

ANATOMY OF XYLEM AND PHLOEM IN STEMS AND ROOTS OF *POPULUS SIBIRICA* AND *ULMUS PUMILA* FROM SEMI-ARID STEPPE IN MONGOLIA

ANATOMIJA KSILEMA IN FLOEMA DEBEL IN KORENIN DREVESNIH VRST *POPULUS SIBIRICA* IN *ULMUS PUMILA* IZ POLSUHE STEPE V MONGOLIJI

Anastazija Dimitrova^{1*}, Angela Balzano^{2*}, Katarina Čufar², Gabriella S. Scippa¹, Maks Merela², Antonio Montagnoli³, Batkhuu Nyam-Osor⁴, Enkhchimeg Tsedensodnom Chingee⁴, Donato Chiatante³

UDK članka: 630*811.18:630*811.28

Original scientific article / Izvirni znanstveni članek

Received / Prispelo: 13.7.2023

Accepted / Sprejeto: 29.8.2023

Abstract / Izvleček

Abstract: The present study focuses on the cambium and the anatomy of secondary tissues (xylem – wood and phloem) of Siberian poplar (*Populus sibirica*) and Siberian elm (*Ulmus pumila*) grown in a plantation in the semi-arid Mongolian steppe. Stem and root microcores from both species were collected and subsequently processed to obtain cross-sections for light microscopy by paraffin embedding, sectioning with a rotary microtome, and staining with safranin and astra blue. The results present the anatomy of the secondary xylem and phloem of stems and roots of both species, along with the characteristics of the youngest xylem and phloem annual rings. We discuss the critical aspects which need to be considered when using the microcoring methodology, along with the need for further studies on wood and phloem formation of less-commonly studied tree species and their characteristics when grown in semi-arid environments.

Keywords: Siberian poplar (*Populus sibirica*), Siberian elm (*Ulmus pumila*), cambium, xylem, phloem, stem, root, microcore

Izvleček: Raziskali smo kambij in anatomijo sekundarnih tkiv (ksilema–lesa in floema) sibirskega topola (*Populus sibirica*) in sibirskega bresta (*Ulmus pumila*) s plantaže v polsuhi stepi v Mongoliji. Tkivo za analize (mikro izvrtke) smo odvzeli iz debel in korenin dreves. Mikro izvrtke smo vklopili v parafin in z rotacijskim mikrotomom narezali preprate prečnih prerezov, ki smo jih obarvali z barviloma safranin in astra modro in pregledali pod svetlobnim mikroskopom. Rezultati predstavljajo anatomijo ksilema in floema debel in korenin obeh vrst. Posebno pozornost smo posvetili prikazu značilnosti najmlajših letnih prirastkov ksilema in floema. V razpravi poudarjamo kritične vidike metodologije odvzema, priprave mikro izvrtkov in anatomskih preparatov, ter potrebo po nadaljevanju raziskav nastajanja ter lastnosti lesa in floema manj znanih drevesnih vrst iz polsuhih okolij.

Ključne besede: sibirski topol (*Populus sibirica*), sibirski brest (*Ulmus pumila*), kambij, ksilem, floem, deblo, korenina, mikro izvrtke

1 INTRODUCTION

1 UVOD

Secondary tissues in trees, secondary xylem and secondary phloem, are produced by the vascular cambium. Studies of cambial activity and xylem and phloem formation are therefore essential for a better understanding of cambial phenology, the subsequent formation processes and characteristics of the secondary tissues. Combined, they provide information on the general health and growth potential of trees, the tree responses to changing

¹ University of Molise, Department of Biosciences and Territory, Contrada Fonte Lappone SnC, 86090 Pesche, Italy

² Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za lesarstvo, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

³ University of Insubria, Department of Biotechnology and Life Science, Via Dunant 3, 21100 Varese, Italy

⁴ National University of Mongolia, Laboratory of Forest Genetics and Ecophysiology, School of Engineering and Applied Sciences, 14201 Ulaanbaatar, Mongolia

* e-mail: anastazijadimitrova@hotmail.com, angela.balzano@bf.uni-lj.si

* A. Dimitrova and A. Balzano contributed equally to this work

environmental conditions, and tree plasticity, in terms of adapting their growth and the structure of xylem and phloem to the given environmental conditions and ensuring their optimal functioning (Rossi et al., 2006a; Gričar & Čufar, 2008; Prislan et al., 2013b; Balzano et al., 2018).

In the present context, the term 'cambium' is used to describe the cambial zone, which consists of several cell layers, including actively dividing cambial initials with phloem and xylem mother cells (Catesson et al., 1994; Larson, 1994; Lachaud et al., 1999; Gričar & Čufar, 2007). The study of cambial activity, and its productivity, usually involves collecting tissues from a living tree, fixation and preparation of tissues, cutting and staining of thin sections, observation under a microscope, image analysis and quantification (counting and measuring) of cambial cells in radial rows (e.g., Prislan et al., 2009; Balzano et al., 2022; Prislan et al., 2022). Studies can also include an analysis of cambial cell morphology, cell content and the developmental stages of their derivatives. Studying actively dividing or dormant cambium cells can be also based on observation of their ultrastructure, focusing on the presence, appearance, and frequency of cell organelles. Such analyses require a demanding fixation with solutions like glutaraldehyde and osmium tetroxide and observation with a transmission electron microscope (Prislan et al., 2011; 2013a; 2016).

