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DOI
10.1016/0166-5162(94)90002-7

Publication date
1994

Published in
International journal of coal geology

International Journal of Coal Geology, 25 (1994) 1–25
Elsevier Science B.V., Amsterdam

Anatomy of recent and peatified *Calluna vulgaris* stems: implications for coal maceral formation

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(Received September 9, 1992; revised version accepted June 23, 1993)

ABSTRACT

Anatomical characteristics of peatified *Calluna vulgaris* stems isolated from a selection of Northwest European raised bog peat deposits were compared with intact stems in order to reveal anatomical modifications caused by the peatification process. Four main decomposition processes were discerned: (1) loss of structural integrity of cell walls and cell inclusions; (2) gelification and swelling of cell walls; (3) discolouration of cell walls; and (4) deposition of matter in cell lumina.

Fibre-tracheids and wood parenchyma appear to be the most affected cell types. The two main decomposition trends discerned in these tissues were related to different fungal types. Precipitation of decay products was mainly observed in vessels. Bark tissues, primary xylem and medullary parenchyma were characterized by an excellent anatomical preservation. In the first stages of peatification a rapid decrease in cell contents was observed in bark tissues. Swelling and gelification was mainly seen in cell walls of the cambium and vessels.

We conclude that different types of cell walls respond differently to decay conditions. This is probably related to differences in the original cell wall chemistry. A high intra- and intercellular variability in degree of preservation was sometimes observed in identical tissues. The microscopic variability arising during peatification of wood and bark testifies to the heterogeneity of coal macerals derived from these tissues.

INTRODUCTION

Northwest European raised bog peat deposits are characterized by a complex mixture of partially preserved plant parts originating from different peat-forming plant species. Preservation and accumulation of plant remains in raised bog deposits is mainly caused by climatic conditions such as high rainfall and low temperatures. As a consequence, waterlogged organic deposits are a common feature, especially in shallow basins. These waterlogged deposits are poor in oxygen, which inhibits the growth of aerobic fungi and bacteria with the consequence that biodegradation of the incorporated plant remains is retarded (Ingram, 1983). Another factor which contributes to the preserving character of raised bog deposits is the high acidity of the groundwater (pH

0166-5162/94/$07.00
SSDI: 0166-5162(93)EO030-0

3–4), which is caused, at least in part, by several *Sphagnum* (Bog Moss) species, which adsorb calcium and magnesium and release protons into the water (Clymo and Hayward, 1982). Due to high acidity, microbial decay is further inhibited. In spite of these preserving conditions, anatomical and chemical modifications, due to microbial activity, have been observed in a selection of peatified raised bog plants (Van der Heijden et al., 1990, 1991). The modifications are caused by the aerobic microbial activities in the uppermost layer of the deposit and within aerobic microenvironments in the anaerobic peat and the activities of facultative anaerobic fungi and bacteria deeper in the deposit (Clymo and Hayward, 1982).

To date, little is known about the effects of the biodegradation process in raised bog peat deposits on the anatomy and chemistry of plant cell walls. The study of these early fossilisation processes is important in that it provides a better insight into the diagenetic pathways of individual plant cell walls and thus the formation of coal maceral precursors. Furthermore, recognizing bias in the fossil record will lead to an improvement in the reconstruction of past vegetations.

During the past few decades, microscopic techniques have become an important tool in peat and coal research. Although some earlier anatomical studies have been performed on northwest European peat deposits (Puffe and Grosse-Brauckmann, 1963; Grosse-Brauckmann and Puffe, 1964), most recent work has been done on the anatomical characterisation of subtropical peat deposits (Cohen and Spackman, 1972, 1977, 1980; Cohen, 1983; Cohen et al., 1989; Rollins et al., 1991), coal (Spackman, 1958; Stach et al., 1982; Taylor and Liu, 1987), isolated peatified and coalified wood (Barghoorn and Spackman, 1950; Spackman and Barghoorn, 1966; Ting, 1977; Russell, 1984; Stout, 1985; Stout and Spackman, 1987, 1989; Blanchette et al., 1991), fossil charcoal (Scott and Jones, 1991) and other peat-forming plant parts (Stewart and Follett, 1966; Cohen and Spackman, 1972, 1980; Cohen et al., 1987; Boon et al., 1989). Studies on the correlation between the anatomical and chemical characteristics of peatified plant tissues have been performed mostly on wood or complex peat (Barghoorn and Spackman, 1950; Philp et al., 1982; Cohen and Andrejko, 1984; Hedges et al., 1985; Stout, 1988; Hatcher et al., 1989; Stout et al., 1989; Van der Heijden et al., 1991; Obst et al., 1991; Rollins et al., 1991; Van der Heijden et al., in prep.). However, most of these studies deal with a mixture of taxonomic entities, with the consequence that interspecific variabilities in chemistry and anatomy have to be taken into account when interpreting the data. We tried to overcome this problem by studying the diagenesis of a single plant species [*Calluna vulgaris* (L.) Hull], a strategy which was also followed by Cohen and Spackman (1980). As a consequence, interspecific variabilities are ruled out.

Samples from different localities and depths provided insight into the variability induced by depositional environment and burial time. In order to trace

the heterogeneity of the decomposition processes within a single peat layer, multiple samples were investigated and compared with each other.

