drilled into the stem provide an easy entry point for oxygen, drilling would lead to a rapid read heart formation (probably noticeable within a single vegetation period) in the area surrounding of the holes.

If red heart is formed because oxygen enters and diffuses through the stem, one would expect:

H1 Higher oxygen concentrations in trees with red heart than in trees without

H2 In large beech trees the dead uncolored heart has generally very low oxygen concentrations

H3 Oxygen penetrating the stem through previously drilled holes in the stem initiates local red heart formation in the tissue surrounding the hole.

It has also been suggested that heartwood forms in trees when oxygen concentrations are too low to support sapwood respiration. If this is correct, then:

H4 Oxygen levels along the stem cross-section should be sufficient to support aerobic respiration for the living cells under normal condition. They can however drop to very low levels under certain circumstances, especially in the inner sapwood area.

If bacteria and fungi participate in red heart formation, it was presumed that:

H5 Sterilized light wood does not darken, while non-sterilized light wood does and non-sterilized red heart shows further discoloration after exposure.

These hypotheses were tested by long-term measurement of oxygen concentrations in standing beech stems (Chapter 3.4.2), by point measurements of oxygen in stems with and without red heart (Chapter 3.4.1), by drilling holes as entry points for oxygen and observing color changes in the surrounding wood (Chapter 3.4.5), and by observing color changes in

wood stored under different oxygen concentrations and sterilized and non-sterilized wood (Chapter 3.4.4).

3.3 Material and Methods

3.3.1 Preliminary experiments

Holes of different depths were drilled into a block of freshly cut beech wood (9 x 10 x 6 cm), which included both sapwood and red heartwood. Aluminum tubes were pounded into the holes as in later field experiments. The tubes were closed gas-tightly with silicone septa (Chapter 2.3.7.1.2) through which the oxygen sensors were inserted. For the first test (A), the aluminum tubes were inserted normal to the direction of gas diffusion, in the second test (B) parallel to the direction of diffusion, resembling the setup und natural conditions in the standing tree (Fig. 30). The block was sealed gas-impermeable on four sides, one side was exposed to the ambient air and one side was flushed with either pure oxygen or nitrogen. If there is no oxygen diffusion along the metal tubes, an oxygen gradient should be measured by the sensors in the tubes, as was found (Fig. 31).

Results of the preliminary experiments clearly indicate that the insertion method for oxygen sensors was gas-tight and suitable to measure in-stem concentrations.

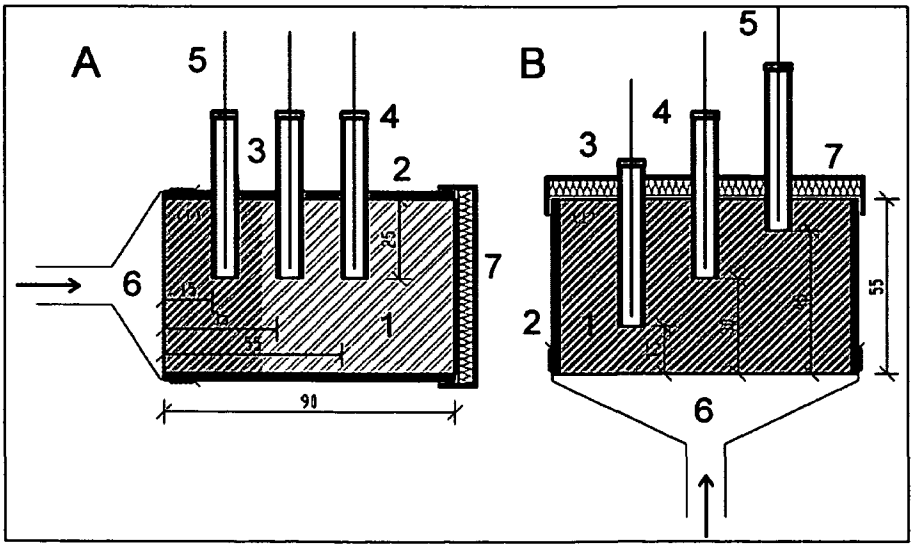

Fig. 30: Preliminary experiment to test oxygen diffusion in beech wood. A: sensors normal to, B: sensors parallel to the direction of diffusion, resembling natural conditions. The block (1) was sealed gas-impermeable with acrylic sealing compound on four sides (2). Thick-walled aluminum tubes (3) were pounded into previously drilled holes and sealed off with silicone septa (4). Oxygen sensors (5) were inserted through the septa into the tubes. A gas concentration gradient is built through flooding of a setup (6) with wet nitrogen or pure oxygen. The side exposed to the ambient atmosphere (7) is protected from desiccation with wet cotton and aluminum foil. Measures in the graph are mm.

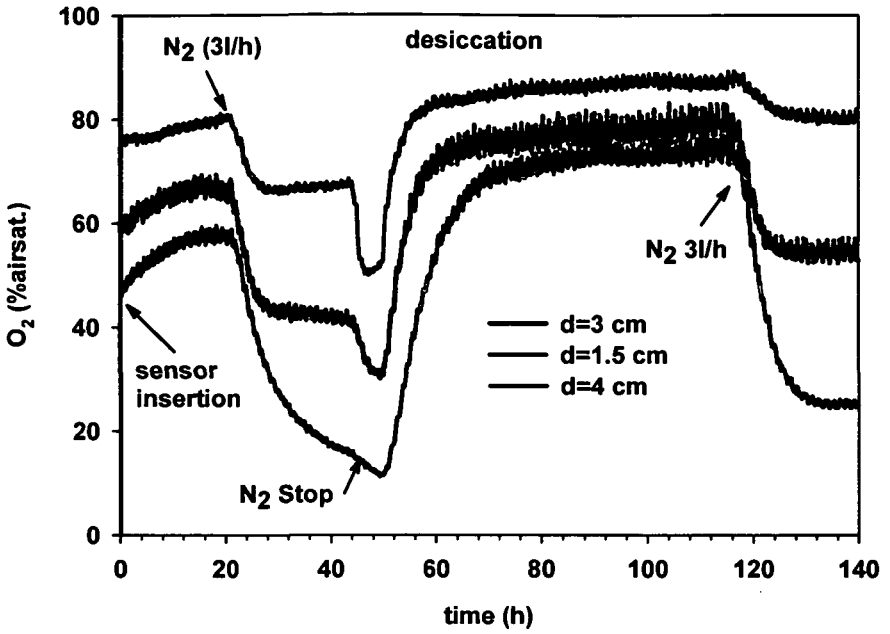

Fig. 31: Measured oxygen profile of a beech wood block (Experiment B): An oxygen gradient was recorded almost immediately after inserting the sensors. Following the external gradient the measured oxygen concentration decreased with distance from the side exposed to the ambient air. When one side was flushed with pure nitrogen, oxygen started to decline at all depths. As the wood slowly dried, the speed of diffusion increased because of the higher permeability of dry wood (Chapter 1). A second flooding with nitrogen gave similar results.

3.3.2 Plant material and field site

Eighteen mature beech trees were selected from a natural forest in Wolfsgraben near Purkersdorf, Lower Austria (48° 9′ 0′′ N, 16 ° 7′ 0′′E, 323 m a.s.l.). The experimental site is part of the Biosphere Reserve Wienerwald and is located on a westward slope. Tree diameters at breast height were between 28 cm and 58 cm. Before the experiments, the trees were cored with an increment borer (diameter Ø = 8.5 mm) along the tree radii at several heights to determine the existence and the dimensions of red heart in the stem (Tab. 3). Five trees with colored heartwood (Nr. 1, 2, 4, 16, and 18) and six trees without colored heartwood in the core (Nr. 3, 6, 7, 10, 11, 12) were selected for oxygen measurements at different radial depths. To investigate the influence of exposure to various levels of oxygen on the formation of red heartwood, cores from seven trees, which did not show any clear evidence of heartwood formation (Nr. 5, 8, 9, 13, 14, 15, and 17) were selected for color comparison tests and anatomical examinations in the laboratory (Chapter 3.4.4 and 3.4.5). The holes in these individuals were not sealed after coring, to investigate the influence of ambient oxygen levels on the formation of red heartwood in situ. During two growing seasons, from April to October 2005 and from April to September 2006, the concentration of oxygen in the stems was determined once a week (Chapter 3.4.1). Additional long-term measurements to examine the diurnal variations in the oxygen concentrations, accompanied

by sap-flow measurements were made between June and October 2005 and from April to September 2006 (Chapter 3.4.2). For the duration of the experiments, local micro climate was recorded on site.

Ind. Nr.	Heartwood (yes/no/ambiguous)	Drilling hole depth (cm)	Sapwood depth (cm)	DBH (cm)	Height of drilling hole (cm)	Special trait
1	yes	20.5	17	38	96	
2	yes	22	17	45	95	Lower part of stem injured
4	yes	28	18.5	51	69	Bifurcation
16	yes	26	21	53	71	Stem cross-section not circular
18	yes	17.5	15	37	85	Dark heartwood
3	no	22	22	34	92	Intense sap effluence after insertion of tube
6	no	22	22	33	110	
7	no	22	22	41	105	
10	no	28	28	59	89	
11	no	18	18	35	104	
12	no	24.5	24.5	56	104	Tree located in a ravine, near the waterline
5	ambiguous	21.5	n.a.			
8	ambiguous	23	n.a.	42	91	
9	ambiguous	15	n.a.	29	92	
13	ambiguous	25.5	n.a.	55	109	
14	ambiguous	25.5	n.a.	52	91	
15	ambiguous	21	n.a.	39	100	Intense sap effluence after insertion of tube
17	ambiguous	24	n.a.	49	87	

Tab. 3: Dimensions and special traits of the beeches selected for oxygen measurements

3.3.3 Oxygen probe tube installation

Several metal tubes of various lengths were installed permanently in each of the eleven trees selected for oxygen measurement. Iron tubes (diameter Ø = 9 mm, wall thickness Ø = 1.5 mm) were cut into specified length and treated with adhesive grease (TecLine, Beko GmbH, Monheim, Germany) to avoid oxidation. The tubes were pounded into the drilling hole to the desired depths, but leaving the last the inner 1 cm of the hole free, which results in an area of ca 4 cm^2 of wood exposed to the gas space. A smaller tube (Ø = 5 mm, Ø = 1 mm) with the same length was inserted into the initial tube to decrease the overall gas space in the drilling

hole, which should result in a faster exchange of oxygen between wood and the gas space. Each tube was protruding ca. 2 cm from the stem and was sealed with a silicone rubber septum, which allowed the small gas space inside the tube to be accessed periodically with a needle-tipped optode oxygen probe. The septum was attached to the tube with superglue and epoxy-resin based 2-component adhesive (UHU Endfest 300, UHU GmbH, Bühl/Baden, Germany). Between and during the measurements the septum was additionally luted with polymer sealant (Teroson, Henkel, Heidelberg, Germany). In 2006 stainless steel tubes of similar dimensions were installed to replace the previously installed iron tubes, in order to avoid oxidation.

3.3.4 Oxygen measurements

Oxygen concentrations in the stem were measured using the novel Microx system with micro-optode sensors (Microx TX3-AOT, Presens GmbH, Regensburg, Germany; Chapter 3.2.1). The fiber-optical sensors connected to the oxygen meter were housed in thin needles (length l = 5 - 20 cm, diameter Ø = 1 mm), allowing easy access to the headspace behind the septum and minimizing the influx of atmospheric oxygen into the tube before and during the measurement. Oxygen was measured in % / air saturation (i.e. 100% = 20.9% [O_2]). Within-stem O_2 readings stabilized within 1 - 5 min. of insertion through the septum. The temperature sensor (Pt-1000) needed for temperature compensation of the oxygen measurement was inserted into holes (l = 25 mm Ø = 5 mm) drilled into each stem.

All probes were calibrated in the laboratory shortly before the actual measurement using a two-point calibration scheme with a N_2 gas standard (0% / air saturation) and ambient air (100% / air saturation). Readings of ambient air O_2 (corrected for temperature) between within-stem O_2-readings allowed for frequent checks of the calibration in the field. During long-term measurements the validity of the calibration was additionally checked in the almost O_2-free headspace above a saturated Na_2SO_3-solution. In 2006 pressurized N_2 from a portable container was used to check the calibration, to re-calibrate in the field, and for additional leak testing (Chapter 2.3.7.2). For short time measurements the oxygen meter was connected to a laptop-PC, and oxygen values were recorded directly in 5 sec. intervals. To determine the diurnal variations in the oxygen concentration in the stem, up to four different depths in the sap- and heartwood of a single tree were monitored continuously for several days, using a CR10X datalogger (Campbell Scientific, Logan, UT, USA) to trigger the oxygen measurement in 60 sec. intervals, and to read and store the analogue output signal of the TX3-AOT. Datalogger and oxygen meters were powered by 12V DC lead-acid rechargeable battery packs (Sealake LTD, Zhejiang, China). A detailed description of the measurement of oxygen with an optode sensor can be found in Chapter 2.3.1 and in (Gansert *et al.*, 2001)

3.3.5 Reliability testing of the measuring equipment

A potential source of errors during continuous oxygen measurements was the potentially sensitive electronic equipment (oxygen meter, datalogger) that was stored for long times in the field. Although the electronic equipment was sheltered in a waterproof box it was sometimes exposed to extreme changes in temperature and longer periods of high humidity and the boxes were sometimes colonized by insects. Tests of the reliability of the equipment were conducted during the continuous oxygen measurements in beech nr.1 in fall 2005 (Fig.32).

Measurements were conducted in four depths and the equipment was split into two independent systems. Each system consisted of two oxygen meters connected to a datalogger. Both setups were powered by separate battery packs. The sensors of system 1 were installed at 2 cm and 5 cm, the sensors of system 2 at 10 cm and 21 cm. Sudden drops and long periods of zero oxygen were recorded with both independent systems, thus the rapid changes in oxygen and the long periods of zero oxygen were not artificial.

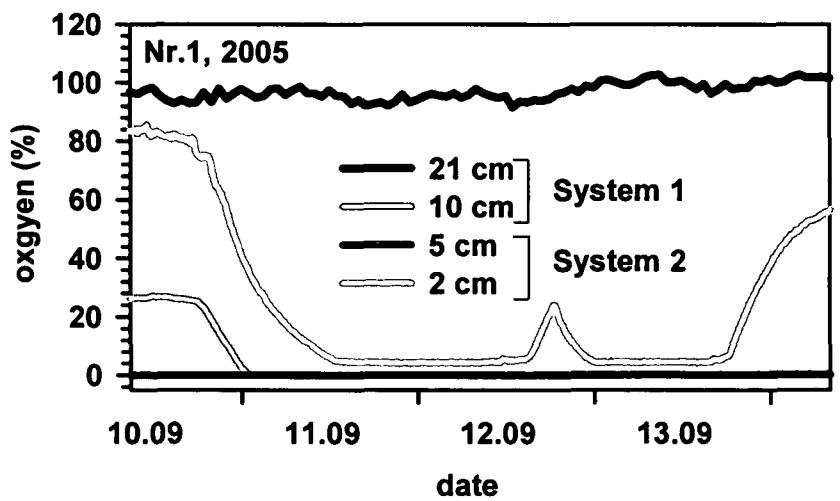

Fig. 32: Oxygen measurement with 2 independent systems. Oxygen was measured in beech Nr.1 in September 2005. System 1 measured at 2 cm and 5 cm, system 2 at 10 cm and 21 cm. Rapid changes in oxygen were recorded in sensors measured completely independently proving that the oscillations and sudden drops were not artificial.

3.3.6 Sapflow measurement

Granier-type sapflow sensors (Chapter 2.3.2.2) were used to monitor the sapflow in three different depths of the outermost sapwood (5 - 25 mm, 25 - 45 mm, and 45 - 65 mm). The sensors were constructed following the original modifications of (Granier, 1985; Granier, 1987) and were installed in August 2005 in two trees used for continuous O_2 measurement (Nr.1 and Nr.18). An additional sensor pair was installed in tree Nr.10 in April 2006. Each

Granier sensor consisted of two probes, made of thin syringes (length l = 25 mm, diameter Ø = 1 mm) housing fine-wire copper-constantan thermocouples. The upper probe's syringe was additionally coiled with constantan wire for its whole length, functioning as a heater element with a total resistance of 16.5 Ω. The two thermocouples were connected differentially so that the measured voltage difference represented the actual temperature difference between the thermocouples. Each probe was inserted into a stainless steel or aluminum tube (l = 2 cm, Ø = 2 mm) filled with thermally conductive paste, which was inserted into a drilled hole in the stem. For the installation in 4.5 cm and 6.5 cm depth, wider access holes of 2.5 cm and 4.5 cm length and 10 mm diameter had to be drilled to be able to install the metal tubes and sensors at the desired depth. The two sensors were installed with 10 cm distance and ca. 20 - 30 cm above the iron tubes for oxygen measurement. All sensor pairs were insulated with polyurethane foam and sheltered with small PVC boxes. To avoid additional heating by absorbed sunlight a reflective metal foil was wrapped around the whole setup. Three sensor pairs were connected in series to a heater circuit supplying a constant current of 120 mA, and to a CR10 datalogger (Campbell Scientific, Logan, UT, USA) for data collection. Temperature difference was measured in 60 sec. intervals, means stored every 15 min. Datalogger and heater were powered by a car battery (12V DC, 55Ah) and enclosed in a lockable weather-proof box. The standard Granier calibration (Granier, 1985) was used to convert the temperature difference between the heated and unheated probes to sap flux density (g m^{-2} s^{-1}).