The secondary xylem in hardwoods (dicotyledons) consists of vessels, various types of fibres, axial parenchyma, and rays. These major components vary widely among species (Wheeler et al., 1989). During wood formation, the new cells formed by the cambium go through several stages of differentiation. In the first phase, they have a thin, non-lignified primary cell wall and undergo expansion to reach the final form and size. This phase is followed by deposition and lignification of the cell walls, and finally, programmed cell death (Prislan et al., 2009).

The secondary phloem in hardwoods consists of sieve tubes with companion cells, axial parenchyma, phloem rays and phloem fibres (Čufar, 2006; Prislan et al., 2012; Crivellaro & Schweingruber, 2015; Gričar et al., 2016). Some taxa, e.g., *Fagus*, do not form phloem fibres but contain sclereids which are formed during secondary processes in older bark (Čufar, 2006; Prislan et al., 2012). After the cambium produces the phloem, we can follow

the cell differentiation, maturation and secondary changes, and observe the non-collapsed and conducting phloem which is able to transport products of photosynthesis. The older (outer) phloem is identified as collapsed and non-conductive, and over time it undergoes considerable changes (Prislan et al., 2019). In temperate species we can usually define at least one annual growth ring in phloem, with early and late phloem and the phloem ring boundary (Prislan et al., 2012).

When studies focus on the characteristics and dynamics of the cambial activity, xylem and phloem formation in response to climatic and environmental changes, it is important to prepare an adequate sampling design, along with an appropriate technique for tissue collection and sample preparation, as described by Prislan et al. (2014a; 2014b; 2022) and Balzano et al. (2022). It is also important to have a thorough knowledge of the anatomy of the secondary tissues of the species in question.

Recently, the need for such knowledge arose in activities focused on species selection for environmental restoration in the semi-arid steppe areas suffering from increasing aridity. In particular, as part of the *Green Belt* project in Mongolia, plantations of the Siberian poplar (*Populus sibirica* Hort. Ex. Taush) and Siberian elm (*Ulmus pumila* L.), have been used to study the effects of irrigation and fertilization on several plant characteristics, i.e., tree growth – stem height and root collar diameter (Byambadorj et al., 2021a), morphophysiological traits – leaf area and leaf biomass (Byambadorj et al., 2021b), above and belowground biomass partitioning (Byambadorj et al., 2022), root biomass distribution (Nyam-Osor et al., 2021), and plasticity of root architecture in *Ulmus pumila* (Montagnoli et al., 2022).

The aim of this study is thus to present the basic anatomy of the vascular cambium, secondary xylem, and secondary phloem from microcores taken from stems and roots of *Populus sibirica* and *Ulmus pumila*, grown under natural conditions (without irrigation or fertilization) in the semi-arid Mongolian steppe. As such, the study presents the first attempt to underline the anatomical and structural characteristics of xylem and phloem of the two species grown in semi-arid conditions. Furthermore, we underline critical aspects related to tissue collection and sample preparation, as part of future

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recommendations for more in depth studies on cambial activity and the development of secondary tissues in challenging environmental conditions.

2 MATERIALS AND METHODS

2 MATERIAL IN METODE

2.1 MATERIALS

2.1 MATERIAL

The *Populus sibirica* Hort. ex Tausch and *Ulmus pumila* L. trees used in this study originate from a plantation–experimental site (area 2 ha) – located in Mongolia (latitude 47°52′15.43″ N, longitude 105°10′46.4″ E, elevation 1130 m a.s.l.) within the forest nursery of the South Korea-Mongolia Joint *Green Belt* Plantation Project in Lun soum, Tuv aimag, Mongolia, 135 km west of Ulaanbaatar, Mongolia (Byambadorj et al., 2021a). In particular, the nursery is located on the right bank of the Tuul River, in a dry-steppe area which is densely populated and greatly degraded by intense livestock grazing. The site had a mean annual temperature of 0.7°C and total annual sum of precipitation 196 mm (period 2000-2019) as recorded by the Lun soum weather station (Byambadorj et al., 2021a). The mean monthly temperatures were below zero from November to March, with a mean air temperature of the warmest month (July) of 16°C, while that of the coldest month (January) of -22°C. Detailed meteorological data measured during the experiments showed that July-September 2019 was characterized by below average precipitation and above average temperatures (Byambadorj et al., 2021a).

As part of the *Green Belt* project, an experimental design aiming to evaluate the performance of *Populus sibirica* and *Ulmus pumila* under different combination of fertilization and irrigation treatments was established (for detailed overview of the experimental design, see Byambadorja et al., 2021a; 2021b). Briefly, as preparatory work, after growing them in a greenhouse, two-year-old *Populus sibirica* saplings (grown from 20 cm cuttings) and *Ulmus pumila* seedlings (grown from seeds) were acclimated in an open nursery and transplanted in open plantations in May 2011. After one month of open-field acclimatization with sufficient watering, the pre-selected combination of fertilization and irrigation treatment was applied. In the autumn of 2019, the trees had reached 10 years of

age with a height of 180 cm and 190 cm, and root collar diameter of 3.7 cm and 5.6 cm, for *Ulmus* and *Populus*, respectively (Byambadorj et al., 2021a).

