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The formation of a ligno-suberised layer and necrophylactic periderm in beech bark (*Fagus sylvatica* L.)

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Summary

Beech (*Fagus sylvatica* L.) bark was wounded in early April of 1993 and tissue changes followed on days 7, 14, 21, 28, 35, 42, 49, 56, 84, 112, and 140. In 7 days, tissue at the wound surface became necrotic and discoloured. In 14 days the walls of the parenchyma cells immediately underneath the necrotic tissue became thickened and after 21 days became lignified. In 28 days these lignified cells showed intracellular suberisation. In 42 days the ligno-suberised layer was continuous with the phellem of the superficial periderm. In 35 days we first noted differentiation of the necrophylactic phellogen under the ligno-suberised layer. In 49 days suberised phellem of the necrophylactic periderm differentiated. At 112 days the phellem of the necrophylactic periderm had coalesced with that of the surface periderm. In 140 days abscission of the wound rhytidome began.

The formation of a ligno-suberised layer and the necrophylactic periderm started in the nonconducting phloem under the basal region of the wound and proceeded from there in two directions: toward the tissue under the original periderm and along the sclerified rays toward the cambium. Sclerified phloem rays protruding into the xylem rays did not prevent the formation of a ligno-suberised layer and necrophylactic periderm in beech. It is supposed that the ligno-suberised layer and the necrophylactic periderm in European beech is generated from living cells extant at the time of wounding as well as from recent phloic derivatives of the vascular cambium.

Key words: Bark, wound response, ligno-suberised layer, necrophylactic periderm, *Fagus sylvatica*

1. Introduction

The formation of new periderm in the mechanically injured or infected living bark restores continuous periderm (ESAU 1965). It is represented by a group of necrophylactic periderms (MULLICK & JENSEN 1973) which always occur immediately below the impervious ligno-suberised layer (BIGGS 1985 a, b, RITTINGER et al. 1987, WOODWARD & PEARCE 1988, OVEN & TORELLI 1994). Early formation of the ligno-suberised layer provides a barrier to further moisture loss and microbial invasion of living bark and hence maintains the conditions for the formation of necrophylactic periderm (BIGGS 1992, WOODWARD 1992). Its formation is a non-specific active response and always occurs when bark dies, no matter what the cause (MULLICK 1975, 1977).

The specific anatomy of European beech bark has been repeatedly investigated (HOLDHEIDE 1951, WHITMORE 1962, BRAUN 1976, KUČERA et al. 1980) and considered as a possible feature predisposing beech to bark disease (BRAUN 1976, 1977). In European beech attacked by *Cryptococcus fagi* BÄR. bark cracks along the sclerified rays to the cambium and it is suggested that the presence of the rays themselves may prevent the formation of a necrophylactic (wound) periderm (BRAUN 1976, 1977). On the contrary, in American beech *(Fagus grandifolia)* it was observed that neither sclerified phloem rays nor large groups of sclereids interrupted the necrophylactic periderm (OSTROFSKY 1982, OSTROFSKY & BLANCHARD 1982). These findings imply that European beech bark responds differently than does American beech. Information on the ligno-suberised layer is also missing.

In comparison with the wound reactions of European beech xylem (cf. PEARCE 1990, SCHMITT & LIESE 1993, TORELLI & OVEN 1996), the bark response to injury is relatively poorly known. We present the sequence of anatomical and histochemical changes in European beech bark after mechanical wounding.