LOCATION, MATERIALS AND METHODS

Location and samples

Fresh *Calluna* stems were collected from plants growing in the neighbourhood of the Meerstalblok deposit. Holocene (MB) samples of highly decomposed *Calluna* stems were isolated from a dark *Sphagnum* peat (Schwarztorf) layer (240 cm below the surface) taken from the Meerstalblok peat deposit (52°41′N, 7°02′E), an area located in the central part of the Bargerveen. This is a natural bog reserve southeast of Emmen in the Drente Province, The Netherlands (Dupont, 1986). Additional samples of sub-recent *Calluna* stems were isolated at 20 and 40 cm depths from Irish raised bog surface cores from Clara Bog, Co. Offaly, Ireland (53°23′N, 7°37′E). More details about the samples are presented in Table 1. Additional botanical and paleobotanical research, including species identification, was performed by Van der Molen et al. (1988).

Anatomical procedures

Fresh and peatified samples were fixed in Craf III (Chromic-VI-acid:acetic acid:formaldehyde:aqua dest=30:20:10:40), subsequently dehydrated in an ethanol-n-butylalcohol series, embedded in glycol-methacrylate, sectioned at 5 μm with glass knives, stained with Schiff reagens (basic fuchsin in aqua dest and hydrochloric acid) and counterstained with aqueous methylene blue. Sudan IV and iodine in potassium-hydroxyde were used for the identification of lipids and starchs, respectively. In order to obtain smooth surfaces of soft peatified tissues for scanning electron microscopy, a methodology was used according to the idea of Kovach (1989). Fresh and peatified samples were

TABLE 1

List of investigated samples

Sample	Location	Depth (cm)	Age[1]	Number of stems investigated	Degree of oxidity of peat-layer
CB20 (sub-recent)	Clarabog Ireland	20	1927–1936 A.D.	1	+
CB40 (sub-recent)	Clarabog, Ireland	40	1772–1878 A.D.	3	–
MB (Holocene)	Meerstalblok, The Netherlands	240	5500–6000 B.P.	5	±

[1]Data according to Dupont (1986) and Van der Molen et al. (1988).

dehydrated in an alcohol series and subsequently embedded in Spurr resin using the procedure described by Spurr (1969). A scanning surface was obtained by sectioning the embedded tissue with glass knives and removing the polymerised resin from the cut surface with a solvent (2.5 g potassium-hydroxyde solubilised in 50 ml dehydrated ethanol and 50 ml methanol). In this way, a smooth scanning surface as well as light microscopic sections, corresponding to the SEM view, were obtained. After solubilisation of the Spurr resin, samples were critical point-dried with liquid carbon-dioxide, sputter coated for about three minutes with gold and subsequently studied on an ISI-DS130 scanning electron microscope.

ANATOMICAL DESCRIPTIONS

Peatification of bark tissues

The recent periderm (Plate I, 1), composed of a variable number of radially aligned cells, is divided into phellogen (cork cambium) and phellem (cork). The phellem cells, which gradually become larger externally, show

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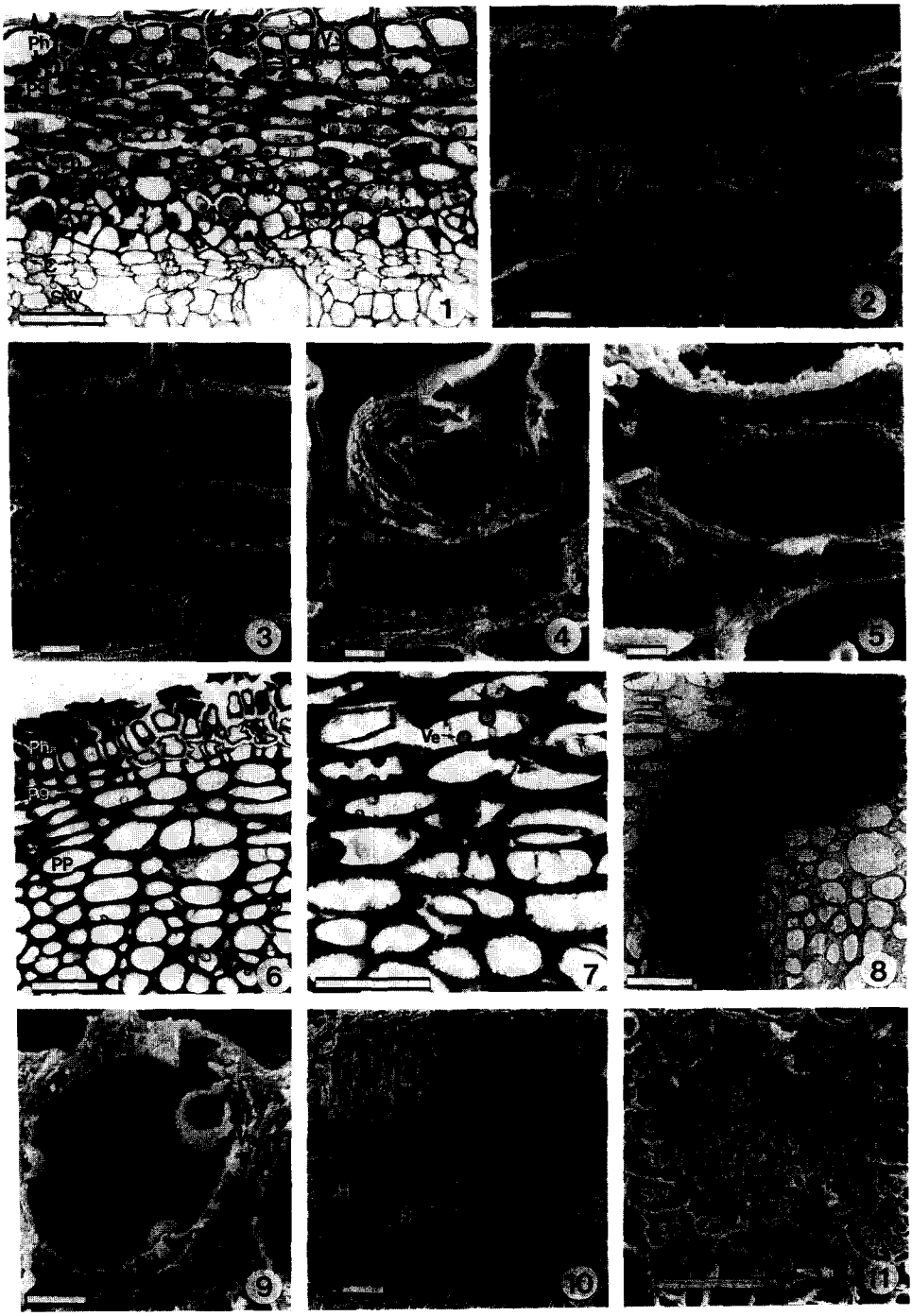
PLATE I