2.1 SAMPLING AND SAMPLE PREPARATION

2.1 VZORČENJE IN PRIPRAVA VZORCEV

In the present study, we investigated the samples coming from eight trees (four from the poplar and four from the elm) which were grown under natural conditions, i.e., without any fertilization and irrigation treatment. For each tree, four microcores were sampled on 20 October 2019; two were taken from the stem (5 cm above ground) and two from the root (5 cm below ground) using a Trephor tool (Rossi et al., 2006b).

The microcores were initially stored in FAA (mixture of formalin, acetic acid and ethanol) and then dehydrated in gradient ethanol series (70, 90, 95 and 100%), infiltrated with bio-clear (D-limonene) and embedded in paraffin blocks using a Leica TP1020-1 tissue processor (Nussloch, Germany). Using a semi-automatic rotary microtome (RM 2245, Leica, Nussloch), cross-sections (9 µm thick) were obtained, and subsequently stained with safranin (0.04%) and astra blue (0.15%) water solution (Prislan et al., 2013a; Balzano et al., 2022; Prislan et al., 2022). The samples were mounted in Euparal (Bioquip Rancho Domingez, CA, USA) and observed under a light microscope – transmitted light mode Zeiss Axio Imager A.2 light microscope (Carl Zeiss Microscopy, White Plains, NY, USA), while images were acquired with a Zeiss AxioCam 712 color (Carl Zeiss Microscopy GmbH, Jena, Germany).

On the transverse sections of each specimen, we identified the non-collapsed phloem and the phloem growth rings including early and late phloem and measured their respective widths. We also recorded the number of cambium cells, the width of the cambium and made observations on the stage of its productivity. On the xylem side, we examined and measured the tissues with the newly formed cells in the enlargement stage, with cells in the secondary cell wall deposition and lignification phase, and the tissue where the cells were mature. We also measured the width of the most recently formed xylem growth ring and the rings formed in one or more previous years.

3 RESULTS

3 REZULTATI

3.1 XYLEM AND PHLOEM STRUCTURE OF *Populus sibirica*

3.1 ZGRADBA KSILEMA IN FLOEMA SIBIRSKEGA TOPOLA *Populus sibirica*

The wood of the stem of *Populus sibirica* has the typical anatomy of poplar with the following features observed on cross-section: wood diffuse porous, growth ring boundaries distinct, tangential diameter of vessel lumina 50 – 100 µm, fibres generally thin-walled, and rays exclusively uniseriate (Wheeler, 2011; InsideWood, 2023) (Fig. 1a, 2a). The growth ring boundaries were characterized by

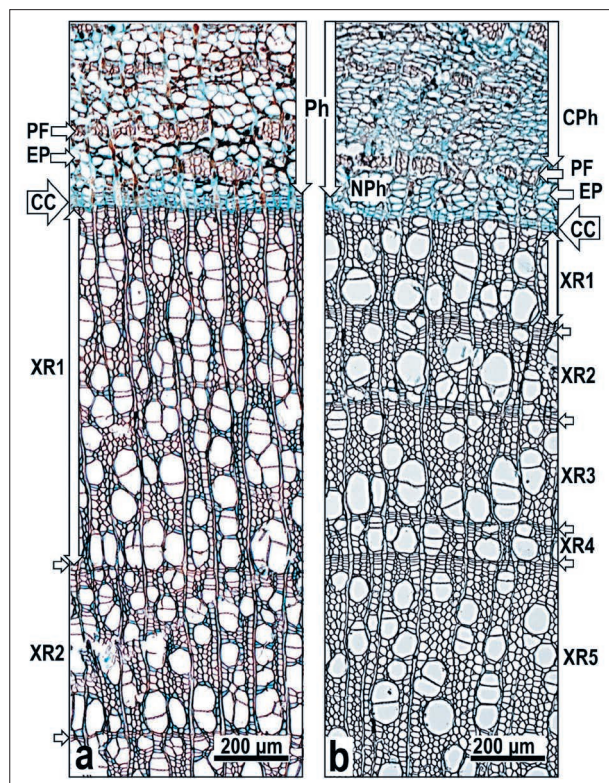


Figure 1. Secondary tissues of *Populus sibirica*, overview: (a) stem, (b) root. CC- cambial cells, XR1... XR5–xylem rings, arrows pointing to growth ring boundaries, Ph-phloem, EP-early phloem (sieve tubes), PF-phloem fibres, NPh – non-collapsed (conductive) phloem, CPh – collapsed phloem.

