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Differences in xylem and phloem structure in living stumps of Norway spruce (*Picea abies* (L.) Karst.) and silver fir (*Abies alba* Mill.)

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Summary – Living stumps reported in many tree species were the first, circumstantial evidence of root grafting. Although stump overgrowth has been extensively studied in various tree species, anatomical observations have mainly focused on the xylem part. Here, we investigated the xylem and phloem structure in living stumps of two conifer species: Norway spruce (*Picea abies* (L.) Karst.) and silver fir (*Abies alba* Mill.). The mechanism of wound closure was similar in the two species, however, there were differences in the structure of wound tissues in wood and bark. Immediately after the injury, a callus consisting of parenchymal cells was formed, followed by wound-wood with wide increments. In addition, the wood structure and morphology of tracheids were changed. Tracheids lost their typical elongated and tapering structure; instead, numerous crooked and forked tracheids were present. Traumatic resin canals were present in both species but were more abundant in *P. abies*, which also had normal resin canals. While the structure of the xylem growth rings eventually normalized in both species, the width of the annual xylem increments was diminished. In both species, the typical structure of annual phloem increment was lost in the non-collapsed phloem and sclereids were very abundant in the collapsed phloem. In *A. alba*, the collapsed phloem was rich in swollen parenchyma cells containing blue-stained contents, while in *P. abies*, numerous large traumatic resin canals were conspicuous. The study showed that similar patterns of vascular tissue restoration exist in *A. alba* and *P. abies* in response to tree felling. However, there are certain species-specific peculiarities in the wood and phloem anatomical response, which might affect long-term stump functioning and survival.

Keywords – callus, overgrowth tissue, maceration, parenchyma, root grafting, sieve cells, tracheids, traumatic resin canals.

Introduction

Natural root grafting (i.e., root anastomosis) is a common phenomenon in woody perennial species in different habitats worldwide (Graham & Bormann 1966) and has been detected in more than 200 tree species (Quer *et al.* 2022). It results from the fusion of root vascular systems via vascular cambium (in brief, cambium) contact during secondary growth (Lev-Yadun & Sprugel 2011). The most important factors for root grafting are the proximity of trees and the number of roots per tree, in addition to stand density, age, and size of trees and roots (Quer *et al.* 2020). The proximity between trees increases the possibility of two roots crossing and promotes mechanical abrasion and root friction due to wind fluctuations, leading to root grafting (Quer *et al.* 2020).

Most natural root grafting occurs within the root system of the same individual, due to proximity and compatibility (self-grafting) but it can also occur with other spatially close individuals of the same tree species (intraspecific

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grafting) or between individuals of different tree species (interspecific grafting). Root grafts enable trees to exchange water, nutrients, and photosynthetic products and thus influence tree growth and physiology (Bader & Leuzinger 2019; Quer *et al.* 2020). Lev-Yadun & Sprugel (2011) suggested that, in addition to their physiological importance and generally better mechanical anchorage, there are other benefits of natural root grafts, which are of two types: (i) benefits to the grafted plants that occur before one of them loses its canopy, such as an increased potential for reproductive success, the 'dear enemy' phenomenon (a weak neighbour may still be a better rival than a possibly stronger one that could replace it), an increased chemical defence arsenal and, consequently, resistance of the trees to various herbivores and pathogens, and the acquisition of beneficial fungi or microorganisms from the grafted neighbour despite the risk of pathogen transmission via root grafts and (ii) advantages for the remaining intact tree or for a tree that has lost its crown but whose roots still occupy an underground territory close to the intact grafted individual, thus partially protecting it from competition for water, minerals and light with another tree. In a grafted root system of a tree that has lost its original crown, the genotype lives on and still has a chance to resume canopy growth and reproduction in certain taxa (Lev-Yadun & Sprugel 2011). Root grafting is thus another underestimated pathway for resource exchange, in addition to the indirect connection of root systems through mycorrhizal and suckering networks via fungal hyphae or directly through parental roots. However, the evidence for wood-wide-web (i.e., a common mycorrhizal network) is rather scanty and controversial (Robinson *et al.* 2024). Nevertheless, all these possible connections can influence the dynamics of forest stands by enabling the exchange of resources between trees (Quer *et al.* 2020). The ecological significance of root grafting in forest stands was demonstrated by Quer *et al.* (2022) by showing the community response of grafted trees rather than a response as individual trees in competition for resources.

Living stumps were the first, circumstantial evidence of root grafting (Graham & Bormann 1966; Liphschitz *et al.* 1987). Stumps are parts of the trunk that remain after the tree has been felled. In the case of a grafted root system, the vascular meristems of such stumps can remain alive for decades and continue the growth and healing processes by using water and resources transported via root fusions from connected living trees (Schweingruber 2007). Often, only part of the stump circumference is alive. Stumps can continue to grow for decades; 30–40 years or even longer is quite common (Liphschitz *et al.* 1987). Lanner (1961), for example, reported living stumps from almost 90-year-old *Pseudotsuga menziesii* and 60-year-old *Abies magnifica*.

With living stumps, cambial activity resumes each year, resulting in annual layers of xylem and phloem increments. Nevertheless, the absence of a living crown has significant effects on the tree hormone system (concentrations and pathways), leading to various physiological and/or anatomical responses (Taiz & Zeiger 2006). Living stumps have been reported in many tree species. For example, the structure of overgrowth of stumps has been well described in *Pseudotsuga menziesii* and the genera *Abies*, *Picea*, *Pinus*, and *Tsuga* (Lanner 1961; Davidson 1963; Graham & Bormann 1966; Schultz 1972; Liphschitz *et al.* 1987; Zajączkowska 2014b). Immediately below the cut surface, the width and structure of the xylem and phloem increments, as well as the morphology, orientation, and arrangement of the xylem and phloem cells, are significantly altered (Schweingruber 2007). The exposed cambium of the stumps reacts to the injury with increased activity near the wound edge and begins to form a callus that grows over the cut surface. The process of overgrowth of the injury is generally explained as the formation of de-differentiated callus tissue, in which vascular tissue eventually forms again. The altered wood formation patterns are reflected in the xylem structure in terms of cell morphology and orientation (Liphschitz *et al.* 1987).

The intensive growth and accumulation of newly deposited xylem tissue in wound spindles seem to be the most effective mechanism for tree stem regeneration to restore its biomechanical and transport functions (Zajączkowska 2014a). Overgrowth of stumps has also been used as a model to study cellular reorganisation and the mechanisms responsible for changes in cell polarity during the process of vascular tissue differentiation from originally disorganised parenchymatous cells (Zajączkowska 2014b). Zajączkowska (2014b) suggested that the ray-like structures may act as organising centres in the morphogenetic field responsible for the differentiation of the overgrown tissue. The occurrence of circular vascular patterns was described earlier in branch junctions (Lev-Yadun & Aloni 1990), around buds (Aloni & Wolf 1984; Hejnowicz & Kurczyńska 1987), in roots (Fink 1982), in callus and in various types of tissue reorientations (Sachs & Cohen 1982), in woundings (Liphschitz & Mendel 1987), and in tumours (Tsoumis *et al.* 1988). Later, other wound-induced anatomical changes occur in the wood, such as the formation of tangential rows of traumatic resin canals, which occur even in species that normally do not have resin canals, such as the genus *Abies*. However, traumatic resin canals are generally more abundant in tree species with normal resin canals, e.g., *Larix* and *Picea* (Schweingruber 2007).

Although stump overgrowth has been extensively studied in various tree species, anatomical observations have mainly focused on the xylem part, while knowledge of the bark tissues is sparse, despite their importance in the overgrowth process of injury. Older phloem cells undergo numerous age-related changes, which include the collapse of sieve elements, the proliferation of parenchyma cells, sclereid formation, ray dilatation, and formation of phellogen. (Angyalossy *et al.* 2016). Such normal spatio-temporal changes in the formation of bark tissues and the morphology of phloem cells pose limitations when studying the development of phloem/bark structures during later stump overgrowth, since some information may be lost over time due to these age-related secondary processes. This study aimed to evaluate and compare the xylem and phloem structure in living stumps of two conifer species: Norway spruce (*Picea abies* (L.) Karst.) and silver fir (*Abies alba* Mill.). The wood anatomy of these two species is similar but they differ in the presence of normal resin canals (spruce), ray composition and fine structure, such as pit types in cross-fields (Grosser 1977). There are also differences in bark structure, especially in non-conducting or collapsed phloem (Holdheide 1951).

Material and methods

Study site characteristics and sample collection

The sampling was conducted at two nearby locations in the Savinja Valley in Slovenia: Tabor (46°14′04″, 15°00′28″, 330 m a.s.l.) and Polzela (46°18′29″, 15°3′19″, 390 m a.s.l.). The sites are less than 9 km apart. The first stand belongs to the *Vaccinio myrtilli-Carpinetum betuli* forest association (limestone soils), with *Abies alba*, *Fagus sylvatica*, *Picea abies*, *Castanea sativa*, *Acer pseudoplatanus*, *Prunus avium*, *Quercus petraea* and *Pinus sylvestris* as the predominant tree species. The second stand belongs to the *Hacquetio-Fagetum* forest association (alluvial loam soils), with *Fagus sylvatica*, *Picea abies* and *Acer pseudoplatanus* as the predominant tree species. The even-aged mixed forests at both locations are privately owned and managed through sustainable, close-to-nature, and multifunctional forest practices, which have been formally used in Slovenia for over 50 years.

The climate at both sites is humid continental. The average monthly mean temperature and precipitation data obtained from Medlog, the nearest weather station, which reflects the weather conditions in the period 2019–2023, are shown in Fig. 1. The mean annual temperature is 11.7°C and the mean annual precipitation is 1192 mm.

The sampling of living tissues from partly overgrown stumps of *A. alba* and *P. abies* was performed in September of 2021, i.e., at the end of the growing season. Three stumps with living overgrowing tissue were selected for each species (Fig. 2). At the first location, all three living stumps of *A. alba* and one stump of *P. abies* were sampled, while in the second location, two stumps of *P. abies* were sampled.

We measured the diameters and heights of the sampled *A. alba* and *P. abies* stumps. The data are presented in Table 1. A chainsaw was used to remove part of the overgrown tissue and tissue formed before tree felling from the stumps. The cut-out parts of the stump that were intended for further analysis (Fig. 2a) were wedge-shaped. They were stored in 70% ethanol and taken to the laboratory.