3.3.7 Microclimate

Air temperature and relative humidity were measured in the canopy of the experimental site using a HMP35C sensor (Campbell Scientific, Logan, UT, USA) protected from direct sunlight by a radiation shield. Soil water potential was measured with a tensiometer probe (Theta probe ML2, UMS GmbH, Munich, Germany) buried in the soil at a depth of 30. All data were measured in 60 sec. intervals and recorded with a CR10X datalogger (Campbell Scientific, Logan, UT, USA). Sensors and datalogger were powered by 12V DC lead-acid rechargeable battery packs (Sealake LTD, Zhejiang, China) and enclosed in a weather-proof box. Additional precipitation data was provided by the provincial government of Lower Austria. Precipitation data (daily totals in mm/day) was recorded in one hour intervals by an automated measuring station located in a nearby village (Laab im Walde, Lower Austria) in a distance of approximately 3.7 km to the measuring site.

3.3.8 Color-measurements

The cores of seven trees (Nr. 5, 8, 9, 13, 14, 15, 17) where taken with an increment borer and cut in half with a band saw. Two sets of cores, (l = 22 - 25 cm), were harvested: the first set in June 2005, the second one in November 2005. Both inner core surfaces were cleaned of shavings with a razor blade, rinsed with clean water, and treated with an antifungal agent (Micropur forte, Katadyn, Wallisellen, Switzerland). Both outer core surfaces were marked in 1 cm segments with a water-proof permanent marker. Initial measurements and treatments were conducted within one-three days after sampling to avoid aging of the surface. Color intensities at wavelengths between 400 nm and 700 nm of both core halves were measured in each 1-cm segment under ambient temperature and humidity in 10-nm wave-length bands with a double beam spectral photometer with continuous illumination (CODEC 400, Phyma GmbH, Gaaden, Austria). The photometer illuminated the probe with D65 standard light through an aperture (6 mm) in a 45° angle and recorded the reflected light spectrum using the HDD-method. From the obtained data L*a*b*-values were calculated according to the CIELAB standard, described in detail in by Beckwith (1979) and Brunner *et al.* (1990), and used for comparison of wood color. In the L*a*b*-system L* represents the lightness (0 = black, 100 = white), a* the position on the (X-) axis from red (+) to green (-), and b* the position on the (Y-) axis from yellow (+) to blue (-) of a measured color (Fig. 34). From the initial L*a*b*-values additional color parameters were derived:

$$C^* = (a^{*2} + b^{*2})^{0.5}$$

$$h^* = 180/\pi \cdot \arctan (b^*/a^*)$$

$$\Delta E^* = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{0.5}$$

C* is defined as the chroma or saturation, where 0 represents only grayish colors and 60 represents very vivid colors. h* is the hue of a color, where 0 or 360° is red, 90° is yellow, 180° is green and 270° is blue. All Δ-values were calculated by subtracting the values of the initial measurement from the values of the final measurement after exposure. ΔE* is the overall color difference between measurements. (Brunner *et al.*, 1990; Resch *et al.*, 2000).

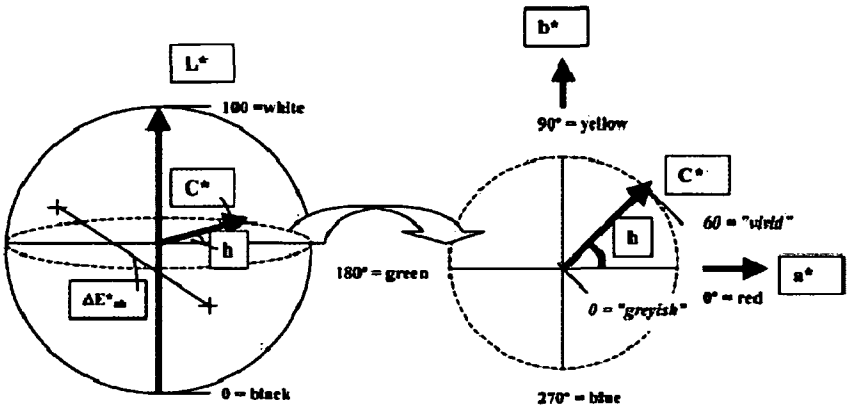

Fig. 33: The CIELAB color space and its transformation into the cylindrical color space L*C*h. Left: The color sphere where the circle of cross section at L*=50 is specified. The color difference (ΔE) is the distance between two colors within the color sphere. Right: Cross section at L*=50 showing the axis from green to red (a*) and from blue to yellow (b*), saturation (C*) and hue (h*) (Sundqvist, 2002).

After the initial color measurement the core halves were split and stored above wet filter paper in desiccators with variable oxygen concentrations (0%, 20%, 50% and 100%). The core halves from the first set were exposed to 100% and 0%, while the halves of the second set were exposed to 20%, 50%, and 100%. To test the influence of microbial activity in the discoloration process of beech wood, one half of the cores from the second set were sterilized in an autoclave before storage. Cores taken in June were split 1:1 between desiccators with 100% and 0%. Cores from November (both sterilized and non-sterilized) were cut into 8 cm sections, split and distributed so that core fragments from different depths were equally exposed in all of the three different oxygen concentrations.

Additional tests were conduced with small wood blocks cut from freshly cut lumber. Four trees previously used for oxygen measurements were felled in January 2006 and several wood disks were cut from the basal 2 m of each stem to investigate the distribution of discolored wood, the effect of coring and the abundance of micro organisms in wood of different colors. Several small blocks (6 - 8 x 2 x 1 cm) were cut from sapwood and discolored heartwood with a saw. After initial color measurement the blocks were divided into red heart (RH) and sapwood samples (SW) and the half of each group was sterilized in an autoclave. Sterilized (S) and non-sterilized (C) samples of SW and RH were split equally among two desiccators and exposed for two months to either 0% or 100% oxygen.

Oxygen levels in the desiccators were established by flooding the desiccators with various amounts of gaseous nitrogen and monitored with an oxygen meter (Microx TX3-AOT, Presens GmbH, Regensburg, Germany). The two desiccators at 100% O_2 were in contact with the ambient atmosphere, the others were sealed air-tight for the duration of the experiment. The bottom of the 0% desiccator was additionally filled with a saturated solution

of Na_2SO_3 to bind any oxygen diffusing into the container from outside. All desiccators were stored in the dark under ambient temperature for the duration of the experiment. Oxygen concentration was checked in weekly intervals with an oxygen meter and, when necessary, corrected by additional flooding with N_2. Oxygen concentrations in the 0% desiccator flooded with N_2 was very low for the duration of the whole experiment and never exceeded 3%. After one week of storage the two containers holding the first set of cores had to be opened to apply a stronger solution of the antifungal agent. After three month of storage color measurements were repeated.

3.3.9 Presence of micro-organisms in wood

To investigate the possible influence of bacterial and fungal activity on the discoloration of beech wood, endophytes were isolated from wood pieces to quantify presence and to compare the composition of endophytes between colored and non-colored beech wood. The samples used for isolation were obtained from wood disks of four trees (Nr.1*, 3, 5[a] and 15[a] - * denotes trees with colored heartwood, [a] trees with ambiguous colored heartwood -) cut in January 2006. Each tree was cut at the base, and a section of approximately 1m was cut out of the stem at the position where the tubes for oxygen measurements were installed. The section was cut into several disks 10 - 15 cm thick, which were labeled from bottom to crown. The disks were photographed for documentation and samples from unaffected sapwood (sw), red heart (rh), uncolored heart (uh) and wood affected by coring (wound heart, wh) were processed from disks where differences in wood coloration allowed a clear distinction between colored and uncolored parts (Fig.34).

From each sample two shavings were extracted under sterile conditions and transferred onto two different nutrient agar plates containing a mixture of standard agar and streptomycinsulfate to inhibit bacterial growth. A week later, two shavings were extracted from the same sample blocks used a week before, but only standard agar without streptomycinsulfate was used. All plates were incubated at room temperature for four to five weeks and then checked for fungal and bacterial growth at the Institute of Forest Pathology (University of Natural Resources and Applied Life Sciences, Vienna).

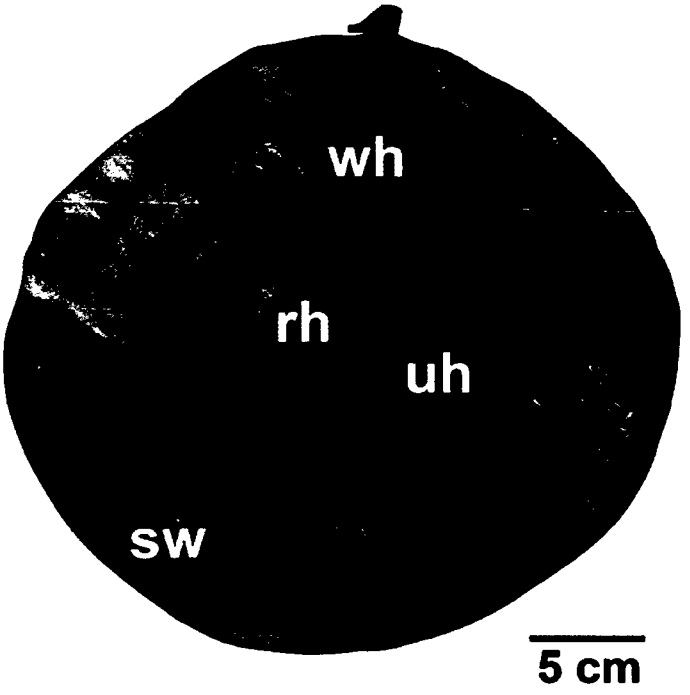

Fig. 34: Example of cut wood disk used for microbiological examinations. Small wood blocks from witch the shaving were extracted were cut from the distinctly discolored parts. sw = sapwood, uh = uncolored heart, rh = red heart, wh = wound heart. The same disks were used for the extraction of wood blocks for color measurements (Chapter 3.4.3)

3.4 Results

3.4.1 Periodical oxygen measurements

In both 2005 and 2006 oxygen values spread widely (Fig.35). A comparison between different depth showed that oxygen was lower at 5 cm than at 14 - 25 cm in trees without red heart on two out of 27 measurement dates (t-test, $p<0.05$) and once significantly higher at 5 cm than at 12 - 20 cm ($p<0.05$). In sapwood (5 cm) oxygen concentrations were significantly higher in trees with than in trees without red heart on only four out of 27 measurement dates ($p<0.05$). Oxygen concentrations at greater depth also tended to be higher in trees with red heart, though differences were not significant. Throughout the year oxygen concentrations fluctuated less in deeper layers than at 5 cm. In general, however, measured concentrations differed widely between trees and between measurement dates.

In 2005 oxygen in Nr. 1*, 2*, 6, 10, 11 showed more frequent and stronger fluctuations at 5 cm than in the center. In 2006 fluctuations were very high in both the peripheral regions and in the center without distinction. In 2006, when stainless steel tubes were used, only seven measurements were conducted to test if data was comparable with data from 2005 when iron

tubes were used. Oxygen was also measured in a previously untested specimen (Nr.19) which featured no red heart.

Oxygen levels were similar at both depths in trees Nr. 4* and 10 in 2005 and in Nr. 2*, 11, 12 and 19 in 2006, in all other trees oxygen levels differed noticeably between 5 cm and stem center.

Oxygen levels in the center generally exceeded those at 5 cm in Nr. 1*, 10 and 16* in 2005 and in Nr. 4*, 7, 6, and 14 in 2006. In Nr. 11, 6 and 12 that relation was reversed in 2005. In all cases except Nr. 1, 10 and 16* at 26 cm oxygen levels (at both depths) dropped noticeably during summer 2005 and remained low until September. In 2006 no trend was observed. In 2005 low values were determined during periods of heavy rain. In most cases oxygen dropped with the onset of rain and remained low for some time, irrespective of successive changes in weather. In Nr. 4*, 6, 7, 10, 12 and 16* periods of low oxygen were interrupted by sudden peaks. Those peaks were often recorded in several individuals at the same time and during periods of warm dry weather (e.g. 20.07, 02.09, 07.09, 14.09, 23.09 and 07.10). In two cases, however, peaks occurred during periods of cold weather and heavy rainfall (03.08, 10.08). In Nr. 4*, 7, 11 and 18* oxygen increased again in late September and early October in 2005. In 2006 a similar rise in oxygen was found in 5 cm in Nr. 12 and 7 and in 12-20 cm in Nr. 2*, 4*, 6, 7, and 11 (Fig.35). An overview of 2005 weather conditions is given in Fig.36.

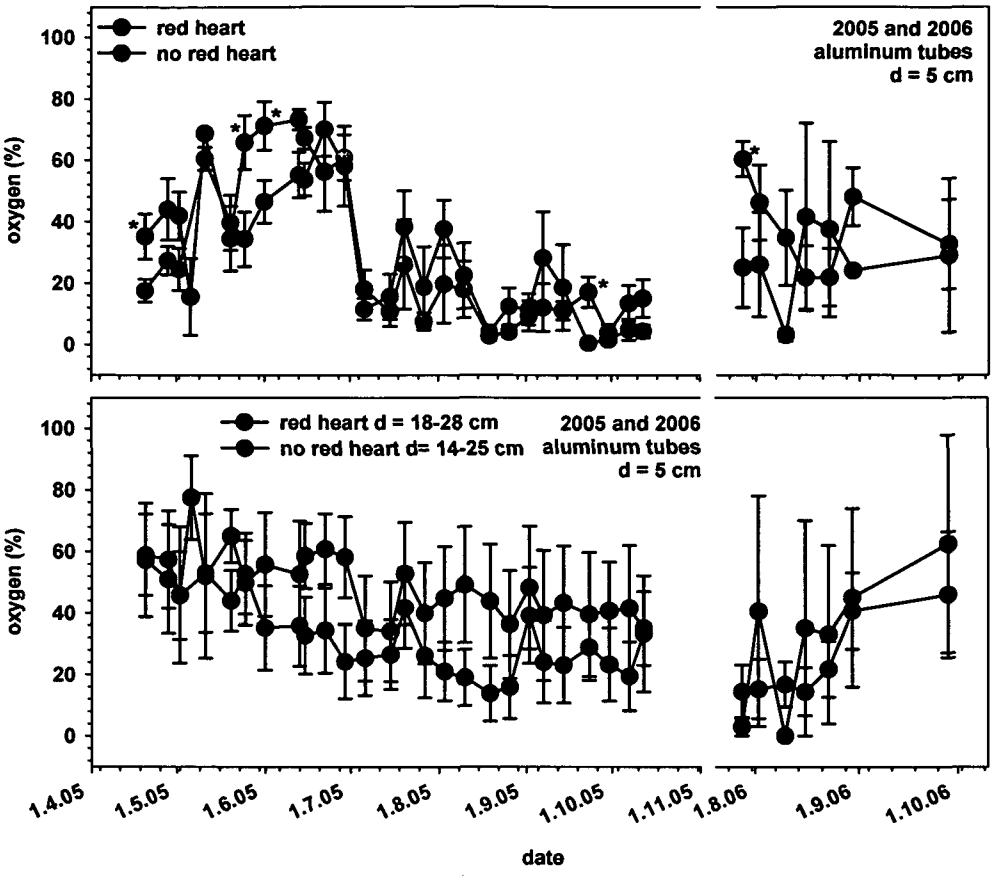

Fig. 35: Results of short term oxygen measurements in beech in 2005 and 2006. Symbols represents mean ± SE; * indicates significant differences (t-test, $p < 0.05$). Differences between the two depths were only found on 20.04 and 23.09 in 2005 and only in trees without red heart.

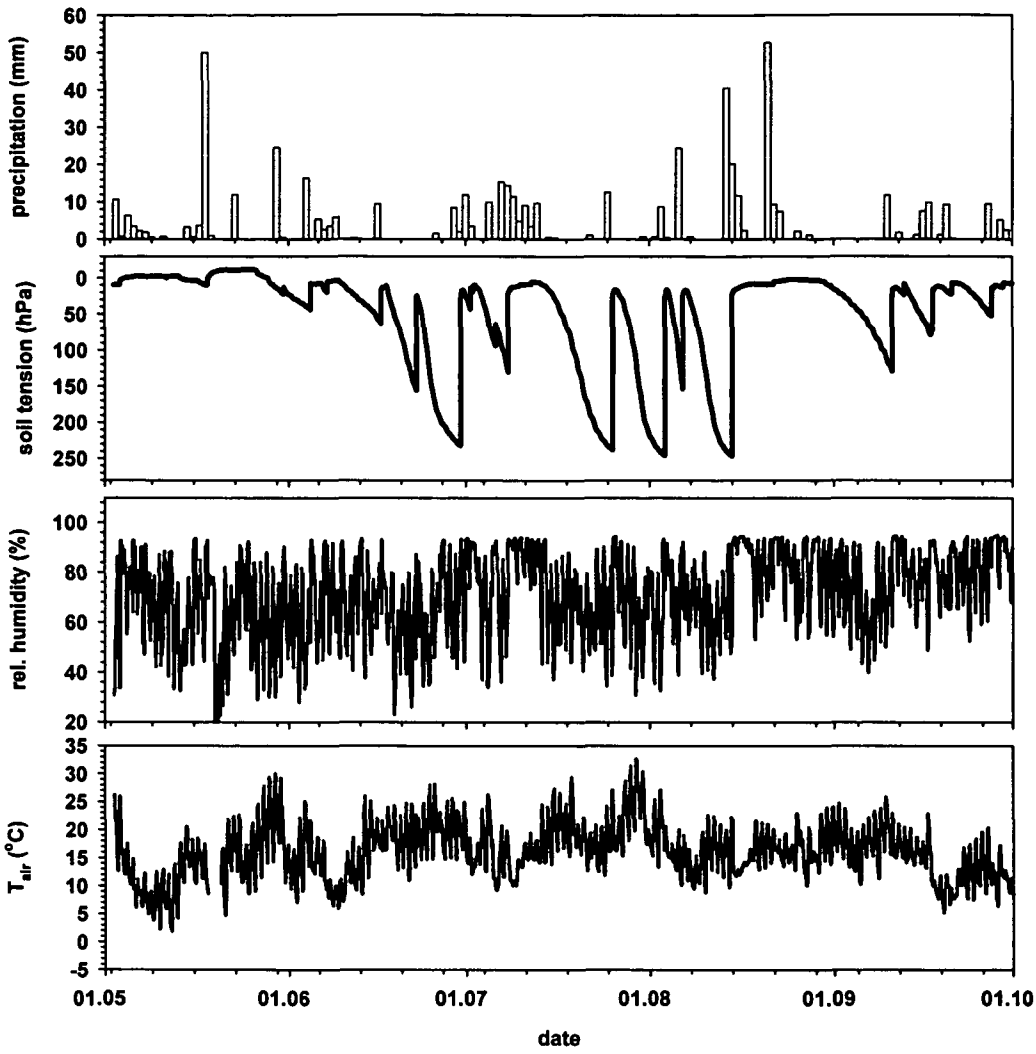

Fig. 36: Climate recorded from April to September 2005. Air temperature, relative humidity and soil tension were recorded onsite in one hour intervals. Precipitation was recorded approximately 3km from the site in Laab im Walde, Lower Austria. Each bar represents a daily total in mm.