Slika 1. *Populus sibirica*, pregled sekundarnih tkiv: (a) deblo, (b) korenina. CC – celice kambija, XR1... XR5 – ksilemske branike, puščice kažejo letnice – meje med branikami, Ph – floem, EP – rani floem (sitaste cevi), PF – floemska vlakna, NPh – nekolabirani (prevodni) floem, CPh – kolabirani floem.

radially flattened fibres arranged in radial rows. Although axial parenchyma is usually absent, rare, or appears in marginal or seemingly marginal bands in poplars (InsideWood, 2023), we noted abundant paratracheal scanty axial parenchyma (Fig. 2). However, its presence would need to be further confirmed in radial or tangential sections. The wood of the roots had slightly larger vessels and less distinct growth ring boundaries (Fig. 1 b, 2b) than the wood of the stem, likely due to the occurrence of density fluctuations. In the stem, the width of the xylem ring averaged 986 µm in 2019 and 740 µm in 2018; the corresponding values in the root were 268 and 212 µm. Tension wood was present in the xylem of both stems and roots, consisting of fibres with cell walls containing a blue stained gelatinous layer.

The cambium of stems and roots had an average of four and three cambium cells per radial file, respectively, and the cambium was not productive.

The phloem of stems of *Populus sibirica* consists of sieve tubes with companion cells, axial pa-

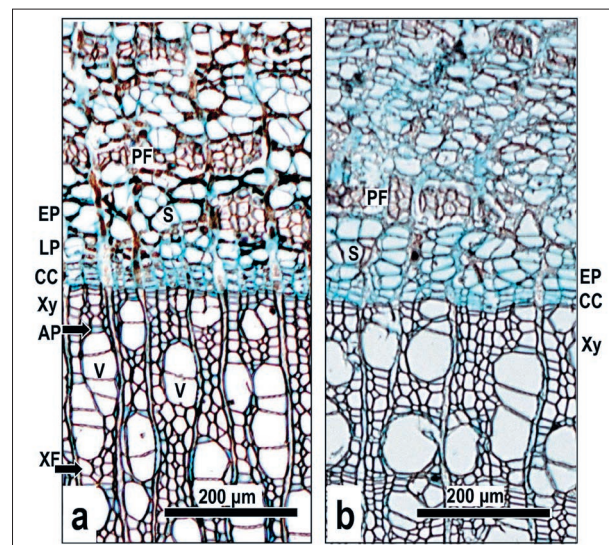


Figure 2. Cambium and secondary tissues of *Populus sibirica*, detailed view: (a) stem and (b) root. CC – cambial cells, Xy – xylem, AP – axial parenchyma, V – vessel, XF – xylem fibre, Ph – phloem, EP – early phloem, LP – late phloem, PF – phloem fibres, S – sieve tubes.

Slika 2. *Populus sibirica*, kambij in sekundarna tkiva, podroben pogled: (a) deblo in (b) korenina. CC – celice kambija, Xy – ksilem, AP – aksialni parenhim, V – traheja, XF – ksilemsko vlakno, Ph – floem, EP – rani floem, LP – kasni floem, PF – floemska vlakna, S – sitaste cevi.

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renchyma, phloem fibres between the early and late phloem, and phloem rays. We could distinguish between non-collapsed (conducting) phloem and collapsed (non-conducting) phloem (Fig. 1 and 2). In the non-collapsed phloem, we could distinguish between early phloem with larger sieve tubes and late phloem in which the sieve tubes had smaller diameters. This is consistent with the phloem structure of *Populus tremula* described by Gričar (2019) where early and late phloem were divided by groups of fibres arranged in tangential bands. Moreover, it was possible to detect the phloem growth ring boundary that delineates the youngest increment in *Populus tremula* (Gričar, 2019), whereas in our case the growth ring boundary could not be detected in the phloem of *Populus sibirica*. We could not detect late phloem in the phloem of the roots of *Populus sibirica*, while the non-collapsed phloem was narrower than that in the stem (Fig. 2).

3.2 XYLEM AND PHLOEM STRUCTURE OF *Ulmus pumila*

3.2 ZGRADBA KSILEMA IN FLOEMA SIBIRKEGA BRESTA *Ulmus pumila*

The wood of the stem of *Ulmus pumila* has the typical anatomy of elms with the following features observed on cross-sections: wood ring porous, growth ring boundaries distinct, tangential diameter of early wood vessel lumina 100 – 200 µm, late wood vessels in tangential bands, vessel clusters common, tyloses common, vascular/vasicentric tracheids present, fibres thin- to thick-walled, axial parenchyma vasicentric, confluent and in marginal or in seemingly marginal bands with 3 – 4 cells per parenchyma strand, larger rays commonly 4 – 10-seriate, rays of two distinct sizes (InsideWood, 2023) (Fig. 3, 4).

Most of the fibres in the wood of the stem and root stained blue, possibly indicating the abundant presence of tension wood (Fig. 3, 4) which is formed to reinforce or change the position of the stem, branch or the root. The wood of the roots had less distinct growth ring boundaries and more earlywood vessels than the wood of the stem (Fig. 3 a, 4 a, b). In the stems of trees of *Ulmus*, the width of the last xylem ring was around 1.5 mm. In the roots intra-annual density fluctuations (IADFs) cannot be excluded (Fig. 4b).

The cambium of stems and roots had an average of 6.7 and 6 cells per radial file, and the cambium was not productive.