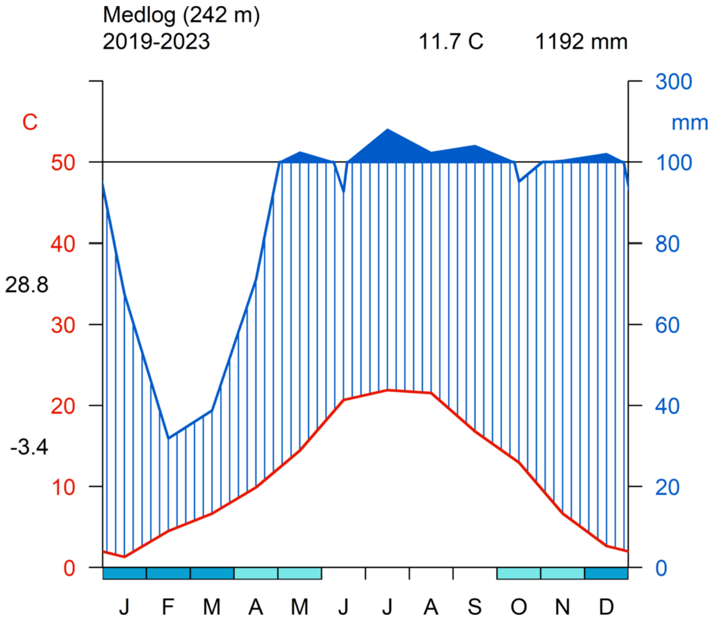

Fig. 1. Walter–Lieth climate diagram for the Medlog meteorological station of the Environmental Agency of the Republic of Slovenia spanning the period 2019–2023. The nearest station is located approximately 15 km from both sampling sites. The values at the top of the diagrams show the long-term annual mean temperature and the total annual amount of precipitation. The value at the top left of the temperature axis is the mean value of the average daily maximum temperature of the warmest month; the value at the bottom of the same axis is the mean value of the average daily minimum temperature of the coldest month. The horizontal black line at 100 mm and 50°C illustrates the threshold value above which precipitation scales by a factor of 10. The blue line represents the annual cycle of monthly precipitation. The red line shows the annual cycle of the monthly mean temperature. The area with the blue shaded lines illustrates the humid conditions below the threshold. The blue filled area shows the humid conditions above the threshold (excess water).

Tissue preparation for light microscopy and data analysis

In the laboratory, using a chisel and hammer, small pieces of wood and bark were extracted from the sawn part of the stems for further histological and cytological analysis (Fig. 2b). They contained tissues formed before and after tree felling. These pieces were kept for a week in 70% ethanol. Tissue processing steps for the preparation of cross-sections from the samples were then started (for details, see Prislan *et al.* 2022). Transverse, radial, and tangential sections of the tissues were prepared for further processing. Embedding samples in hydrophobic paraffin required the removal of water from the tissues, which was achieved by immersing the samples in a series of ethanol solutions of increasing concentration (70, 90 and 95%) until pure, water-free alcohol was reached, after which the samples were infiltrated with a clearing solvent UltraClear (J.T. Baker) and finally with paraffin heated to 60°C. The samples were then embedded in paraffin, followed by cutting 8–12-μm-thick permanent sections using a Leica RM 2245 rotary microtome (Leica Microsystems) and staining with a safranin (Merck) (0.04%) and Astra Blue (Sigma-Aldrich) (0.15%) water mixture. Sections were mounted in Euparal (Waldeck).

For maceration of the wood and wound wood of the stumps, we followed the procedure developed by Franklin (1945) and described by Helmling *et al.* (2016). Equal parts of glacial acetic acid (99%) and a 30% solution of H_2O_2 were used for maceration. The maceration process lasted 48 hours at a temperature of 80°C. As soon as the samples were bleached, the test tubes were shaken to separate the cells. The maceration process was completed once the tissue was dissolved. A filter from Bad Heilbrunner® Naturheilmittel was used for the separation of the macerated

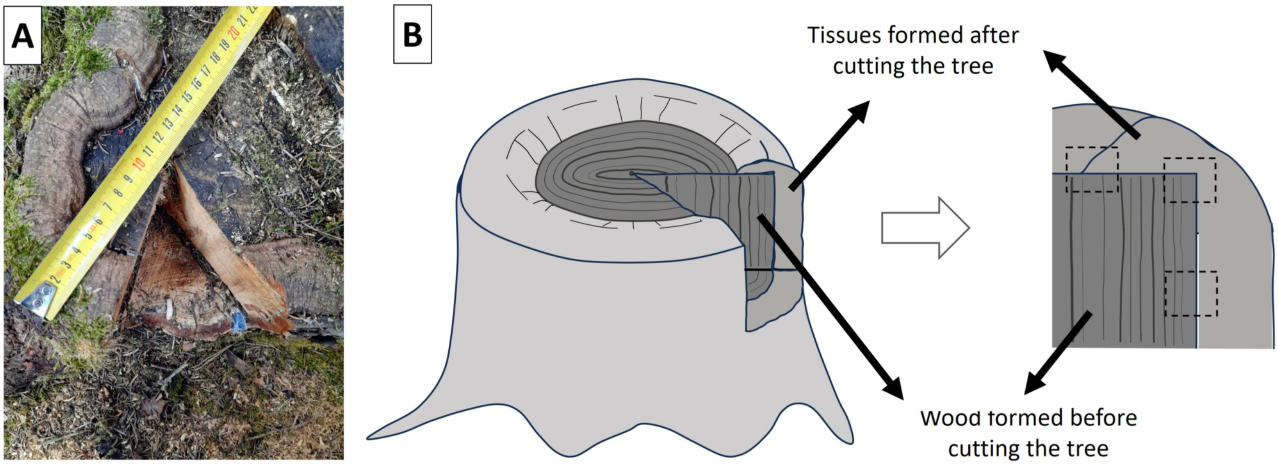

Fig. 2. (a) Sampled living stump. (b) Schematic illustration of stump sampling. Light grey colour indicates tissues formed after tree cutting, dark grey indicates wood formed before tree cutting. The dashed squares denote the position of the collection of wood and bark tissues for histological observations. The location on the circumference of the tree stump, where the differences in the anatomy of the xylem rings before the tree was felled and the youngest xylem rings formed after the tree was felled, can be observed. Other locations were chosen at the edge of the felled stump and at the top of the overgrowth, where the newly formed tissue deviated from the longitudinal axis. At these locations, callus tissue was formed immediately after the injury.

Table 1. Average diameter and height of sampled *Abies alba* and *Picea abies* stumps.

Sample	Stump diameter (cm)	Stump height (cm)
Abies alba no. 1	43	13
Abies alba no. 2	28	12
Abies alba no. 3	17	4
Picea abies no. 1	12	7
Picea abies no. 2	33	19
Picea abies no. 3	30	16

tissue from the liquid chemicals. The macerate was rinsed with 96% ethanol. The separated cells were then stained with a safranin and Astra blue water mixture and mounted in Euparal, as explained above.

All sections and macerates were observed under an Olympus BX51 light microscope using transmitted and polarised light modes. Histometric observations were performed at 4 and 10× magnifications using the Nikon NIS-Elements Basic Research version 2.3 image analysis system.

Phloem anatomy of *A. alba* and *P. abies*

The terminology for bark anatomy in this paper follows Trockenbrodt (1990) and Angyalossy *et al.* (2016). Since the bark structure is less commonly known than that of wood (Shtein *et al.* 2023), we briefly describe below the phloem anatomy of *A. alba* and *P. abies*.

In 'normal' phloem, the structure of non-collapsed phloem in *A. alba* and *P. abies* is relatively simple and consists of living, vertically oriented sieve cells and various types of parenchyma cells, which can include crystals, tannins, resins, and lipids. The contents of these ergastic substances increase in older portions of the bark (Rosner *et al.* 2001; Jyske *et al.* 2015). *A. alba* has only uniseriate rays, consisting of parenchyma cells and Strasburger cells, whereas fusiform

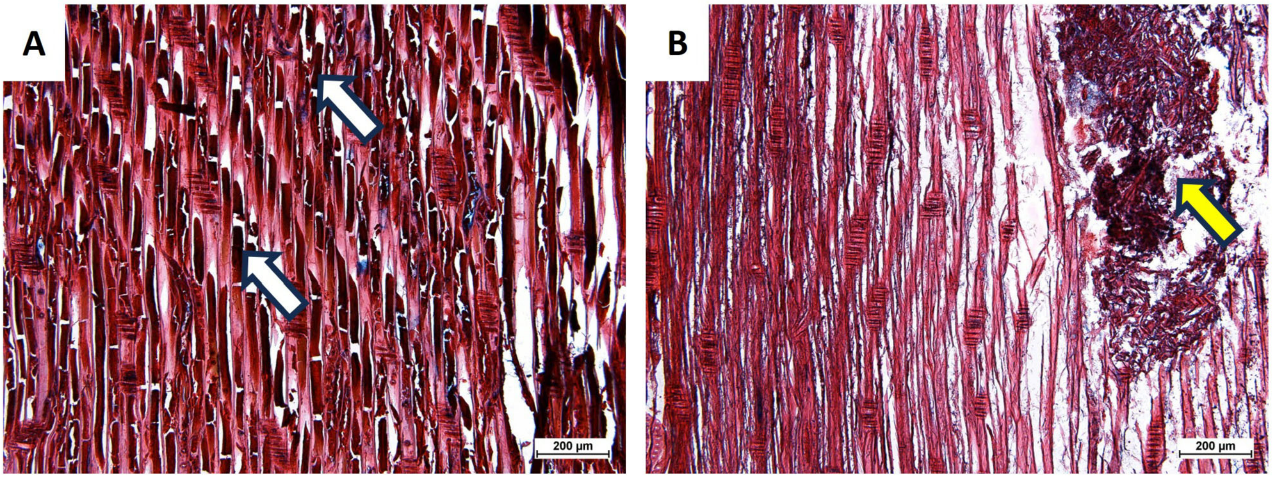

Fig. 3. Longitudinal sections presenting polyphenols (white arrows) in tracheid lumens (a) and process of decomposition (yellow arrow) of wood formed before tree cutting in *A. alba* (b).

rays containing radial resin canals are also present in *P. abies*. Strassburger cells are specialized cells of phloem rays or axial parenchyma that are morphologically and physiologically similar to sieve cells (Angyalossy *et al.* 2016).