3.4.2 Continuous long-term oxygen measurements

Continuous long-term measurements were made in trees Nr. 1*, 3, 10, 18* in 2005 and in trees Nr. 10 and 16* in 2006 (Fig.37 - 42). Differences in oxygen content in different depths were found in all individuals. Oxygen oscillated in a diurnal rhythm and reached its extreme usually between early morning and noon, generally somewhat before the peak in sapflow. Air and stem temperature reached their highest values in the afternoon. Changes in oxygen concentration were not always synchronous and sometimes changed in amplitude and frequency during measurement. Diurnal changes between 2% and 40% were recorded and higher amplitudes were more frequently found in the outer sapwood than in the heartwood. In tree Nr. 10 a gradient in amplitudes declining from sapwood to pith was recorded in 2006

(Fig.38). Maxima were also recorded later in deeper layers. In Tree Nr.16 a similar phenomenon was recorded, but oxygen at 5 cm exceeded 2 cm (Fig.41).

Sudden increases and decreases were found in all individuals (except in beech Nr.3) at various depths (Fig.43). These drops occurred mostly during times of heavy rainfall and were often accompanied by rapid increases in soil moisture. A rapid decrease was often followed by a rapid increase back to the previous level. In some cases, however, oxygen remained low for a longer period (up to 4 weeks). In September 2005 and from April to September 2006 sapflow was recorded. No obvious relationship between the sudden decline in oxygen and sapflow was found (Fig.44). Also the increase of oxygen after sudden declines occasionally happened during times of zero sapflow, and a reduction of sapflow (e.g. during longer periods of cold and rainy weather) did not necessarily lead to a decline in oxygen. Similar to the results of the periodical measurements (Chapter 3.4.1) the trees showed rather individual patterns of oxygen distribution:

In trees Nr.1* and 18* (Fig.42) oxygen in the heartwood and the outer sapwood (2 cm) was relatively high with heartwood exceeding sapwood at 5 cm. Tree Nr.3 showed a similar oxygen distribution where heartwood also exceeded sapwood at 5 cm. In trees Nr. 10 and 16* oxygen decreased from bark to pith in 2006 (Fig.38 and 41), however in 2005 the gradient was variable and even was reversed in tree Nr.10 when concentrations were highest in the heartwood and lowest at 2 cm (Fig.37). In May 2005 oxygen in 2 cm was lower than at 5 cm and 10 cm. Long periods of anoxia (~0%) where recorded at all depths, but no tree showed constant anoxia in either sapwood or heartwood. Low values were often recorded during periods of heavy rainfall in July and August 2005 and 2006.

In tree Nr.1* concentrations were around ambient levels in the heartwood (depth d = 18 cm) and oscillated between 98% and 100%. While oxygen remained around 0% at 5 cm, it fluctuated between 12% and 42% in 10 cm and between 78% and 98% in the outer sapwood region (d = 2 cm). A sudden drop at 2 cm from 80 to 4% in 20h and at 10 cm from 25 to 0% in 5h was recorded on 13.09 during a period of heavy rainfall (soil tension was 13 hPa one hour before the decrease in oxygen was recorded). Oxygen at 2 cm slowly increased back to 58% during a dry period in late September. Heartwood oxygen remained unchanged during heavy rainfall.

In tree Nr.3 heartwood oxygen (d = 22 cm) declined slowly and continuously from 87% to 66%, without any diurnal fluctuations. Oxygen at 5 cm fluctuated between 25% and 76%.

In tree Nr.10: Heartwood oxygen (d = 28 cm in 2005, d = 12-20 cm in 2006) fluctuated between 69% and 71% in 2005 and between 0% and 50% in 2006. Oxygen at 5 cm fluctuated between 22% and 45% in 2005 and between 0% and 70% in 2006 and at 15 cm between 50% and 61% in 2005 and at 10 cm between 0% and 100%. Oxygen in the outer sapwood (d = 2 cm) fluctuated between 0% and 50% in 2005 and between 0% and 80% in

2006. Several sudden drops and peaks of variable magnitude were recorded in 2005 and 2006. Longer periods of anoxia (O_2 = 0%) were recorded at 2 cm (2005) and 5 cm (2006).

In tree Nr.16* oxygen fluctuated in the heartwood (d = 12-20 cm) between 0% and 20%, at 10 cm between 10% and 90%, at 5 cm between 15% and 90% and in the outer sapwood (d=2 cm) between 0% and 95%. Several sudden drops and peaks of variable magnitude were recorded. Longer periods of anoxia were recorded at d = 12-20 cm and at d = 2 cm. An example of diurnal oxygen variations of Nr. 16 is given in Fig. 41.

In tree Nr. 18* (Fig. 42) oxygen in the heartwood (d = 18 cm) fluctuated between 0% and 95%,at 8 cm between 0% and 25%, at 5 cm between 0% and 70% and in the outer sapwood (d = 2 cm) between 0% and 90%. Several sudden drops and peaks of variable magnitude were recorded. Longer periods of anoxia were recorded in all four depths.

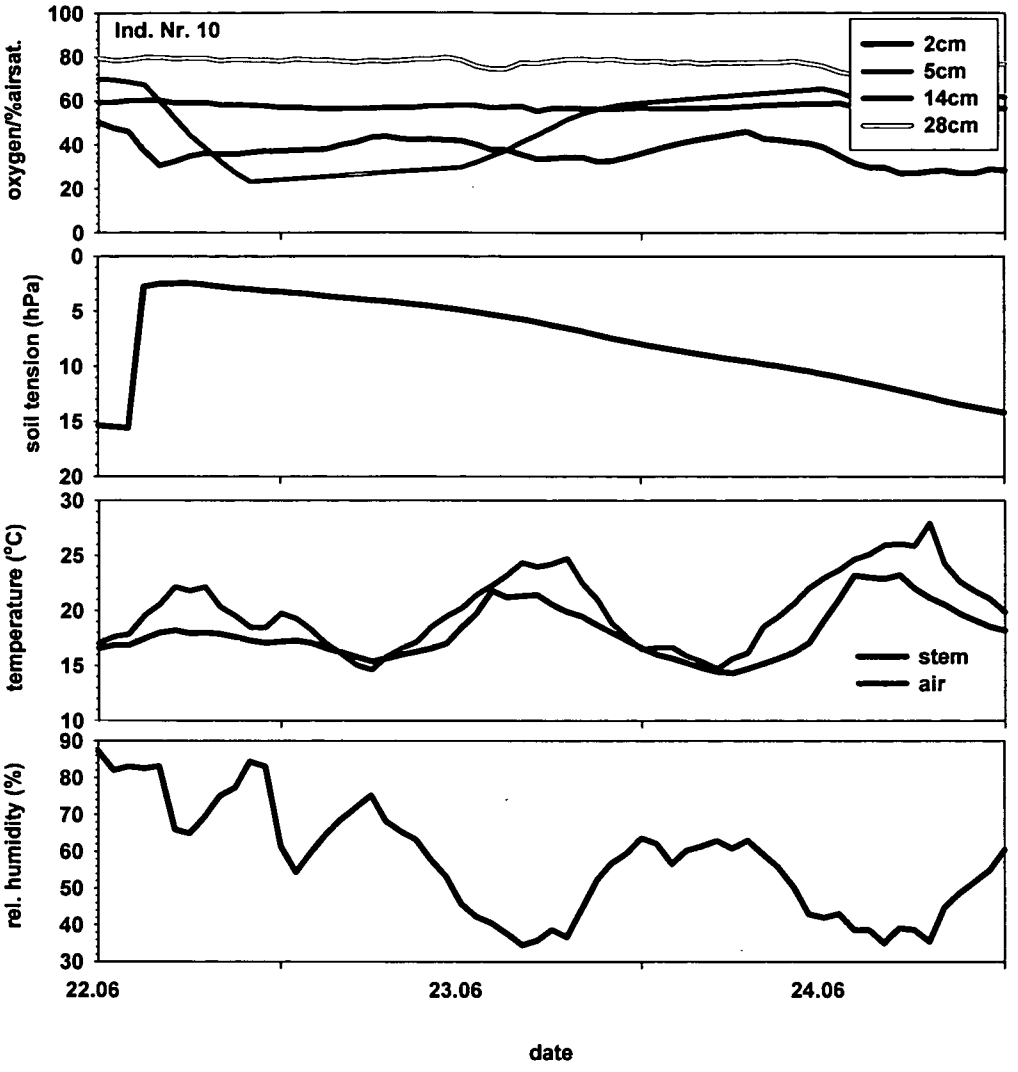

Fig. 37: Diurnal variations in oxygen recorded in tree Nr.10 from 22.06 to 24.06.2005. The solid lines are the oxygen concentrations measured in one hour intervals (d = 2, 5, 14 and 28 cm).

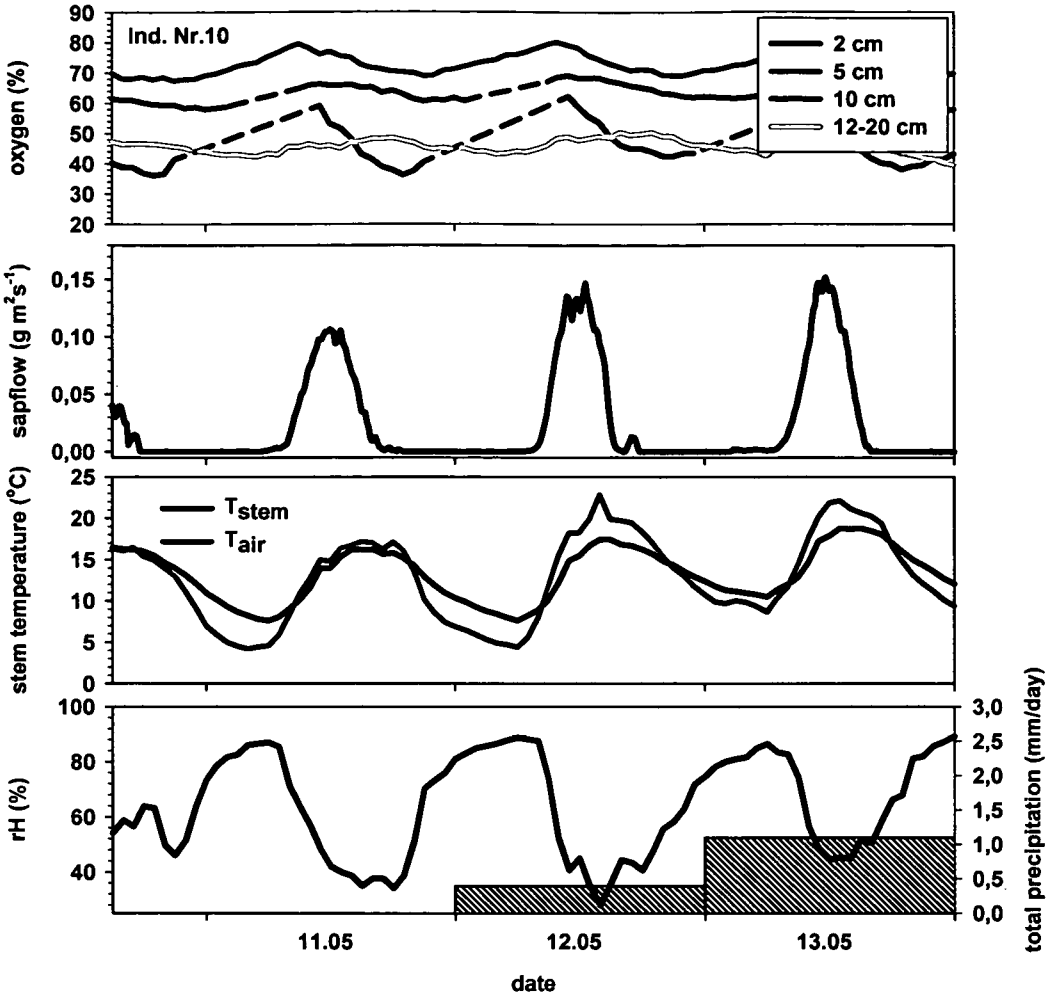

Fig. 38: Diurnal variations in oxygen recorded in tree Nr.10 from 10.05 to 13.05.2006. Oxygen concentrations were measured in one hour intervals at 2, 5, 10 and 12 - 20 cm. Dashed lines bridge intervals when measurements were interrupted. Sapflow was measured in the outer 2 cm of sapwood. Concentrations decreased towards the center, except for the outer sapwood at 2 cm. Streaked bars are total precipitation (mm/day) recorded in a nearby weather station.

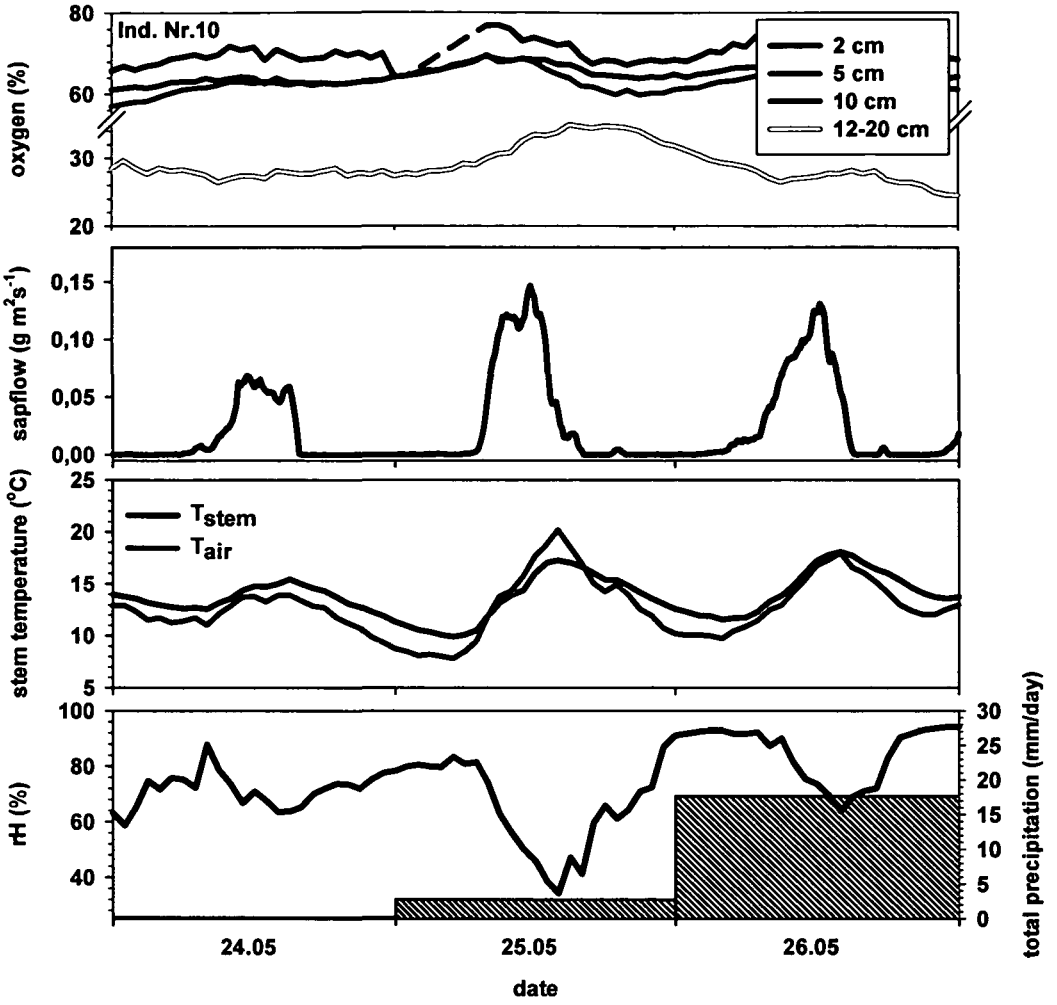

Fig. 39: Diurnal variations in stem oxygen in tree Nr.10 recorded from 25.05 to 26.05.2006. Oxygen concentrations were measured in one hour intervals at 2, 5, 10 and 12 - 20 cm. Dashed lines bridge intervals when measurements were interrupted. Sapflow was measured in the outer 2 cm of sapwood. Concentrations decreased towards the center. Streaked bars are daily precipitation totals (mm/day) recorded in a nearby weather station.