The phloem of *Ulmus pumila* consists of early and late phloem sieve tubes with companion cells, axial parenchyma, and phloem rays (Fig. 3). Fibre sclereids (Fig. 3b) and mucilage (slime) cells (Fig. 3a) are observed in the older phloem, as described by Holdheide (1951) in *Ulmus scabra*. In *Ulmus pumila*

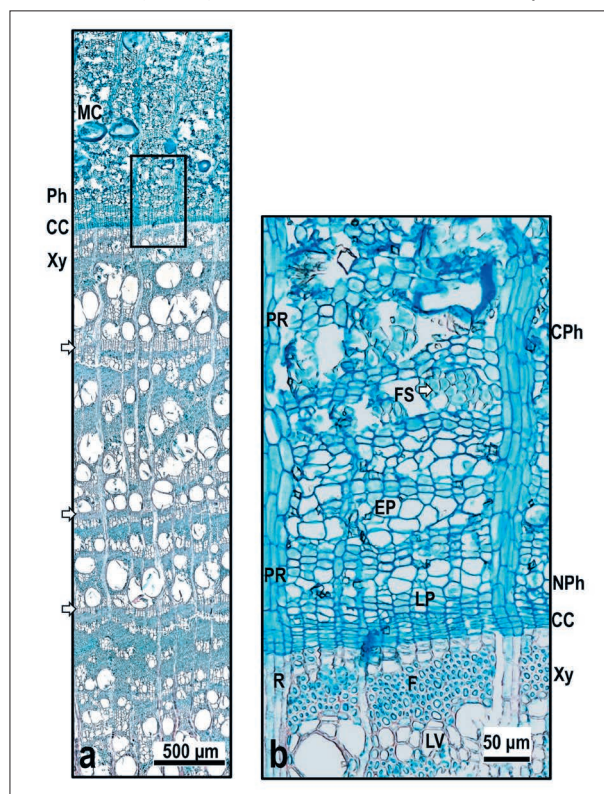


Figure 3. Secondary tissues of *Ulmus pumila* stem: (a) overview, (b) detail of image a. CC – cambial cells, Xy – xylem, arrows pointing to growth ring boundaries, Ph – phloem, LV – latewood vessel, F – xylem fibre, EP – early phloem (sieve cell), R – xylem ray, NPh – non-collapsed (conductive) phloem, CPh – collapsed phloem, LP – late phloem, FS – fibre sclereid, PR – phloem ray, MC – mucilage (slime) cell.

Slika 3. Sekundarna tkiva debla *Ulmus pumila*: (a) pregled, (b) podroben pogled slike a. CC – celice kambija, Xy – ksilem, puščice kažejo na letnice, Ph – floem, LV – traheje kasnega lesa, F – ksilemsko vlakno, R – ksilemski trak, NPh – nekolabirani (prevodni) floem, CPh – kolabirani floem, LP – kasni floem, EP – rani floem (sitasta cev), PR – floemski trak, FS – vlaknasta sklereida, MC – sluzna celica.

we could distinguish between non-collapsed (conducting) phloem and collapsed (non-conducting) phloem (Fig. 3, 4). In the non-collapsed phloem, we could recognize early phloem with larger sieve tubes and late phloem where the sieve tubes had smaller diameters. However, we were unable to identify clear growth ring boundaries and therefore could not accurately determine the width of the last formed phloem annual increment. The first thick walled fibre sclereids were observed at a distance of about 300 µm from the cambium (Fig. 3b) in phloem tissue that may have been formed in the previous year.

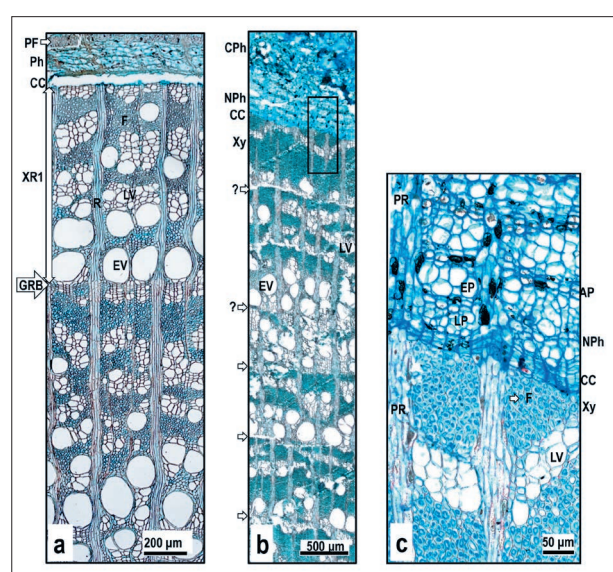


Figure 4. Secondary tissues of *Ulmus pumila* root: (a) normal structure of xylem ring (XR1) below cambium (CC) with clear tree ring boundary, (b) overview (cross-section of the microcore) where some growth ring boundaries are not clear (?), (c) detail from b. EV – early wood vessel, LV late wood vessel, R – ray, F – fibre, AP – axial parenchyma; for other abbreviations see Figs. 1 to 3.