Light and scanning electron microscopic photographs of recent and peatified bark tissues and cambial zone (transverse sections). *C*=vascular cambium; *H*=fungal hyphae; *Pg*=phellogen; *Ph*=phellem; *PP*=phloem parenchyma; *PW*=primary wall; *SPh*=secondary phloem; *SW*=secondary wall; *SXy*=secondary xylem; *TI*=tanniniferous cell inclusion; *Ve*=vesicle. Scale bar=30 μm (1), 20 μm (6–8, 10, 11), 2.5 μm (2–5, 9).

1. Recent and mature bark, showing periderm, phloem parenchyma and vascular cambium.
2. Recent phellogen and inner phellem cells.
3. Recent phloem parenchyma cells showing fibrous cell walls. An internal layer lines the lumina(!).
4. Peatified phellogen and phellem (Holocene MB sample). The inner cell wall of the phellogen is swollen(!).
5. Peatified phloem parenchyma cells (Holocene MB sample), showing the preserved internal cell wall layer(!).
6. Peatified bark (Holocene MB sample). The outer phellem cells still contain conspicuous cell inclusions, while those of the inner phellem elements are gone.
7. Peatified phloem parenchyma (sub-recent CB40 sample), showing preserved and modified vesicles.
8. Peatified phloem parenchyma (sub-recent CB20 sample), showing empty cells and cells with perforated inclusions.
9. Peatified phloem parenchyma cell (Holocene MB sample), showing preserved vesicle membranes.
10. Peatified bark tissues (Holocene MB sample), showing severe fungal infection in the cell lumina of the phloem parenchyma.
11. Peatified vascular cambium (Holocene MB sample), showing conspicuous swollen cell walls.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conspicuous tanniniferous cell inclusions. The shape of these bodies is variable: spherical, ring, bean and vesicular sponge-shaped bodies occur frequently, while the outer phellem cells in particular are often completely filled with tannin blocks. The periderm cell wall architecture is complex and difficult to reveal using light microscopic (LM) and scanning electron microscopic (SEM) techniques. Usually, a highly suberized primary cell wall layer is discernible from a secondary layer. The latter appears rather complex, consisting of lamellae superimposed on the suberized layers (Plate I, 2). The secondary walls of the outer phellem elements are lined with tanniniferous material (Plate I, 1, 2) and therefore hardly visible by LM. According to Fahn (1982), this might be due to migration of tannin material from the lumina into the secondary wall. Under the phellem, a thin-walled, non-suberized, phellogen cell layer has formed (Plate I, 1, 2). The lumina of the cells within this meristematic layer usually contain globular, bean, sponge or irregular-shaped bodies. The tanniniferous contents of the periderm cells show a natural brownish colour, whereas the primary and secondary walls are unstained.

The anatomical characteristics of the peatified samples isolated from different depths are very similar. The cell walls of the outer phellem cells are intact and do not show any change in natural stain, while the lumina are still filled with cell inclusions (Plate I, 6). The cell walls of the inner phellem elements are still intact but sometimes appear slightly swollen. The natural stain has become brown during peatification. The cell contents have disappeared in most cells (Plate I, 6), although granules, filaments and ring, sponge, glossy or irregular-shaped bodies are sometimes preserved. The phellogen cells, which are thin walled in recent tissues, show a swollen internal cell wall (Plate I, 4). These cells are usually empty (Plate I, 6), although sporadically, irregularly shaped and glossy structureless bodies are observed.

Peatification of phloem

Internal to the periderm, a thick layer of primary and secondary phloem is distinguished, which consists mainly of phloem parenchyma (Plate I, 1). The external cell layers are, due to stem dilatation, composed of tangentially elongated cells with intercellular spaces filled with fibrous material (Plate I, 3). An internal cell wall layer lining the lumen is often quite distinct (Plate I, 3). Inwards, the phloem parenchyma become gradually less flattened, while the cell walls are much thinner. The cell contents consist of globular and bean-shaped bodies and vesicles which show a natural brown stain. The vesicles are sometimes fused, forming sponge-shaped structures. Sieve element fields, situated in particular near the cambium, are underdeveloped. They are discernible from the phloem parenchyma by their smaller size and the lack of cell inclusions. The cell walls of the phloem elements are unstained.