Results

XYLEM

We could not determine the age of the sampled trees at the time of their felling, based on the counting of the number of xylem rings, because the wood was already too decomposed at that time (Fig. 3f). For a similar reason, we could not determine the age of newly formed xylem in one of the *A. alba* stumps. We assessed that it was over 10 years old. The other two *A. alba* stumps were 6 and 12 years old, respectively. The *P. abies* stumps had grown 12, 19, and 29 years after tree felling.

Investigation of wood and bark samples from *A. alba* and *P. abies* taken several years after injury showed that the mechanism of wound closure explained below, was similar in the two species, however, there were differences in the structure of wound tissues in wood and bark. A callus consisting of parenchymal cells formed immediately after the injury (Fig. 4). In both species, the structure of the wound wood, especially in the immediate vicinity of the callus, was greatly changed (Figs 4 and 5). In particular: (1) cambial productivity was accelerated, which was reflected in wide xylem growth rings (Fig. 4a,b); (2) the typical axial orientation of the axial tracheids was absent as well as the radial orientation of the rays (Fig. 5a,b); (3) spindles of parenchyma cells were visible, indicating the beginnings of rays; they were surrounded by circularly arranged axial tracheids (Fig. 5c,d) and (4) traumatic resin canals were present (Fig. 5b). Because of all these anatomical changes, the border between wood formed before and after the injury/tree felling was very distinct (Fig. 4a,b).

The structure of the xylem growth rings eventually normalized in both species, with the width of the annual xylem growth rings changing from distinctly wide (Fig. 5a,b) to very narrow (Fig. 6a,b), composed of fewer than 10 cell layers. Discontinuous xylem growth rings were also present (Fig. 6c). The main wood-anatomical difference between the two conifer species in the response to tree felling was observed in the frequency of formation of traumatic resin canals. In *P. abies*, which has normal resin canals, traumatic resin canals were abundant and present in nearly every xylem growth ring formed after the injury, whereas in *A. alba* their presence was very rare (Fig. 6a,b). Traumatic resin canals were present in the wood of *P. abies* years after tree cutting and, in combination with reduced xylem growth rings,

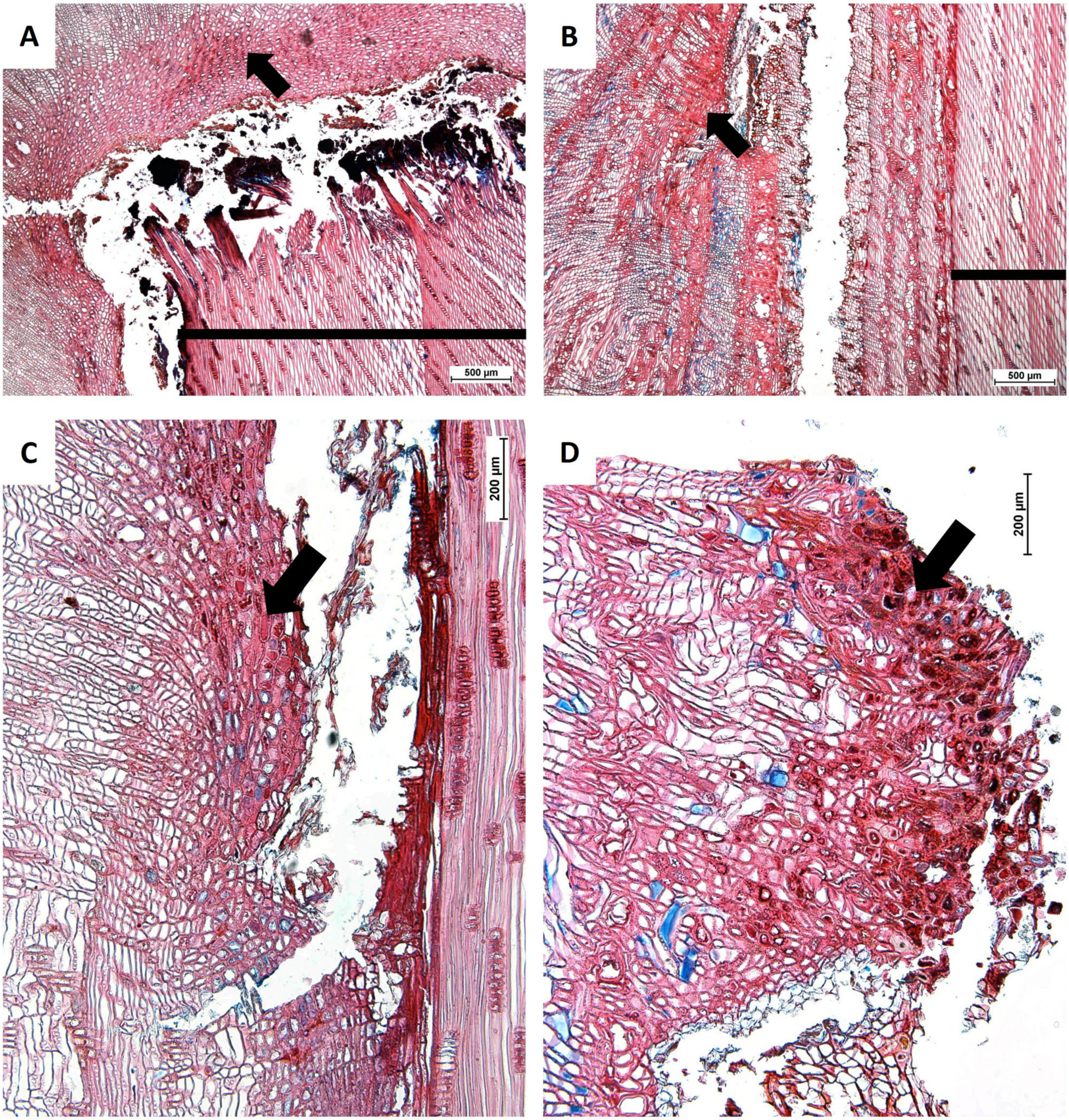

Fig. 4. Longitudinal sections of the xylem tissues in tree stumps of *Abies alba* (a, c) and *Picea abies* (b, d). Xylem formed before (black lines) and after tree felling, in which callus (black arrows) is clearly visible on the border in both *A. alba* (a, c) and *P. abies* (b, d).

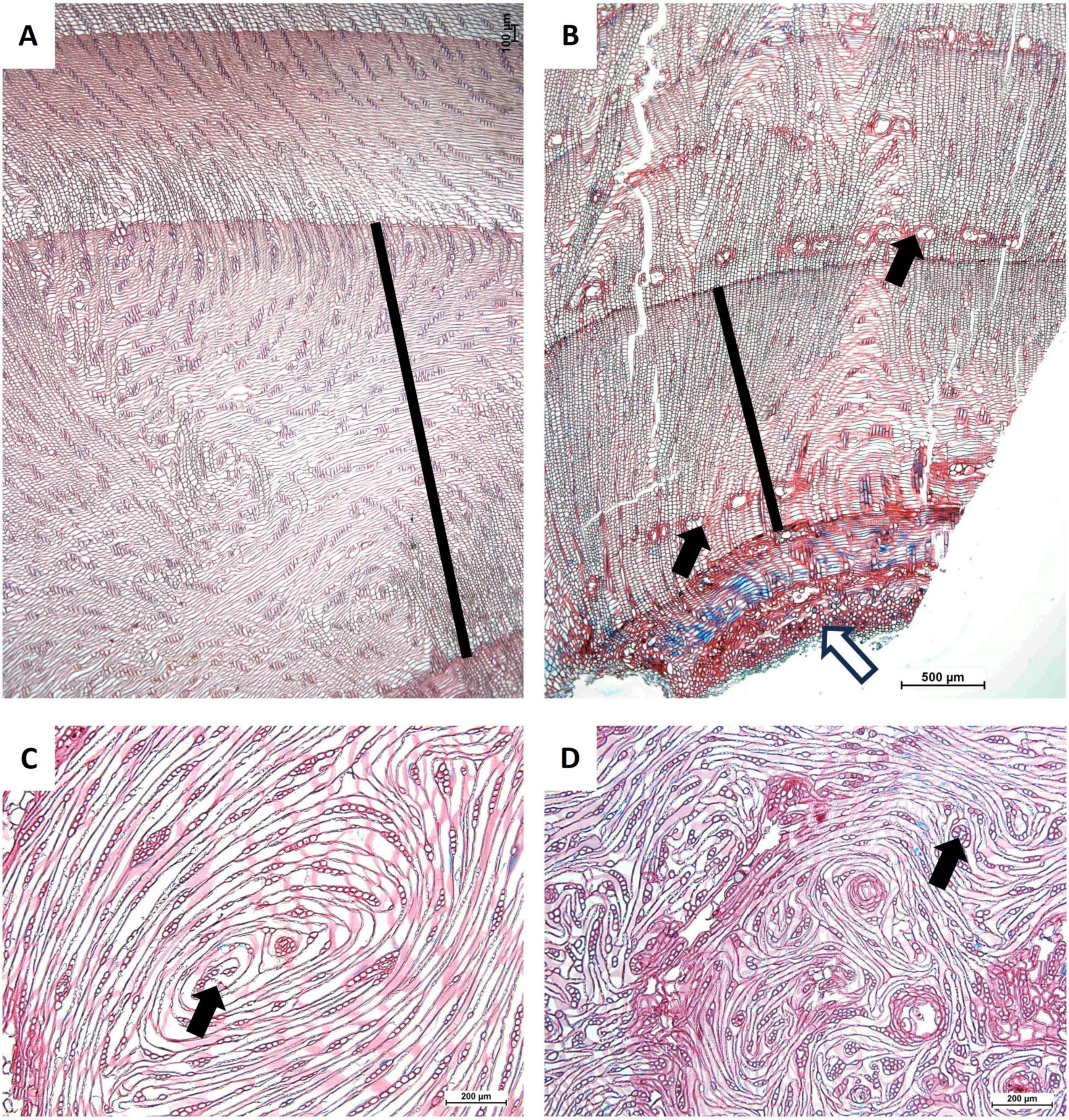

Fig. 5. Microscopic sections of overgrown tissue in *Abies alba* (a, c) and *Picea abies* (b, d). Accelerated xylem growth (black line) in the immediate years after tree cutting in transverse sections of *A. alba* (a) and of *P. abies* (b). Callus cells (white arrow) and traumatic resin canals (black arrows) in wound tissue of *P. abies* (b). Tangential sections showing spindles of parenchyma cells indicate the beginnings of rays in both *A. alba* (c) and *P. abies* (d).