Slika 4. Sekundarna tkiva korenine *Ulmus pumila*: (a) normalna struktura ksilemske branike (XR1) pod kambijem (CC) z razločno letnico (TRB), (b) pregled tkiv (prečni prerez mikro izvrtka), kjer nekatere meje med branikami niso jasne (?), (c) podrobnosti iz slike b (okvir). EV – traheja ranega lesa, LV traheja kasnega lesa, R – trak, F – vlakno, AP – aksialni parenhim; za ostale okrajšave glej slike 1–3.

4 DISCUSSION

4 RAZPRAVA

The microcoring technique has proven effective for the study of wood, cambium, and phloem, including their anatomy and stage of development at the end of the vegetation period in both species and in the particular environment. The method is time consuming and requires a sophisticated procedure from the removal from the tree to sample preparation and production of sections of appropriate quality for microscopy and image analysis. As a result, numerous technical challenges can be encountered during all steps of the analysis. Samples are typically collected from forest trees, which are often located in remote areas. Therefore, tissues must be efficiently fixed and appropriately stored before section preparation can begin. Cutting is usually complicated by the fact that plant tissue is generally heterogeneous and fragile (Balzano et al., 2022). These limitations can be exacerbated when we sample species for which there are few or no studies of wood and bark anatomy, and when we work in difficult, remote environments, as in our case in semi-arid areas in the Mongolian steppe, far from the laboratory. The amount of material available for analyses may also limit our ability to make high quality microscopic preparations, as we usually do not have replicates to replace damaged microcores. In addition, we rely mainly on cross sections, as we do not have enough material to make radial or tangential sections as well. All these factors may affect the results of the study.

The present study was conducted on two tree species, *Populus sibirica* and *Ulmus pumila*, whose wood and bark anatomy are less well known, especially when the trees grow in a harsh environment under semi-arid conditions, in addition to occasional very cold winter and very hot summer conditions. The wood and phloem anatomy could be compared with the anatomy of other species of *Populus* and *Ulmus* like InsideWood (2023) for wood, and Holdheide (1951) and Gričar (2019) for phloem.

The study involved sampling in remote areas with a relatively large team assigned to perform the various steps of the experimental work. Although two technical replicates of microcores from the stem and root of each tree were taken, the material was limited and the samples often did not contain enough wood due to the thicker bark. This also

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caused challenges during the fixation of the samples.

In view of the above, we recommend for future studies a rapid evaluation of the material and a prioritization of high-quality cross-sections that can provide sufficient information. This is especially important when working with less frequently studied species. Indeed, most previous studies have focused on the more widespread tree species (e.g., *Fagus sylvatica* L., *Pinus sylvestris* L., *Quercus robur* L., etc.) from less remote sites in temperate climates (e.g., Van der Werf et al., 2007; Michelot et al., 2012; Giagli et al., 2016; Martinez del Castillo et al., 2016).

However, our study has shown that the analysed tree tissues contain a lot of information if we know how to extract and interpret it.

Impending climatic challenges require expansion of studies to areas of increasing aridity (e.g., Liu et al., 2022), as such changes will inevitably affect cambium productivity and wood and phloem formation. This raises the need for better understanding of how a particular species behaves in a semiarid climate and how this climate affects tissue development and structure. Another aspect of interest is the selection of plant tissues. Microcoring is usually performed on stems, often focusing on the anatomy and formation of the wood. However, the phloem is often not included in such studies, as most focus on the aboveground portion of the tree, such as the stem at breast height, which is more accessible. Root sampling presents a list of technical and logistical challenges (Freschet et al., 2021). However, as the above- and belowground tree parts, i.e., stem and root, play distinct roles in plant establishment and performance (Byambadorj et al., 2022), understanding their combined response to growing conditions could provide more in-depth information about plant plasticity and adaptive potential.

Given the projected climatic challenges, afforestation and reforestation measures are considered extremely important. However, these costly and complicated actions require appropriate and context-specific species selection. Therefore, in order to understand the anatomy of wood, which represents a large proportion of biomass, and bark, which is a smaller proportion of the biomass but performs critical functions for photosynthate

transport (non-collapsed phloem) and protective function (collapsed phloem and outer bark), it is of particular value to know their anatomical characteristics as well as the potential changes in tissue due to management actions and/or changing climatic conditions.

The present study highlights the similarities and differences between the wood anatomy of the stem and roots of *Populus sibirica* and *Ulmus pumila*. Although certain technical challenges have hindered a more thorough analysis, i.e., small number of replicates, damage to some samples due to extended storage in FAA, thick bark, and the increased presence of tension wood, the descriptive approach and characterization of the wood, cambium, and phloem that we have used provides the basis for expanding the analyses. To the best of our knowledge, this is the first study to attempt to characterize the secondary tissues of *Populus sibirica* and *Ulmus pumila* growing in semi-arid areas.

5 CONCLUSIONS

5 ZAKLJUČKI

The present study summarizes the anatomy of secondary xylem and phloem in the stems and roots of two tree species less known in this context, *Populus sibirica* and *Ulmus pumila*. Observations were made on samples collected from the trees in the plantation in the Mongolian semi-arid steppe on October 20, 2019, when temperatures dropped below zero and the growing season had ended. The samples contained cambium that was no longer producing new cells and a narrow cambial zone. Most of the cells in the secondary xylem and phloem produced during the current growing season were already mature. In the wood of the stems of *Populus* we found some tension wood where the cell walls of the fibres contained a non-lignified G-layer. In both species the phloem increment contained early and late phloem, but no clear growth ring boundaries. Because of the timing of tissue sampling, it was possible to see tissues formed in the last growth period, while the process of xylem and phloem formation was not evident.