The cell walls of the peatified phloem parenchyma are still intact, but they

have been naturally stained brown and appear sometimes slightly swollen. The innermost cell wall layer is still distinctly delineated from the external cell wall layers (Plate I, 5). The former layer is sometimes covered with empty globular bodies, which may be remains of recent vesicle membranes or bacteria (Plate I, 9). Most parenchyma cells of the Holocene (MB) and subrecent stems (CB20) appear empty (Plate I, 6), although sometimes granular cell inclusions are observed. In the latter sample, sporadically areas are observed with cells containing a dark brown perforated precipitate (Plate I, 8). The precipitates are also found in the lumina of the adjacent xylem fibre-tracheids and ray parenchyma, although no such inclusions are seen in the adjacent periderm cells. In the CB40 samples, the lumina contain numerous cell inclusions such as brown-stained granules and vesicles, which often line the cell walls (Plate I, 7). Fungal hyphae, seen in cross section as small ring-shaped bodies, are observed in all samples in variable amounts (Plate I, 10). No direct cell wall penetration by fungal hyphae is observed.

Peatification of the cambial zone

The recent cambial zone is often composed of several layers of radially flattened thin-walled cells without any visible cell contents (Plate I, 1).

The peatified cell walls of the cambial zone are preserved but appear significantly swollen and are therefore hardly distinguishable from the external phloem parenchyma cells (Plate I, 11). The walls have been naturally stained brown during peatification.

Peatification of the secondary xylem

The recent semi ring-porous wood is in the form of a continuous cylinder, including vessel elements, fibre-tracheids and uniseriate medullary ray cells (Plate III, 1; Metcalfe and Chalk, 1950).

The cell wall patterns of the peatified wood elements are mostly well preserved (Plate II, 8; Plate III, 3, 8) and no significant compression has occurred. Changes in local cell wall architecture have occurred in all tissues. Sometimes, tissues are lost leaving large empty spaces (Plate III, 9). Because of the fact that these empty areas usually contain specific cell wall fragments (e.g. only pit remainders, Plate III, 9), they are probably not an artifact of preparation. The decomposition characteristics such as described below are remarkably similar among the samples derived from the same peat layer.

Vessel comparison

Observed in cross section, the recent vessels are rather small with only slightly secondary thickened cell walls (Plate III, 21). In longitudinal section, the bordered pits and scalariform perforation plates, are the most conspicu-

ous structures (Plate II, 1). No tyloses are developed, which agrees with the observations of Metcalfe and Chalk (1950).

The peatified vessels show a wide range of different anatomical modifications. Concerning the cell wall architecture, five different stages are discerned which, in Fig. 2c, are arranged in two different decomposition pathways:

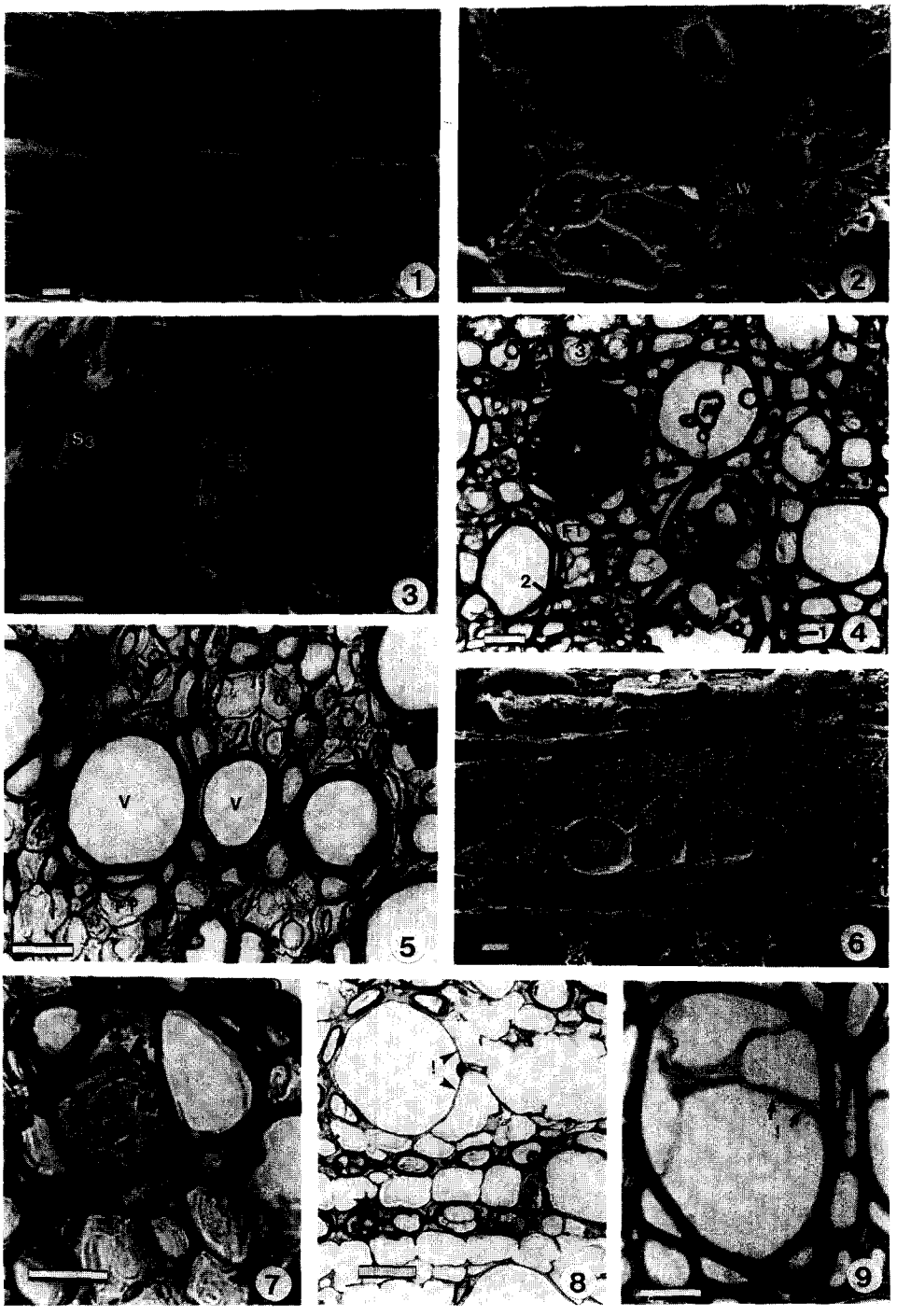
(1) CML (middle lamella and primary wall) and secondary wall are intact and more or less discernible with LM and SEM techniques (Plate II, 2, 4). In the CB20 sample, the non modified cell walls appear unstained while in the CB40 and Holocene (MB) samples the cell walls appear brownish.