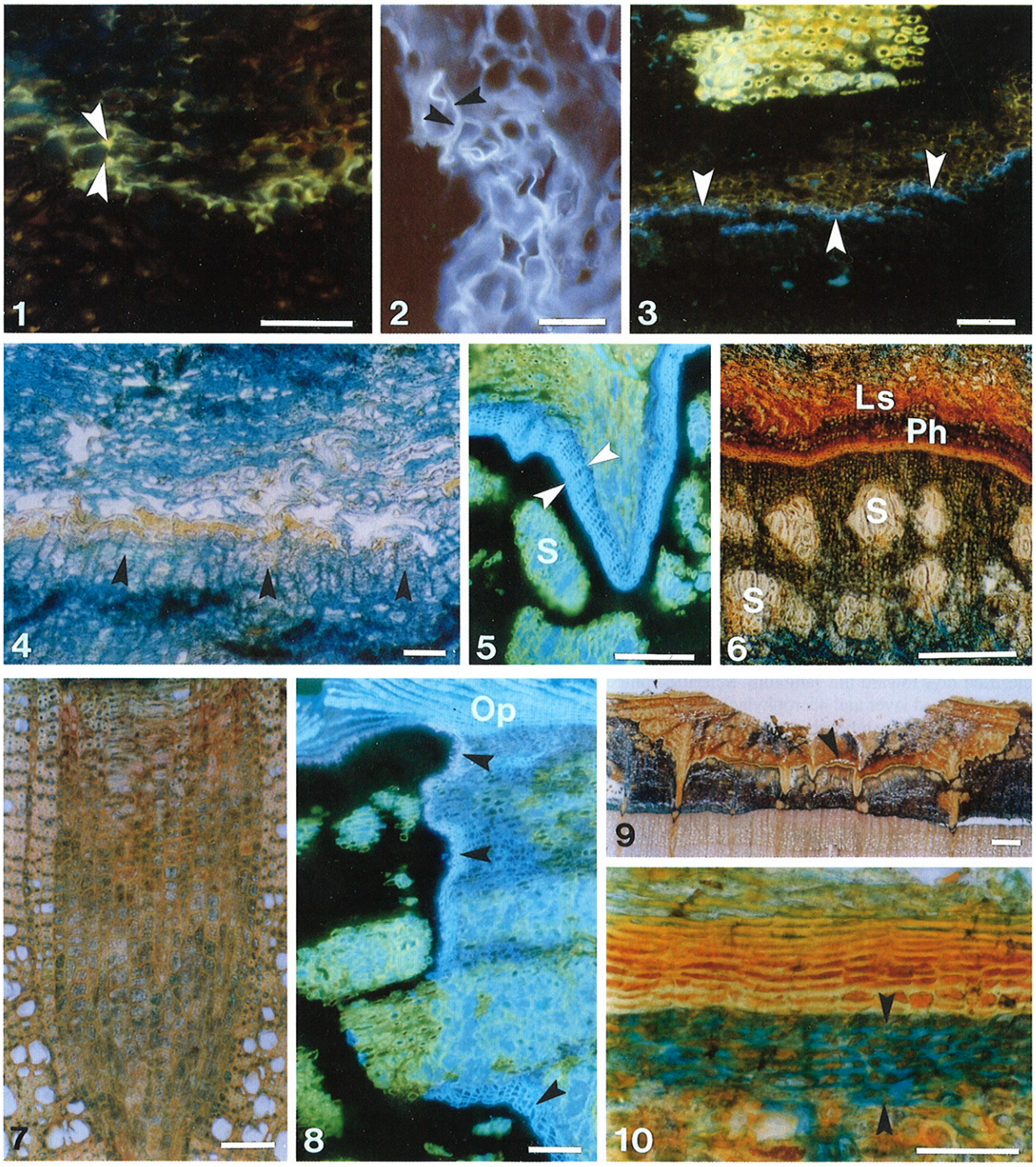

Fig. 1–9. *Fagus sylvatica* L. Cross-sections of wounded bark.

– 1. Day 21; lignification of thickened parenchyma cell walls is marked by arrows; Acridin Red – Chrysoidin/Astra blue (ACA); UV; bar = 100 µm.

– 2. Day 28; suberin encrusted onto the previously lignified walls of individual parenchyma cells (arrows); Phluoroglucinol + HCl; UV; bar = 50 µm.

– 3. Day 35; the continuous ligno-suberised layer (arrows) formed in the nonconducting phloem under the base of the wound; ACA; UV; bar = 100 µm.

2. Materials and methods

On April 7, 1993, we drilled 11 wounds, 1–2 mm deep, with a 5 mm borer into an approximately 50 year old beech, thus wounding the original superficial periderm and noncounducting phloem. The lowest wound was 70 cm above the ground; the others spiraled around at distances of 10 cm. Sampling was acropetal; we took one sample on day 7, 14, 21, 28, 35, 42, 49, 56, 84, 112, and 140 after wounding, consisting of bark with the cambium and xylem. We also took as a control an intact sample.