Despite the difficulty of sampling in the remote areas and some technical problems in preparing samples for microscopy, we have shown that microscopic examination of the basic anatomy of the

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tissues of interest was possible in the stems and roots of the two species.

As far as we know, the results presented here are the first attempt to characterize xylem and phloem structure in a specific time of wood and phloem formation of *Populus sibirica* and *Ulmus pumila* at the end of the vegetation period in the semi-arid region of Mongolia. This study also provides information for planning future studies focusing on the seasonal dynamics of secondary xylem and phloem formation of the two species, which would provide valuable information on their potential for adaptation to the challenging climatic conditions.

6 SUMMARY

6 POVZETEK

Proučili smo kambij (v tem prispevku z besedo kambij označujemo kambijevo cono s kambijevimi inicialkami ter ksilemskimi in floemskimi materinskimi celicami) in anatomijo ksilema – lesa in floema sibirskega topola (*Populus sibirica*) in sibirskega bresta (*Ulmus pumila*) v okviru širše raziskave, namenjene preučevanju plastičnosti in sposobnosti prilagajanja obeh vrst na podnebne in okoljske spremembe. Vzorčenje oz. odvzem mikro izvrtkov je bilo opravljeno na plantaži v Mongoliji, v stepi s polsuhim podnebjem. Tkivo za analize (mikro izvrtke) smo odvzeli iz debel in korenin dreves obeh vrst 19.10. 2019, pri čemer smo v tej študiji raziskali samo vzorce iz kontrolne skupine (brez gnojenja in namakanja). Preparati prečnih prerezov tkiv za svetlobno mikroskopijo so bili po vklapljanju mikro izvrtkov v parafin narezani z rotacijskim mikrotomom ter obarvani z barviloma safranin in astra modro.

Les debela *Populus sibirica* ima anatomijo lesa, kot je značilna za rod *Populus* z naslednjimi znaki, vidnimi na prečnem prerezu: les je difuzno porozen, prirastne plasti so razločne, tangencialni premer trahej je 50–100 µm, vlakna so večinoma tankostenska, trakovi so izključno enoredni (Wheeler et al., 2011; InsideWood, 2023) (sliki 1a in 2a). Proučeni topol je imel v nasprotju z opisi v literaturi tudi dokaj obilen aksialni parenhim (slika 2). Les korenin je imel nekoliko večje traheje in manj izrazite branike (sliki 1b in 2b) kot les debela. Les debela je imel okoli 1 mm široke branike, v lesu korenin pa so bile te široke okoli 0,25 mm. V ksilemu korenin so

bile letnice manj izrazite, zato ne izključujemo prisotnosti »lažnih branik« oz. gostotnih variacij znotraj letnih prirastnih plasti.

V kambiju debela smo v povprečju našli 4 in v kambiju korenin 3 celice na radialni niz. V nobenem primeru nismo zasledili produkcije novih celic.

Floem debela *Populus sibirica* je sestavljen iz sitastih cevi s celicami spremljevalkami, aksialnega parenhima, floemskih vlaken in floemskih trakov. Razlikovali smo lahko med nekolabiranim (prevodnim) floemom in kolabiranim (neprevodnim) floemom (sliki 1 in 2). V nekolabiranem floemu smo lahko prepoznali rani floem s sitastimi cevmi večjih premerov in kasni floem, v katerem so imele sitaste cevi manjši premer. Meje med prirastnimi plastmi (floemske letnice) nismo mogli določiti. Anatomija floema *Populus sibirica* je večinoma podobna kot pri trepetliki (*Populus tremula*), ki jo je podrobno opisala Gričar (2019), kjer so rani in kasni floem delile skupine vlaken, razporejene v tangencialnih pasovih. Poleg tega je bilo pri *Populus tremula* mogoče zaznati mejo floemske branike (Gričar, 2019), medtem ko v našem primeru meje prirastnih plasti v najmlajšem floemu *Populus sibirica* ni bilo mogoče zaznati. V floemu korenin *Populus sibirica* nismo mogli zaznati kasnega floema, nekolabirani floem pa je bil ožji od tistega v floemu debela (slika 2).

Les debela sibirskega bresta (*Ulmus pumila*) ima značilno anatomijo lesa brestov z naslednjimi znaki, ki jih lahko vidimo na prečnem prerezu: les je venčasto porozen, letnice so izrazite, tangencialni premer trahej ranega lesa je 100–200 µm, traheje kasnega lesa so grupirane v tangencialnih skupinah, traheje so pogosto otiljene, prisotne so vaskularne/vazicentrične traheide, vlakna so tankostena do debelostena, aksialni parenhim je paratrahealen, vazicentričen, zlivajoč in v marginalnih ali navidezno marginalnih pasovih s 3–4 celicami na parenhimski pramen, trakovi so dveh različnih velikosti, večji trakovi so običajno 4 do 10-redni (InsideWood, 2023) (sliki 3 in 4).