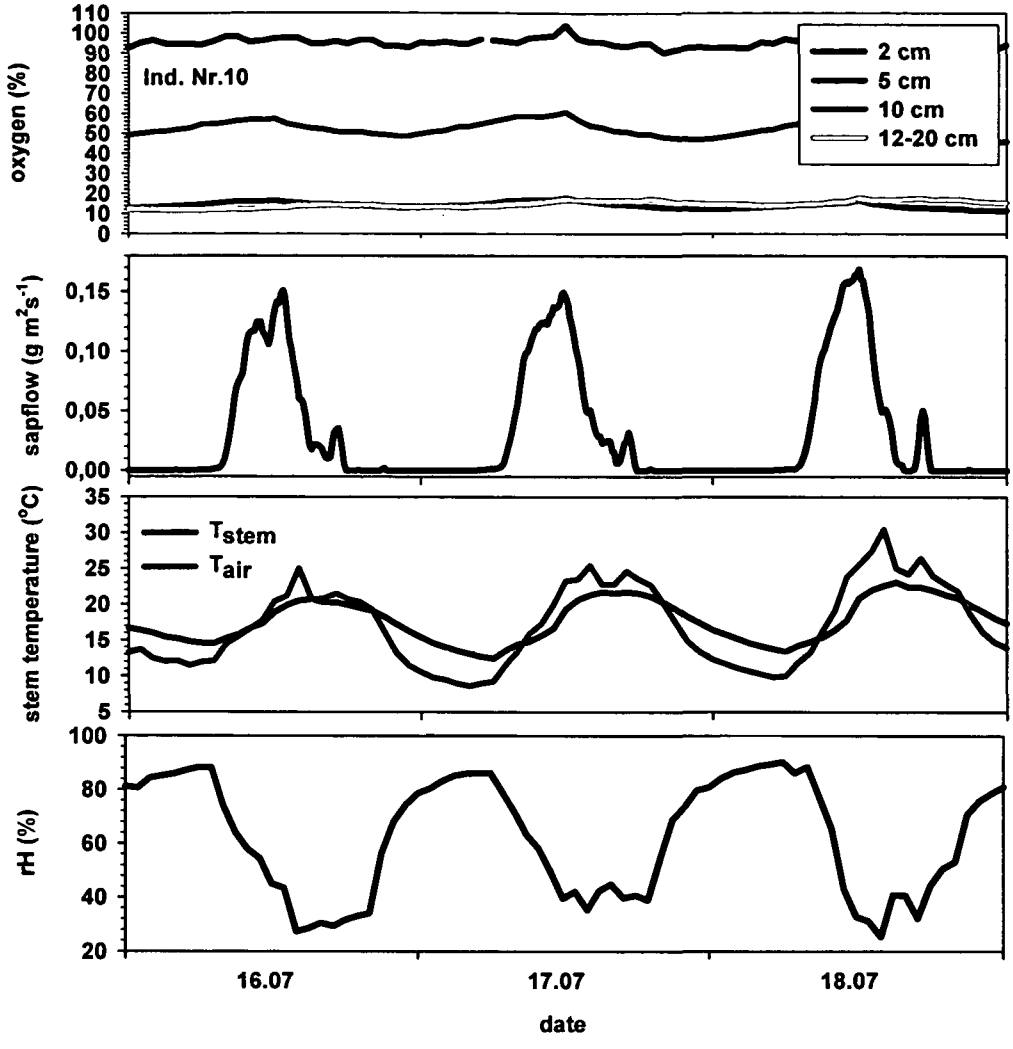

Fig. 40: Diurnal variations in oxygen recorded in tree Nr.10 from 16.07 to 18.07.2006. Oxygen concentrations were measured in one hour intervals at 2, 5, 10 and 12 - 20 cm. Dashed lines bridge intervals when measurements were interrupted. Sapflow was measured in the outer 2 cm of sapwood. Oxygen decreased towards the center and was particularly low at ≥ 10 cm. For that period no precipitation date was available.

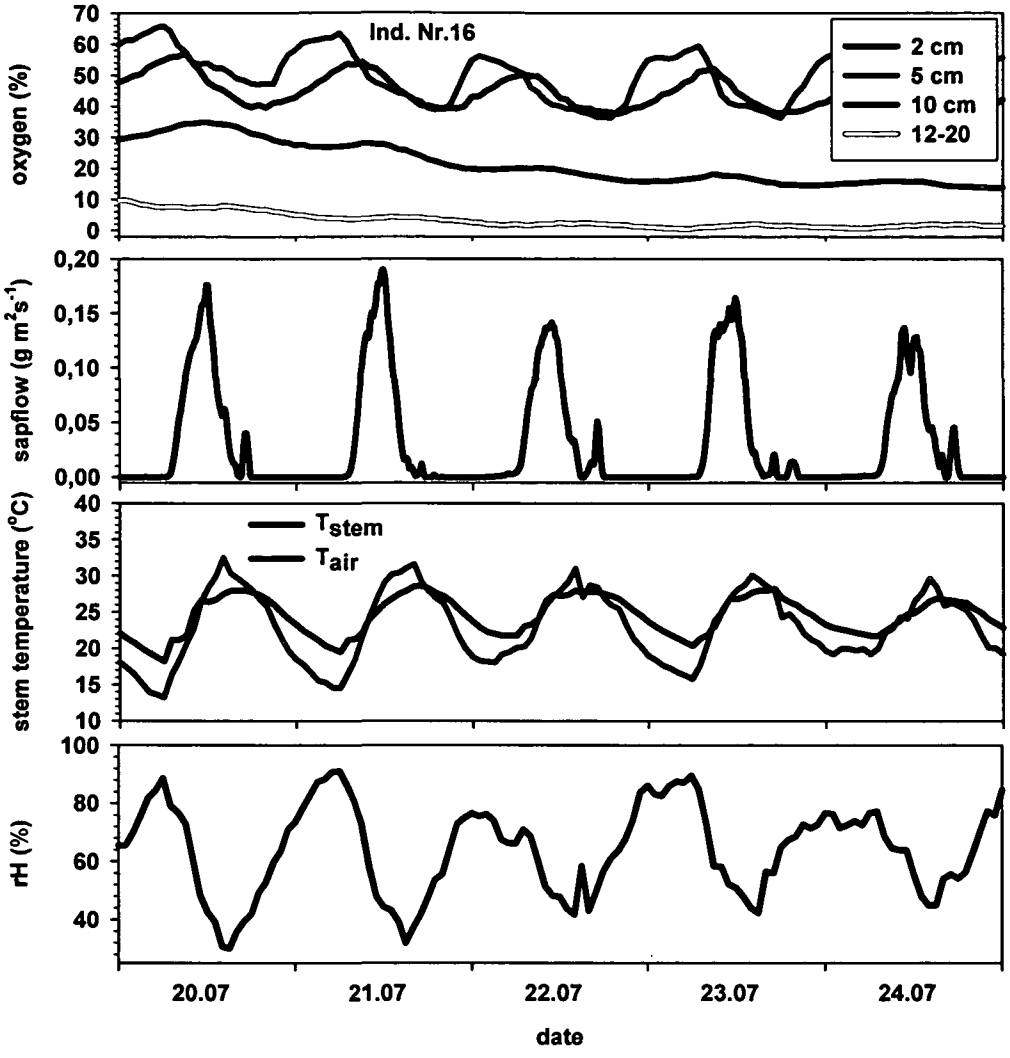

Fig. 41: Diurnal variations in oxygen recorded in tree Nr.16* from 20.07 to 24.07.2006. Oxygen concentrations were measured in one hour intervals at 2, 5, 10 and 12 - 20 cm. Dashed lines bridge intervals when measurements were interrupted. Sapflow was measured in the outer 2 cm of sapwood.

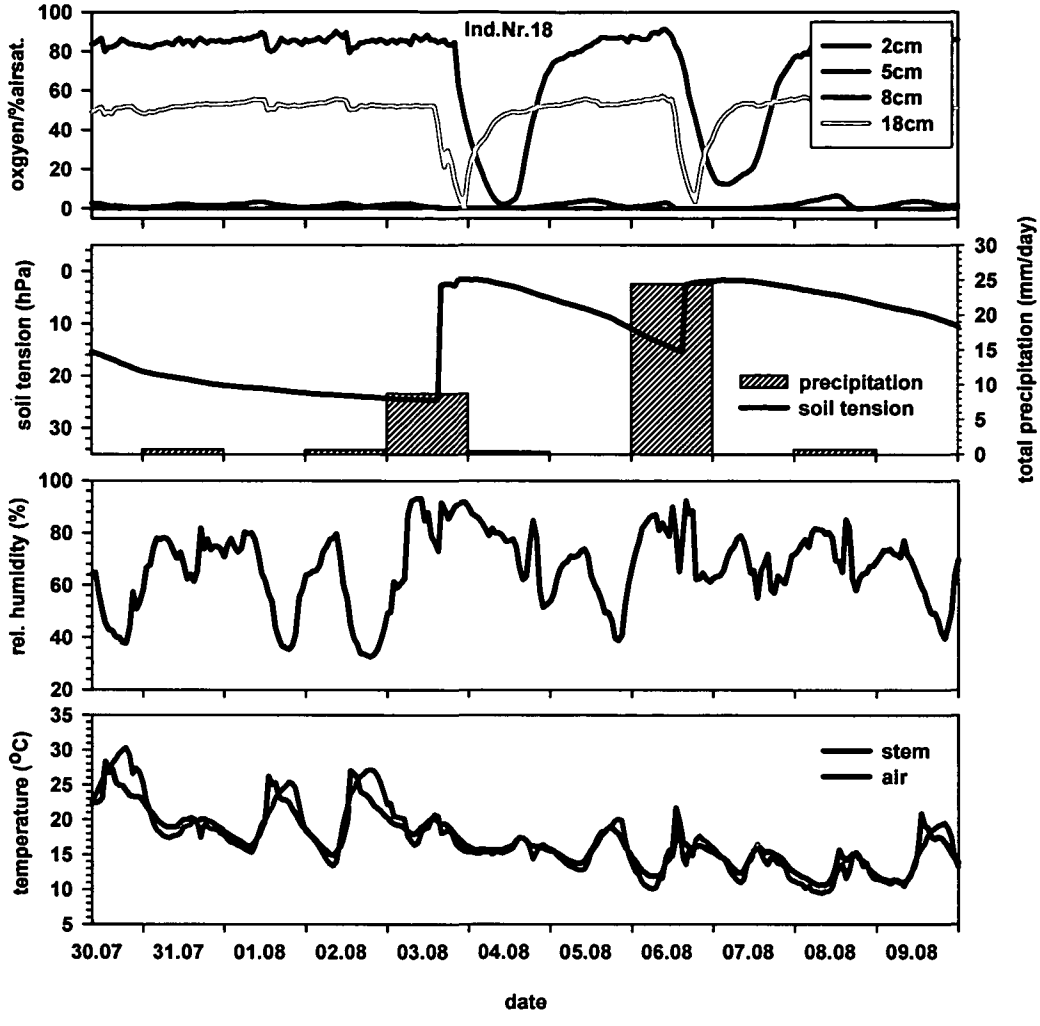

Fig. 42: Diurnal variations in oxygen recorded in tree Nr.18* from 30.07 to 09.08.2005. Oxygen decreased from the outer sapwood to the heartwood. Concentrations at 5 and 8 cm oscillated around 0%. Rapid decreases in oxygen at 2 and 18 cm were recorded on 03.08 and 06.08. Both incidents followed a rapid increase in soil tension after rain. In this example, concentrations increased again rapidly and returned to pre-values after one or two days. Streaked bars are daily precipitation totals (mm/day) recorded in a nearby weather station.

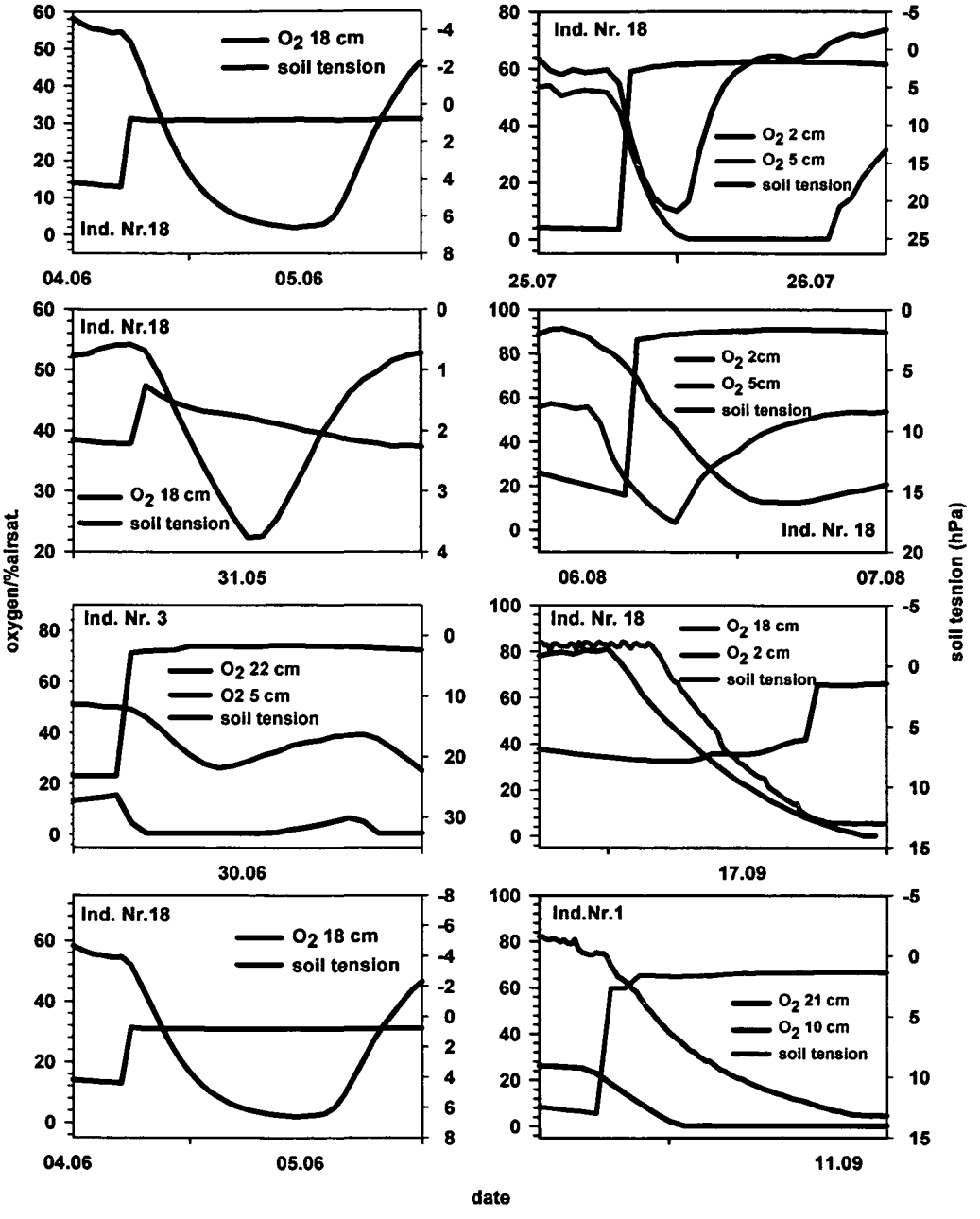

Fig. 43: Examples of rapid decreases in oxygen concentrations measured in 2005, which generally followed rain, as illustrated by an increase in soil tension. In one event (tree 1 on 10.09), however, an increase in soil water potential was recorded hours after the decrease in oxygen concentration.