The tissues were fixed in a solution of formaldehyde, acetic acid, and ethanol and embedded with Polyethylenglycol 1500 (GERLACH 1969). Cross-sections were prepared at 25 µm with a sliding microtome. We employed light microscopy and a fluorescent microscopic technique in combination with improved and modified histochemical methods for detection of lignification and suberisation. We used: (a) a polychromatic combination of the stains Acridin red – Chrysoidin/Astra blue (ACA), (b) the quenching autofluorescence technique (BIGGS 1984; 1985 a) based on the selective use of Phluoroglucinol + HCl or Toluidine blue O and Sudan black B (JENSEN 1962). Extraction procedures additionally confirmed the presence of suberin: chlorine dioxide for the extraction of polyphenols, KOH for the saponification of suberin, acetone for soluble lipids (PEARCE & WOODWARD 1986). After extraction, the samples were processed by methods (a) and (b). An Olympus BH2 reflected light flurorescence microscope with mercury burner HBO 100W, exciter filter UG-1 and dichroic mirror U (DM-400 + L-420) was used for observations in ultraviolet spectrum (UV).

The sequence of events is demonstrated by low power colour photomicrographs (Figs. 1–10). Additionally, details are shown on black and white figures (Figs. 11–18).

3. Results

Day 7. The parenchyma cells were necrotic and discoloured at the wound surface. Brown polyphenolic deposits were present in the lumina of parenchyma cells and could be extracted with chlorine dioxide. Sclereids showed no change.

Day 14. First anatomical changes occurred in the nonconducting phloem below the wound base. Walls of parenchyma cells were thickened under the necrotic tissue.

Day 21. The thickened parenchyma cell walls were lignified; this was particularly noticeable in the corners of walls (Fig. 1). Parenchyma cell walls were also thickened under the necrotic tissue below the original periderm and along the sclerified ray.

Day 28. Deposition of lignin continued in the nonconducting phloem at the basal region of the wound; intracellular suberin appeared in individual lignified cells (Fig. 2). Individual parenchyma cells with thickened walls showed lignification in tissue under the original periderm and at the sclerified rays.

Day 35. Further suberisation led to a continuous ligno-suberised layer in the nonconducting phloem (Fig. 3). The cell lumina of the ligno-saberised layer were filled with polyphenols. Immediately underneath the layer there were cell walls, indicating the differentiation of a new phellogen (Figs. 4, 11). Suberin accrusted onto individual lignified walls of parenchyma cells in the tissue under the original periderm and along the sclerified ray (Figs. 12 a, b). The depression of the xylem ray was filled by derivatives of the cambium, resembling callus cells (Figs. 13 a, b).

Day 42. Phloic derivatives of the cambium occurred under the sclerified phloem ray (Fig. 14). The ligno-suberised layer was continuous with the suberised phellem cells of the original periderm. It also arose under the sclerified phloem rays (Fig. 15). Sclerified tissue did not suberise. The differentiation of a new phellogen below the ligno-suberised layer proceeded into the tissue under original periderm and along the sclerified rays.

Day 49. Activity of new phellogen resulted in the formation of a necrophylactic periderm surrounding the sclerified phloem rays (Figs. 5, 16). The necrophylactic

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– 4. Day 35; divisions of parenchyma cells (arrows) indicated differentiation of new phellogen below the ligno-suberised layer; ACA; bar = 100 µm.

– 5. Day 49; suberised phellem (arrows) of the necrophylactic periderm surrounding the sclerified phloem ray; S = newly formed sclereids in the phelloderm; ACA; UV; bar = 100 µm.

– 6. Day 56; the phelloderm of the necrophylactic periderm is more than 20 cells thick. Phelloderm cells have transformed into nests of sclereids. Ls = ligno-suberised layer; Ph = a phellem of necrophylactic periderm; S = sclereids; ACA; bar = 100 µm.

– 7. Day 56; disorganised callus in the indentation of the xylem ray; ACA; bar = 100 µm.

– 8. Day 112; the suberised phellem of necrophylactic periderm (arrows) has merged with that of the original periderm (Op); ACA; UV; bar = 100 µm.

– 9. Day 140; the situation at the end of the experiment. Note the different depths of necrophylactic periderm formation. Tangential fissure (arrow) indicated the site of abscission of wound-associated rhytidome; ACA; bar = 300 µm.

Fig. 10. *Fagus sylvatica* L. Cross-section of periderm in intact bark. A phelloderm (between arrows) of the original periderm comprises up to 6 cells (see also Fig. 6); ACA; bar = 100 µm.