Večina vlaken v lesu debela in korenine se je pri sibirskem brestu obarvala modro, kar verjetno kaže na obilno prisotnost tenzijskega lesa (sliki 3 in 4), ki omogoča usmerjanje položaja debela, veje ali korenine. Les korenin *Ulmus pumila* je imel manj izrazite letnice in več trahej ranega lesa kot les debela (slike 3a, 4a, 4b). V deblih preiskanih dreves je bila povprečna širina zadnje branike približno 1,5 mm.

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V koreninah je bil prirastek nekoliko manjši, znotraj branik pa so se pojavljale gostotne variacije (IADF) (slika 4b).

Kambij debel in korenin je imel v povprečju 6–7 celic v radialnem nizu in ni bil več produktiven.

Floem *Ulmus pumila* sestavljajo sitaste cevi s celicami spremljevalkami, aksialni parenhim in floemski trakovi (slika 3). Običajnih floemskih vlaken nismo zaznali, v starejšem floemu pa smo opazili sklereide (slika 3b) in sluzne celice (slika 3a). To se ujema z opažanji, ki jih je objavil (Holdheide, 1951), ki je poročal, da floem *Ulmus scabra* nima vlaken, ima pa številne vlaknaste sklereide s podobnim videzom, vendar različno ontogenijo kot floemska vlakna (Čufar, 2006; Prislán et al., 2019). Holdheide (1951) poroča tudi o številnih sluznih celicah v starejšem floemskem tkivu *Ulmus scabra*.

Pri *Ulmus pumila* smo lahko razlikovali med nekolabiranim (prevodnim) floemom in kolabiranim (neprevodnim) floemom (sliki 3 in 4). V nekolabiranem floemu smo lahko razlikovali med ranim floemom s sitastimi cevmi večjih premerov in kasnim floemom, v katerem so imele sitaste cevi manjši tangencialni premer. V floemskih prirastnih plasteh nismo mogli določiti letnic (mej med dvema floemskima branikama) zato tudi nismo mogli natančno izmeriti širine zadnje nastale floemske branike. Prve debelostene vlaknaste sklereide smo opazili na razdalji približno 300 µm od kambija, najverjetneje v floemu, nastalem v predhodnem letu 2018 (slika 3b), kar je v skladu z ugotovitvami, ki jih je objavil Holdheide (1951).

Predstavljamo tudi nekatere ključne tehnične in logistične vidike, ki jih je treba upoštevati pri izvedbi terensko in laboratorijsko zahtevne raziskave. Čeprav smo odvzeli po več vzorcev iz debla in korenin posameznega drevesa, je bilo v nekaterih primerih tkivo poškodovano zaradi narave materiala, postopka vzorčenja in dolgotrajnega hranjenja mikroizvrtkov v fiksirnem sredstvu FAA (mešanice formalina, očetne kisline in alkohola). Kljub tehničnim težavam so izdelani preparati omogočili preučevanje osnovne anatomije in razvojno fazo lesa in floema, nastalega v tekočem letu.

Pri obeh vrstah so branike lesa vsebovale večino zrele celice. Floemski prirastek je pri obeh vrstah vseboval rani in kasni floem, nismo pa mogli razmejiti prirastnih plasti v floemu. Zaradi časa odvzema je bilo mogoče videti le tkiva, ki so nastala

v zadnji rastni sezoni, zato bo v prihodnje potrebno dobro časovno načrtovano vzorčenje, osredotočeno na študij dinamike kambijeve aktivnosti ter procesa nastajanja ksilema in floema.

V predstavljeni študiji smo se osredotočili na kvalitativne podatke, vendar so proučeni preparati uporabni tudi za merjenje oz. analizo slike tkiv, s čimer bi lahko pridobili tudi kvantitativne podatke, in s tem povečali vrednost pridobljenih informacij o lesu in skorji proučenih vrst.

ACKNOWLEDGEMENTS

ZAHVALA

The study and the time spent at the University of Ljubljana were supported by the PhD fellowship for AD from the University of Molise. AM and DC acknowledge the Department of Biotechnology and Life Science at the University of Insubria for the support in the joint research project. The authors also thank the staff of the Korea-Mongolia Joint *Green Belt* Plantation Project and the members of the Laboratory of Forest Genetics and Ecophysiology from the National University of Mongolia for their assistance in the field and laboratory works. The work of AB, MM and KČ and the laboratory equipment were funded by the Programme P4-0015 of the Slovenian Research and Innovation Agency ARIS. We thank Luka Krže for his great support in the laboratory. We thank Darja Vranjek and Paul Steed for editing the Slovene and English language, and two reviewers whose comments helped us to improve the original version of the manuscript.

Author contributions: AM, BN-O, GSS, and DC conceived and designed the project and the study plan. BN-O, ETC, DC, and AM were responsible for tissue collection; AD and AB, with the assistance of MM, performed the laboratory analyses (tissue preparation, microscopy, and measurements) and organized the results; KČ, AB, and AD drafted and wrote the manuscript. All co-authors contributed to the writing; they also read and approved the final version of the manuscript. AD and AB contributed equally to this work.

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