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Wound callus formation – a microscopic study on poplar (*Populus tremula* L. x *Populus tremuloides* Michx.)

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Summary

Poplar trees were mechanically wounded and the subsequently built parenchymatic callus tissue and wound cambium as well as the developing lateral wound callus were investigated by light microscopy. Two strategies of callus formation were observed. One of them is characterized by four steps: i- formation of a parenchymatic tissue at the wound edge; ii- formation of a cambium within the parenchymatic zone as a tangential extension of the undisturbed vascular cambium; iii- release of radially oriented xylem and phloem cells; iiiii- formation of cambial cells with greater parallel orientation to the wound edge. The other strategy can be subdivided into three steps: i-formation of parenchymatic cells at the wound edge; ii- establishment of a wound cambium preferably within the differentiated phloem tissue; iii- formation of wound xylem and phloem cells, with the tendency to cover the wound laterally. Both strategies may occur within the same tree or even at different portions of one and the same wound.

Introduction

Trees respond to mechanical injury both anatomically and biochemically. Depending on the extent and date of wounding, the reactions can either be restricted closely around the wound or can affect larger portions of the surrounding tissue. They show a large variety and have been subject of numerous investigations on macroscopic and microscopic levels (e.g. BUNTROCK, 1989; LIESE and DUJESIEFKEN, 1996; PEARCE, 2000; SCHMITT and LIESE, 1990, 1992a, 1992b; SHIGO, 1984).

Wound reactions restricted to the bark start with the production of phenolic substances close to the wound and the formation of a ligno-suberized layer around the wound to protect the unaffected tissues against pathogen attack and water loss. Subsequently, a necrophylactic periderm develops (BIGGS et al., 1984; BIGGS, 1985; MULLICK, 1977; OVEN et al., 1999; TROCKENBRODT and LIESE, 1991; TROCKENBRODT, 1994). Wounds up into the cambial region often lead to the formation of callus tissue at the edges (FINK, 1999; GRÜNWALD et al., 2002; LIESE and DUJESIEFKEN, 1989). Wounds extending into the xylem lead to a sequence of reactions, which are often described by the CODIT-model (Compartmentalization Of Decay/Damage In Trees) (BAUCH et al., 1980; LIESE and DUJESIEFKEN, 1996; SHIGO, 1977, 1984). The macro- and microscopically most prominent mechanism involved in compartmentalization, as mainly revealed for hardwoods, is the formation of a so called boundary layer or reaction zone mostly characterized by the synthesis of phenolic compounds and vessel occlusions (e.g. PEARCE, 2000; SCHMITT and LIESE, 1990). Wounding during the period of wood formation by partially removing the bark may lead to the formation of surface callus tissue (DOLEY and LEYTON, 1970; JAKES and HEXNEROVA, 1939; MCDUGALL, BLANCHETTE, 1996; MCQUILKIN, 1950; NOEL, 1968; NOVITSKAYA, 1998; SHARPLES and GUNNERY, 1933; SHORTLE and SHIGO, 1978; STOBBE et al., 2002; WARREN WILSON and WARREN WILSON, 1984). For poplar, some

research was conducted on wound reactions in the bark focusing on the formation of the ligno-suberized zone and the formation of a new periderm as well as a new bark (KAUFERT, 1937; SOE, 1959; TROCKENBRODT and LIESE, 1991). Additionally, the influence of pressure on tissue differentiation on longitudinal bark strips, separated from the bole during early spring and held under different humid conditions, was subject of a detailed study (BROWN and SAX, 1962). The present paper describes on a light microscopic level the regeneration pattern along the lateral edges of stem wounds extending into the xylem. Particular emphasis is given to the role of the bark tissue entering in callus formation and the regeneration of the vascular cambium.