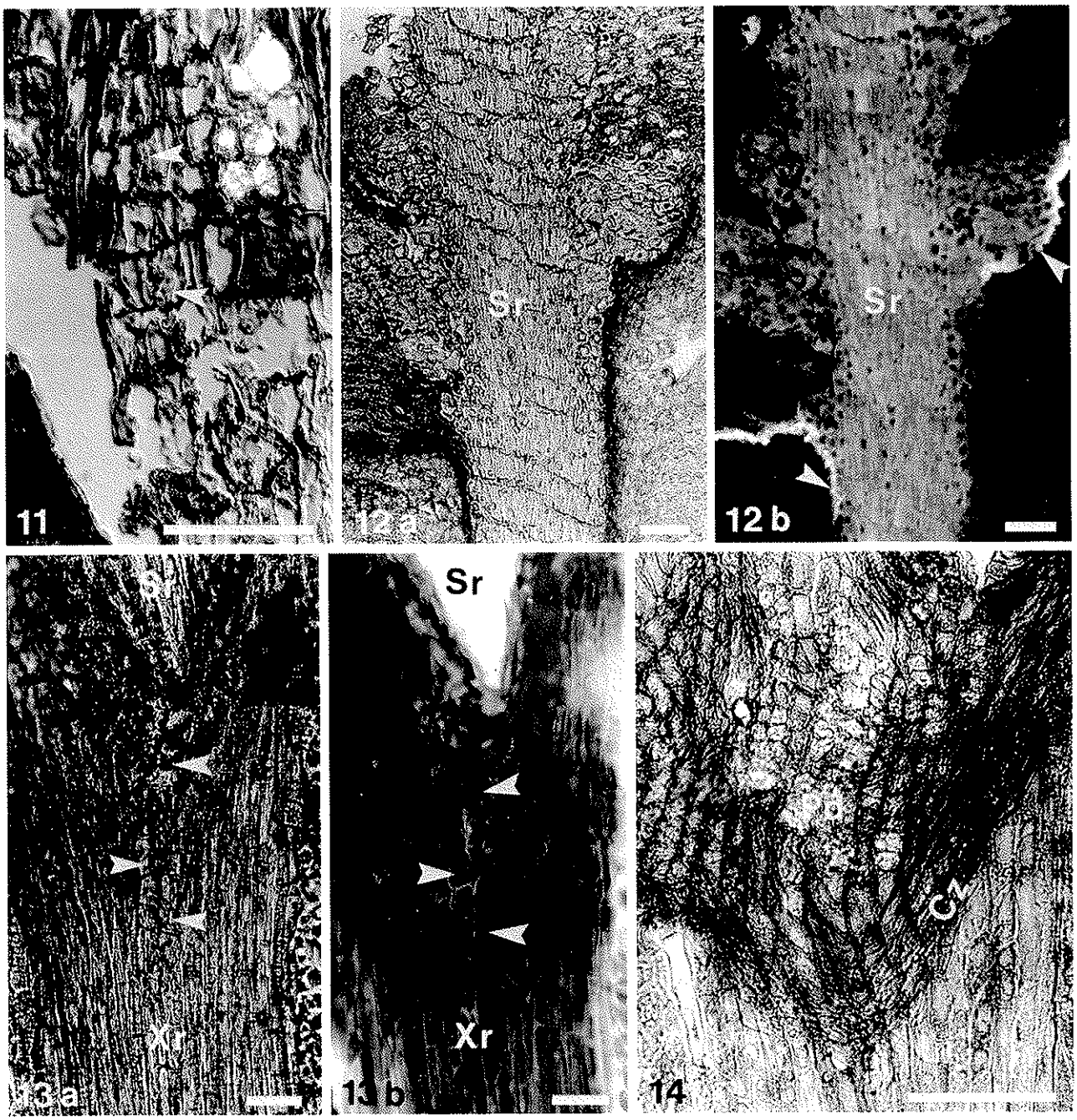

Fig. 11–18. *Fagus sylvatica* L. Radial (Fig. 11) and cross-sections (Figs. 12–18) of wounded bark.

– 11. Day 35; radial-section of dividing parenchyma cells below the ligno-suberised layer (see Fig. 4.). Arrows point at new cell walls; Toluidine blue O; bar = 100 µm.