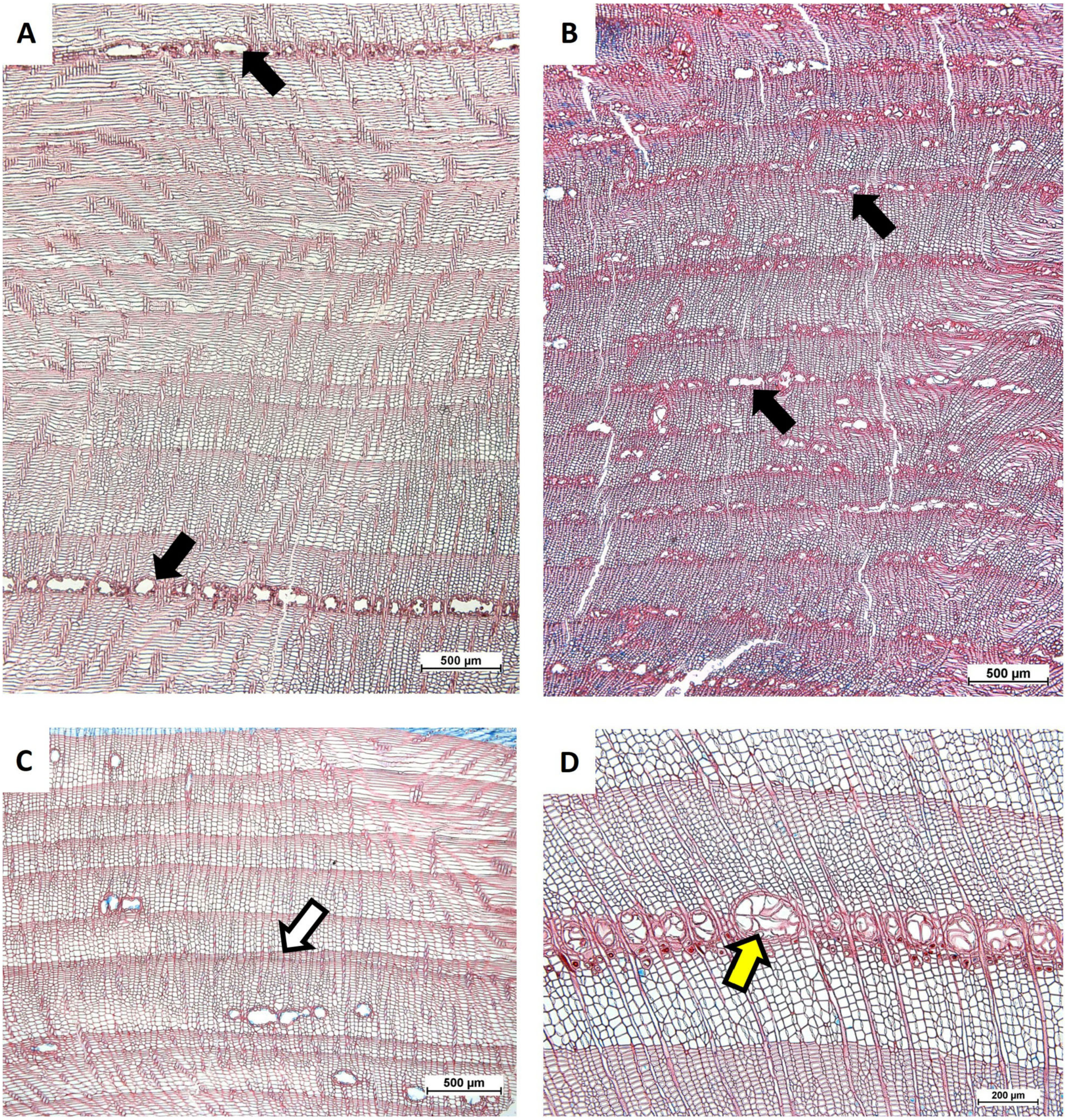

Fig. 6. Transverse sections of the overgrown tissue. (a, b) Scarcity of traumatic resin canals (black arrows) in *Abies alba* (a) compared to *Picea abies* (b). (c) Narrow xylem growth rings, including discontinuous growth rings (white arrow) in the years following tree felling in *P. abies*. (d) Presence of tylosoids (yellow arrow) in traumatic resin canals in *P. abies*.

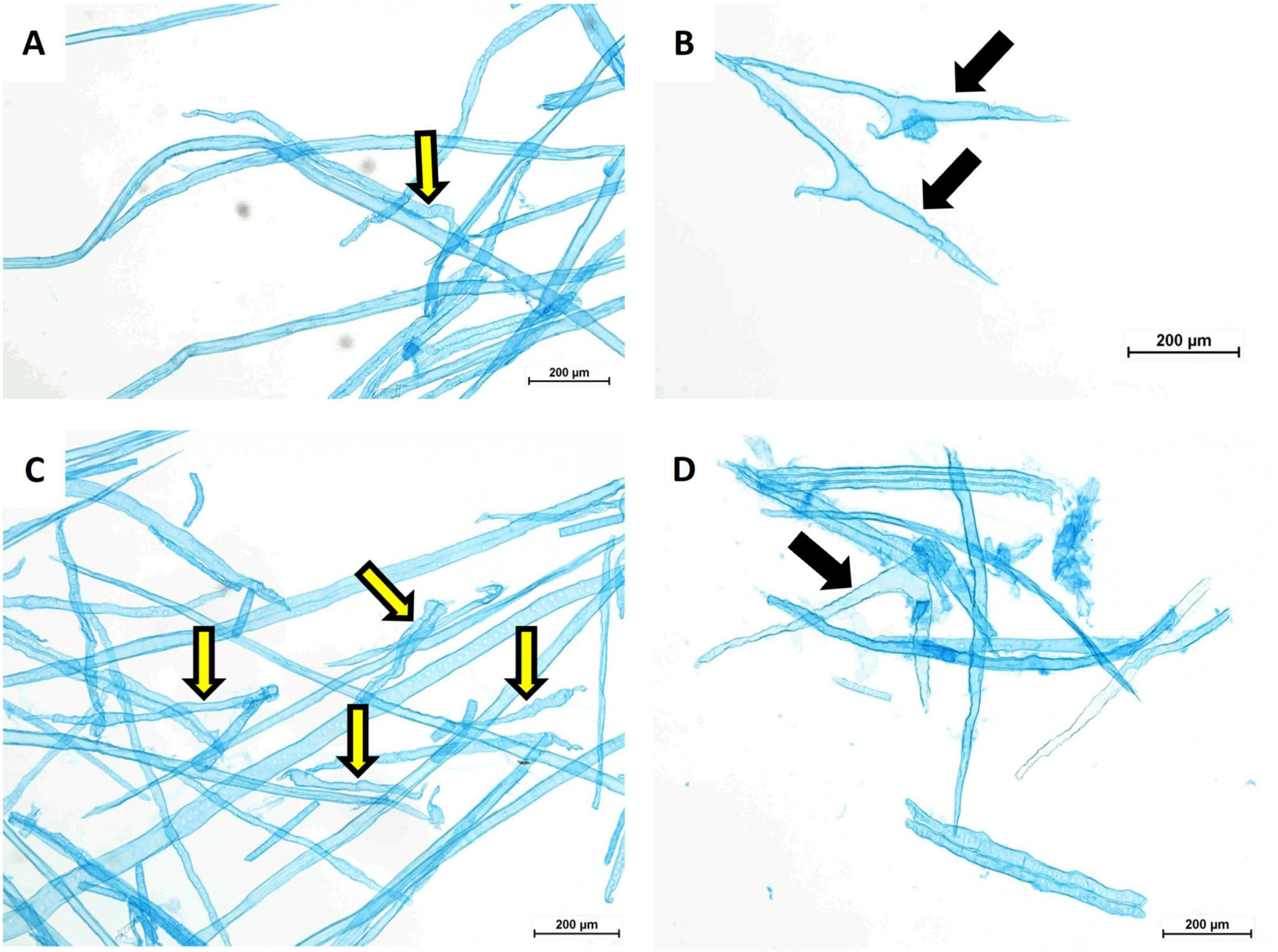

Fig. 7. Maceration of the wood tissue formed after the tree felling in *Abies alba* (a, c) and *Picea abies* (b, d). (a, c) Changed morphology of tracheids (yellow arrows). (b, d) A common presence of crooked and forked tracheids (black arrows).

was an additional sign of tree injury. They often contain tylosoids, i.e., a proliferation of a thin-walled epithelial cell into an intercellular canal (Fig. 6d) (Richter *et al.* 2004).

Maceration of the wood tissue formed after tree felling showed a changed morphology of the wood cells. Tracheids generally lost their typical elongated and tapering structure (Fig. 7a,b). Instead, numerous crooked and forked tracheids were present in both species (Fig. 7b,d).

PHLOEM

Tree felling resulted in an altered structure of the phloem in both species (Figs 8–10). Sclereids were very abundant in the collapsed phloem in both species (Fig. 8a,b). However, species-specific differences in response to injury were also distinctive. In *A. alba*, the collapsed phloem was rich in swollen parenchyma cells containing blue-stained contents (Fig. 9a), while numerous large traumatic resin canals were conspicuous in *P. abies* (Figs 8d and 9b).