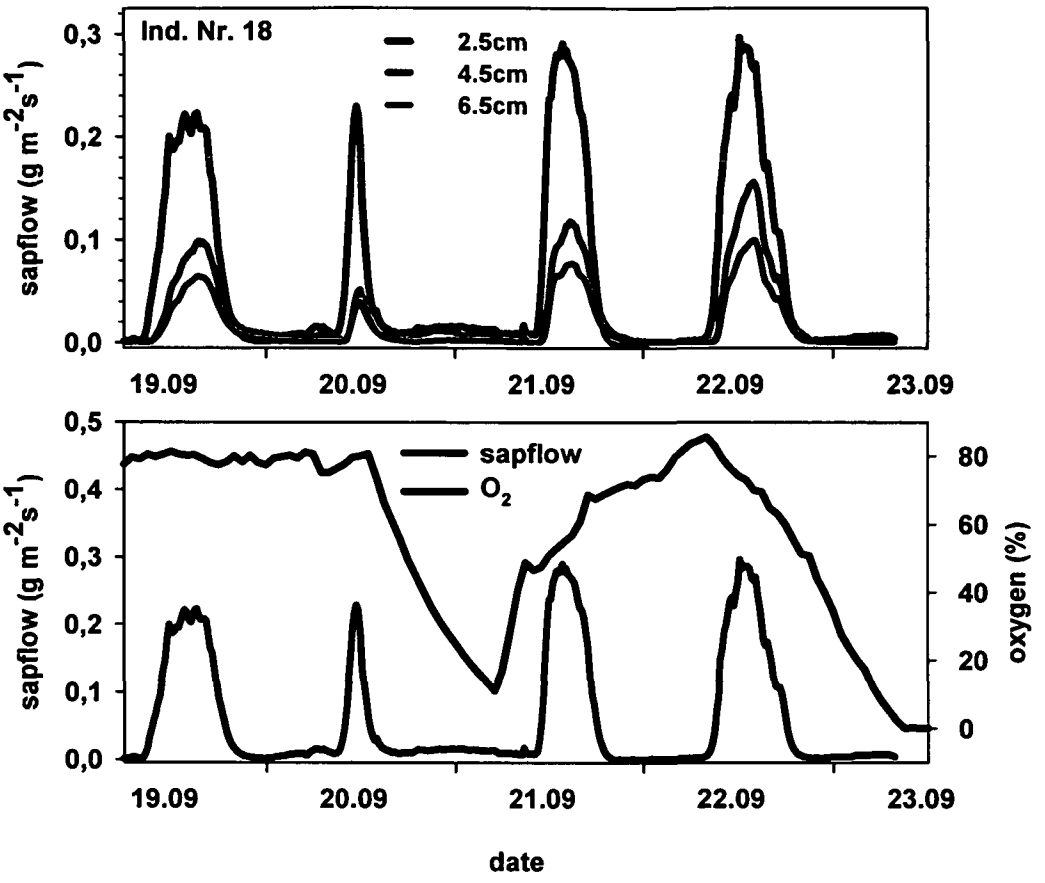

Fig. 44: Oxygen concentrations and sapflow in tree Nr.18, September 2005. Sapflow was measured at 2.5, 4.5 and 6.5 cm depths. The lower part of the graph shows that sudden decreases in oxygen could occur at times of peak sapflow and oxygen increased without sapflow.

3.4.3 Color-measurements

3.4.3.1 First set of cores

The first set of cores was harvested in June 2005 and exposed from June 2005 to September 2005. In the first set (C_0 = core halves exposed to 0% oxygen, C_{100} = core halves exposed to 100%) lightness before exposure (L_i^*) ranged from 27.0 to 52.6 and after exposure (L_f^*) from 21.0 to 53.5, redness a_i^* from 7.2 to 20.1 and a_f^* from 7.7 to 21.9, yellowness b_i^* from 20.2 to 31.4 and b_f^* from 10.2 to 22.3, saturation C_i^* from 24.2 to 34.3 and C_f^* from 16.7 to 29., and hue h_i^* from 46.9 to 74.3 and h_f^* from 32.8 to 68.2.

Mean color values ($L_{i,f}^*$, $a_{i,f}^*$, $b_{i,f}^*$, $C_{i,f}^*$, $h_{i,f}^*$), standard deviations, the overall color change (ΔE^*), and significances for t-tests between and among the two groups are given in Table 4.

After three month of exposure, the core halves exposed to 100% O_2 seemed darkened and greyish and were also abundantly interspersed with dark brown spots (Fig.45). Wood exposed to 0% O_2 did not appear darkened, but had a slightly reddish tone. This difference in

color change is reflected in the ΔE*-values of the two groups, which showed a generally stronger discoloration in C_{100} than in C_0, with a significant difference ($p < 0.01$) between C_0 and C_{100} in the outer sections I and II (Fig.46).

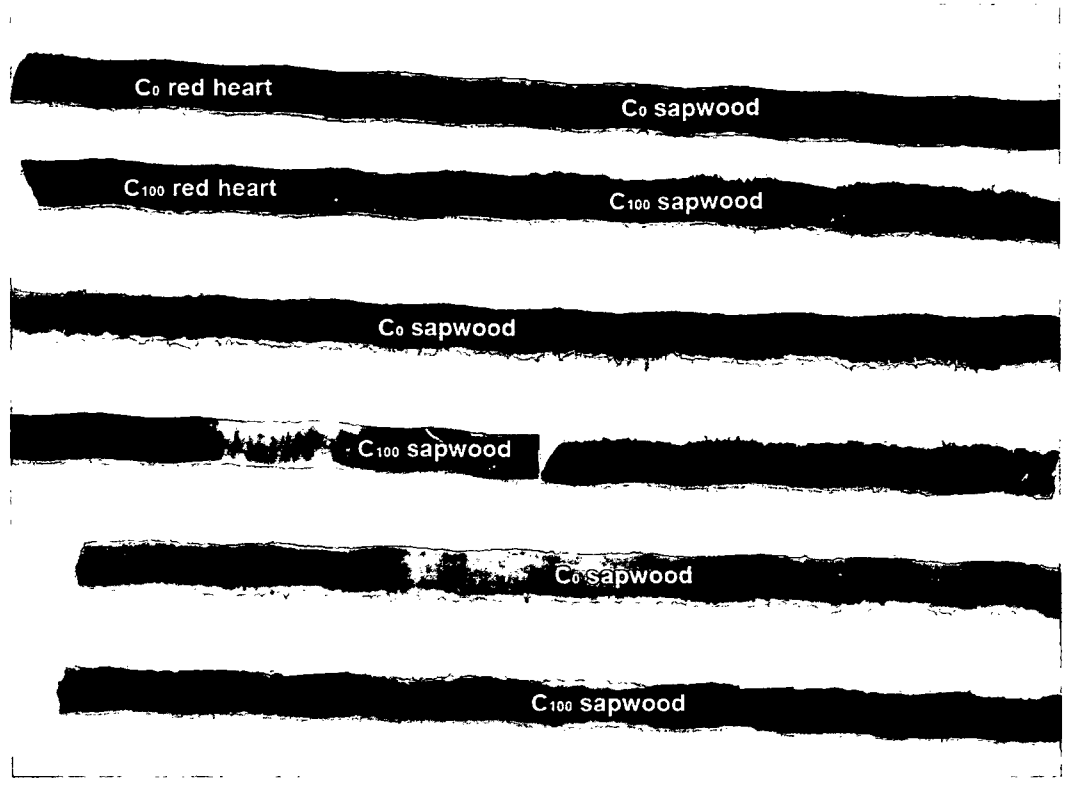

Fig. 45: Core halves after 3 month exposure to either ambient O_2 concentrations (100% = C_{100}) or 0% O_2 (C_0). Red heart regions were also affected and darkened during exposure.

Lightness (L*) declined significantly during storage at 0 and 100% O_2, in sections I and II of C_{100}, and declined significantly more in C_{100} than in C_0. The process of darkening was stronger in the first two sections, which consisted of more peripheral wood than the third section, which was mostly colored heartwood.

Redness (a*) differed significantly ($p<0.01$) between C_0 and C_{100} in all three sections. While a* increased in C_0 in all three sections, it decreased in all sections of C_{100}, particularly in sections II and III. At the beginning of the experiment a*-values in both groups increased from bark to pith and were especially high in section III, where the cores were also darker in comparison to the first two sections. After three month of exposure to oxygen the differences between the sections diminished, while differences in a* between initial and final measurements increased in wood stored at 0% O_2. Section I of C_0 still had lower a*-values than the inner two sections after exposure.

Yellowness (b*) decreased in both groups. Although the differences between initial and final measurements were significant ($p<0.01$) in both groups and in all sections, there was no

difference found C_0 and C_{100}. b* decreased from bark to pith in both groups before exposure, and in C_0 also after the exposure,.

Color saturation (C*) declined during the exposure in C_0 and C_{100}. As expected the change was stronger and significant in all sections in C_{100}, while in C_0 the change was significant only in sections I and III. Since C* is a parameter derived from both a* and b*, the decrease found in C_{100} was related to the strong decrease in a*. The decrease in saturation was strongest in section I and II of C_{100}.

Also related to the changes in color represented by a* and b* were the changes in hue (h*), another parameter derived from those two values. A significant ($p<0.01$) change from yellow towards red was found in C_0 in all three sections, again likely related to the increase in a* values, recorded in all sections of C_0. Section I of C_{100} also changed from yellow towards red, while sections II and III changed from red towards yellow. This also corresponds to the decrease in a*, which was exceptionally strong in the first two sections of C_{100}, while the decrease in b* was equal in all sections. Δh^* was significantly higher in all sections exposed to 100% than in wood exposed to 0%.. The decrease in h* was less intense in the inner than in the outer sections, especially in C_{100}.

The color index parameter ΔE^* summarizes all discoloration during storage and was significantly higher in C_{100} than in C_0 in sections I ($p<0.01$) and II ($p<0.05$).

	C_{0i} (x ± SD)	C_{100i} (x ± SD)	C_{0f} (x ± SD)	C_{100f} (x ± SD)	p (C_{0i} vs. C_{0f})	p (C_{100i} vs. C_{100f})	p (ΔC_0 vs. ΔC_{100})
L* I	47.6 ± 3.3	46.2 ± 5.3	42.2 ± 7.0	32.8 ± 6.1	**.027**	**.000**	**.004**
L* II	39.3 ± 7.3	40.2 ± 7.6	36.4 ± 5.2	30.2 ± 6.7	.086	**.020**	**.005**
L* III	39.1 ± 4.3	39.8 ± 4.6	35.7 ± 5.2	31.3 ± 7.6	.084	**.027**	.133
a* I	10.8 ± 3.4	11.0 ± 2.2	13.6 ± 2.7	8.9 ± 1.0	**.010**	.100	**.000**
a* II	14.6 ± 3.1	15.0 ± 2.4	16.9 ± 3.2	9.1 ± 0.7	**.005**	**.001**	**.001**
a* III	15.1 ± 2.3	15.5 ± 2.1	16.4 ± 1.6	9.3 ± 1.1	.145	**.000**	**.000**
b* I	25.6 ± 2.4	26.8 ± 3.1	18.0 ± 3.8	19.0 ± 1.7	**.000**	**.000**	.858
b* II	24.5 ± 2.5	26.7 ± 3.6	17.1 ± 3.9	19.0 ± 2.9	**.001**	**.000**	.749
b* III	23.9 ± 1.9	25.2 ± 2.8	16.3 ± 2.6	19.2 ± 2.3	**.000**	**.007**	.383
C*I	27.9 ± 3.3	29.1 ± 2.8	22.8 ± 3.6	21.2 ± 1.6	**.000**	**.000**	**.029**
C*II	28.7 ± 2.4	30.8 ± 2.9	24.3 ± 4.4	21.2 ± 2.9	**.008**	**.000**	**.016**
C*III	28.3 ± 2.4	29.7 ± 3.3	23.2 ± 2.9	21.5 ± 2.4	**.001**	**.002**	.120
h* I	67.6 ± 5.1	67.6 ± 4.6	52.5 ± 7.9	64.3 ± 3.4	**.001**	**.011**	**.002**
h* II	59.3 ± 6.3	60.4 ± 6.1	45.2 ± 6.5	63.6 ± 2.4	**.001**	.144	**.000**
h* III	57.8 ± 3.9	58.3 ± 2.4	44.6 ± 2.9	64.0 ± 2.9	**.000**	**.003**	**.000**
ΔE* I			10.3 ± 4.4	16.0 ± 3.7			**.008**
ΔE* II			8.9 ± 3.7	14.5 ± 3.5			**.013**
ΔE* III			9.3 ± 2.4	13.6 ± 5.8			.149

Tab. 4: Mean (n = 7), and SD for all color indices (L*: lightness, a*: redness, b*: yellowness, C*: chroma, h*: hue, ΔE*: overall color change) for the first set of cores exposed to 0 and 100% (i.e., ambient) O_2. C_{0i} and C_{100i} are samples prior to exposure, C_{0f} and C_{100f} are the same samples after exposure, Significant differences (paired t-test, p<0.05) are in bold numbers.

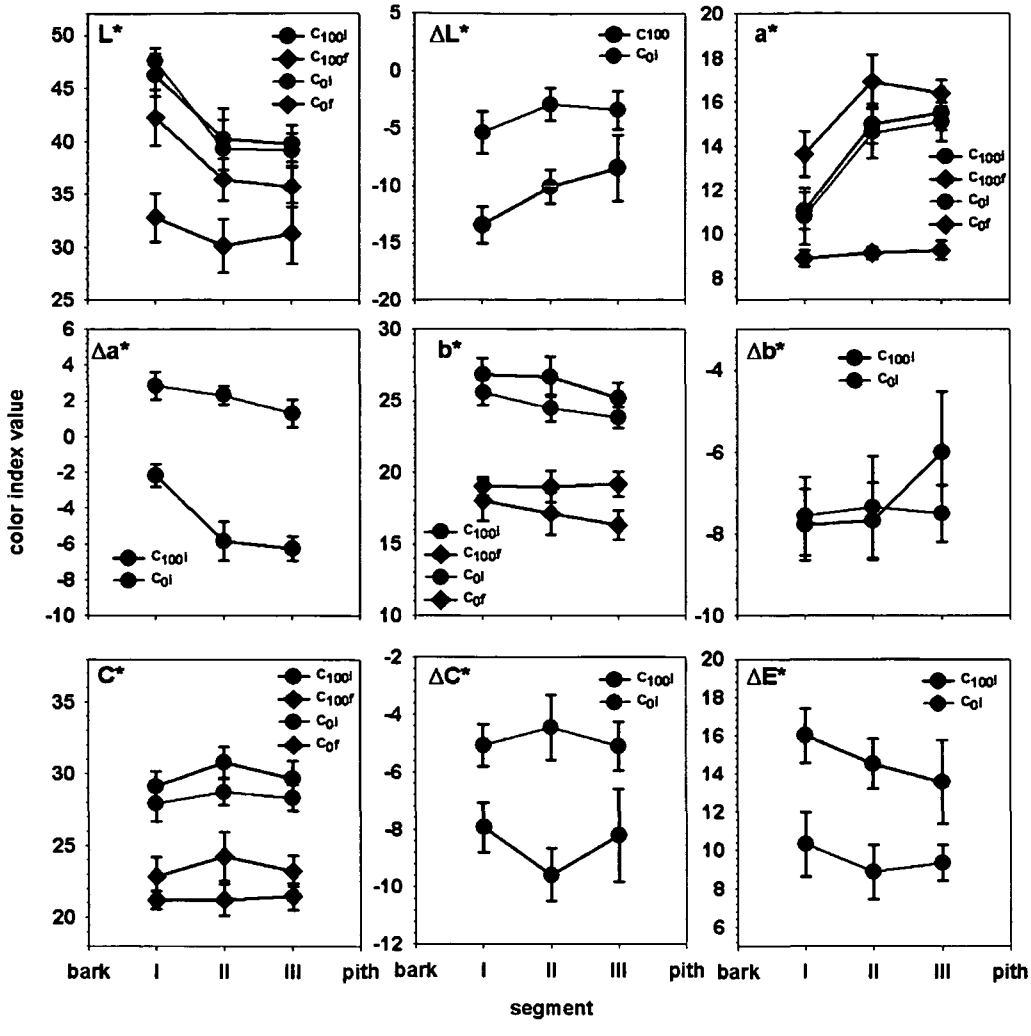

Fig. 46: Color index values (L*: lightness, a*: redness, b*: yellowness, C*: hue, plus Δ-values and ΔE*: overall discoloration) of core halves before (indexed i) and after (indexed f) exposure to 0% (C_0) and 100% (C_{100}) O_2 for three months. Symbols are mean (n = 7) ± SE. Sections I to III are 8-cm sections from the cambium towards the center.

3.4.3.2 Second set of cores

These cores were harvested in November 2005 and exposed from November 2005 – January 2006 (Fig.47). In sterilized samples (S) lightness prior to exposure (L_i*) ranged from 22 to 61.5 and after exposure (L_f*) from 10.1 to 36.2, redness a_i* from 1.5 to 15.3 and a_f* from 5.8 to 16.2, yellowness b_i* from 21.1 to 36.3 and b_f* from 8.6 to 27.1, saturation C_i* from 21.3 to 38 and C_f* from 10.7 to 29.8, and hue h_i* from 61 to 86.7 and h_f* from 43.4 to 67.

In un-sterilized control samples (C) lightness L_i* ranged from 24.7 to 61.1 and L_f* from 9.2 to 34.8, redness a_i* from 1.7 to 14.9 and a_f* from 2.7 to 14.1, yellowness b_i* from 20.9 to 36.5 and b_f* from 4.1 to 29.1, saturation C_i* from 22.5 to 38.2 and C_f* from 5.3 to 31, hue h_i* from 63.6 to 83.2 and h_f* from 47.7 to 75.9.

Cores were sterilized after the initial measurements and results from the final measurements were probably biased by sterilization itself. During steaming wood logs are exposed to high levels of pressure, heat and moisture, which causes substantial discolorations in beech wood (Riehl *et al.*, 2002). Because this process strongly resembles the method of sterilization in an autoclave applied in this study, color was most likely altered by sterilization itself and a valid comparison between sterilized (S) and control samples (C) was not assured and thus not considered.

Color changes during exposure (Δ) were calculated by subtracting the initial measurement from the values after exposure (Fig. 3 and 4). Mean color values ± SD ($L_{i,f}^*$, $a_{i,f}^*$, $b_{i,f}^*$, $C_{i,f}^*$, $h_{i,f}^*$), the corresponding Δ-values, and significances for the t-tests and ANOVA are given in Tab. 5.

Three month after the initial measurement, both groups (S, C) seemed darkened and grayish in compared to their appearance before the exposure. All samples were abundantly interspersed with dark brown spots. The core halves exposed at 100% O_2 were more darkened than cores exposed at 50% and 20%. Sterilized and non-sterilized samples did not obviously differ to the naked eye.

