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ORIGINAL ARTICLE

Wood formation in urban Norway maple trees studied by the micro-coring method

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Abstract

Intra-annual wood formation in healthy and affected Norway maple (*Acer platanoides* L.) street trees in the City of Ljubljana, Slovenia, was studied by the micro-coring method. Samples were taken with a Trephor instrument at weekly intervals during the 2005 growing season. The beginning of wood formation corresponded to the initiation of cambial activity in the middle of April in all investigated Norway maple trees. In healthy trees, the period of wood formation was finished in mid-September, persisting 7 weeks longer than in affected ones. Small destructivity and sufficient quality of micro-cores confirmed that sampling with a Trephor is a convenient method for the study of wood formation in diffuse-porous hardwoods, growing in the harsh conditions of an urban environment.

Keywords: Norway maple (*Acer platanoides*); Xylem; Cambial activity; Cell differentiation; Urban tree; Micro-core

Introduction

Wood formation in trees is a periodic process, initiated by cell division in the vascular cambium, followed by differentiation of cambial derivatives. The differentiation of these cells involves four major steps: cell expansion followed by the ordered deposition of a thick, multilayered secondary cell wall, lignification, and cell death (Plomion et al., 2001). These processes are regulated by genetic and environmental factors (Lauchaud et al., 1999; Aloni et al., 2000; Savidge, 2000; Sundberg et al., 2000). The physiology of cambial activity and the cellular phenology of xylem growth ring formation have been intensively investigated in saplings and various forest tree species (Wodzicki, 1971; Denne and Dodd, 1981; Funada et al., 1990; Larson, 1994; Schmitt et al., 2004; Deslauriers and Morin, 2005; Frankenstein et al., 2005), but there have been only a few studies done on urban trees (Schmitt et al., 1998; Bühler et al., 2006). It is known that the specific site conditions in urban areas, characterized by pollution, de-icing salts contamination, water stress, soil compaction, wounding, and limited root space result in altered wood structure, reduced growth and vitality, or even increased mortality of amenity trees (Eckstein et al., 1976; Petersen et al., 1982; Oven and Levanič, 2001; Oven, 2004), but the influence of the harsh urban environment on the duration of cambial activity and wood formation in urban trees is only fragmentarily understood.

Various methods are available for studying the seasonal dynamics of wood formation at high-level resolution, such as dendrometers (Deslauriers et al., 2003a; Mäkinen et al., 2003), the pinning method (Wolter, 1968; Schmitt et al., 2000; Schmitt et al., 2004), and micro-sampling, which enables direct

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observation of the tissues between differentiated secondary phloem and xylem (Wodzicki, 1971; Antonova and Stasova, 1993, 1997; Bäucker et al., 1998; Gindl and Grabner, 2000; Deslauriers et al., 2003b; Deslauriers and Morin, 2005; Rossi et al., 2006a).

The objective of this research was to apply a Trephor micro-coring instrument to describe wood formation in diffuse-porous Norway maple trees. We assessed the suitability of samples obtained for examination of cambial activity and cell differentiation with a light microscope, the duration of the wood formation period in healthy and affected street trees, and the damage caused to the tree when sampling.

Material and methods

We sampled Norway maples (*Acer platanoides* L.) growing in the city of Ljubljana (323 m a.s.l.), Slovenia. Two groups of six trees were selected according to the vitality of the crown (Fig. 1). Trees in the control group had dense, healthy, and normally developed crowns, with enough space for root growth. Trees in the affected group had limited root space between the pavement and the street, a crown in poor condition with many dead branches, and leaves revealing symptoms of pollution by de-icing salts.

Small micro-cores (1.8 mm in diameter) of inner phloem, cambium, and outer xylem were taken with a prototype Trephor instrument at weekly intervals from the middle of April until the middle of October 2005. Sampling positions were arranged along the stem in a semi-helical pattern. The lowest sample was located 1.2 m above the ground. Before sampling, the rhytidome and part of the outer living bark were removed to reduce the compression and damage to cambial tissue. This step was important in May and June, when regions of the cambium and radially expanding cells were very wide and many samples were damaged due to the mechanical weakness of these tissues.

Fig. 1. Two lines of Norway maple with different crown vitalities. Affected trees are on the left and healthy ones on the right side of the pavement.

Experience showed that after the insertion of the Trephor piercing head into the tree with a hammer, pushing the instrument toward the stem and rotating it left and right was necessary to separate the micro-core from the stem. The instrument was then removed by rotating like a corkscrew as described by Rossi et al. (2006a). When sampled correctly, only one micro-core was sufficient per tree on each sampling date. Since the samples could break in the following steps of the histological procedure, we always took two micro-cores.

The diameter of the trees was measured at breast height and trees were cored for age determination at the end of the 2005 growing season. Additionally, three wounds induced by the Trephor were removed with a chisel and hammer in one experimental tree in order to assess the damage produced during sampling.

Micro-cores were immediately fixed in formaldehyde–ethanol–acetic acid (FAA) solution for 1 week, and then stored in 50% ethanol. Tissues were oriented and put in a Leica TP 1020-1 tissue processor for dehydration in a graded series of ethanol (70%, 90%, 95%, and 100%) and D-limonene (Bio-Clear) for paraffin infiltration (Johansen, 1940; Rossi et al., 2006a).

For light microscopy, permanent cross sections 8–14 μm in thickness were prepared on a Leica RM 2245 rotary microtome, using disposable Feather N35H blades. For better adhesion of the sections, slides were previously treated with albumin. Sections were dried at 70 °C for 1 h and cleaned of residual paraffin by immersing the slides in D-limonene and ethanol. They were stained with safranin (0.5 g in 100 ml 96% ethanol) and astra blue (0.5 g in 100 ml distilled water) solution to distinguish among the different phases of cell development, and finally mounted in Euparal (Chroma, 3C-239).

A Nikon Eclipse 800E light microscope (bright field and polarized light) and Lucia G 4.8 image analysis system were used for anatomical observation of cambial and xylem cells. Fibres were counted along three radial files. If vessels interrupted the radial files of the fibres, we continued by counting the cells in one of the adjacent cell files. At least three radial files of xylem and cambial cells were counted in each sample and then averaged.

The beginning of cambial activity was defined by an increased number of cambial cells in spring and cessation by a decreased number of them in autumn. At that time, there were no xylem cells in post-cambial growth adjacent to the cambium. We determined the onset of wood formation as the point at which the number of cambial cells increased and cessation when the latest formed xylem cells were fully developed.