In the non-collapsed part of the phloem, the typical structure of the annual phloem growth ring was lost (Fig. 10). No tangential band of axial parenchyma separating early- and late-phloem parts was visible. Axial parenchyma cells seemed to be randomly scattered in non-collapsed phloem. In addition, the distinction between larger early phloem

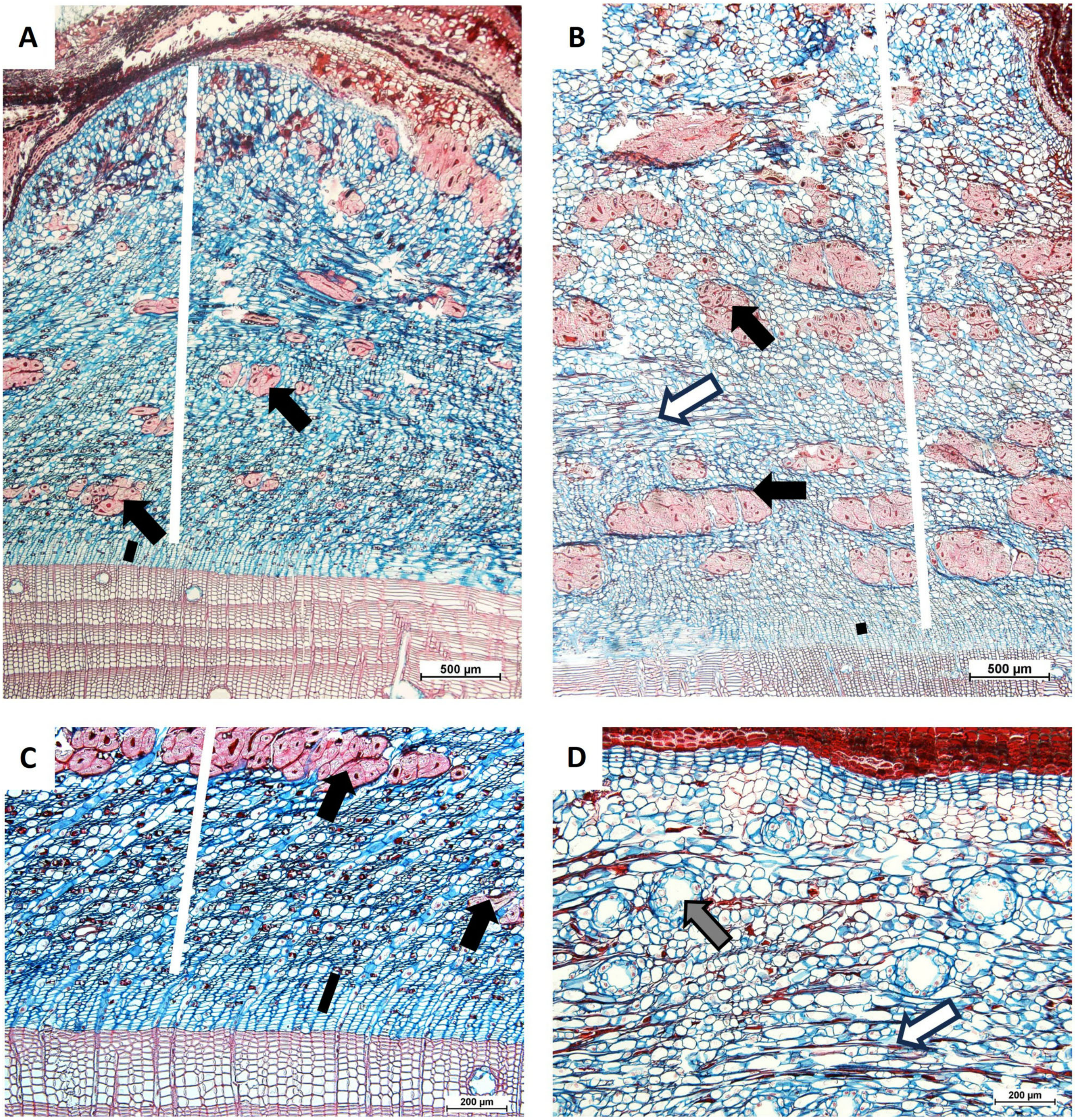

Fig. 8. Transverse sections of bark tissues. Living phloem composed of collapsed (white lines) and non-collapsed parts (black lines) in *Abies alba* (a, c) and *Picea abies* (b, d). Numerous sclereids (black arrows) were formed in the collapsed part of the phloem in both species (black arrows in a–c), whereas numerous traumatic resin canals (grey arrow) were present in *P. abies* (d). In the collapsed phloem, a change in cell orientation (white arrows) is visible (b, d).

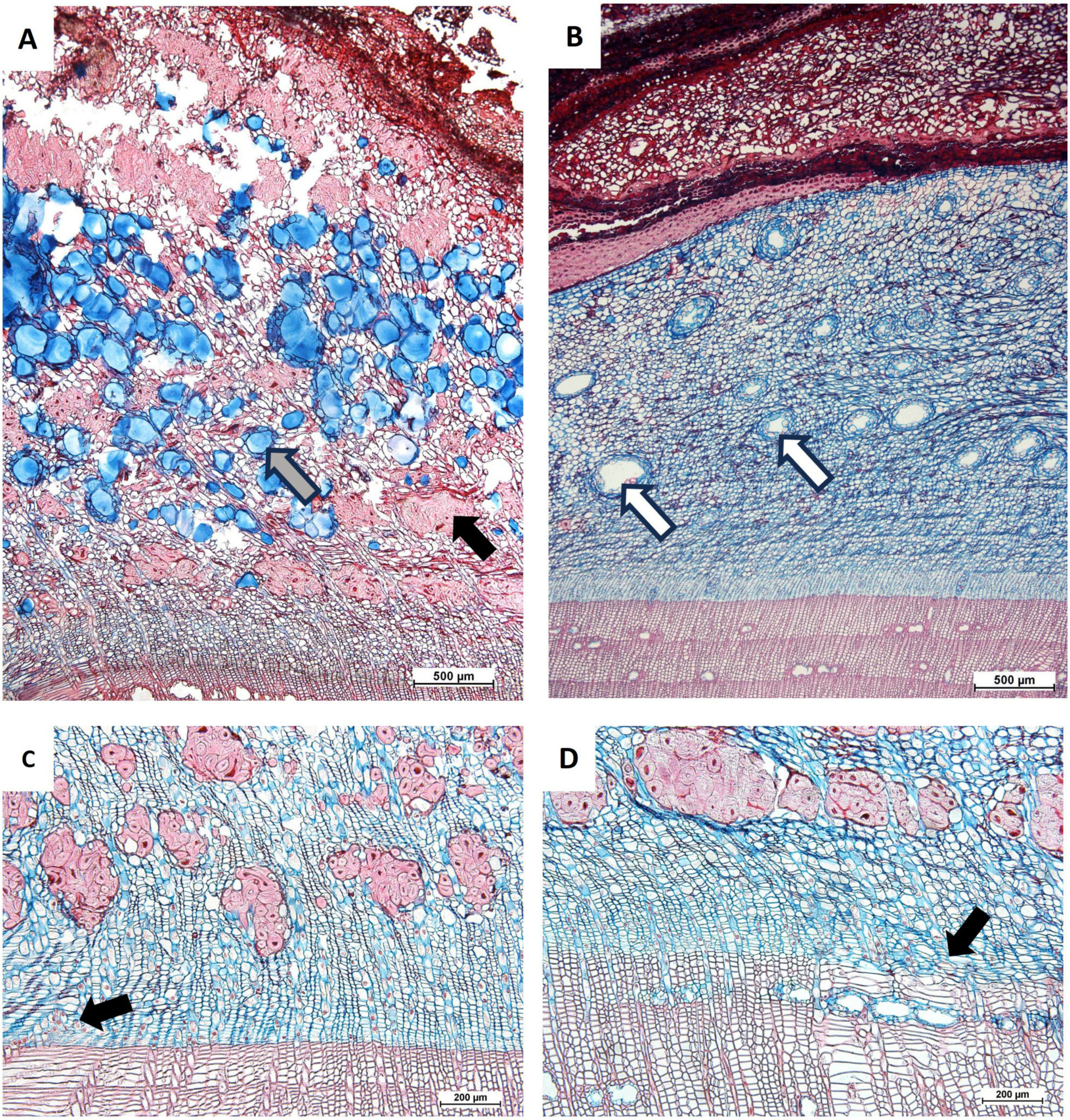

Fig. 9. Transverse sections of bark tissues. (a) Swollen parenchyma cells containing blue-stained contents (grey arrow) and sclereids (black arrow) in the collapsed phloem of *Abies alba*. (b) Numerous traumatic resin canals (white arrows) in the collapsed phloem of *Picea abies*. (c, d) Changed tissue orientation (black arrow) in wood, cambium and phloem from transverse to longitudinal in *A. alba* (c) and *P. abies* (d).

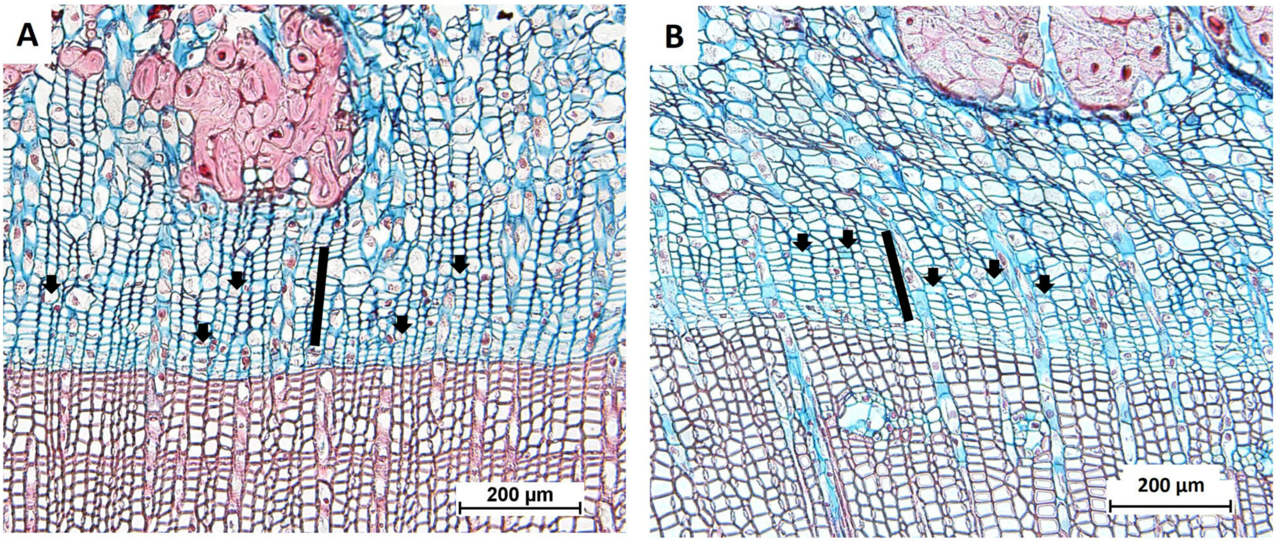

Fig. 10. Transverse sections of the non-collapsed part of the phloem (black lines) in *A. alba* (a) and *P. abies* (b). Randomly scattered axial parenchyma cells (black arrows).

and smaller late phloem sieve cells was not possible because their size did not decrease, as is common in non-damaged trees of both species. Since Strasburger cells are not easily distinguished from parenchyma cells, we did not examine the potential changes in their morphology or distribution. In *A. alba*, we detected fusiform rays containing radial (traumatic) resin canals, which are not present in the 'normal' bark of this species (Fig. 9a). Similarly to wood, a changed tissue orientation from transverse to longitudinal was also clearly visible in the phloem, in both rays and axially oriented cells (Fig. 9c, d). In the collapsed phloem, a loss of cell arrangement was preserved for many years despite the effect of secondary changes on the phloem cells (Fig. 8b, d).