(2) (Fig. 2c, A1). CML and secondary wall are discernible by SEM, although the secondary wall is swollen (Plate II, 3, 4). The swellings are sometimes quite local and may be related to fungal hyphae nearby (Plate II, 3).

PLATE II

Light and scanning electron microscopic photographs of recent and peatified secondary xylem elements. *BP*=bordered pit; *CML*=compound middle lamella; *FT*=fibre-tracheid; *H*=fungal hyphae; *SP*=scalariform perforation plate; *SW*=secondary wall; *TV*=tylose vessel; *V*=vessel; *WP*=wood parenchyma. Scale bar=10 μm (4, 5, 7–9), 5 μm (1–3, 6).

1. Longitudinal section of a recent vessel, showing a scalariform perforation plate and bordered pits.
2. Transverse section of a peatified vessel and fibre-tracheid (Holocene MB sample). The secondary wall of the vessel is preserved, whereas the secondary wall of the fibre-tracheid is highly degraded; the S_3 layer, CML and the bordered pits are preserved.
3. Transverse section of a peatified vessel, fibre-tracheids and wood parenchyma cells (Holocene MB sample). The secondary cell wall of the vessel is locally swollen. The CML and S_3 layer of both the fibre-tracheids and the wood parenchyma cells are preserved whereas the S_1 and S_2 layers are vanished.
4. Transverse section of secondary xylem (sub-recent CB40 sample), showing vessels with preserved and swollen secondary cell walls. Some vessels show ring-shaped protrusions, which are continuous with the modified cell wall. Preserved vessels with granular precipitates are also common. The numbers refer to different stages of decomposition such as observed in the fibre tracheids: *1*, secondary cell wall and CML preserved; *2*, S_1/S_2 layer with irregular-shaped cavities; *3*, CML and S_3 preserved. The S_1/S_2 layer has vanished for the larger part (see also text).
5. Transverse section of peatified secondary xylem (Holocene MB sample). The primary and secondary wall of the vessels are fused and appear significantly swollen.
6. Longitudinal section of a peatified vessel (Holocene MB sample), showing tylose vessels surrounded by granular precipitates.
7. Transverse section of a highly degraded vessel (Holocene MB sample). The cell wall system is vanished and the decomposition products appear as a glossy gel in the cell lumen.
8. Transverse section of the secondary xylem (sub-recent CB20 sample), showing highly degraded vessels in which only the CML is preserved(!).
9. Transverse section of a peatified vessel (sub-recent CB40 sample), showing long filaments which are continuous with the secondary wall(!).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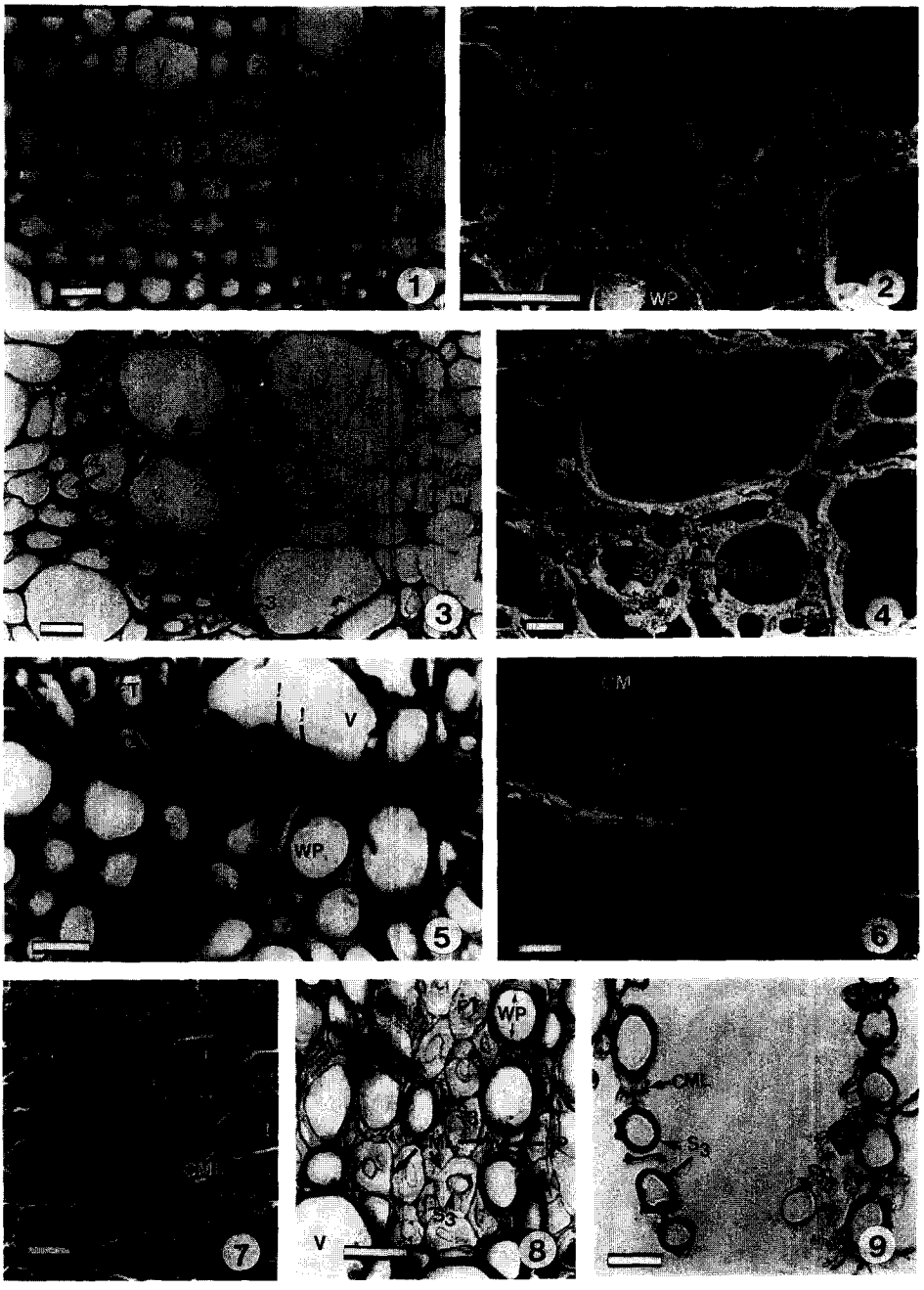
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CM
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P1
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S3
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8
CML
S3
9