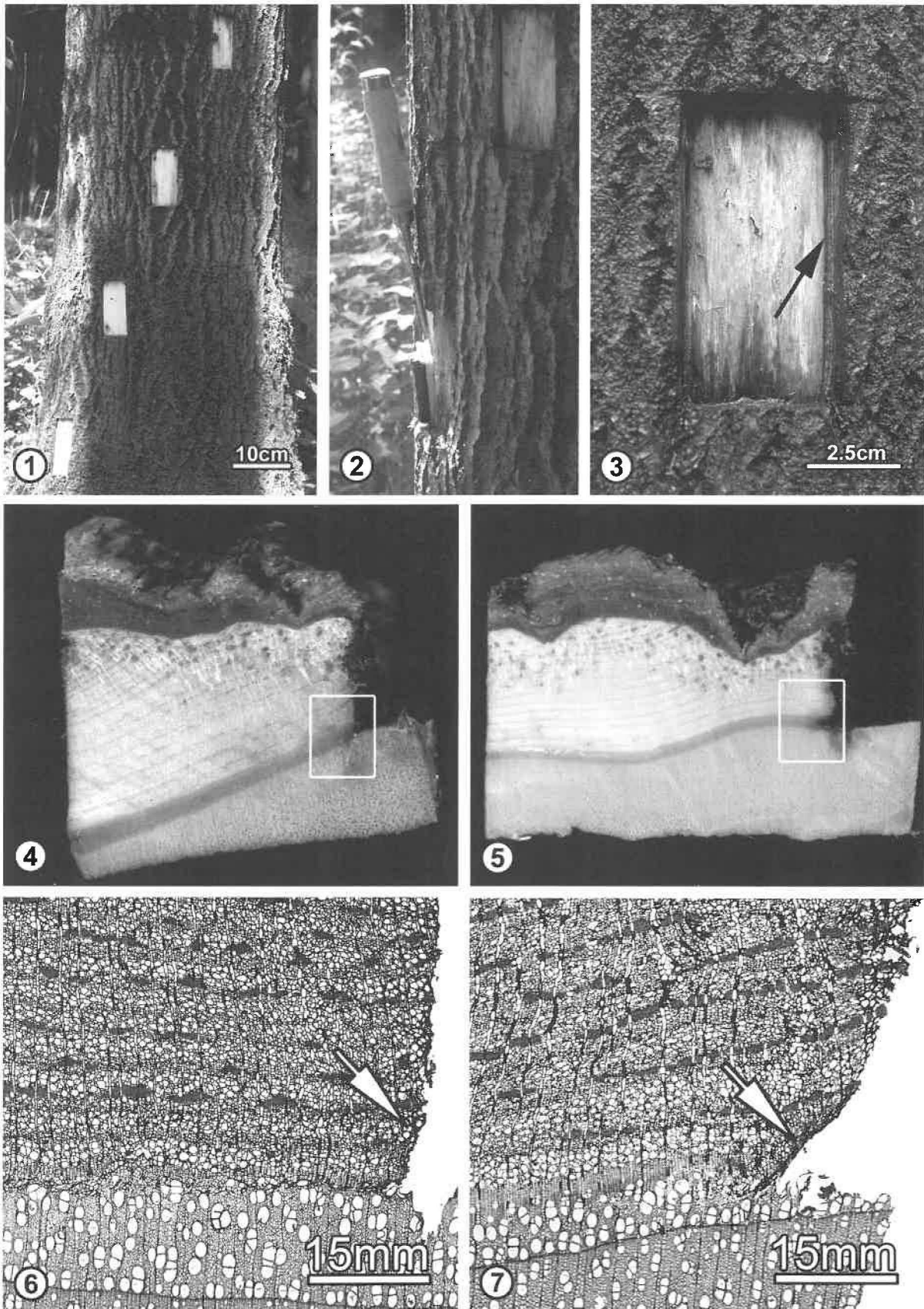
Material and methods

Investigations were carried out using four 25-year old poplar trees (*Populus tremula* L. x *Populus tremuloides* Michx.) of a plantation in the northeast of Hamburg. Eight wounds were set helically around each stem on 27 May 2002. 32 rectangles of bark of 5 x 10 cm² were removed from the trunks using a saw; the wounds were separated 15 cm vertically (Fig. 1, Tab. 1). The edges of the debarked areas were smoothed and the wound surfaces scraped with a razor blade to remove remnants of the cambium and the differentiating xylem. This procedure was carried out to avoid reactions on the wound surface, which might affect tissue portions on the wound edges.