– 12. Day 35; section treated with phluoroglucinol + HCl photographed under bright field (a) and ultraviolet (b) illumination revealed the position of the ligno-suberised layer (arrows). Its formation started in the nonconducting phloem (see Fig. 3) and proceeded from there in two directions: toward the tissue under the original periderm and along the sclerified rays (Sr) toward the cambium; bar = 100 µm.

Figs. 13 and 14. Response of the cambial zone under the sclerified phloem ray.

– 13. Day 35; unstained section photographed under bright field (a) and ultraviolet (b) illumination. The indentation in the xylem ray is filled by callus cells (arrows); Sr = sclerified phloem ray; Xr = xylem ray; bar = 100 µm.

– 14. Day 42; phloic derivatives (Pd) between the xylem ray and sclerified phloem ray; Cz = cambial zone; ACA; bar = 100 µm.

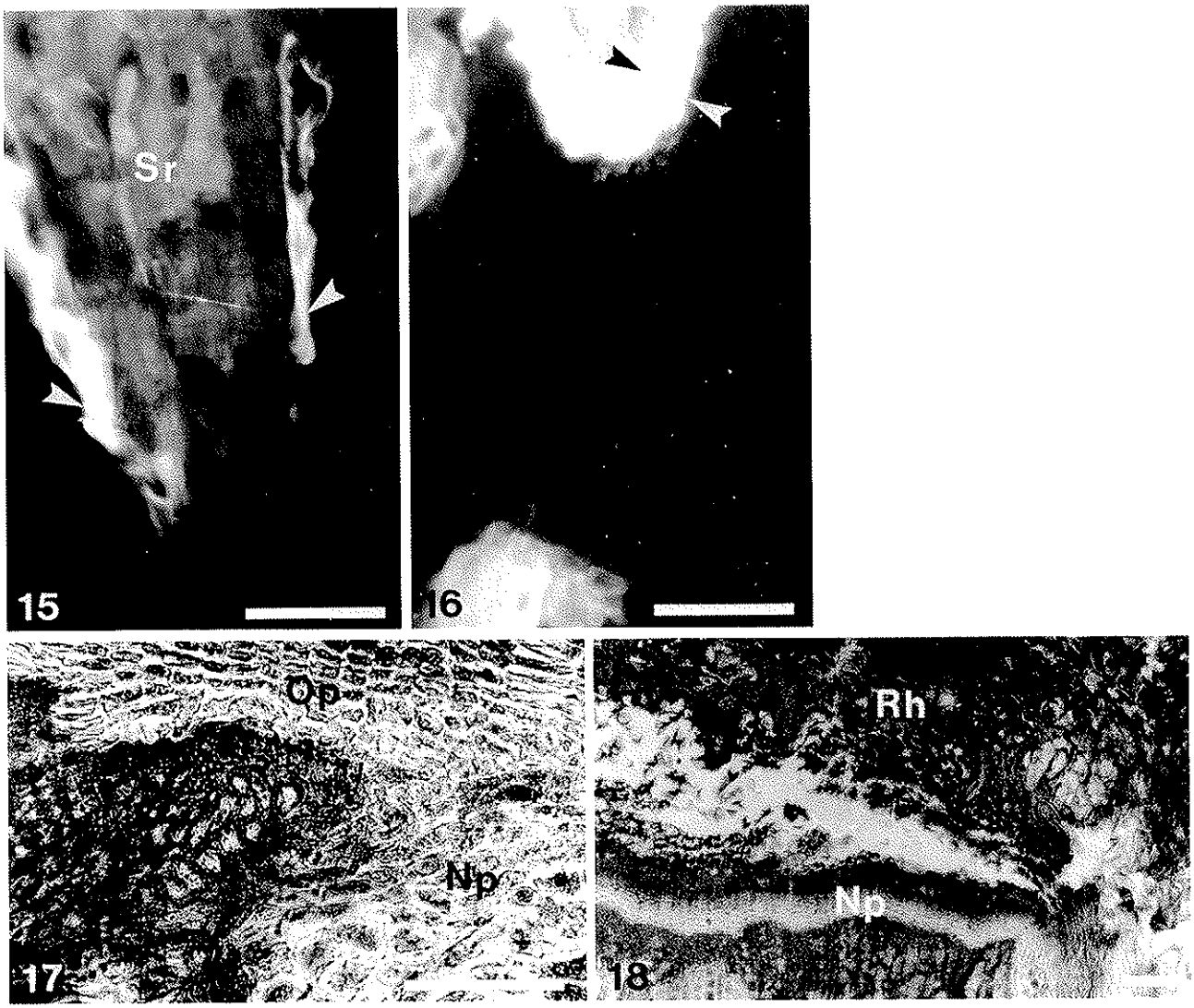

Fig. 15. Day 42; the occurrence of the ligno-suberised layer (arrows) at the tapered end of a sclerified phloem ray (Sr); Phluoroglucinol + HCl; UV; bar = 100 μm.