The darkening was reflected by a decrease in L*-values, found in both the sterilized (S) and the non-sterilized (C) group, and at all three oxygen levels (100%, 50%, 20%). Generally, the L*-values of the second set were noticeably lower than those from the first set of cores. The cores of both groups (S, C) darkened significantly more at 100% oxygen than at 50% and 20% ($p<0.05$), but no difference was found between 50% and 20% oxygen. Although differences in L* between initial and the final measurement were found in both groups and at all three oxygen levels ($p<0.05$), groups S and C did not differ at any oxygen level.

Redness a* increased at 100%, 50% and 20% in group C ($p<0.05$), and at 20% in group S ($p<0.01$). This was different from results of the first experiment, where a* increased only in cores exposed to 0%, but decreased at 100%. In group S the increase between initial and the final measurement was equally strong at all three oxygen levels, while in C there was no increase in a* at 100% and 50% and only the cores exposed at 20% showed significant reddening ($p<0.05$). No difference in reddening (Δa*) was found between sterile and control samples.

Yellowness b* decreased in all cores of both groups, with results similar to the first set of cores. In group S the decrease in b* was more intense at 100% than at 50% ($p<0.01$), but no difference between 100% and 20% or 50% and 20% was found. In group C discoloration at 100% was significantly higher than at 50% and 20% ($P<0.05$). No difference between cores at 50% and at 20% was found. There was no effect of sterilization on Δb* at any oxygen level.

Chroma C* decreased, i.e., color became less vivid and more gray, in both groups and at all oxygen levels, similar to though slightly higher than in the first set of cores. Since C* was calculated from a* and b*, the influence of the strong decrease in b* found in both groups reflected on C*. In group C C* decreased significantly more at 100% than at 50% and 20% ($p<0.01$), but there was no difference between 50% and 20%. In group S no difference between oxygen levels was found. Groups S and C differed only at 100% ($p<0.05$).

Changes in hue (h*) were similar to those observed in the first set of cores, although h* was slightly higher during experiments with the second set. Negative h*-values represent a change from yellow to red. In both groups oxygen levels had no effect on Δh^*. In sterilized samples the change from yellow to red was stronger at all oxygen levels ($p<0.05$).

Overall discoloration (ΔE^*) was stronger at 100% than at 50% and 20% in C and S ($p<0.01$). There was no difference between 50% and 20% in any of the two groups. Also no differences were found between S and C at any oxygen level.

	O_2 (%)	S	C	p (C vs. S)		O_2 (%)	S	C	p (C vs. S)
L_i^*	100	44.8 ± 5.6	45.5 ± 5.1	.804	Δb^*	100	**-13.1 ± 2.2**	**-15.6 ± 2.5**	.054
	50	41.8 ± 8.1	42.7 ± 7.9	.822		50	**-8.8 ± 3.6**	**-11.2 ± 3.3**	.185
	20	44.9 ± 7.4	44.5 ± 7.6	.912		20	**-11.8 ± 2.9**	**-8.9 ± 2.6**	.062
L_f^*	100	17.3 ± 2.6	17.6 ± 3.7	.084	C_i^*	100	28.9 ± 1.2	29.5 ± 1.3	.380
	50	22.2 ± 4.9	23.6 ± 3.0	.499		50	29.6 ± 3.6	30.2 ± 3.1	.737
	20	23.6 ± 6.4	23.0 ± 5.1	.850		20	31.6 ± 2.2	30.8 ± 1.6	.399
ΔL^*	100	**-27.6 ± 4.6**	**-27.9 ± 3.1**	.861	C_f^*	100	18.0 ± 2.3	14.8 ± 3.0	**.029**
	50	**-19.6 ± 4.5**	**-19.1 ± 7.0**	.869		50	22.8 ± 4.7	19.8 ± 3.6	.167
	20	**-21.5 ± 2.2**	**-21.5 ± 5.6**	.990		20	21.6 ± 3.7	23.7 ± 3.0	.231
a_i^*	100	6.0 ± 2.2	5.8 ± 2.3	.890	ΔC^*	100	**-11.0 ± 3.0**	**-14.7 ± 2.8**	**.021**
	50	7.8 ± 3.8	7.4 ± 3.5	.826		50	**-6.8 ± 4.4**	**-10.4 ± 3.6**	.092
	20	7.8 ± 3.0	7.2 ± 2.7	.650		20	**-9.5 ± 3.6**	**-7.0 ± 2.6**	.143
a_f^*	100	9.7 ± 1.5	6.5 ± 1.6	**.001**	h_i^*	100	78.1 ± 4.4	78.7 ± 4.3	.793
	50	11.6 ± 1.7	8.4 ±1.9	**.003**		50	74.8 ± 7.0	75.8 ± 6.7	.790
	20	11.5 ± 1.4	11.1 ±1.1	.576		20	75.6 ± 5.4	76.4 ± 5.3	.783
Δa^*	100	3.7 ± 3.4	**0.7 ± 2.2**	.055	h_f^*	100	57.4 ± 2.7	63.6 ± 5.3	**.011**
	50	3.8 ± 4.3	**1.0 ± 3.0**	.152		50	59.0 ± 4.0	64.9 ± 3.5	**.007**
	20	**4.0 ± 4.0**	**4.0 ± 2.1**	.986		20	57.2 ± 3.7	61.6 ± 4.1	**.049**
b_i^*	100	28.2 ± 1.2	28.8 ± 1.2	.333	Δh^*	100	**-20.7 ± 5.3**	**-15.1 ± 3.5**	**.026**
	50	28.4 ± 3.3	29.1 ± 3.0	.656		50	**-15.9 ± 3.9**	**-10.8 ± 4.2**	**.026**
	20	30.4 ± 2.1	29.7 ± 1.8	.502		20	**-18.8 ± 3.4**	**-14.8 ± 2.9**	**.027**
b_f^*	100	15.1 ±1.9	13.2 ± 2.8	.131	ΔE	100	31.2 ± 4.2	32.3 ± 2.8	.660
	50	19.6 ± 4.7	17.9 ± 3.2	.407		50	22.8 ± 3.6	22.8 ± 6.9	.997
	20	18.2 ± 3.7	20.9 ± 3.2	.156		20	25.6 ± 1.6	24.0 ± 5.4	.464

Tab. 5: Mean (n = 8) and SD for all color indices (L*: lightness, a*: redness, b*: yellowness, C*: chroma, h*: hue, ΔE*: overall color change) before (i) and after (f) exposure of sterilized (S) and non-sterilized (C) samples to three different oxygen concentrations (100%, 50%, 20%). Bold numbers in Δ indicate a significant effect of exposure (i.e., difference of i and f-values, paired t-test), underlined numbers in Δ indicate that oxygen levels had a significant effect on color changes (Δ, ANOVA), p-values in the last column compare color changes between sterilized and non-sterilized samples (t-test).

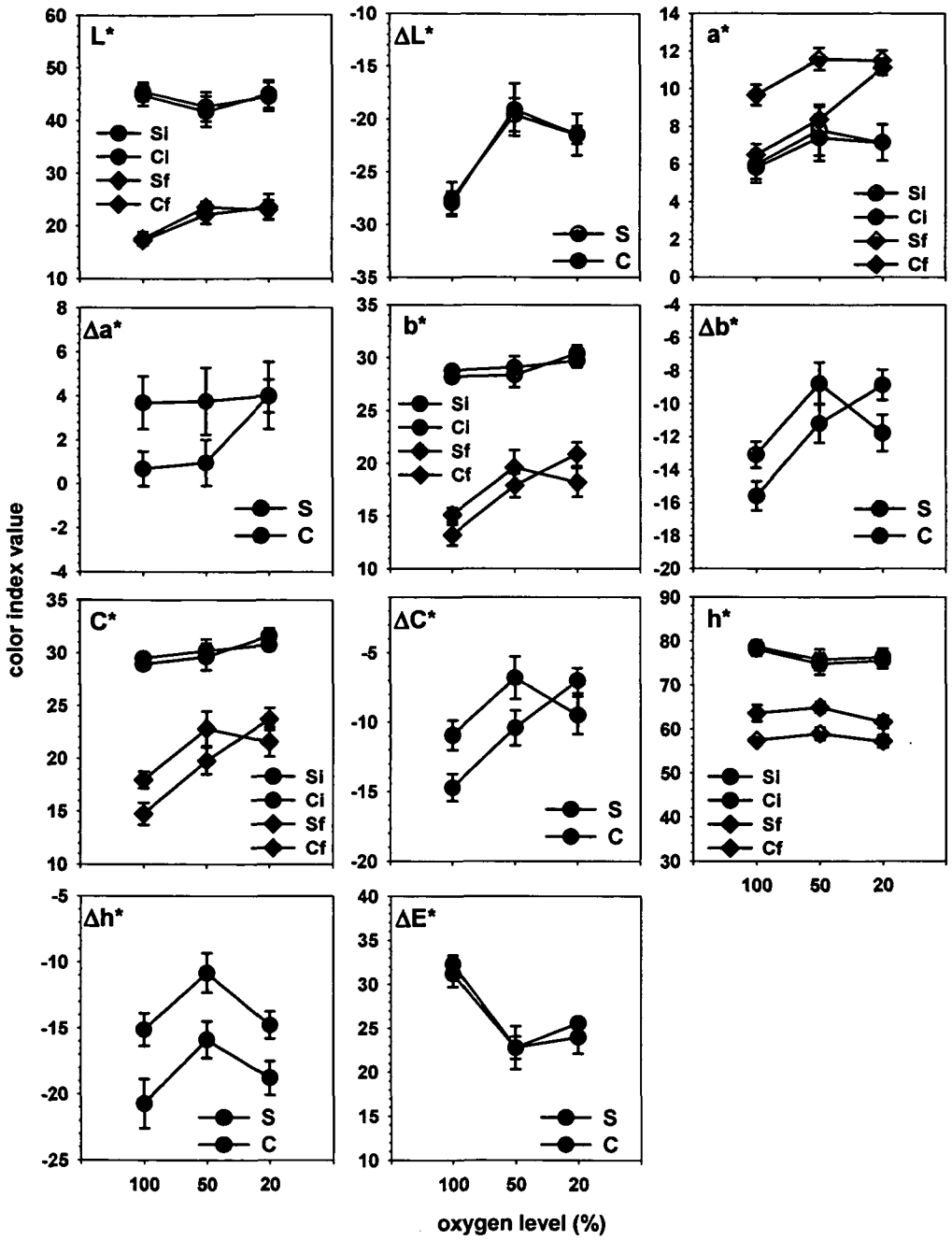

Fig. 47: Comparison of color indices (L*: lightness, a*: redness, b*: yellowness, C*: chroma, h*: hue, ΔE*: overall color change) before (i) and after (f) exposure of sterilized (S) and non-sterilized (C) samples to three different oxygen concentrations (100%, 50%, 20%). Dots are mean values (n = 8), error bars are SE.

3.4.3.3 Wood blocks

Wood blocks were harvested in January 2006 and exposed from January – April 2006. Color index values and their changes (Δ-values) are given in Tab.6. Fig.48 shows differences in color index Δ-values in the sapwood (S) and the red heart (RH) of both groups (S, C) and both oxygen concentrations (0, 100).

	C0 SW	C100 SW	C0 RH	C100 RH	S0 SW	S100SW	S0 RH	S100 RH
n	37	32	16	19	23	25	22	25
L_i^*	48.0 ± 2.1	49.1 ± 3.1	33.4 ± 5.1	33. ± 4.3	56.5 ± 2.5	57.6 ± 2.8	32.3 ± 4.5	35.2 ± 3.7
L_f^*	43.0 ± 3.9	32.7 ± 6.0	32.5 ± 4.6	25.3 ± 3.0	45.1 ± 2.3	39.4 ± 2.8	28.2 ± 3.1	26.6 ± 3.4
ΔL*	**-5.1 ± 3.6**	**-16.4 ± 5.9**	**-0.9 ± 1.9**	**-7.9 ± 3.7**	**-11.4 ± 0.9**	**-18.1 ± 2.4**	-3.7 ± 2.7	**-8.6 ± 2.5**
a_i^*	15.7 ± 1.3	15.2 ± 1.1	16.4 ± 1.0	16.3 ± 1.6	14.8 ± 2.1	13.8 ± 1.4	17.1 ± 1.1	16.6 ± 1.2
a_f^*	14.5 ± 2.4	12.6 ± 3.0	18.0 ± 2.2	12.1 ± 1.7	18.6 ± 2.0	16.6 ± 1.0	16.8 ± 2.1	18.0 ± 1.6
Δa*	-1.2 ± 1.7	**-2.5 ± 3.4**	**1.6 ± 1.7**	**-4.2 ± 1.2**	**3.9 ± 1.2**	**2.7 ± 1.5**	-0.2 ± 1.5	**1.5 ± 1.1**
b_i^*	32 ± 2.7	31.8 ± 3.6	25.5 ± 4.0	24.0 ± 2.4	37.7 ± 2.4	36.7 ± 2.1	25.1 ± 2.8	24.7 ± 2.3
b_f^*	31.8 ± 4.0	22.1 ± 7.1	28.4 ± 6.3	14.8 ± 2.2	35.4 ± 4.8	27.2 ± 1.8	23.1 ± 4.4	25.3 ± 3.3
Δb*	-0.2 ± 2.8	**-9.7 ± 4.7**	**2.9 ± 4.1**	**-9.2 ± 2.2**	**-2.3 ± 3.6**	**-9.5 ± 2.3**	**-2.1 ± 2.7**	0.6 ± 4.1
C_i^*	35.7 ± 2.5	35.3 ± 3.3	30.4 ± 3.6	29.1 ± 2.6	40.1 ± 2.6	38.7 ± 2.1	30.4 ± 2.7	29.7 ± 2.4
C_f^*	35.0 ± 4.0	25.5 ± 7.6	33.7 ± 6.2	19.2 ± 2.6	40.1 ± 5.1	32.2 ± 1.9	28.6 ± 4.5	31.2 ± 3.0
ΔC*	-0.7 ± 3.0	**-9.8 ± 5.5**	**3.3 ± 4.0**	**-9.9 ± 2.3**	0.0 ± 3.4	**-6.4 ± 2.3**	**-1.8 ± 3.0**	**1.4 ± 3.4**
h_i^*	63.8 ± 2.8	64.3 ± 3.2	56.8 ± 4.3	55.9 ± 2.2	69.9 ± 2.9	70.3 ± 2.3	55.7 ± 2.6	56.1 ± 2.0
h_f^*	65.4 ± 3.9	59.2 ± 3.5	57.0 ± 4.2	50.7 ± 2.6	62.3 ± 2.1	56.3 ± 1.6	53.3 ± 2.9	54.2 ± 4.0
Δh*	**1.6 ± 2.3**	**-5.1 ± 2.2**	0.3 ± 3.5	**-5.2 ± 2.4**	**-7.5 ± 3.3**	**-14.0 ± 1.9**	**-2.3 ± 1.7**	-1.8 ± 4.9
ΔE*	6.6 ± 2.5	19.6 ± 7.4	5.2 ± 2.6	13.0 ± 3.8	12.8 ± 1.6	20.8 ± 2.7	5.4 ± 2.3	9.6 ± 2.9

Tab. 6: Mean and SD of lightness (L*), redness (a*), yellowness (b*), chroma (C*), hue (h*), and overall color change (ΔE*) of sterilized (S) and non-sterilized (C) blocks of sapwood (SW) and red heartwood (RH) before (i) and after (f) exposure to 0% and 100% O_2. Bold numbers in Δ indicate a significant effect of exposure (i.e., difference of i and f-values, paired t-test), underlined numbers in Δ indicate that sterilization had a significant effect on color changes (ANOVA).

Similar to the cores in the two previous experiments the wood blocks of both groups (S, C) were darkened and grayish compared to their appearance before the exposure. The wood blocks of C100 and S100 had abundantly dark brown spots, whereas blocks of S0 had few spots and appeared less darkened than blocks exposed at 100%. The blocks of C0 did not seem affected by discoloration at all. The color index values obtained from wood blocks were similar to results from the measurements with core halves.

Lightness L* was reduced in all groups during exposure ($p<0.01$) except in sterilized red heart samples exposed at 0%. It decreased stronger at 100% than at 0% in all groups ($p<0.01$). A significant difference between S and C was found in sapwood and red heart at 0% ($p<0.01$).

Redness a* was decreased in sapwood and heartwood of C100 and was increased in red heart of C0 and S100 and in the sapwood of S0 and S100 ($p<0.01$). A stronger decrease in a* at 100% compared with 0% was found in sapwood ($p<0.05$) and heartwood ($p<0.01$) of C100, a stronger increase in 100% compared with 0% in red heart of S 100 ($p<0.01$). Only in S0 a* was higher at 0% compared with 100% ($p<0.05$). A difference between sterilized and non-sterilized samples was found in all groups except red heart at 0% ($p<0.01$).

Yellowness b* was decreased in the sapwood of C100, S0 and S100 and in the red heart of C100 and S0, and was increased in the red heart of C0 ($p<0.01$). b* showed a stronger decrease in samples exposed at 100% compared with 0% ($p<0.01$) except in sterilized red heart where it was reversed ($p<0.05$). Differences between sterilized and non-sterilized blocks were found in the red heart at 100% and 0% ($p<0.01$).

Chroma C* was reduced (i.e, a shift from vivid to grayish colors) in the sapwood of S0, C100, and S100 and in the red heart of C100 ($p<0.01$). C* was increased in the red heart of C0 and S100 ($p<0.01$). C* showed a stronger decrease at 100% compared to 0% in all groups except the sterilized red heart. A difference between sterilized and non-sterilized samples was found in the red heart at 0% and 100% ($p<0.01$).