For light microscopy, samples of 5 x 3 x 1 cm³ were collected from the lateral wound edge after 7, 14, 21, 28, 35, 42, 56, and 70 days of response. Spot checks were additionally taken at the end of the vegetation period 2003. The wound tissue was removed with a chisel and a razor blade (Fig. 2, 3, Tab. 1). The samples were cut into smaller cubes of 4 x 4 x 4 mm³, fixed for at least 24h in a phosphate-buffered solution of 37% formaldehyde, dehydrated in propanol and embedded in glycol methacrylate (Technovit 7100). 6µm thin transversal sections were cut with a rotary microtome, stained for 1.5h with a standard Giemsa solution and mounted on glass slides using Euparal.

Results

Seven days after wounding, a brownish discolouration in parts of the xylem next to the wound edge became macroscopically visible (Fig. 4, 5, Tab. 1). Light microscopy revealed that phloem parenchyma, undifferentiated xylem and especially cambium cells at the wound edges collapsed and degenerated. In some wounds, proliferations of a few undifferentiated cells occurred in the former cambial zone adjacent to the necrotic tissue (Fig. 4, 5, Tab. 1). Fourteen days after wounding, large isodiametric cells with thin, unoriented new walls were present in the transition zone from xylem to phloem close to the wound edge. These cells mostly dedifferentiated from phloem parenchyma cells, whereas cambial cells and



Tab. 1: (1-3) *Populus*, experimental design; (4 - 7) 7 days after wounding: transverse sections through lateral wound edges; 1) size and arrangement of stem wounds; 2) sampling of callus tissue with a chisel; 3) callus formation at the edge (arrow) 70 days after wounding; 4, 5) degeneration of cells at the wound surface and wound-associated discoloration in the xylem (frames); 6, 7) light micrographs: degeneration of phloem, cambium and xylem cells at the wound surface (arrows); 6 detail of Fig. 4 (frame), 7 detail of Fig. 5 (frame).

undifferentiated cells of the xylem differentiation zone were hardly involved. At the same time, phloem cells adjacent to this tissue along the wound edge showed dedifferentiation indicating a functional change. Repeated oriented divisions led to the formation of an initial discontinuous band of radially flattened cells.

21 days after wounding, a distinct wound callus became microscopically visible (Fig. 1, Tab. 2). The former unoriented parenchymatic callus tissue now showed oriented cell divisions towards the wound surface (Fig. 2, Tab. 2). In most wounds, the above described band of radially flattened cells in the vicinity of the degenerated outermost tissue developed a phellogen, separated from the degenerated outer tissue by a phellem-like zone consisting of large but thin-walled cells. Such a band extended into or crossed the callus tissue up to the former xylem differentiating zone. Within the phloem, present at the time of wounding, differentiated parenchyma cells started to divide leading to radial and tangential groups of flattened cells. In wounds with a nearly unaffected cambium, wood formation was restarted at the transition between the zone with wound-associated modifications and the unaffected tissue.

Within the next 14 days, these wounds showed a regeneration of the cambium as a tangential extension of the undisturbed cambium across the parenchymatous zone. This new vascular cambium was already starting with the production of xylem and phloem cells. In a second group of wounds, showing cell divisions of dedifferentiated phloem cells, these cells fused to a nearly continuous band.

42 days after wounding, the tangential regeneration of the cambium almost reached the callus tip. With progressing elongation it gradually started to form xylem and phloem. The new modified wound xylem, consisting of less and smaller vessels, predominantly showed a radial orientation (Fig. 3, Tab. 2). In the second group of wounds, the bands of dividing cells within the differentiated phloem fused to a wound cambium, which appeared semicircular in outline by including parts of the preexisting phloem. At this stage, the wound cambium did not contact the vascular cambium present before wounding. The formation and lignification of secondary cell walls in the wound tissue started in modified phloem fibres (Fig. 1, Tab. 3).