Fig. 16. Day 42; Phellem of the necrophylactic periderm (between arrows) under the tapered end of the sclerified phloem ray; Phluoroglucinol + HCl; UV; bar = 100 μm.

Fig. 17. Day 112; detail of Fig. 8. The phelloderm cells of the original periderm (Op) were involved in formation of the necrophylactic periderm (Np); ACA; bar = 100 μm.

Fig. 18. Day 140; detail of Fig. 9. The separation of wound-associated rhytidome (Rh) seems to occur between the ligno-suberised layer and the phellem of the necrophylactic periderm (Np); ACA; bar = 100 μm.

periderm consisted of suberised phellem cells and a thick layer of phelloderm cells arranged in a series centripetal to the wound surface. The phelloderm of the necrophylactic periderm contained individual cells differentiating into sclereids (Figs. 5, 6). The depression in the xylem ray, once filled with sclerified phloem ray, was now filled with disorganised and lignified callus (Fig. 7).

Day 56. Phelloderm and phellem cells had also arisen in the tissue under the original periderm.

Day 84. Phellem cells of the necrophylactic periderm were also suberised in the tissue under the original periderm.

Day 112. The phellem of the necrophylactic periderm had coalesced with the phellem of the original periderm (Figs. 8, 17).

Day 140. Between the phellem of the necrophylactic periderm and wound-associated rhytidome tangential cracks occurred, indicating the site of its abscission (Figs. 9, 18). It is likely that separation occurs at the position of a ligno-suberised layer (Fig. 18). The crucial difference between the original and the necrophylactic periderm lies in the phelloderm: it was up to 20 cells wide in the necrophylactic periderm, while in the original it was only 6 cells thick (Fig. 10).

4. Discussion

Our results showed two types of tissue changes in wounded beech bark: the first were associated with the ligno-suberised layer and the second with the necrophylactic periderm formation. Along with the response of phloem parenchyma, we observed a cambial response as well, although the vascular cambium itself was not wounded.

In the bark, a ligno-suberised layer developed from the cells already present at the time of wounding by thickening, lignification and suberisation. It has recently been reported that phloem parenchyma may be somewhat modified before lignification. *Abies amabilis, Tsuga heterophylla, Thuja plicata* (MULLICK 1975), *Picea abies* and *Larix decidua* (BANGERTER 1984) also showed a hypertrophied parenchyma, while *Abies alba* (OVEN & TORELLI 1994) showed a hyperplasia of the ray parenchyma as well. *Salix caprea, Tilia tomentosa* (TROCKENBRODT 1994), *Populus tremula* and *Platanus x acerifolia* (TROCKENBRODT & LIESE 1991) had no hypertrophy or thickening of the cell walls.

In thickened parenchyma cells we observed the occurrence of lignin at the corners of cells. Lignification as a part of bark response was observed in several gymnosperms (KUČERA 1971, MULLICK 1975, BANGERTER 1984, WOODWARD & PEARCE 1988, WAHLSTRÖM & JOHANSSON 1992, OVEN & TORELLI 1994) and angiosperms (BIGGS 1984, 1985a, b, TROCKENBRODT & LIESE 1991, TROCKENBRODT 1994). BIGGS et al. (1984) showed that wound-induced lignin is not typical angiosperm lignin.

Intracellular suberin in beech occurred after the lignification of thickened parenchyma cells. In wounded and infected bark of *Pinus sylvestris* suberisation occurred in different depths below the wound surface before the cell walls became lignified (WAHLSTRÖM & JOHANSSON 1992). In wounded bark of *Populus tremula* and *Platanus x acerifolia* suberisation of lignified cells present at the time of wounding was observed as well (TROCKENBRODT & LIESE 1991).

