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Technical note

Monitoring intra-annual cambial activity based on the periodic collection of twigs – A feasibility study

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ABSTRACT

This study aims at monitoring the cambial activity and xylogenesis along the growing season under Mediterranean climate using a newly designed sampling method based on the periodic collection of twigs from the crown, in order to test its efficacy. Ten species were selected; seven hardwood (*Acer pseudoplatanus* L., *Ceratonia siliqua* L., *Schinus terebinthifolius* Raddi, *Olea europaea* L., *Fraxinus angustifolia* Vahl, *Populus alba* L., *Quercus suber* L.) and three softwood species (*Abies alba* Mill., *Cupressus sempervirens* L., *Pinus pinea* L.). The twigs were collected monthly during one year. After microtome sectioning and staining, the sections were photographed and analyzed to monitor ring development and the period of cambial activity.

The twigs revealed large differences in radial growth, within and among species, which might be associated to their particular position in the tree, to the effect of the different crown microclimates, and/or to the crown conformation and type of leaves. The method showed effectiveness in the study of cambial activity, but limitations in the study of ring development due to impossibility of comparing samples. Improvements to the method are proposed, aiming essentially at minimizing the variability among samples.

Introduction

Dendrochronology has revealed to be of great value in the study of environmental variations, especially in the present context of climatic changes (e.g. Brienen and Zuidema, 2005; Davi et al., 2006). Initially based in interannual variations of tree ring width as indicator of environmental conditions (Fritts and Swetnam, 1989), dendrochronology developed to include other ring variables such as wood density, anatomical features and chemical characteristics (e.g. Collins et al., 2002; García-González and Fonti, 2006; Roig et al., 2006; Helama et al., 2008, Kress et al., 2010).

A considerable number of studies focus on the growth-ring development throughout the growing season (e.g. Gričar and Čufar, 2008; Campelo et al., 2006; De Luis et al., 2007).

There are several sampling methods for ring analysis at an intra-annual scale, all using periodical collection of data from the stem along the growing season: dendrometers, the pinning method (Makinen et al., 2008; Nocetti and Romagnoli, 2008); and the extraction of wood samples containing phloem, cambium and xylem, either as small blocks or using a microsampling with specifically designed or adapted utensils (Forster et al., 2000; Rossi and Menardi, 2006; De Luis et al., 2007; Deslauriers et al., 2008; Gričar and Čufar, 2008; Makinen et al., 2008; Čufar et al., 2011; Krepkowsky et al., 2011).

These methods have disadvantages associated to their impact on the tree, the cost and operational difficulty, as well as limitations concerning the applicability to hardwoods. Most works were developed with softwood species and under climates in which the growing season lasts only a few months but sampling of hardwood species is more difficult because of their wood hardness and anatomical complexity. In climates with variable growing season length, such as in the Mediterranean region, e.g. some species are active all year-round, while others have one or two stops in cambial activity (Cherubini et al., 2003), monitoring requires a larger number of samples per year, and therefore a least impacting method is to be preferred. A method based on the collection of small structures, such as twigs from the crown, has less impact than repeatedly extracting wood pieces from the stem. Moreover, each sampling does not influence the next (e.g. by formation of wound tissue), since different twigs are collected. The collection of samples and the sectioning of twigs for observation is also easier and less expensive.

In this paper we monitor the seasonality of cambial activity and the development of the growth-ring along one growth-season

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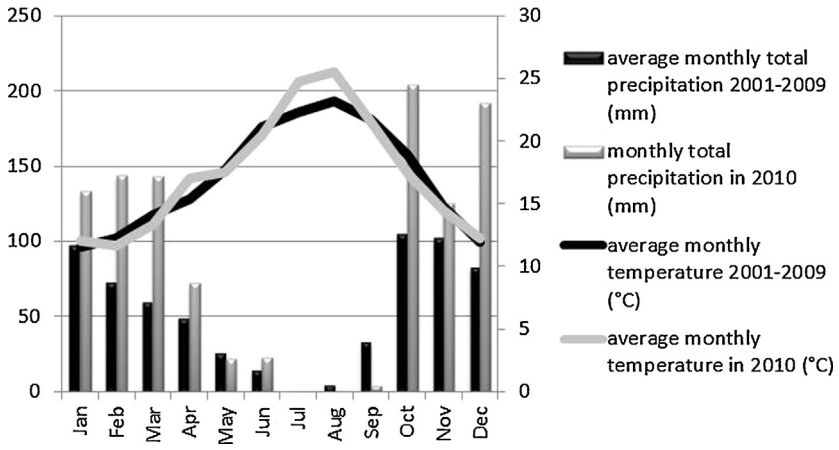

Fig. 1. Tapada da Ajuda's average monthly temperature and total precipitation in the sampling year (2010) and in the period from 2001 to 2009 (average).

on seven hardwood and three softwood species, using a sampling method adapted to the Mediterranean region based on the monthly collection of twigs, in order to test its efficacy.