56 days after wounding, the wound cambium extended inward thus achieving a connection with the structurally unaffected cambium further apart from the wound edge. Along the wound edge, the wound cambium extended exactly to the zone, where wood formation had stopped after setting the wound. This new wound cambium started to develop wound xylem directly adjacent to the preexisting phloem. Dark phenolic deposits were present in numerous phloem parenchyma cells of this area (Fig. 2, Tab. 3). In wounds without included phloem but with a tangential extension of the cambium, additional cambium cells were formed parallel to the lateral wound edge. This cambium subsequently formed phloem and xylem cells starting to continuously cover the wound.

70 days after wounding, two strategies of cambium regeneration and callus formation could be clearly differentiated: accordingly, included preexisting secondary phloem was either present (Fig. 2, 4, Tab. 4) or not (Fig. 1, 3, Tab. 4).

In summary of the microscopic observations, the two strategies can be described as follows:

One strategy is subdivided into four developmental steps:

i- formation of a uniform parenchymatous tissue at the wound edge by division of undifferentiated xylem, phloem as well as cambial cells; ii- formation of a wound cambium within the parenchymatous zone, as an exclusively tangential elongation of the undisturbed cambium further apart from the wound edge; iii- release of strictly radial oriented xylem and phloem cells; iiiii- formation of cambial cells with an increasingly parallel orientation to the perpendicular wound edge, finally leading to the lateral covering of the wound.

The other strategy can be subdivided into three developmental steps:

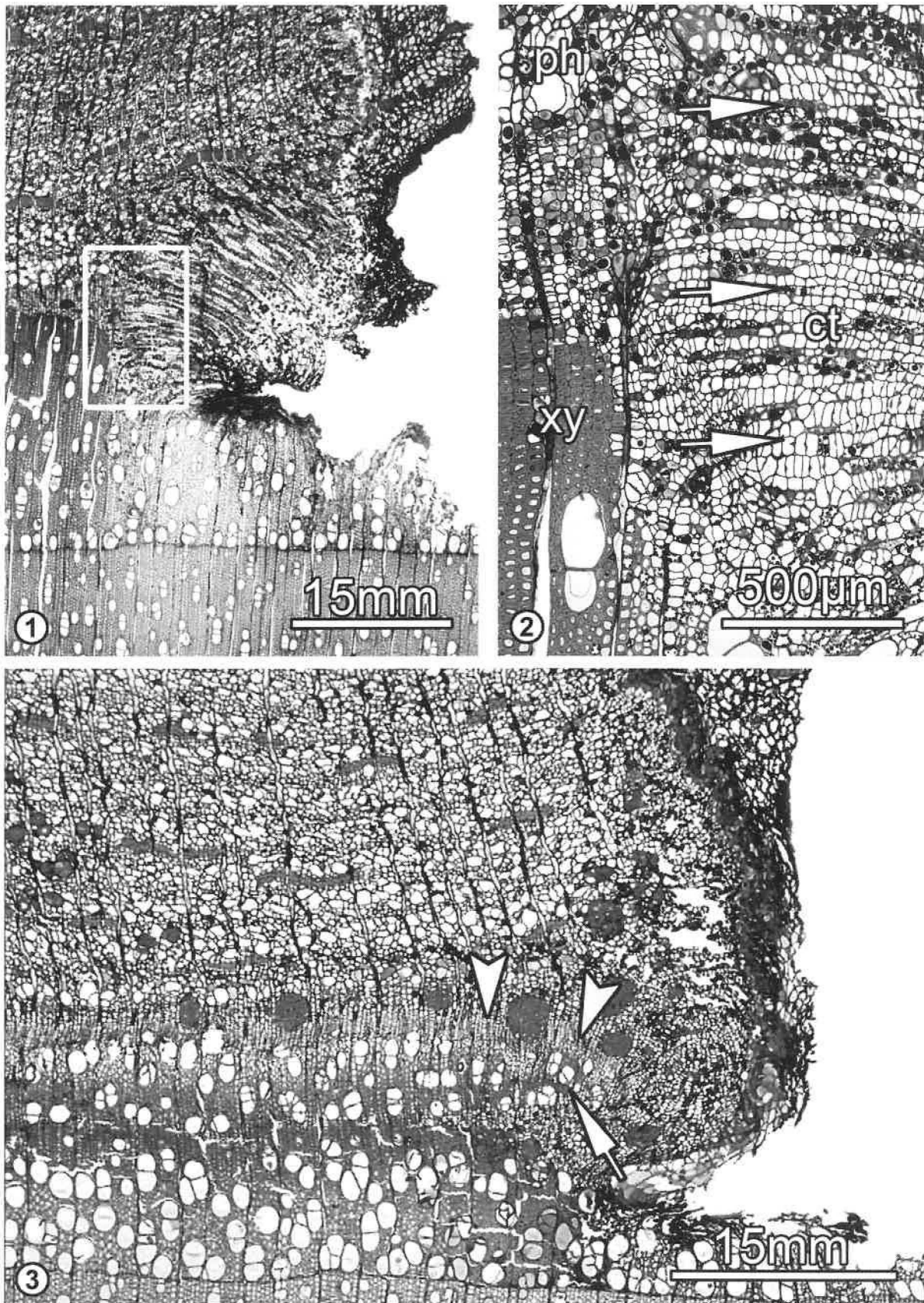
i-formation of parenchymatous cells at the wound edge by division of undifferentiated xylem, phloem as well as cambial cells; ii-formation of a wound cambium preferably within the differentiated phloem tissue, finally semicircular in outline and including parts of the differentiated phloem; iii- beginning formation of wound xylem and phloem cells by the cambium, already with the tendency to cover the wound laterally.