Hue h* was reduced (i.e, a shift from yellow to red) in all groups except sapwood and red heart of C0 ($p<0.01$). In the sapwood of C0 h* was increased ($p<0.01$). h* showed a stronger decrease at 100% compared with 0% ($p<0.01$) in all groups except sterilized red heart. Differences between S and C were found in sapwood exposed to 0% and 100% ($p<0.01$).

All changes in lightness and color between the initial and the final measurement can be summarized using the index value ΔE^*. A stronger overall discoloration was found at 100% compared with 0% in all groups ($p<0.01$). A difference between sterilized and non-sterilized groups was found in the sapwood exposed at 0%.

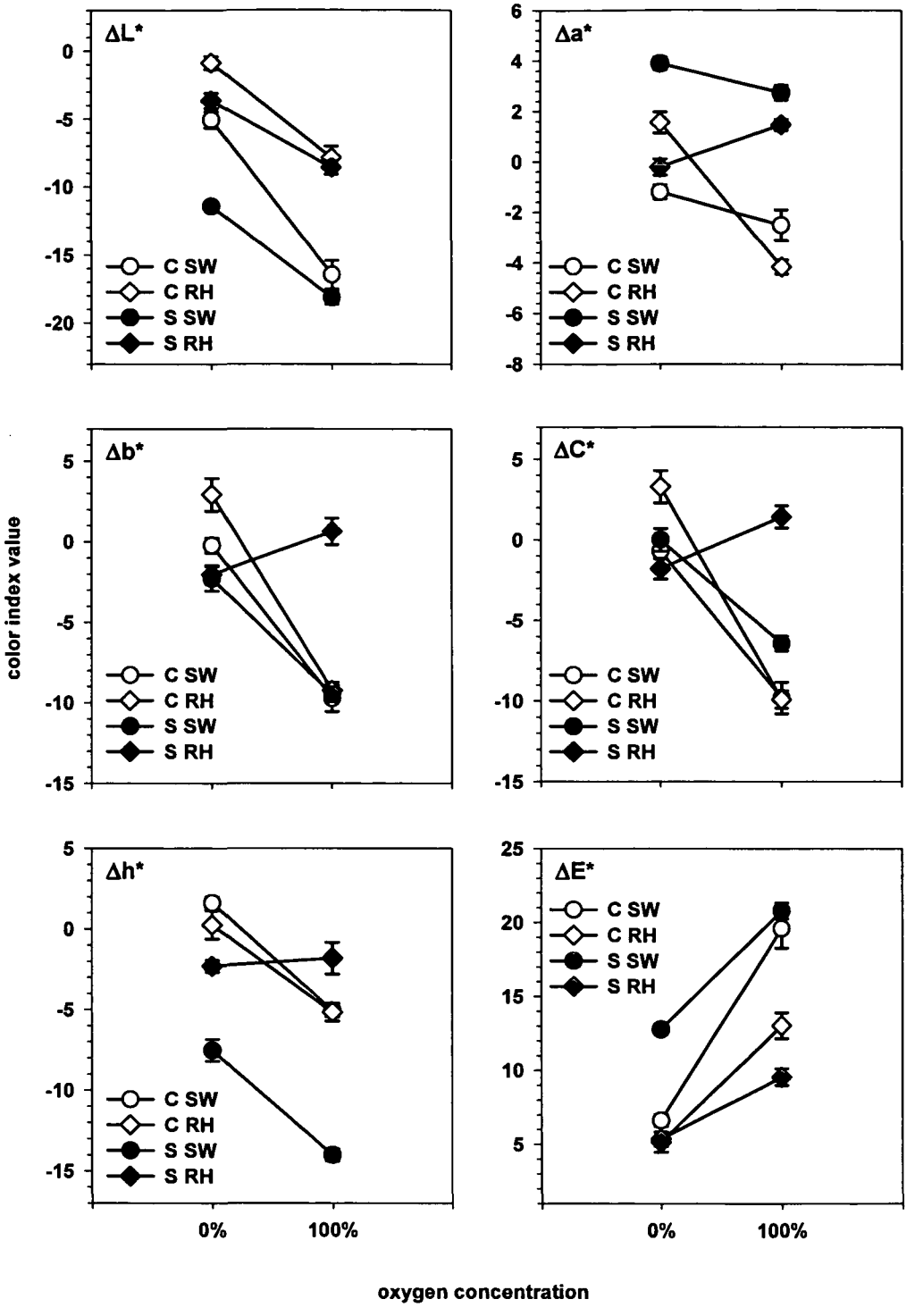

Fig. 48: Comparison of color changes (Δ-values) between sterilized (S) and non-sterilized (C) samples of sapwood (SW) and red heart (RH) exposed at 0% and 100% O_2. Symbols are means (n = 16-37), error bars are SE.

3.4.4 Discolorations on cut wood disks

Seven tree discs (height h = 6 - 7 cm, diameter Ø = 33 - 37 cm) each were cut from trees Nr. 1* and Nr.3 (h: 4 - 9 cm, d: 32 - 35 cm), six from tree Nr.5[a] (h= 5 - 10 cm, Ø = 35 - 40 cm), and four disks from tree Nr.15[a] (h = 7 - 15 cm, Ø = 37 - 40 cm). (* = red heart, [a] = ambiguous colored heart). Discs were photographed to study the spread of discolorations.

Pronounced formations of wound heart were found in the direct vicinity of the inserted metal tubes on the cross-section of trees Nr.1*, 3 and 5[a]. These were blue-black discolorations of great intensity, rather small (ca. 1 - 5 cm) in their tangential extent, and not much wider than the diameter of the inserted iron tubes (Ø = 0.9 cm) at their point of origin in the bark. Towards the pith the discolorations broadened, which resulted in a conical shape on the cross section (Fig.34). The radial extent was also almost identical to the length of the tubes (length l = 5 - 20 cm). In axial direction the discolorations extended to ca. 15 - 20 cm above and below the injury. Smaller wounds such as those from the holes for temperature sensors (l = 2 cm, Ø = 5 mm) caused discolorations of less intensity and dimension. These results correspond to (Schute, 1986) who found wound cores of similar appearance and dimensions associated with gunshot wounds in beech stems where the spatial extend of the discolorations was also correlated to the size of the impurity. Response reactions of the sapwood to injuries were a probably cause of the discolorations, possibly combined with the effect of bacteria and fungi, that entered the tissue after the drilling of the bore holes. The strong bluish color almost certainly resulted from reactions with iron released from the iron tubes, stainless-steel tubes (l = 5 cm) inserted in September 2005 and April 2006 caused no visible discolorations in tree Nr.1* and only a faint discoloration in tree Nr.3.

Leaving boreholes open for ten month resulted in brownish discolorations of similar size as in trees Nr.1 and 3. So far only one of the aerated trees was felled and the discoloration did not correspond to that of red heart and was of very limited extent.

3.4.5 Forest-pathological screening

Of the specimen incubated with streptomycinsulfate 41 out of 56 (73.2%) samples showed no microbial growth (sapwood: 19 samples or 95%, red heart 8 or 80%, wound heart 12 or 55% and uncolored heart 2 or 50%), and bacteria were only found on three (5.4%) samples (one each on uncolored heart, sapwood and wound heart samples) (Tab.7 and 8, Fig.49).

Fomes fometarius, the 'tinder fungus', a basidomycota known for causing beech bark disease in weakened individuals (Butin, 1995), was found in two (3.6%) of the wound heart samples. *Trichoderma sp.*, a mould very common in temperate soil and wood, was found in only one wound heart sample. *Ophiostoma quercus*, an ascomycete and a common sapwood colonizer of hardwood species found on discolored wood (Kim *et al.*, 1998), was

detected on four (7.1%) samples (all wound heart). On five samples (8.9%, one uncolored heart, two red heart and two wound heart samples) unknown endophytic fungi were found.

In the samples incubated on standard agar without streptomycinsulfate bacteria were not suppressed and colonization with endophytic species showed a quite different distribution. Of these samples only 26 (46.4%) were sterile (nine sapwood, three red heart, ten wound heart and four uncolored heart samples), bacteria were found on 21 (37.5%) samples (ten sapwood, two red heart, nine wound heart), whereas *F. fometarius* and *Trichoderma sp.* were not found at all. *O. quercus* was found on five (8.9%) samples (two sapwood, one red heart, two wound heart), and unknown endophytic fungi were also found on five (8.9%) samples (four red heart and one wound heart)

	Fungus		Bacteria			Fungus		Bacteria	
streptomycin	+	-	+	-	streptomycin	+	-	+	-
1/1s I	-	O.querc.	-	+	3/7rh I	-	-	-	-
1/1s II	-	-	-	-	3/7wh II	-	-	-	-
1/1rh I	-	-	-	-	5/5s I	-	-	-	+
1/1rh II	-	Unk.	-	-	5/5s II	-	-	+	+
1/1wh I	-	Unk.	+	-	5/5rh I	-	O. querc.	-	-
1/1wh II	-	-	-	-	5/5rh II	-	Unkn.	-	-
1/4s I	-	-	-	+	5/5wh I	*O. querc.*	-	-	+
1/4s II	-	-	-	+	5/5wh II	*T. sp.*	-	-	+
1/4rh I	-	-	-	+	5/5wh2 fd I	*O. querc.*	-	-	+
1/4rh II	-	-	-	+	5/5wh2 fd II	-	-	-	-
1/4wh I	-	-	-	+	5/5wh2 sd I	*O. querc.*	O. querc.	-	-
1/4wh II	-	-	-	+	5/5wh2 sd II	*O. querc.*	O. querc.	-	-
1/4uh I	-	-	-	-	5/6s I	-	-	-	+
1/4uh II	-	-	-	-	5/6s II	-	-	-	+
3/3s I	-	-	-	-	5/6s knob I	-	-	-	+
3/3s II	-	-	-	-	5/6s*knob*II	-	-	-	+
3/3rh I	-	-	-	-	5/6rh I	Unkn.	Unkn.	-	-
3/3rh II	-	-	-	-	5/6rh II	Unkn.	Unkn.	-	-
3/3wh I	-	-	-	-	5/6wh I	-	-	-	-
3/3wh II	-	-	-	-	5/6wh II	-	-	-	-
3/4s I	-	-	-	-	15/2s I	-	O. querc.	-	-
3/4s II	-	-	-	-	15/2s II	-	-	-	-
3/4wh I	-	-	-	-	15/2s2 I	-	-	-	-
3/4wh II	-	-	-	-	15/2s2 II	-	-	-	+
3/4uh I	Unkn.	-	-	-	15/2wh I	*F. fomet.*	-	-	+
3/4uh II	-	-	+	-	15/2wh II	*F. fomet.*	-	-	+
3/7s I	-	-	-	-	15/2wh2 I	Unkn.	-	-	+
3/7s II	-	-	-	-	15/2wh2 II	Unkn.	-	-	+

Tab. 7: Results of a microbiological screening of wood blocks taken from freshly cut beech trees and grown on agar without or with streptomycin to suppress bacterial growth (s = sapwood, rh = red heart, uh = uncolored heart, fk = wound heart, fd = faint discoloration, sd = strong discoloration, knob = knob tissue, O. querc. = *Ophiostoma quercus*, T. sp. = *Trichoderma sp.*, F. fomet. = *Fomes fometarius*, Unkn. = unknown fungus species).

	n	sterile	Bacteria	*F. fomentarius*	Unknown	*Trichoderma sp.*	O. quercus
+ streptomycin		73.2	5.4	3.6	8.9	1.8	7.1
s	20	95	5	-	-	-	-
uh	4	50	25	-	25	-	-
rh	10	80	-	-	20	-	-
wh	22	54.5	4.5	9.1	9.1	4.5	18.2
- streptomycin		46.4	37.5	-	8.9	-	8.9
s	20	45	50	-	-	-	10
uh	4	100	-	-	-	-	-
rh	10	30	20	-	40	-	10
wh	22	45.5	40.9	-	4.5	-	9.1

Tab. 8: Proportion of samples with endophytic micro-organisms found in different wood s = sapwood, uh = uncolored heartwood, rh = red heart, wh = wound heart. The sum of the percentages sapwood (- streptomycin) exceeded 100 because one sample was infected with both fungus and bacteria.

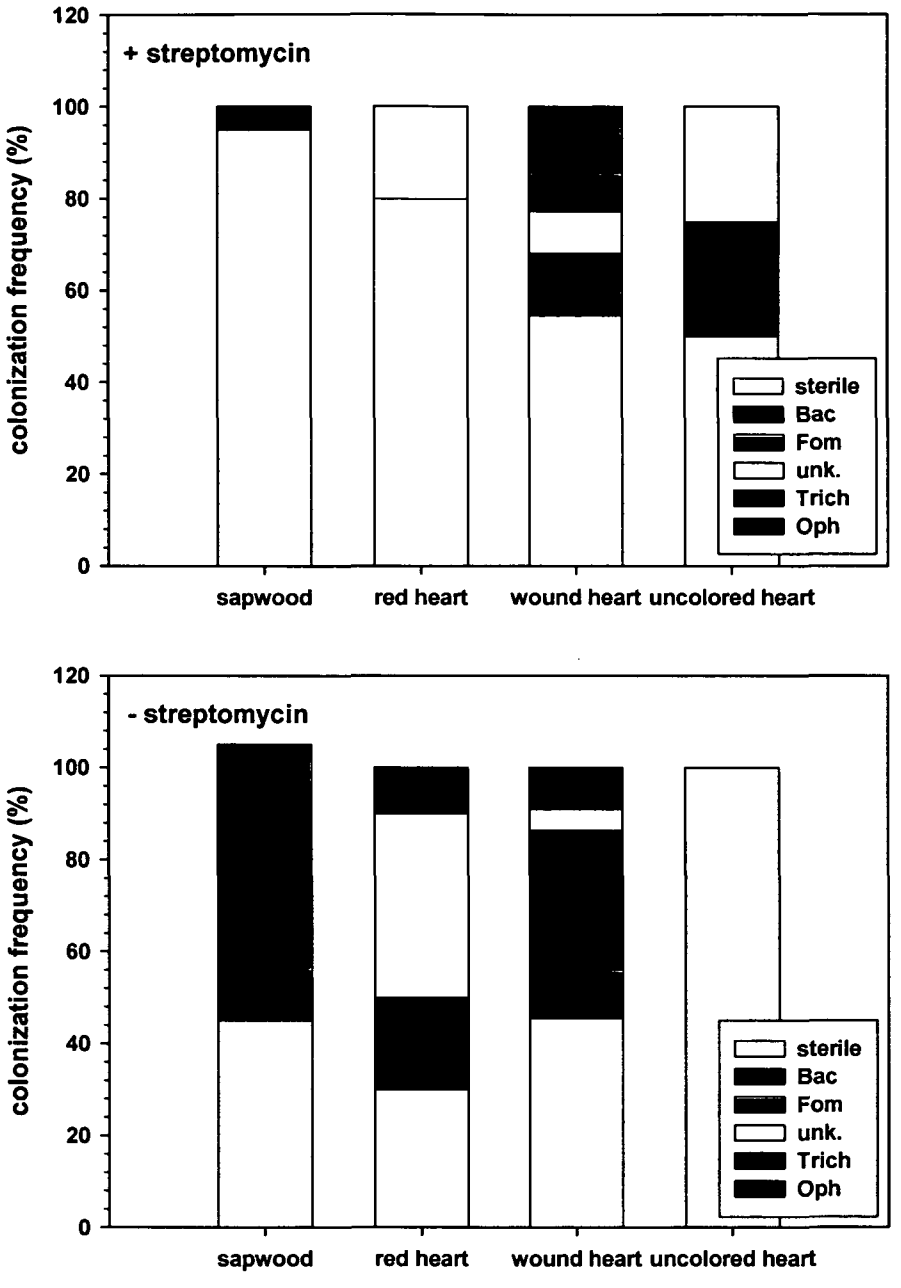

Fig. 49: Frequency of colonization on different wood from beech. Upper the graph: samples exposed with, lower graph without streptomycin. Bac = bacteria, Fom = *Fomes fomentarius*, unk = unknown endophytic fungus, Trich = *Trichoderma sp.*, Oph = *Ophiostoma quercus*.

3.5 Discussion

3.5.1 Uncertainties in oxygen measurements

Beech oxygen measurements were exclusively conducted using metal tube setups which were less susceptible to gas-leaking than anchor jackets (Chapter 2.3.7.1) and suspiciously high values (≥ 100%) were rejected (all oxygen values are in % / air saturation unless stated otherwise). Leakage tests with a portable pressurized N_2-container (Chapter 2.3.7.2), conducted in 2006 showed that all tested setups were completely gas tight. Also Spicer and Halbrook (2005) used a quite similar setup with metal chambers in their study of four temperate species. While stainless steel tubes were used in 2006, measurements in 2005 were conducted with iron tubes treated with adhesive grease to avoid oxidation processes. Though the oxidation of iron may have consumed a small amount of oxygen, this should have been much lower than oxygen consumption by respiration and should be easily compensated by diffusion, and measurements in 2005 and 2006 gave quite similar results.

During continuous long-term measurements in 2005 and 2006 (Chapter 3.4.2) longer periods of 0% oxygen interrupted with sudden peaks were recorded (Fig.43). These periods occurred mostly during heavy rainfall, and were longer and more frequent in 2005 compared with 2006, which could indicate a faint but steady depletion of oxygen by iron oxidation. This is, however, not very likely since periods of low oxygen also occurred in 2006 when stainless steel tubes were used. Frequent testing and re-calibration of the micro sensors and thorough testing of the electronic equipment and setup (Chapter 3.3.5) proved the reliability of the measurements, so that measurement errors can practically be excluded for these data.

