THE EFFECT OF CALCIUM NUTRITION ON
WOOD FORMATION IN POPLAR

Silke Lautner, Elisabeth Windeisen & Jörg Fromm

Dept. of Applied Wood Biology, Life Science Centre, Technische Universität München, Winzerer Strasse 45, 80797 Munich, Germany

SUMMARY

To study the effect of calcium on wood formation, poplar plants (Populus tremula L. x Populus tremuloides Michx.) were grown for six weeks under different calcium regimes. Energy-dispersive X-ray analysis (EDXA) revealed a decrease of calcium ions in the cambial zone under calcium deficiency. Using light microscopy, a strong impact was shown on the cambial and xylem elongation zone under calcium starvation. By Fourier transform infrared (FTIR) spectroscopy performed on wood and bark cells grown under calcium starvation, we detected a reduction of several absorptions, such as methoxy groups from S-lignin. In conclusion, our results show a distinct influence of calcium on wood formation and chemistry. Hence, efficient tree calcium supply can be considered a decisive factor in xylem production.

Key words: Calcium, poplar, wood formation, lignin.

INTRODUCTION

Mineral nutrition is essential for many aspects of plant physiology and is considered to be an important factor in plant growth and development. However, in contrast to annual herbaceous species, where fundamental functions of minerals have already been investigated, our knowledge on the effect of mineral nutrition in trees is still poor. The information is mainly restricted to the effect of fertilization on tree phenotype, such as annual increment in height and diameter (Safford & Czapowskyj 1986; Nilsson & Wasielewski 1987). Research on anatomical and cytological aspects of wood formation has so far focused on nitrogen fertilized (Puech et al., 2000) and on potassium fertilized poplar plants (Wind et al. 2004; Arend et al. 2005). On the basis of those two ions clear effects of mineral nutrition on cambial growth and wood formation could be detected.

Among other nutrients, calcium is one of the so called macronutrients and is essential for plant metabolism. It binds only weakly with water, its uptake occurs passively via the fine root apoplast, and its acropetal oriented transport in the plant occurs almost exclusively via the transpiration stream (Marschner 1995). In contrast to most macronutrients, calcium is mainly found in the cell apoplast, because of the multiple binding sites in the cell wall, especially the carboxyl groups of pectins in the middle lamella. In trees, this calcium binding capability of the middle lamella as well as of the flexible primary cell wall confers strength to developing xylem tissues (Brett & Waldron 1996; Guglielmino et al. 1997). It is also known, that the positive impact of
K, Ca and Mg fertilization on wood formation in coniferous trees is stronger during periods of low precipitation than during periods of high soil water content (Dünisch & Bauch 1994).

However, once calcium is bound to the cell wall, it remains mostly unavailable for further plant metabolism. Another reason for calcium to be mainly localized in the cell apoplast might be the restricted calcium capacity in the protoplast. Free calcium concentrations in the cytoplasm range between 0.1 and 0.2 μM concentrations (Felle et al. 1988; Evans et al. 1991; Hirschi 2004). These low concentrations enable this ion to work as an effective signal transducer upon small changes in its concentrations. Apart from acting as a messenger ion, intracellular calcium also functions as a membrane stabilizer, and therefore acts as a protector against passive ion influx (White & Broadley 2003). Often intracellular calcium is also associated with enzyme-activating processes (Plieth 2005). In angiosperm trees, excess calcium was found to precipitate as calcium oxalate crystals in cell vacuoles (Borchert 1990), whereas in gymnosperm trees calcium oxalate crystals were found mainly in intercellular spaces (Fink 1991a-c). Using secondary ion mass spectrometry, Follet-Gueye et al. (1998) were able to detect a distinct temporary increase in the calcium concentration in the cambium and in the phloem of beech during the period of reactivation in spring, but until today the biochemical mechanism of action of calcium is still not fully understood.

Research on the impact of calcium has revealed multiple functions of this ion in many different processes of plant biology. Concerning wood formation, Du & Yamamoto (2003) obtained results that point to an involvement of calcium in reaction wood formation of Taxodium distichum. Other studies report a reduction of lignification during tracheid differentiation under calcium deficiency, suggesting that Ca plays an important role in the lignification process of compression wood formation (Westernmark 1982; Lohrasebi et al. 1999). Furthermore, it was found that in hypocotyls of Picea abies cell wall deposition is reduced at low calcium concentrations due to inhibition of lignin and noncellulosic polysaccharide deposition (Eklund & Eliasson 1990; Eklund 1991). However, up to some degree IAA could overcome this Ca deficiency (Eklund 1991).