Materials and methods

Description of the site

The sampling was made in Tapada da Ajuda, situated in Lisbon, Portugal (38°42′N; 9°10′W). According to the Koppen' classification, Lisbon is under the influence of a mesothermal humid climate, with a dry season in the summer extending from June to August (Medina, 1973). The soil is characterized by a fine, or medium to fine, texture, derived from tuffs or basalts, frequently with limestone on the inferior horizons, or from calcareous rock (in much less extension).

The Tapada da Ajuda, a former royal game reserve, is now the campus of the School of Agriculture of the University of Lisbon. It extends to an area of 100 ha, integrating various experimental fields and a great diversity of botanical species, and includes a meteorological station.

During the sampling year of 2010, the weather at the Tapada was particularly humid (annual rainfall of 1063.8 mm, compared to the average annual sum for the period 2001–2009 of 652.2 mm) with temperatures within the average of the ten previous years, except for the summer months of July and August, which registered temperatures above the average and no rainfall (Fig. 1).

Description of the method

The sampling was made during 2010 with a monthly frequency. The period of sampling covered the whole growing season, with the first sample collection in March, and following a monthly basis until December. It consisted of the collection of three-year old twigs from three conifer species, *Abies alba* Mill., *Cupressus sempervirens* L.,and *Pinus pinea* L., and seven broadleaf species, *Acer pseudoplatanus* L.; *Ceratonia siliqua* L., *Schinus terebinthifolius* Raddi, *Olea europaea* L., *Fraxinus angustifolia* Vahl, *Populus alba* L., and *Quercus suber* L. The taxonomic classification and leaf cycle of each species is shown in Table 1. Fig. 2 illustrates the crown and twigs of each species. Due to the fact that this was a pilot study a considerable number of species was tested and selected according to their availability and abundance in the Tapada, focusing on species present in the Portuguese landscape. The twigs' age was estimated by the number of scars left by the annual buds formation (Schweingruber, 2007): three scars were counted from the extremity of the twig and the sample was taken between the second and the third scar, as near as possible to the latter. The sampling was made on up to three trees of each species, by collecting randomly three twigs per individual. It was not always possible to follow this sampling scheme, whereby a few number of species were not represented by the same number of samples.

The sampled twigs were inserted in small identified plastic bottles, containing 40% ethanol (Schweingruber, 2007). Cross-sections with 15 micrometers of thickness were cut, using a microtome GSL1 (MICROT L) and stained with a 1:1 solution of safranin and astra blue.

Safranin stains lignin in red, allowing visual distinction of lignified cells, and Astra blue, stains cellulose in blue. When used together, unlignified or less lignified cells remain blue. Each section was impregnated directly on the slide with the safranin and astra blue solution (5 min). The removal of the dye solution excess and cell dehydration was made using a three step wash sequence: tap water, 75% ethanol and 96% ethanol. The stained cross-sections

Table 1
Division, order, family and leaf cycle of the species studied.

	Division Class Order Family	Leaf cycle
Abies alba	Gimnospermae Coniferae Coniferales Pinaceae	Evergreen
Acer pseudoplatanus	Angiospermae Dicotiledoneae Sapindales Aceraceae	Deciduous
Ceratonia siliqua	Angiospermae Dicotiledoneae Rosales Leguminosae	Evergreen
Cupressus. sempervirens	Gimnospermae Coniferae Coniferales Cupressaceae	Evergreen
Fraxinus angustifolia	Angiospermae Dicotiledoneae Contortae Oleaceae	Deciduous
Olea europaea	Angiospermae Dicotiledoneae Lamiales Oleaceae	Evergreen to deciduous
Pinus pinea	Gimnospermae Coniferae Coniferales Pinaceae	Evergreen
Populus alba	Angiospermae Dicotiledoneae Salicales Salicaceae	Deciduous
Quercus suber	Angiospermae Dicotiledoneae Fagales Fagaceae	Evergreen
Schinus terebinthifolius	Angiospermae Dicotiledoneae Sapindales Anacardiaceae	Evergreen