3.5.2 Oxygen in beech stems - implications for red heart formation

In continuous measurements (Chapter 3.4.2) no difference was found in the distribution of oxygen in the stem between trees with and without red heart. Periodical point-measurements (Chapter 3.4.1) showed that in four of the six trees with uncoloured heartwood, oxygen concentrations were < 20% at more than two thirds of measurements, but in no trees with red heart. All but one tree with red heart showed oxygen concentrations > 50% during at least half of the measurements. Oxygen concentrations tended to be somewhat higher in trees with red heart than in trees without, though differences were significant on only 4 out of 21 days (Fig.35). Differences were only found in the peripheral sapwood (depth = 5 cm), where also higher variations were measured in five of eleven trees (Fig.35). The lack of significance could be related to the low sample number, but is certainly also a result of the high variability between trees and between successive measurement dates.

Results show that in trees without red heart the oxygen concentration was around 50% during spring of 2005 and ca 40% in late summer of 2006 (Fig.35), which should be enough

to induce red heart formation, if oxygen is the only requirement. Also, leaving open holes in the stem, which provides a pathway that must result in 100% oxygen concentration in the vicinity of the hole, after ten months resulted in discoloration only along a few cm in axial direction from the hole, and the discoloration (Fig.34) appears to be an effect of microbial infections and perhaps wound reaction, differing from typical red heart. This corresponds with Bosshard (1974) and Schute (1986), who failed to induce red heart in the standing tree and only found wound heart formed in the vicinity of afflicted stem injuries. If, as suggested by the current theory, e.g. (Stuber *et al.*, 2002; Knoke, 2003), oxygen entered through wounds and diffused axially in the central stem, the advance of red heart should be much faster as oxygen would spread and establish a new equilibrium within a matter of hours or days, and not months or years, the time the development of red heart in standing trees apparently takes. Since older beech trees with a larger diameter can have a substantial amount of dead uncolored heartwood (Bosshard, 1974), it is not very plausible that no beech trees exist with uncolored heart surrounded by a ring of red heart, formed by dying parenchyma cells at the sapwood/heartwood border. This suggests that oxygen is not the only factor in red heart formation and that bacteria and fungi are most likely also involved in the process.

3.5.3 Spatial oxygen distribution – implications for transport mechanisms

Distinct diurnal variations in oxygen and substantial differences between various depths were found in all individuals (Chapter 3.4.2). Diurnal variations, with daily amplitudes between 2 and 40% and extremes found between early morning and noon, with the lowest values in the evening. These results corresponded well to the measurements in mature spruce in 2003 (Chapter 2.4.1) and to (Gansert *et al.*, 2001) and emphasize a dependency between oxygen supply and the transpiration stream. However, oxygen decreased and increased after substantial drops even in times of reduced or zero sapflow and restriction of sapflow during the night or long rainy periods did not lead to a depletion of oxygen (Fig.44). This suggests a combination of axial oxygen transport, which supplies the sapwood during daytimes and high sapflow rates, and radial influx through bark, phloem and cambium assuring O_2-supply during the night and at times of low or zero sapflow.

Generally, sapwood O_2 depends on supply, consumption and demand. Consumption depends strongly on temperature, differences between seasons and the distance from the cambium (Lavigne, 1996; Stockfors & Linder, 1998). While axial supply is mostly dependant on the sapflow rate and the availability of oxygen in the soil (Chapter 2.1.1), radial supply depends on diffusion and thus of the permeability of bark and wood, and on the respiration in outer layers, which can be high during growth, especially in the cambium (Stockfors and Linder, 1998). How much growth respiration in the cambial region affects sapwood oxygen supply is unknown, as is the diffusibility of the bark. Generally stem respiration along with the

proportion of living parenchyma is reduced from cambium towards the central stem and is normally highest in the outer sapwood (Pruyn *et al.*, 2002; Pruyn *et al.*, 2004).

So far only Spicer et al. (2005) measured oxygen gradients in the stem of temperate species and found oxygen declining from cambium to the heartwood boundary in *F. americana* and *T. canadensis* during summer, while no substantial gradient existed in *A. rubrum*. In *F. americana* and *Q. rubra* the inner sapwood was significantly lower in O_2 than the outer sapwood.

In this study periodical short-term measurements (Chapter 3.4.1) showed that the spatial distribution of oxygen varied substantially between trees and often was not consistent. Continuous measurements (Chapter 3.4.2) found different the spatial distributions and gradients of oxygen in the stem in the six beech trees measured. In two trees oxygen declined from the peripheral sapwood to the central area of the stem (Fig.40). Amplitudes of the extremes decreased from bark to heartwood with maxima occurring later in the inner stem, similar to the vertical differences in mature spruce. In other trees, oxygen in the outer sapwood (d = 2 cm) was lower than in the central parts, probably due to increased respiration in the area (Fig. 38 and 41). Lower diel changes were found in the central stem, with little or no living cells. This area, including the entire heartwood, can function as oxygen storage, from which oxygen is drawn when oxygen is depleted in the outer sapwood. However, oxygen did not always decline gradually, and in several trees oxygen was e.g. high in both outer sapwood and heartwood, while it was low in 5 and 10 cm depth (Fig.42). This suggests a highly variable pattern of respiration within the stem combined with an overlay of radial and vertical transport mechanisms resulting an a complex and changing oxygen distribution, which could explain the shifting distribution observed in several trees, e.g. in tree Nr.10.

In this study oxygen was only measured during the growth season. A decrease was observed in late June 2005 and lower values where measured during July and August, especially in the sapwood (Fig.35). This corresponds to Eklund (1990, 2000), who recorded the lowest values in spruce during summer, when respiration was highest. An increase was found in the sapwood of four beech trees in September and October 2005, and in the sapwood of two and the central area of five trees in 2006, suggesting that oxygen increased when respiration was reduced in fall.

Stem O_2 concentrations could decline to nearly anoxic conditions and these could prevail for several days, if not weeks. Although parenchyma cells may survive anoxic conditions for a longer period, it may be an energetic disadvantage for a tree to maintain living cells in tissues where oxygen is often insufficient for normal respiration (extremely low concentrations were mostly found in the deepest layers). Active parenchyma cells account for 10-35% vol.% in the secondary xylem of angiosperms. These cells live between two and 150 years, extend to

a sapwood depth of 20 cm and more (Panshin & de Zeeuw, 1980), and require oxygen for aerobic respiration. Failing to find low oxygen concentration (below 5% absolute or 25% / air saturation) led Spicer et al. (2005) to reject the hypothesis that a lack of O_2 may be the physiological cause (or trigger) of heartwood formation, as suggested by (Carrodus, 1971). However, they measured only once in spring and once in summer and since O_2 concentrations can vary substantially between seasons and also on a much shorter time-scale, their data are too few to prove that low O_2 concentrations may not occur also in their study species. This leaves the theory that oxygen deficiency results in heartwood formation open for further investigations.

The sudden and strong decreases in O_2 recorded during continuous measurements were mostly associated with an increase in soil potential (Fig.43) and since oxidation of iron tubes was unlikely and the equipment was thoroughly tested, artifacts can most likely be excluded. Thus two plausible explanations remain:

- If oxygen is mainly supplied via sapflow, high water content in the soil results in lower oxygen diffusion and a successive decline in soil O_2, thus only oxygen depleted sap ascends. However, the decrease was very fast and also occurred during nighttimes without sapflow and high soil water content was not generally related to high stem O_2. Also in many cases the decrease in stem O_2 occurred almost simultaneously to the increase in soil potential, which would be too fast to suggest a transport via the sap stream.

- Wet bark should have a higher diffusion resistance, which could effect wood O_2 concentration within hours (or even shorter). As O_2 also diminished and replenished in times of zero sapflow, the second explanation appears more plausible than the first one, but still the difference in the time it took for O_2 to recover is puzzling. Also measurements showed that periods of low oxygen continued sometimes after soil and bark dried again.

None of the hypotheses can convincingly explain this effect and further investigations of that effect, best under controlled conditions, should be conducted to explain this phenomenon.

3.5.4 Discolorations during storage of beech wood

Red heart is systematically darker, redder and more yellowish compared to peripheral sapwood and uncolored heartwood, which feature a lighter tone (Büren, 2002; Pöhler *et al.*, 2004; Liu *et al.*, 2005; Pöhler *et al.*, 2006) and results from storage experiments (Chapter 3.4.3) partly agree (L* was reduced in all cores, and a* was very variable but generally increased in high O_2) and prove that red heart can be induced during storage by exposure to oxygen, as suggested by Torelli (1984). There is little question that the presence of oxygen is necessary for red heart formation as the chemical transformation is mainly an oxidation (Koch *et al.*, 2000; Koch *et al.*, 2001) and discolorations were stronger in cores exposed to higher oxygen concentrations (Tab.4, Fig.45 and 46). There was no gradual change between

100, 50 and 20% O_2 (Tab.5, Fig.47), probably as a result of inhomogeneous distribution of living parenchyma cells between the three groups. Water content was equally high in all cores during storage thus an effect can be excluded. Discolorations were especially strong in peripheral segments (Fig.46), which probably exhibited a higher ratio of living sapwood. Color changes in wood stored in an anoxic atmosphere were only minor (Tab.4 and 6, Fig.46 and 48) but nevertheless occurred in cores and wood blocks exposed to 0% and in already formed heartwood. This does not correspond to the hypothesis that red heart is only formed by dying sapwood cells exposed to oxygen at the sapwood/heartwood boundary (Zycha, 1948; Paclt, 1953; Necesany, 1966; Ziegler, 1967; Necesany, 1969; Bonsen, 1991), and suggests the contribution of another factor, most likely infection with bacteria and/or fungi.

The comparison of sterilized and non-sterilized cores in the second test was problematic because the initial measurement had to be conducted before sterilization. Sterilization in an autoclave, where samples are exposed high temperature and pressure, is similar to the method of lumber steaming for conservation (Riehl *et al.*, 2002), which also causes discolorations in beech wood. For the experiment with wood blocks (Chapter 3.4.3.3) the color effect of sterilization was subtracted and results showed that although several color values differed between sterilized (S) and control blocks (C) (Tab.6, Fig.48), a significant difference (S > C) in darkening (ΔL^*) and overall discoloration (ΔE^*) of was only found in sapwood and red heart exposed to 0% O_2, and in sapwood exposed to 0% respectively.

Possible micro-organisms, either endophytic or infections during sample processing, were not entirely terminated by antibiotic agents and sterilization. *Trichoderma sp.*, also found in this study on cut wood disks (Chapter 3.4.4), is the dominant fungal species on beech wood under low O_2-conditions (Hendry *et al.*, 2002), and flourishes in laboratory experiments under conditions of mild sterilization which causes the death of parenchyma cells and successively terminates all host defense mechanisms. On excised beech sections mild sterilization causes a 5 to 20-fold increase in fungal spread (Gramss, 1989). Thus endophytic organisms could have survived both sterilization and treatment with antibiotic agents, or even thrived under conditions where potential competitors were suppressed. However, the discoloration at 0% O_2 and of red heart segments was also found on non-sterilized cores. An alternative explanation: (Höster, 1974) described oxidation processes that occurred below the surface after felling and a successive diffusion of discolored products together with evaporating water from inner parts to the surface area where they produce reddish to brownish discolorations.

The fact that red heart segments discolored in all three storage experiments contradicts the hypothesis that red heart is only formed by dying parenchyma cells at the sapwood/heartwood boundary. Red heart formation is, however, strongly influenced by oxygen and is substantially stronger at higher oxygen concentrations. But discolorations occurred also under anaerobic conditions, probably suggesting activity of bacteria and fungi.

To fully elucidate the phenomenon further investigation is required and also a method must be developed that allows sterilization of the samples without altering wood color.

3.5.5 The role of bacteria and fungi in red heart formation

Various studies e.g. (Cosenza *et al.*, 1970; Shigo & Hillis, 1973; Shortle *et al.*, 1978; Chapela & Boddy, 1988b; Schmidt & Mehringer, 1989) describe increased colonization rates of bacteria and fungi in traumatic tissue and wound hearts of beech and other angiosperms, which are believed to contribute largely to discolorations associated with injuries. Although red heart in beech is typically not characterized by fungal growth (Zycha, 1948), in contrast to wound heart (Seeling, 1998), results of this study suggest that microorganisms likely play a role. Although microbiological examinations (Chapter 3.4.5) found no bacteria and fungi in a substantial number of samples, in all types of wood tested (sapwood, uncolored and red heartwood and discolored wound heart) there were at least some samples with bacteria or fungi, i.e., the population of endophytes is not high, but wood is generally not sterile (Tab.7 and 8, Fig.49). As expected the highest infection rate was found in the wound heart (wh) which corresponds to (Schmidt & Mehringer, 1989), who also found abundant bacteria in wound hearts of beech, and with (Cosenza *et al.*, 1970) who found bacteria in discolored and decayed tissue of beech, birch and maple. Infections were also present in the red heart, with bacteria abundant when no streptomycin was added.

The fungi identified were typical endophytes and wound colonizers of native hardwoods (Kirisits, personal commment). According to (Chapela & Boddy, 1988a) micro-organisms colonize adjacent areas either by extension from wood already colonized within the tree, from spores entering through gaps in the bark, or from propagules that remained latent in the wood after a previous infection within bark or wood tissue. Endophytic bacteria and fungi can be present in dormant form in a healthy tree and infection only expresses under certain circumstances such as a change in water content (Griffith & Boddy, 1990; Hendry *et al.*, 2002). The involvement of micro-organisms could explain why the presence of O_2 does not automatically result in red heart and why the spread of red heart is much slower (depending on microbial growth and spread) than O_2 would diffuse. However the growth of the endophytic flora in the stem is likely to be influenced by the oxygen content: An injury followed by oxygen entering through wounds substantially changes the in-stem microenvironment and, next to the open pathway for wound colonizers, could favor the development of specific aerobic or facultative-anaerobic endophytic bacteria (Shortle *et al.*, 1978). Oxygen can also influence fungal growth, which was stimulated by aeration in incubation experiments on beech wood (Hendry *et al.*, 2002), though if fungi were the cause of red heart, their hyphae should be abundant and found in the wood, which was so far not reported. This leaves a role for wounds and tree age, because wounds may be entry points

for oxygen and also for micro-organisms and would explain the positive correlation of tree age with red heart formation found by several authors, e.g. (Walter & Kucera, 1991; Frank, 1996). Old trees are likely to have injuries and have large heartwood area through which oxygen and micro-organisms spread, where oxygen can be stored and through which oxygen diffuses, and have passed a long time for the microbial growth to proceed. Several authors found red heart on stands where tree vigor is negatively influenced by various environmental factors (Chapter 3.1.1.6). Vital trees will show stronger respiration and thus lower O_2 concentration in the inner stem, their sapwood may be thicker and parenchyma cells may be better able to keep microorganisms in check.

4 Conclusion and Outlook

Oxygen measurements using optode sensors produced novel results in beech and in periodical measurements in spruce, but were not entirely suitable for continuous measurements in spruce trees, where volatile resin components substantially influenced the sensor output. The gas-tightness of the system is essential and was not always easy to achieve.

Measurements of axial and radial diffusion velocity of oxygen in the wood of various native species and a model calculated based on the results, showed that the living sapwood may be supplied with enough oxygen for respiration by radial diffusion only, if the water content is not too high, which suggests an important physiological function of wood gas content. However, in this study the permeability of bark and phloem was not considered, and should be included in further experiments. Measurements of oxygen diffusion through bark and phloem layers at variable water content may show additional limitations for the radial oxygen supply of living sapwood.

Oxygen measurements in spruce and beech showed variations in oxygen content depending on height and depth, and changes in spatial and temporal distribution in living stems. Results suggested that both pathways (i.e., radial diffusion and axial transport via the transpiration stream) contribute to the oxygen supply of the living sapwood. The proportion contributed by each pathway was not quantified, which should be further elucidated by manipulative experiments such as wetting of the bark or restricting sapflow by flooding or drought. The hypothesis suggesting that stress induces hypoxia or anoxia in the stem, which consecutively results in increased bark emissions of ethanol and monoterpenes and an increased attractiveness towards bark beetles, could not be confirmed. Experiments were conducted with small spruce trees, where, due to a higher surface/volume ratio, most oxygen supply was probably by radial diffusion. Possibly, studying the impact of water stress on stem oxygen in larger individuals, preferably in species without resin, would yield different results, but also in cut logs where the oxygen concentration was low no substantial ethanol emission was recorded.

The predominantly theory that oxygen is the only influencing factor in red heart formation in beech was falsified by oxygen measurements in the living stem and storage experiments under different conditions. Results suggested that red heart formation in beech more likely results from a combination of elevated oxygen concentrations in the stem with increased microbial activity, although no strong evidence for the latter was produced in this study. This partly accounts on color measurements, were sterilization itself strongly influenced sample color. Thus color measurements and microbiological examinations should be repeated with a larger sample size to improve the results, and bacteria should also be identified. A promising

approach to study the involvement of micro organisms would also be an inoculation experiment, where sterilized wood is infected with specific bacteria associated with discolorations in standing trees.

For the management of beech forests to produce valuable uncolored timber, understanding the formation of red wood is important, so that silvicultural measures can be taken to avoid conditions resulting in red heart or to identify standing trees with red heart, so that these can be logged early and other trees spared. If the hypothesis presented in this study is correct and a combination of oxygen plus micro-organisms induces red heart formation, there may not be very much forest management can do to beyond current management practices to avoid stem injuries and harvest at a size before red heart formation is likely to occur.

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6 Index of Tables

7 Index of Figures

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Publikationen und Vorträge

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