Discussion

The present study on callus formation in poplar revealed different ways of vascular cambium regeneration and subsequent callus development. In principle, two strategies were defined by several morphologically characteristic developmental steps. Both strategies may occur within the same tree or even at different portions of one and the same wound.

The first step towards a wound callus, viz. the formation of large, thin-walled parenchymatous cells along the wound edge, originating from undifferentiated xylem cells, vascular cambium and parenchymatous phloem cells, is common to both strategies. The un-oriented, isodiametric callus cells mainly derive from parenchymatous phloem cells after dedifferentiation. Undifferentiated xylem and cambium cells are not contributing to that extension, as similarly observed in *Fraxinus excelsior* L. stems (DOLEY and LEYTON, 1970). SWARBRICK (1927) also showed that callus in girdled apple trees almost entirely originate from the phloem. However, other observations (BUCCIARELLI et al., 1999; SHARPLES and GUNNERY, 1933) led to the assumption that xylem elements, namely the vascular rays as well as cambial cells are mainly responsible for callus formation. In other tree species like *Fagus sylvatica* L. and *Quercus robur* L., the secondary phloem does not play a role in the development of a lateral wound callus (FINK, 1999; GRÜNWARD et al., 2002; NOEL, 1968, 1970). Such a contradiction may depend either on species-specific reactions or the mode and time of wounding. Especially the mode of wounding seems to have a strong selective effect on the tissue mainly contributing to callus formation. According to SOE (1959), the callus tissue in *Populus deltoides*, scored by cutting with a knife through the bark and often into the underlying xylem, originates mainly from cells of the vascular rays. The present study on poplar with large-scale wounds revealed a dominant role of the secondary phloem in the initiation of callus tissue.