At the last stage of ligno-suberised layer formation we observed insoluble polyphenolic deposits in cell lumina. It was confirmed that accumulation of polyphenols is biochemically associated with suberisation (KOLATTUKUDY 1984). In American beech inoculation of wounds with the fungus resulted in reduced soluble phenolic levels in bark sectioned nearest the wound, and increased levels in sections nearest the vascular cambium (OSTROFSKY et al. 1984).

Immediately under the ligno-suberised layer new cell walls were visible indicating the occurrence of new phellogen. It is reported that new phellogen arises as a result of remeristemization of parenchyma cells (BIGGS 1992, WOODWARD 1992). We never observed the occurrence of new phellogen in sections where thickened and lignified cells were not suberised. Additive divisions of new phellogen give rise to the phellem and phelloderm of necrophylactic periderm. In the further course of the experiment the suberisation of phellem was observed. Studies of *Salix caprea, Tilia tomentosa* (TROCKENBRODT 1994), *Populus tremula* and *Platanus x acerifolia* (TROCKENBRODT & LIESE 1991), *Picea sitchensis* (WOODWARD & PEARCE 1988), *Pinus sylvestris* (WAHLSTRÖM & JOHANSSON 1992), *Abies alba* (OVEN & TORELLI 1994), a larger number of tree species studied by BIGGS (1984), and also those of different plant organs (RITTINGER et al. 1987) all confirm this sequence of events.

However, the origin and location of the ligno-suberised layer and the necrophylactic periderm were specifically determined by the broad sclerified phloem rays in beech bark. We observed that the development of a ligno-suberised layer and the underlying necrophylactic periderm under the tapered ends of sclerified phloem rays was associated with the response of the vascular cambium. By intensive production of phloic and xylem derivatives of cambium the sclerified phloem ray was replaced from the indentation in the xylem ray. During this process, the depression in the xylem rays was filled with callus which later undergoes lignification. Recent phloic derivatives of the cambium under the tapered end of sclerified ray seem to be involved in the formation of a ligno-suberised layer as well as the differentiation of new phellogen, and hence the necrophylactic periderm. We hypothesise that the ligno-suberised layer and the necrophylactic periderm in European beech is generated from living cells extant at the time of wounding as well as from recent phloic derivatives of the vascular cambium. OSTROFSKY (1982) and OSTROFSKY & BLANCHARD (1982) observed similar ontogeny of necrophylactic periderm in the wounded and infected bark of American beech. Mullick (1977) reported that wounding of bark may trigger a cambial response even though the cambium is not directly wounded, but the reasons for this are unknown. It is likely that such a response is triggered by the decrease of bark pressure after wounding (cf. VRIES 1876, BROWN & SAX 1962, TORELLI et al. 1990).

The wide phelloderm of the necrophylactic periderm

in beech was the site of intense differentiation of cells into sclereids. It seems that the formation of a broad phelloderm of necrophylactic periderm plays a special role in the restoration of sclerified tissues.

The formation of a ligno-suberised layer and the necrophylactic periderm started in the nonconducting phloem in the basal region of the wound and proceeded from there in two directions: outward in the tissue under the original periderm and along the sclerified rays toward the cambium. Later response in the tissue under the original periderm may be the consequence of a lower vitality of the parenchyma cells (KUČERA 1971, BANGERTER 1984) and a larger area of sclerified tissues in outer, older parts of the bark (OVEN & TORELLI 1994). Quicker desiccation of older segments of the living bark is supposed to affect the depth at which a ligno-suberised layer forms as well (TROCKENBRODT 1994). Later formation of a necrophylactic periderm under the sclerified phloem ray seems to be associated with the onset of the response of the vascular cambium in spring.

Our observations demonstrate that neither phloem rays nor groups of sclereids lessen the possibility of formation of a continuous ligno-suberised layer and necrophylactic periderm in European beech. Regarding the necrophylactic periderm similar results were obtained on American beech (OSTROFSKY 1982, OSTROFSKY & BLANCHARD 1982). On the other hand, BIGGS (1986) observed discontinuities in new periderm in wounded and inoculated samples of peach cultivars at the site of primary phloem fibres.

The sequence of events in beech bark reveal that during the first two months the continuous layer of polyphenol deposits, lignified and suberised cell walls fulfill the temporary role of the periderm. After four months the ligno-suberised layer is no longer needed because a suberised phellem of the necrophylactic periderm differentiated between the ligno-suberised layer and the vascular cambium. This new periderm connects with the extant periderm to form once again continuous layer.

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