In this context, the present work focuses on the effect of calcium on anatomical characteristics of cambial growth and the resulting chemical characteristics of cell walls during wood formation in poplar. The influence of different calcium nutrition on structure and ion contents of the cambial zone was demonstrated by light microscopy and X-ray microanalysis. Investigations on wood cell wall chemistry were performed using FTIR-analysis.

MATERIALS AND METHODS

Plant material
Populus tremula L. x Populus tremuloides Michx. clones T89 were grown for six weeks in hydroponic culture under long day conditions (16 h light / 22°C : 8 h darkness / 18°C). At the beginning of the experiment, all plants were at similar height (5-6 cm). The plants were provided with macro- and micronutrients in a modified Hoagland solution (Hoagland & Arnon, 1950) containing either no Ca^{2+} (0 mM) or full strength Ca^{2+} (5 mM) concentration (four poplar plants per variation). To provide constant oxygen supply, hydroponics were aerated with pumps (Tetratec®AP50, Tetra Werke, Melle, Germany).

X-ray microanalysis
After cutting and immediately freezing in pre-cooled liquid isopentane, 2–4 mm long stem segments were freeze-dried, coated with chromium and examined on a scanning electron mi-
Toluidin blue and 4,5-diaminodifenilidet et 450 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup> and 16 scans. The FTIR spectra were baseline-corrected and normalized on the absorption band of the internal standard (2050 cm<sup>-1</sup>). All presented FTIR spectra are average value spectra of the respective single spectrum.

RESULTS

To examine the effect of calcium on wood production, poplar clone saplings were grown in hydroponics under different calcium supply. After six weeks clear differences in their growth were observed. Compared with poplars grown under full strength Ca-supply (5 mM), variants grown under calcium starvation (0 mM) showed a clearly reduced biomass production and hence a decrease in their shoot and root development (Fig.1). However, poplar saplings grown under excessive calcium supply of 10 mM did not show enhanced biomass production as compared to the full strength (5 mM) Ca supplied trees (data not shown).
**Effect of calcium supply on ion concentrations in the wood forming tissue**

The element composition of the cambial zone was analysed using a scanning electron microscope in combination with an X-ray microanalysis system. In both treatments the most abundant element present in the cambial zone was potassium, but calcium and phosphorous were also evident. Changing calcium nutrition not only caused a shift in the cambial calcium concentration, but also affected the relative content of potassium and of phosphorous. Thus, under calcium starvation a decrease was found in calcium and also in potassium and phosphorous content in the cambial tissue (Fig. 2).

![EDX analysis of the cambial zone in poplar.](image)

**Fig. 2**: EDX analysis of the cambial zone in poplar. Relative calcium, phosphorous and potassium concentrations under different calcium supply, expressed as peak : background values. Presented data are means of $n = 4$ and SD.

**Microscopic analysis**

In order to find out if wood anatomy is affected by different calcium regimes, we performed light microscopic analysis on stem cross sections of different Ca-supplied poplars. The results revealed strong differences in the width of the cambial zone as well as of the xylem differentiating zone. After growing for six weeks under optimal calcium supply (5mM), poplars developed a cambial zone of about seven cell layers in radial direction (Fig. 3a). In contrast, poplars grown for six weeks under calcium starvation (0mM) formed a cambial zone of only three to four cell layers in radial direction and showed secondary cell wall formation in close proximity to the cambial zone (Fig. 3b), which points to a very limited xylem cell elongation zone.