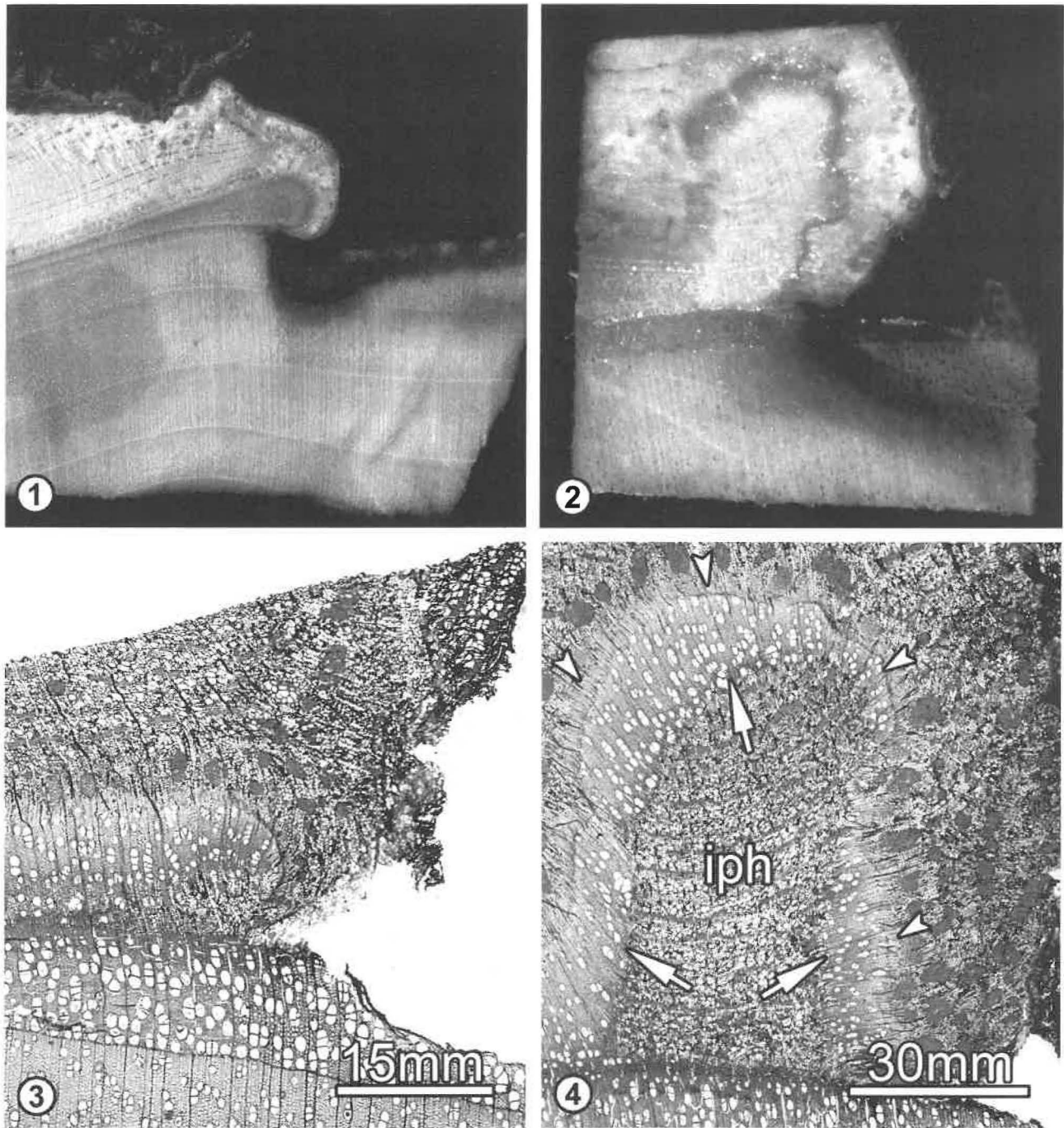
The second step of strategy one, i.e. the formation of a wound cambium within the parenchymatous zone as a tangential extension of the undisturbed cambium further apart, corresponds with previous observations. SOE (1959) reported the formation of a new cambial zone in *Populus deltoides* scores in regions where the callus tissue lies close to the undisturbed cambium. Other studies described the formation of a wound cambium inside the lateral parenchymatous callus tissue (DOLEY and LEYTON, 1970; FINK, 1999; GRÜNWARD et al., 2002; NOEL, 1970; SHARPLES and GUNNERY, 1933). It was also found on a debarked surface that the vascular cambium regenerates within the callus tissue (BROWN and SAX, 1962; NOEL, 1968; STOBBE et al., 2002; WARREN WILSON and WARREN WILSON, 1961, 1984; WARREN WILSON, 1984; ZHENGLI and KEMING, 1988). The physiological mechanism responsible for the formation of a cambium and the factors determining the site of cambium regeneration within a wound-induced callus are still rarely understood. While the young wound cambium of poplar, following strategy one, started to release strictly radially oriented xylem and phloem cells, the cambium continuously extends through the formation of meristematic cells. This cambium, with an increasingly parallel orientation to the wound edge, finally leads to the lateral covering of the wound through phloem and xylem formation. It is mentioned that in some cases tracheary