Discussion

We found that the structure of the newly formed xylem and phloem growth rings in living stumps of *P. abies* and *A. alba* differed from the normal structure of the two tissues, but eventually normalises. Although similar patterns of vascular tissue restoration existed in *A. alba* and *P. abies* in response to tree felling, there were species-specific anatomical adjustments to mechanical wounding in both the xylem and phloem. In the xylem, the traumatic resin canals were more abundant in *P. abies*, which has also normal resin canals. In both species, the typical structure of annual phloem increment was lost in the non-collapsed phloem and sclereids were very abundant in the collapsed phloem. In *A. alba*, the collapsed phloem was rich in swollen parenchyma cells containing blue-stained contents, while in *P. abies*, numerous large traumatic resin canals were conspicuous.

Callus formation and changes in wood anatomy

Wounding of trees during the growing season causes the formation of callus tissue, which develops over the wound surface or parts of it (Kuroda 1986; Liese & Dujesiefken 1996). Callus, followed by the formation of wound tissue, looks like a scab (longitudinal rolls), which starts to develop at the edge of the cut stump, gradually growing towards the centre of the stump, spreading over older xylem tissue formed before the tree was felled. The callus can eventually cover the entire stump and is either produced (1) by cambial cells and cells in the early stages of differentiation or (2) by an abnormal proliferation of parenchyma cells (Kuroda 1986; Oven & Torelli 1999; Gričar *et al.* 2007; Chano *et al.*

2015). The callus consists of parenchyma cells and is thus the initial part of the process of covering wounds, or tissue regeneration, in vascular plants (Stobbe *et al.* 2002). Wound wood is formed afterward, with generally very intense cambial growth, which results in wide growth rings formed to outgrow the damaged and exposed tissues quickly and efficiently (Stobbe *et al.* 2002). Consistent with previous reports (Zajączkowska 2014a), newly developed vascular tissues on the tree stumps were formed as longitudinal spindles or rolls along the perimeter of the stump in an attempt eventually to cover the wound surface. In addition, newly formed xylem growth rings changed their orientation to perpendicular with respect to the wound surface. The disturbances of wood formation near the wound edge were manifested by an oblique orientation of xylem rays with respect to annual ring boundaries (Zajączkowska 2014a). Nevertheless, the process of wound recovery is greatly affected by tree species, the extent of the injury, environmental conditions, and tree vigour and vitality (Shigo & Hillis 1973; Larson 1994).

In *A. alba* and *P. abies*, the structure of the wound-wood differed from that of normal wood, lacking the typical axial orientation and alignment of the tracheids in radial rows. In addition, the morphology of tracheids was changed. They generally lost their typical elongated and tapering structure; instead, numerous crooked and forked tracheids were present. It can be assumed that those changes also occurred in the structure and frequency of bordered pits connecting neighbouring tracheids, although this was not the subject of the current study. Within disorganized tissue, the spindles of the parenchyma cells, ray-like structures, appeared, around which tracheids and traumatic resin canals were arranged in a circle. This is in line with the observations of Zajączkowska (2014b). That author suggested that these ray-like structures represent centres that control the differentiation of cells in the wound wood (Zajączkowska 2014b). Moreover, these local regions with tracheids oriented circularly around the rays are presumably responsible for the progress of tissue differentiation and cell orientation. The intensive growth and accumulation of newly deposited tissue in the wound spindles seems to be the most effective mechanism for tree stem regeneration, to restore its biomechanical and transport functions (Zajączkowska 2014b). Although the structure of wood eventually normalized, growth suppression was clearly visible in all xylem rings formed after the injury and until our sampling.

The formation of traumatic resin canals is also a typical reaction to tree felling or any other tree mechanical injury (Stoffel & Bollschweiler 2008; Gärtner & Heinrich 2009). Extensive formation of traumatic resin canals near wounds is part of a tree's defense mechanism to protect itself from decay and pathogens (Shigo 1984). Traumatic resin canals continue to form in the vicinity of a wound several years after the mechanical injury (Stoffel & Hitz 2008; Stoffel & Klinkmüller 2013). However, the arrangement of the traumatic resin canals is narrowest close to the wound and becomes more dispersed with increasing distance from it (Schneuwly & Stoffel 2008). Their abundance differed between the two species; in *P. abies*, with normal resin canals, the frequency of traumatic resin canals was noticeably higher than in *A. alba*, which has no normal resin canals. This has already been observed by other authors (Stoffel *et al.* 2005; Schweingruber 2007; Schneuwly-Bollschweiler & Schneuwly 2012). The scarcity of traumatic resin canals in *A. alba* is the result of its genetic make-up (Schweingruber 2007). In addition, tylosoids were commonly present in traumatic resin canals. Tylosoids are proliferations of a thin-walled epithelial cell into an intercellular canal, which do not pass through a pit cavity as found in parenchyma–vessel pit membranes in the case of tyloses in angiosperms (Richter *et al.* 2004). Wound-induced tangential bands of traumatic resin canals filled with tylosoids form a barrier to prevent the axial and radial spread of fungi/decay (Morris *et al.* 2020).

Despite the formation of traumatic resin canals years after the tree felling (especially in *P. abies*), the wood structure eventually normalized in terms of tracheid morphology and orientation. The width of the annual xylem growth rings changed from distinctly wide in the vicinity of the callus to very narrow. Discontinuous rings were also present. The substantial reduction of xylem growth rings can be ascribed to the tree felling, i.e., loss of the crown and consequent limited supply of photosynthates to the newly developing tissues and meristematic regions, which is in line with the findings of previous studies (Lanner 1961; Graham & Bormann 1966; Liphschitz *et al.* 1987). Moreover, the reduction in the bark pressure along the wound margin probably leads to a loss of growth-promoting factors (Chano *et al.* 2015).

Changes in phloem anatomy

While the anatomical structure of the youngest non-collapsed secondary phloem is similar in intact tissues of *A. alba* and *P. abies*, the structure of older collapsed secondary phloem generally differs between the two species because of species-specific secondary processes (Abbe & Crafts 1939; Holdheide 1951). Tree felling resulted in an altered structure of non-collapsed and collapsed phloem tissues in both species. These structural changes were similar in the two species in the non-collapsed phloem, while some species-specific changes were observed in the collapsed phloem. This indicates that the response of vascular cambium to the injury was comparable in the two species, while secondary changes in collapsed phloem differed.

Similarly to the arrangement in wood, the typical axial and radial arrangement of cells is absent in the phloem in the years after tree felling, which is seen in the collapsed phloem. Eventually, the typical orientation of phloem cells was restored, i.e., sieve elements and axial parenchyma were vertically aligned, and phloem rays radially. However, a changing tissue orientation from transverse to longitudinal in wood, cambium, and the youngest phloem was clearly visible in both species. Growth ring boundaries between neighbouring phloem increments, which are typical of conifers from temperate and cold environments, were not possible to determine because the size (radial dimension) of sieve cells did not differ between early and late phloem parts. In addition, the typical tangential band of axial parenchyma separating early- and late-phloem parts (Gričar *et al.* 2016) was absent. Instead, parenchyma cells, which were conspicuous by dark stained contents in their lumina, seemed to be randomly scattered throughout the phloem. In *A. alba*, fusiform rays containing radial (traumatic) resin canals, which are not normally present in the bark of this species, were detected. As already mentioned in the results, the Strasburger cells were not distinguished from the parenchyma cells, because without proper sample orientation (longitudinal sections) and observations at higher magnifications (i.e. contact of the Strasburger cells with the sieve cells) the distinction between these two cell types is not reliable (Sauter 1974; Angyalossy *et al.* 2016). The structure of non-collapsed phloem strongly resembled the structure of Mediterranean conifers, which is adapted to Mediterranean conditions, characterized by mild winter conditions and dry summers (Balzano *et al.* 2020).

In the collapsed phloem of *A. alba* and *P. abies*, recognition of growth-ring boundaries is not possible due to age-related changes in phloem cells, which greatly affect cell morphology. Consequently, it was not possible to analyse older phloem growth-ring widths, which would have enabled us to reconstruct the phloem formation dynamics in the years before and after tree felling. In wood, a clear transition was evident in both species, from very wide xylem growth rings adjacent to the callus followed by narrow rings. In the phloem, such information could not be obtained. However, since the changed structure of the youngest phloem indicates that typical phloem formation dynamics are still lost more than 10 years after tree felling, it can be assumed to be the typical reaction of cambium to wounding. It is assumed that phloem growth is accelerated immediately after tree cutting in an attempt to close the wound quickly and re-establish the conducting system (Oven & Torelli 1994; Stobbe *et al.* 2002). Such a reaction was observed in *Q. pubescens* trees exposed to a fire event (Gričar *et al.* 2020). Since there is a huge gap in the literature on this topic, more studies are needed to understand traumatic bark formation in different tree species, especially because all trees experience some kind of mechanical damage (for various reasons) during their lifetime (Shtein *et al.* 2023). Thick outer bark (rhytidome) acts as a very good insulation layer for the sensitive inner tissues of bark and cambium and therefore protects them against external abiotic and biotic factors (Gričar 2013). In this respect, the role of periderms (structure and formation), which was not part of the current study, should also be included. In both studied species, sclereids were very abundant in the collapsed part of the phloem. In normal collapsed phloem, sclereids are generally more common in *A. alba* than in *P. abies* (Holdheide 1951). It can be inferred that their richness in *P. abies* is connected to tree felling. Species-specific differences in response to injury included the formation of traumatic resin canals in *P. abies*, which are normally not present in this species, and enlarged parenchyma cells containing blue-stained contents in *A. alba*. Specialization of parenchyma cells to synthesize and accumulate various ergastic substances, such as oils or slimes, is typical of *A. alba* older collapsed phloem (Holdheide 1951), while their abundance generally increases with age or may be stimulated by wounding (Torelli *et al.* 1996). Barks are frequently rich in secondary metabolites (e.g., crystals, mucilages, tannins, resins, lipids), which may be stored in special cells (i.e., secretory cells), cavities or canals that are often of high diagnostic value for families, genera or individual species (Angyalossy *et al.* 2016).