The swellings have been naturally gold-brown stained, while, in case of the sub-recent sample (CB20) the non-altered remainder of the cell wall is unstained.

(3) (Fig. 2c, A2). The secondary wall is fused with the CML and therefore hardly discernible separately using LM and SEM. The fused cell walls are often significantly swollen (Plate II, 5). This stage is only present in the Holocene (MB) samples.

(4) (Fig. 2c, A3). The secondary cell wall and CML have disappeared or have partially disappeared. Cell wall derived material is spread out in the lumen and is observed as granular or glossy precipitates (Plate II, 7). Sporadically, the vessels have not maintained their original shape but are somewhat collapsed. This stage is only present in the Holocene (MB) samples.

(5) (Fig. 2c, B1–B2). Highly degraded vessels, in which only parts of the CML are preserved, while the secondary cell wall has gone. The vessels are usually surrounded by highly decomposed fibre-tracheids (Plate II, 8). This

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PLATE III

Light and scanning electron microscopic photographs of recent and peatified secondary xylem elements. *Bp*=bordered pit; *CML*=compound middle lamella; *FT*=fibre-tracheid; *Pl*=plastid; *SW*=secondary wall; *V*=vessel; *WP*=wood parenchyma. Scale bar=2.5 μm (4, 6), 5 μm (2, 7), 10 μm (1, 3, 5, 8, 9).

1. Transverse section of recent secondary xylem, showing vessels, fibre-tracheids and wood parenchyma.
2. Transverse section of recent fibre-tracheids and wood parenchyma, showing different cell wall layers.
3. Transverse section of secondary xylem (sub-recent CB20 sample), showing fibre-tracheids in different stages of decomposition (see text): *1*, CML and secondary wall are preserved; *2*, CML and S_3 layers are preserved, whereas the S_1/S_2 layer shows irregular-shaped cavities; *3*, the CML and S_3 layer is preserved whereas the S_1/S_2 layer has vanished; *4*, the secondary cell wall is gradually thinned, beginning at the lumen and progressing outward to the middle lamella. Most vessels are well preserved.
4. Transverse section of peatified fibre-tracheids (Holocene sample), showing circular cavities in the S_1/S_2 layers of the secondary wall(!).
5. Transverse section of the border between spring- and summerwood (sub-recent CB20 sample). Some fibre-tracheid, wood parenchyma and vessel elements are significantly flattened and deformed(!). The dark-stained mass probably originates from gelified xylary elements.
6. Longitudinal section of a peatified fibre-tracheid (Holocene sample), showing a spirally oriented fungal hyphae superimposed on the S_3 layer(!).
7. Transverse section of peatified fibre-tracheids (Holocene MB sample). The cell lumina are filled with perforated granular precipitates(!).
8. Peatified secondary xylem (Holocene MB sample). In both fibre-tracheids and wood parenchyma, the combined S_1/S_2 layer has vanished, whereas the compound middle lamella and S_3 layer is preserved. The cell lumen of the wood parenchyma is empty.
9. Wood parenchyma elements (sub-recent CB40 sample), showing a preservation of S_3 layers, which are heavily swollen and dark stained. Fibre-tracheids and vessels are degraded, although parts of the CML and bordered pits are preserved.

stage is only present in the CB20 sample, but is also sporadically seen in the CB40 samples.