Tab. 2: *Populus*, transverse sections through lateral wound edges, light micrographs; 21 days after wounding: 1) formation of a parenchymatous callus tissue; 2) detail of Fig. 1 showing callus tissue (ct) with oriented cell formation (arrows), originating from phloem (ph), cambium and undifferentiated xylem cells, differentiated xylem (xy); 42 days after wounding: 3) cambium regeneration within the callus tissue as a tangential continuation of the undisturbed cambium (arrowheads) and deposition of new xylem cells (arrow).



Tab. 3: *Populus*, transverse sections through lateral wound edges, light micrographs; 42 days after wounding: 1) formation of a wound cambium within the differentiated phloem tissue and semicircular in outline (arrows), included phloem (iph); 56 days after wounding: 2) formation of wound xylem (arrowheads) and wound phloem cells by the wound cambium (wc), included phloem (iph).



Tab. 4: Populus, 70 days after wounding; strategy one: 1, 3) formation of additional cambium cells as a tangential elongation of the unaffected cambium and along the lateral side of the wound with subsequent formation of xylem and phloem cells continuously covering the wound; strategy two: 2, 4) formation of xylem (arrows) and phloem cells through the newly formed cambium (arrowheads) around a portion of included phloem (iph) also continuously covering the wound; transversal sections, macroscopic view (1, 2), light micrographs (3, 4).

elements and sieve tube strands appear before any cambium has formed and that not all differentiated cells derive from the cambium (WARREN WILSON and WARREN WILSON, 1984), as variously observed in this study. A continuing extension of the cambium within a lateral callus with simultaneous xylem formation by previously formed wound cambium segments has not been reported yet. SHARPLES and GUNNERY (1933) found that in a callus on an exposed wood surface of *Hibiscus* the earliest formed cambium had begun to function normally before the cambial cylinder was completely closed. In most cases it is reported that xylem differentiation starts after a vascular

cambium has formed (e.g. GRÜNWARD et al., 2002; NOEL, 1970; SOE, 1959; WARREN WILSON and WARREN WILSON, 1984).

The second strategy is characterized by the formation of a wound cambium through dedifferentiation of mature secondary phloem cells followed by redifferentiation into a cambial tissue. Its semicircular outline consequently included parts of the differentiated phloem. No such observations were found in the literature.

It is a well-known phenomenon that cells in the secondary phloem are able to differentiate into meristematic cells to form a new periderm during rhytidom formation. Additionally, the formation of a necro-

phylactic periderm due to wounding demonstrates the capability of differentiation of secondary phloem parenchyma cells into a meristematic tissue (e.g. BIGGS et al., 1984; BIGGS, 1985; MULLICK, 1977; OVEN et al., 1999; TROCKENBRODT and LIESE, 1991; TROCKENBRODT, 1994).

During early developmental stages, it was impossible to exactly predict whether a phellogen or a vascular cambium would develop at the sites of redifferentiation. There are several hypotheses referring to what induces and what determines the site of cambium formation within a wound-associated callus (e.g. ALONI, 2004; DOLEY and LEYTON, 1970; SHARPLES and GUNNERY, 1933; WARREN WILSON and WARREN WILSON, 1961). Future studies should focus on the relationships between structural details as observed in the present work and mechanisms described in these hypotheses such as the involvement of hormones (ALONI, 2004), including the "gradient induction hypothesis" of WARREN WILSON and WARREN WILSON (1961), and other hypotheses based on histological observations, such as the "free surface" hypothesis (BERTRAND, 1884; VÖCHTING, 1892) and the "cambial ring" hypothesis (JANSE, 1921; SNOW, 1942).

In conclusion, the present study revealed two strategies of wound cambium development in poplar. Both strategies, which are mainly defined by the position of the wound cambium at early developmental stages, aim at the formation of a wound callus starting to cover the wound. The factors guiding these processes on the cell and the tissue level have yet to be determined.

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