Hormonal regulation of overgrown tissue in stumps

Tree growth and cell orientation are affected by polar auxin transport, which induces periclinal division in the cambium (Aloni 2021). Auxin, which is synthesised in apical meristems of shoots and actively growing leaves, is transported basipetally in the stem and acropetally in the roots toward root apices (Aloni 2021). Since this transport pathway is interrupted in living stumps, Zajączkowska (2014b) concluded that the polarity of auxin transport is reversed and takes place from source (i.e., apical stem meristems and leaves) in the neighbouring tree via the grafted roots to the living stump. According to this author (Zajączkowska 2014b), the spindles of the parenchyma cells, around which tracheids and traumatic resin canals are arranged in a circle, are probably the result of the opposite direction to the original basipetal auxin transport (Sachs & Cohen 1982). These circular structures, which are formed at the early stages of tissue differentiation in wound wood, are the result of an intrusive growth of axial cells around the precursors of the rays. They are the initial stage of tissue organization, which gradually develops into a highly ordered axially polar structure (Zajączkowska 2014b). Auxin flux, and probably also other signals controlling vascular differentiation, seems to follow a circular route, indicating that differentiation is a response of individual cells to the flux rather than the gradient or concentration of the hormonal signals (Sachs & Cohen 1982).

The formation of traumatic resin canals due to wounding in conifers is regulated by hormones to ensure chemical defence against insects and pathogens (Franceschi *et al.* 2005). Wounding and ethylene can mediate traumatic resin canal formation induced by jasmonates (Hudgins & Franceschi 2004). In conifers, the resin duct epithelial cells produce oleoresin terpenoids, which protect the tree from insects and their associated pathogens (Bouwmeester 2019; Celedon & Bohlmann 2019).

Physiology of living stumps

Knowledge about the biology of living stumps is generally scarce in the literature. It is assumed that root grafting occurs before the tree is felled, while the transport system for carbohydrates from the photosynthetically active tree to the stump is maintained by the fusion of the root vascular systems (Lanner 1961). However, there is a lack of combined studies linking structural traits with the physiological functions of tissues in living stumps. So far, there is only one study on water transport in the xylem based on measurements of sap flow in *Agathis australis* (Bader & Leuzinger 2019), which provides insight into the hydraulic function of a living stump without a crown. It has been shown that there is a close physiological coupling of a living stump with the two neighbouring trees. Although the living stump revealed a strongly reduced sap flow in the xylem, its diurnal pattern was exactly the opposite compared to the neighbouring intact *A. australis* trees. Sap flow in the stump was minimised during the day and maximised at night, while the opposite was true for the intact trees. In addition, the measured respiration rates showed a similar metabolic activity between the host trees and the living stump (Bader & Leuzinger 2019). As no anatomical observations of the stump tissues were performed, the authors presumed (1) that horizontal sap flow in the *A. australis* stump was via existing vascular rays and (2) that flow patterns were highly dependent on the physiology of the host trees and not on the atmosphere, which is the normal case in the plants via the stomata in the leaves. Tissue anatomy was not part of the aforementioned study by Bader & Leuzinger (2019), however, it can be surmised that the anatomy of the xylem and phloem in the *A. australis* stump was altered, similar to all tree species studied. In particular, the amount of parenchyma in wound wood of conifers is increased by the formation of callus tissue, axial parenchyma, and larger and more numerous rays (Arbellay *et al.* 2014), which is important for (1) promoting compartmentalisation and wound closure (Shigo 1984), (2) the refilling of embolised conduits (Hölttä *et al.* 2006; Nardini *et al.* 2011) and (3) inducing defence responses (Hudgins & Franceschi 2004). In addition, rays are important for the reduction of notch stresses at the wound margins and for the mechanical properties (Burgert *et al.* 1999; Arbellay *et al.* 2014).

Based on Bader & Leuzinger (2019), who hypothesise that the survival of living stumps depends primarily on the physiology of the host trees rather than the atmosphere, and reports of the presence of living stumps in different locations in the world with contrasting environmental conditions (dry and wet habitats) (Graham & Bormann 1966; Lev-Yadun & Sprugel 2011), it seems premature to speculate on optimal environmental conditions that allow the survival of living stumps in trees of a non-resprouting taxon with root grafts. The physiological interactions with the host trees reported by Bader & Leuzinger (2019) indicate that such hydrological couplings are more complex than previously thought and point to the need to combine structural and physiological observations on the living stumps in the future.

Effect of root grafting on forest dynamics and management

Previous studies have shown that root grafts can be formed in young and old trees, but the proximity of trees and the number of roots per tree increase the number of root grafts per tree, probably because these factors increase the possibility of root contacts (Quer *et al.* 2020). The spatial arrangement of local trees and root density near grafted trees might therefore help better predict the probability of root grafting. It can also be assumed that the frequency of root grafting is species-specific, and might partly explain why certain tree species, such as firs, can survive for many years in the understory. Root grafting could reduce intraspecific competition for soil resources and light, and allow grafted trees to persist under conditions in which un-grafted trees would be removed. Root grafting should thus be considered in forest management, since it can influence forest dynamics by modulating competition relationships between trees, and tree species, or constitute a way for diseases to spread (Quer *et al.* 2020, 2022). Moreover, awareness of root grafting is important for common silvicultural practices, such as thinning. For example, if thinning is performed to release tree growth and reduce competition for resources among neighbouring trees, the remaining trees may not benefit from this intervention if living stumps of the removed trees take away part of the carbohydrates from residual trees, causing slower productivity of treated stands (Ruel *et al.* 2003; Fraser *et al.* 2005). Finally, soil properties that determine root system architecture can also increase root density in particular soil areas, leading to root grafting. On the one hand, shallow or stoney soils, in which roots are constrained to a thin layer of soil, promote root encounter. On the other hand, mechanical abrasion of root bark and meristematic tissues of trees swaying in the wind, which is greater in sandy soils, promotes the fusion of the root's vascular tissues and subsequent graft formation (Quer *et al.* 2020).

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References

Abbe LB, Crafts AS. 1939. Phloem of white pine and other coniferous species. *Bot. Gaz.* 100: 695–722.

Aloni R. 2021. *Vascular differentiation and plant hormones*. Springer Nature, Cham.

Aloni R, Wolf A. 1984. Suppressed buds embedded in the bark across the bole and the occurrence of their circular vessels in *Ficus religiosa*. *Am. J. Bot.* 71: 1060–1066. DOI: 10.1002/j.1537-2197.1984.tb11958.x.

Angyalossy V, Pace MR, Evert RF, Marcati CR, Oskolski AA, Terrazas T, Kotina E, Lens F, Mazzoni-Viveiros SC, Angeles G, Machado SR, Crivellaro A, Rao KS, Junikka L, Nikolaeva N, Baas P. 2016. IAWA list of microscopic bark features. *IAWA J.* 37: 517–615. DOI: 10.1163/22941932-20160151.

Arbellay E, Stoffel M, Sutherland EK, Smith KT, Falk DA. 2014. Changes in tracheid and ray traits in fire scars of North American conifers and their ecophysiological implications. *Ann. Bot.* 114: 223–232. DOI: 10.1093/aob/mcu112.

Bader MKF, Leuzinger S. 2019. Hydraulic coupling of a leafless Kauri tree remnant to conspecific hosts. *iScience* 19: 1238–1247. DOI: 10.1016/j.isci.2019.05.009.

Balzano A, De Micco V, Čufar K, De Luis M, Gričar J. 2020. Intra-seasonal trends in phloem traits in *Pinus* spp. from drought-prone environments. *IAWA J.* 41: 219–235. DOI: 10.1163/22941932-00002112.

Bouwmeester H. 2019. Dissecting the pine tree green chemical factory. *J. Exp. Bot.* 70: 4–6. DOI: 10.1093/jxb/ery407.

Burgert I, Bernasconi A, Eckstein D. 1999. Evidence for the strength function of rays in living trees. *Holz Roh Werkstoff* 57: 397–399. DOI: 10.1007/s001070050367.

Celedon JM, Bohlmann J. 2019. Oleoresin defenses in conifers: chemical diversity, terpene synthases and limitations of oleoresin defense under climate change. *New Phytol.* 224: 1444–1463. DOI: 10.1111/nph.15984.

Chano V, López R, Pita P, Collada C, Soto Á. 2015. Proliferation of axial parenchymatic xylem cells is a key step in wound closure of girdled stems in *Pinus canariensis*. *BMC Plant Biol.* 15: 64. DOI: 10.1186/s12870-015-0447-z.

Davidson DW. 1963. Living stumps of *Tsuga canadensis* (L.) Carr. (Hemlock) in Northern New Jersey. *Bull. Torrey Bot. Club* 90: 204–207. DOI: 10.2307/2482756.

Fink S. 1982. Adventitious root primordia — the cause of abnormally broad xylem rays in hard- and softwoods. *IAWA J.* 3: 31–38. DOI: 10.1163/22941932-90000744.

Franceschi VR, Krokene P, Christiansen E, Krekling T. 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytol.* 167: 353–376. DOI: 10.1111/j.1469-8137.2005.01436.x.

Franklin GL. 1945. Preparation of thin sections of synthetic resins and wood-resin composites, and a new macerating method for wood. *Nature* 155: 51.

Fraser EC, Lieffers VJ, Landhäusser SM. 2005. Age, stand density, and tree size as factors in root and basal grafting of lodgepole pine. *Can. J. Bot.* 83: 983–988. DOI: 10.1139/b05-048.

Gärtner H, Heinrich I. 2009. The formation of traumatic rows of resin ducts in *Larix decidua* and *Picea abies* (Pinaceae) as a result of wounding experiments in the dormant season. *IAWA J.* 30: 199–215. DOI: 10.1163/22941932-90000215.

Graham JBF, Bormann FH. 1966. Natural root grafts. *Bot. Rev.* 32: 255–292.