![Effect of different Ca supply on the anatomy of cambial cells and expanding xylem cells.](image)

**Fig. 3**: Effect of different Ca supply on the anatomy of cambial cells and expanding xylem cells. Light microscopy of stem cross sections of poplar grown under full strength Ca supply (5 mM, a) in comparison with no Ca supply (0 mM, b) revealed that under Ca limiting conditions the cambial as well as the xylem differentiation zone lacks 2-3 cell layers in radial direction. Secondary cell wall formation starts closer to the cambial zone. Abbreviations: cz - cambial zone; p – phloem; x – xylem differentiation zone.
**FTIR spectroscopy**
To compare FTIR spectra of poplar wood and bark grown under optimal nutrient supply with those of poplar grown under calcium starvation we chose the wavenumber range between 4000 cm\(^{-1}\) and 450 cm\(^{-1}\). In the xylem a reduction of the methoxy groups was detected at wavenumber 1325 cm\(^{-1}\); in comparison to the aromatic skeletal vibrations of lignin at wavenumber 1505 cm\(^{-1}\) in trees, grown under calcium starvation (Fig. 4a). Wavenumber 1325 cm\(^{-1}\) is representing the C-O vibration of aryl-acyl-ether, namely of methoxy groups of S-lignin. Therefore, a reduction of S-lignin content could be detected in poplars grown under calcium starvation. Moreover, other absorption bands also showed lower intensity in wood cells produced under calcium starvation, such as typical bands of acetyl groups, e.g. wavenumbers 1740 cm\(^{-1}\) and 1380 cm\(^{-1}\). In addition, we also observed Ca-dependent changes in the bark with a distinct reduction in the absorption rate under calcium starvation. Remarkably, a significant reduction of wavenumber 1640 cm\(^{-1}\) occurred in the bark tissue under calcium starvation in comparison to the control plants (Fig.4b). For lignin, this absorption band represents the conjugated C=O stretch vibrations (e.g. in aryl ketones).

![FTIR spectra](image)

**Fig. 4:** FTIR spectra of poplar wood (a) and bark cells (b) grown under different Ca nutrition \((n=5)\). Under calcium starvation a reduction of methoxy-groups of S-lignin (wavenumber 1325 cm\(^{-1}\)) and typical bands of acetyl groups (wavenumbers 1740 cm\(^{-1}\) and 1380 cm\(^{-1}\)) became obvious in xylem cell walls. In the bark tissue a strong decrease of conjugated C=O stretch vibrations (1640 cm\(^{-1}\)) was observed under Ca stress compared to full strength Ca supply.
DISCUSSION

The aim of this study was to analyse the effect of calcium nutrition on anatomical and chemical aspects of wood formation. Using light microscopy, EDX-analysis and FTIR spectroscopy we found changes in both chemical and anatomical characteristics of poplar stem tissue cells. Chemical analysis revealed a reduction of S-lignin concentration in the xylem cell walls of poplar grown under calcium starvation. Since the ratio of S- and G- lignin in poplar trees is known to be stable (Hu et al. 1999), we suggest that over all lignin concentration was lower in poplar grown under Ca deficiency compared with the poplar grown under optimal calcium supply. Even though the exact effect of calcium on the lignin biosynthesis pathway is not well understood, our results do confirm earlier findings of Eklund & Eliasson (1990) and Westermark (1982), who report a dependency of lignin biosynthesis on Ca-supply in trees. Apoplastic peroxidases could play a key role in this effect, since several apoplastic peroxidases are known to bind to pectin in their Ca-induced conformation (Penel & Greppin 1996). Since the cell corners and the middle lamellae which are rich in calcium pectate are the first sites in the cell wall to be lignified. Ca-pectate-bound peroxidases might play a notable role in the spatial control of lignin deposition. Hence, changes in calcium concentration might modulate the location of peroxidases (Carpin et al. 2001; Boerjan et al. 2003) and thus lignin biosynthesis.

In our study we also observed a strong reduction in the width of the cambial zone under calcium deficiency. Earlier studies have shown that insufficient mineral supply of potassium evokes similar responses of a reduced cambial formation in poplar (Wind et al. 2004). The authors observed a reduction of cambial cell layers in radial direction and also a shift of secondary cell wall formation starting closer at the cambial initial cells. Since we have now detected similar characteristics under insufficient calcium supply, this effect may be due to general nutrition stress and therefore can not be regarded as being calcium specific.

Taken together, the results presented here indicate a possible regulatory role of calcium in poplar wood formation; however, the crucial processes that mediate and facilitate the effect of this ion on xylem development are yet far from clear. For further understanding of the precise function of calcium in xylemogenesis extensive molecular studies on Ca channels and receptors in the wood producing cambial zone would be necessary.

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REFERENCES