The modified and preserved vessels are empty or contain filaments, tyloses, dense granular material, or glossy structureless or ring-shaped bodies (Plate II, 4) or fungal hyphae (Plate II, 2, 3). In Plate II, 4 (CB40), occlusions are visible in non degraded vessels, pointing to migration of decomposition products. In spite of the fact that tyloses are not present in recent vessels they are sometimes observed in the Holocene (MB) and CB40 samples (Plate II, 6). The tylose vesicles are empty and usually surrounded by granular unstained debris. A remarkable characteristic, which is especially found in the vessels of the CB40 samples, are long filaments which appear to originate from the secondary cell wall (Plate II, 9). They are not related with tyloses. In the latter samples conspicuous ring-shaped inclusions, sometimes connected with the swollen secondary cell wall and protruding into the lumen, are also frequently observed (Plate II, 4). These structures are probably related with fungal hyphae.

Fibre-tracheid comparison

Between the uniseriate rays, 2–8 layers of fibre-tracheids are distinguishable (Plate III, 1). The fibre-tracheids are more or less square-shaped in cross section, with a diameter ranging from 6–10 μm in summerwood to 9–16 μm in springwood. The cell walls show a distinct division into a compound middle lamella, combined S_1/S_2, and an inner S_3 layer (Plate III, 2). Bordered pits between single fibre-tracheids are conspicuous but variable in number. The secondary xylem elements found directly beneath the cambium show less developed secondary cell wall thickenings (Plate I, 1) because of the fact that they are less mature.

The state of preservation of the fibre-tracheids in the peatified wood samples is rather complex and highly variable. The following stages are distinguished which in Fig. 2a are arranged in two different decomposition pathways:

(1) The fibre-tracheids show an excellent preservation of CML and secondary cell walls (Plate II, 4; Plate III, 3). This stage is seen in the sub-recent (CB20 and CB40) but not in the Holocene (MB) samples. In the CB20 sample the preserved fibre-tracheids occur in the central parts of the stem, whereas in the CB40 samples, preservation of fibre-tracheids is seen in the younger wood. The preserved secondary cell walls are unstained (CB20) or are brown (CB40), while in the latter sample the pit area between the single elements show a much darker stain.

(2) (Fig. 2a, B1–B3). The degraded fibre tracheids show a gradual thinning of the secondary cell wall, beginning at the lumen and progressing outward to the middle lamella (Plate III, 3). As a result, most degraded fibre-tracheids only show a preservation of CML. Sometimes, only the cell corners

are preserved. This decomposition pathway is very prominent in the CB20 sample, although the B2 stage is also sporadically seen in the Holocene (MB) wood. It is remarkable that the cell wall remains of even the most decomposed fibre-tracheids of the CB20 wood are unstained.

(3) (Fig. 2a, A1). CML, S_3 and the combined S_1/S_2 layer are present. The S_1/S_2 layers are affected by fungal hyphae, which penetrate the S_1/S_2 layer in longitudinal direction, thus creating circular cavities (Plate II, 4; Plate III, 3, 4). In longitudinal section, the hyphae are spirally oriented and usually superimposed on the S_3 layer (Plate III, 6). This decomposition stage is prominent in the sub-recent (CB40) but rare in the Holocene (MB) and the CB20 stems.

(4) (Fig. 2a, A2) Both the S_1 and S_2 have disappeared, whereas the CML and the S_3 layer are preserved (Plate II, 2–4; Plate III, 3, 8). A remarkable feature observed in the middle lamella (CML) region is the occurrence of highly resistant lens-shaped bodies, which are the remains of bordered pits. After decomposition of the combined S_1/S_2 layer, the shape of the bordered pit is preserved, due to the preservation of the S_3 layer, which covers both pit-canal and cavity (Plate II, 2). The S_3 layer appears pale and does not react with histochemical stains. This decomposition stage is the most common observed in the CB40 and the Holocene (MB) samples, but it appears rare in the CB20 wood. Sometimes, the cell lumen is filled with a granular precipitate, showing conspicuous channels (Plate III, 7). These kinds of peatified fibre-tracheids are often found in groups, but their occurrence is rare and they have been only found in the Holocene (MB) samples.

(5) The CML, S_1, S_2 and S_3 layers have disappeared, whereas the lens-shaped pits are preserved (Plate III, 9). This is sporadically seen in the sub-recent (CB40) and the Holocene (MB) samples.

The decomposition stages described above are observed in close proximity of each other. In all samples no differences in degree of decomposition is observed between spring and summerwood elements, although in the latest developed springwood elements of the sub-recent CB20 sample severe modifications have occurred (Plate III, 5); the fibre-tracheids have lost their structure and cell wall material is gelified and swollen. This results in a homogeneous fibrous mass, which has been naturally stained brown. The adjacent fibre-tracheids and vessels are sometimes compressed, although their cell wall structure is intact.

Wood parenchyma comparison

The recent uniseriate medullary rays are numerous and consist, observed in cross section, of somewhat radially elongated cells with secondary cell wall thickenings which are more conspicuous in the tangential walls (Plate III, 1, 2). The cell wall is divided into a CML (compound middle lamella: middle lamella and primary wall), compound S_1/S_2 layer and an inner S_3 layer (Plate

III, 2). The latter layer is difficult to distinguish using LM and SEM techniques. The wood parenchyma of especially the younger twigs often contain chloroplasts which are globular or bean-shaped.

The secondary cell walls of the peatified sub-recent samples are usually well preserved, while no such preservation is seen in the Holocene (MB) samples. The following decomposition stages are discerned, which in Fig. 2b are arranged in two different decomposition pathways:

(1) (Fig. 2b, B1–B2). In case of the CB20 sample, most degraded wood parenchyma elements show a gradual thinning of the secondary cell wall, beginning at the lumen and progressing outward to the middle lamella (Plate III, 3).