Gričar J. 2013. Influence of applied temperature on cambial activity and cell differentiation in *Quercus sessiliflora* and *Acer pseudoplatanus* of different ages. *Drvna Indust.* 64: 95–105.

Gričar J, Hafner P, Lavrič M, Ferlan M, Ogrinc N, Krajnc B, Eler K, Vodnik D. 2020. Post-fire effects on development of leaves and secondary vascular tissues in *Quercus pubescens*. *Tree Physiol.* 40: 796–809. DOI: 10.1093/treephys/tpaa030.

Gričar J, Prislan P, De Luis M, Novak K, Longares LA, Martinez del Castillo E, Čufar K. 2016. Lack of annual periodicity in cambial production of phloem in trees from Mediterranean areas. *IAWA J.* 37: 332–348. DOI: 10.1163/22941932-20160138.

Gričar J, Zupančič M, Čufar K, Oven P. 2007. Wood formation in Norway spruce studied by pinning technique and intact tissue sampling method. *Wood Res.* 52: 1–9.

Grosser D. 1977. *Die Hölzes Mitteleuropas*. Springer, Berlin.

Hejnowicz Z, Kurczyńska E. 1987. Occurrence of circular vessels above axillary buds in stems of woody plants. *Acta Soc. Bot. Pol.* 56: 415–419.

Helmling S, Olbrich A, Tepe L, Koch G. 2016. Qualitative and quantitative characteristics of macerated vessels of 23 mixed tropical hardwood (MTH) species: a data collection for the identification of wood species in pulp and paper. *Holzforschung* 70: 839–844. DOI: 10.1515/hf-2015-0195.

Holdheide W. 1951. Anatomie mitteleuropäischer Gehölzrinden. In: Freud H (ed.), *Handbuch der Mikroskopie in der Technik*: 193–367. Umschau Verlag, Frankfurt am Main.

Hölttä T, Vesala T, Perämäki M, Nikinmaa E. 2006. Refilling of embolised conduits as a consequence of 'Münch water' circulation. *Funct. Plant Biol.* 33: 949–959. DOI: 10.1071/FP06108.

Hudgins JW, Franceschi VR. 2004. Methyl jasmonate-induced ethylene production is responsible for conifer phloem defense responses and reprogramming of stem cambial zone for traumatic resin duct formation. *Plant Physiol.* 135: 2134–2149. DOI: 10.1104/pp.103.037929.

Jyske TM, Suuronen J-P, Pranovich AV, Laakso T, Watanabe U, Kuroda K, Abe H. 2015. Seasonal variation in formation, structure, and chemical properties of phloem in *Picea abies* as studied by novel microtechniques. *Planta* 242: 613–629. DOI: 10.1007/s00425-015-2347-8.

Kuroda K. 1986. Wound effects on cytodifferentiation in the secondary xylem of woody plants. *Wood Res.* 72: 67–118.

Lanner RM. 1961. Living stumps in the Sierra Nevada. *Ecology* 42: 170–173.

Larson PR. 1994. *The vascular cambium: development and structure*. Springer, Berlin.

Lev-Yadun S, Aloni R. 1990. Vascular differentiation in branch junctions of trees: circular patterns and functional significance. *Trees* 4: 49–54. DOI: 10.1007/BF00226240.

Lev-Yadun S, Sprugel D. 2011. Why should trees have natural root grafts? *Tree Physiol.* 31: 575–578. DOI: 10.1093/treephys/tpr061.

Liese W, Dujesiefken D. 1996. Wound reactions of trees. In: Raychaudhuri SP, Maramorosch K (eds.), *Forest trees and palms: diseases and control*: 21–35. Science Publishers, Lebanon, PA.

Liphschitz N, Bonneh O, Mendel Z. 1987. Living stumps — circumstantial evidence for root grafting in *Pinus halepensis* and *P. brutia* plantations in Israel. *Isr. J. Bot.* 36: 41–43.

Liphschitz N, Mendel Z. 1987. Histological studies of *Pinus halepensis* stem xylem affected by *Matsucoccus josephi* (Homoptera: Margarodidae). *IAWA J.* 8: 369–376. DOI: 10.1163/22941932-90000457.

Morris H, Hietala AM, Jansen S, Ribera J, Rosner S, Salmeia KA, Schwarze F. 2020. Using the CODIT model to explain secondary metabolites of xylem in defence systems of temperate trees against decay fungi. *Ann. Bot.* 125: 701–720. DOI: 10.1093/aob/mcz138.

Nardini A, Lo Gullo MA, Salleo S. 2011. Refilling embolized xylem conduits: is it a matter of phloem unloading? *Plant Sci.* 180: 604–611. DOI: 10.1016/j.plantsci.2010.12.011.

Oven P, Torelli N. 1994. Wound response of the bark in healthy and declining silver firs (*Abies alba*). *IAWA J.* 15: 407–415. DOI: 10.1163/22941932-90001375.

Oven P, Torelli N. 1999. Response of the cambial zone in conifers to wounding. *Phyton — Ann. Rei Bot.* 39: 133–137.

Prislan P, del Castillo EM, Skoberne G, Špenko N, Gričar J. 2022. Sample preparation protocol for wood and phloem formation analyses. *Dendrochronologia* 73: 125959. DOI: 10.1016/j.dendro.2022.125959.

Quer E, Baldy V, DesRochers A. 2020. Ecological drivers of root grafting in balsam fir natural stands. *For. Ecol. Manage.* 475: 118388. DOI: 10.1016/j.foreco.2020.118388.

Quer E, Helluy M, Baldy V, DesRochers A. 2022. Does natural root grafting make trees better competitors? *Oikos*: e09666. DOI: 10.1111/oik.09666.

Richter HG, Grosser D, Heinz I, Gasson PE. 2004. IAWA list of microscopic features for softwood identification. *IAWA J.* 25: 1–70. DOI: 10.1163/22941932-90000349.

Rosner S, Baier P, Kikuta S. 2001. Osmotic potential of Norway spruce [*Picea abies* (L.) Karst.] secondary phloem in relation to anatomy. *Trees* 15: 472–482. DOI: 10.1007/s00468-001-0131-9.

Ruel J-C, Larouche C, Achim A. 2003. Changes in root morphology after precommercial thinning in balsam fir stands. *Can. J. For. Res.* 33: 2452–2459. DOI: 10.1139/x03-178.

Sachs T, Cohen D. 1982. Circular vessels and the control of vascular differentiation in plants. *Differentiation* 21: 22–26.

Sauter JJ. 1974. Structure and physiology of Strasburger cells. *Ber. Dtsch. Bot. Gesells.* 87: 327–336.

Schneuwly-Bollschweiler M, Schneuwly DM. 2012. How fast do European conifers overgrow wounds inflicted by rockfall? *Tree Physiol.* 32: 968–975. DOI: 10.1093/treephys/tps059.

Schneuwly DM, Stoffel M. 2008. Tree-ring based reconstruction of the seasonal timing, major events and origin of rockfall on a case-study slope in the Swiss Alps. *Nat. Hazards Earth Syst. Sci.* 8: 203–211. DOI: 10.5194/nhess-8-203-2008.

Schultz RP. 1972. Intraspecific root grafting in Slash pine. *Bot. Gaz.* 133: 26–29.

Schweingruber FH. 2007. *Wood structure and environment*. Springer, Berlin.

Shigo AL. 1984. Compartmentalization: a conceptual framework for understanding how trees grow and defend themselves. *Annu. Rev. Phytopathol.* 22: 189–214.

Shigo AL, Hillis WE. 1973. Heartwood, discolored wood, and microorganisms in living trees. *Annu. Rev. Phytopathol.* 11: 197–222.

Shtein I, Gričar J, Lev-Yadun S, Oskolski A, Pace MR, Rosell JA, Crivellaro A. 2023. Priorities for bark anatomical research: study venues and open questions. *Plants* 12: 1985. DOI: 10.3390/plants12101985.

Stobbe H, Schmitt U, Eckstein D, Dujesiefken D. 2002. Developmental stages and fine structure of surface callus formed after debarking of living lime trees (*Tilia* sp.). *Ann. Bot.* 89: 773–782. DOI: 10.1093/aob/mcf137.

Stoffel M, Hitz OM. 2008. Rockfall and snow avalanche impacts leave different anatomical signatures in tree rings of juvenile *Larix decidua*. *Tree Physiol.* 28: 1713–1720. DOI: 10.1093/treephys/28.8.1713.

Stoffel M, Bollschweiler M. 2008. Tree-ring analysis in natural hazards research — an overview. *Nat. Hazards Earth Syst. Sci.* 8: 187–202. DOI: 10.5194/nhess-8-187-2008.

Stoffel M, Klinkmüller M. 2013. 3D analysis of anatomical reactions in conifers after mechanical wounding: first qualitative insights from X-ray computed tomography. *Trees* 27: 1805–1811. DOI: 10.1007/s00468-013-0900-2.

Stoffel M, Schneuwly D, Bollschweiler M, Lièvre I, Delaloye R, Myint M, Monbaron M. 2005. Analyzing rockfall activity (1600–2002) in a protection forest — a case study using dendrogeomorphology. *Geomorphology* 68: 224–241. DOI: 10.1016/j.geomorph.2004.11.017.

Taiz L, Zeiger E. 2006. *Plant physiology*. Sinauer Associates, Sunderland, MA.

Torelli N, Cufar K, Oven P. 1996. Bioelectrical characterization of tree conditions and slime cells in the bark as possible symptoms of silver fir decline. *Phyton — Ann. Rei Bot. Horn* 36: 35–38.

Trockenbrodt M. 1990. Survey and discussion of the terminology used in bark anatomy. *IAWA Bull.* n.s. 11: 141–166. DOI: 10.1163/22941932-90000511.

Tsoumis G, Kezos N, Fanariotou I, Voulgaridis E, Passialis C. 1988. Characteristics of Briarwood. *Holzforschung* 42: 71–77. DOI: 10.1515/hfsg.1988.42.2.71.

Zajączkowska U. 2014a. Regeneration of Scots pine stem after wounding. *IAWA J.* 35: 270–280. DOI: 10.1163/22941932-00000065.

Zajączkowska U. 2014b. Overgrowth of Douglas fir (*Pseudotsuga menziesii* Franco) stumps with regenerative tissue as an example of cell ordering and tissue reorganization. *Planta* 240: 1203–1211. DOI: 10.1007/s00425-014-2142-y.

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