(2) (Fig. 2b, A1–A2). The decomposed wood parenchyma elements from the sub-recent (CB40) and Holocene (MB) samples show a preservation of CML and S_3 layer. The latter is usually somewhat swollen and dark brown-stained. The secondary cell wall (S_1/S_2) has disappeared (Plate II, 3; Plate III, 8). Usually the pit canal, which is continuous with the S_3 layer, is preserved and can be seen as a filament connecting adjacent parenchyma elements (Plate III, 8).

(3) (Fig. 2b, A3). The compound middle lamella and secondary walls (CML, S_1, S_2) are degraded, while the swollen S_3 layer is preserved (Plate III, 9). This stage is most commonly found in the CB40 sample.

In all samples studied, the chloroplasts are decomposed for the most part and the cells appear empty (Plate III, 8, 9). Cell inclusions are sometimes observed (Plate III, 9), although, the relationship with the native chloroplasts is obscure.

Peatification of primary xylem and medullary parenchyma

The recent primary xylem (Plate IV, 1) consists of irregularly oriented vessels with spiral- or net-shaped secondary cell walls (Plate IV, 3), wood parenchyma, and primary fibre-tracheids all with secondary cell walls. The recent medullary parenchyma (Plate IV, 1), found directly below the primary xylem, consist of large isodiametric thin-walled cells.

The cell walls of the peatified primary xylem elements, such as primary vessels, parenchyma and fibre-tracheids, are well preserved (Plate IV, 2). Usually, the spirally thickened secondary wall is preserved also (Plate IV, 33). It is sometimes difficult with LM techniques to distinguish the CML from the secondary walls due to gelification and fusion of both layers. Highly gelified elements are sometimes observed, especially in the Holocene (MB) samples. The decomposed cell walls appear then as single, structureless bodies filling up the entire space that was occupied by the former primary xylem elements (Plate IV, 2). The natural stain of the cell walls is brown in case of

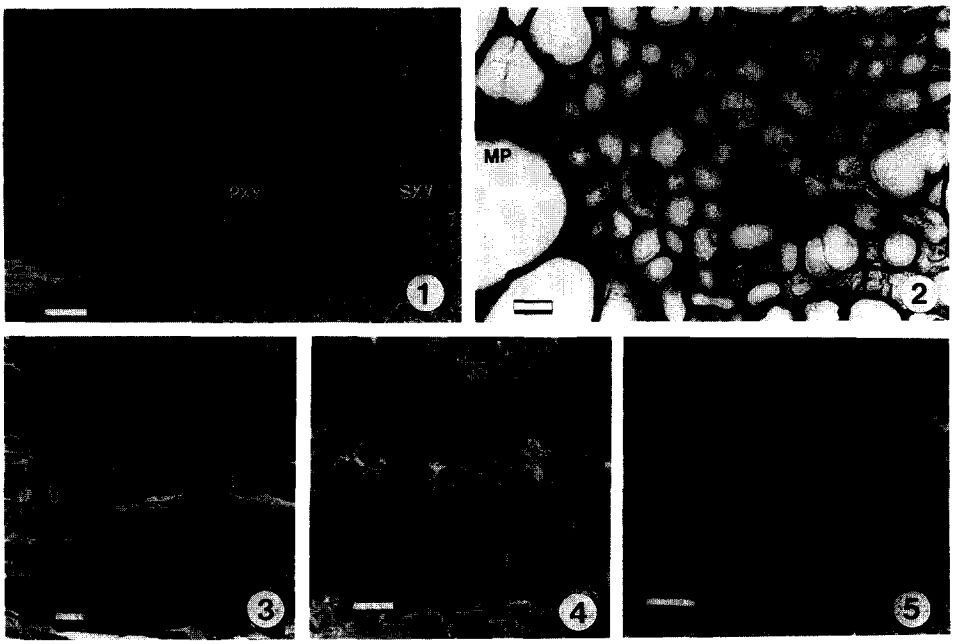

PLATE IV

Light and scanning electron microscopic photographs of recent and peatified primary xylem and medullary parenchyma. *H*=fungal hyphae; *MP*=medullary parenchyma; *PXy*=primary xylem; *SXy*=secondary xylem; *V*=vessel. Scale bar=20 μm (1, 5), 10 μm (2), 5 μm (3), 2.5 μm (4).

1. Transverse section of recent secondary xylem, primary xylem and medullary parenchyma.
2. Transverse section of peatified primary and secondary xylem (Holocene MB sample). The cell walls of the primary xylem elements are preserved. Some elements are gelified and fused, appearing as a glossy gel(!).
3. Longitudinal section of recent primary xylem, showing a vessel element with spiral secondary cell wall thickening(!).
4. Longitudinal section of peatified primary xylem (Holocene MB sample), showing a vessel with preserved spiral secondary wall thickenings(!).
5. Longitudinal section of peatified medullary parenchyma (Holocene MB sample), showing preserved cells infected by fungal hyphae.

the Holocene (MB) and CB40 samples, while the cell walls of the CB20 sample are unstained.

The medullary parenchyma cells exhibit an excellent preservation (Plate IV, 5). Although the cell walls have been stained brown in the Holocene (MB) and sub-recent (CB40) samples, no significant changes in cell wall architecture is observed. Sometimes fungal hyphae are observed in the lumina (Plate IV, 5).

DISCUSSION

Because of the high variability in degree of preservation between cell walls of identical tissues, it is possible to arrange the stages of decomposition in directions of transformation. The different degradation pathways of both bark and xylary tissues are presented schematically in Figs. 1 and 2. No significant differences in decomposition characteristics were observed among the samples isolated from the same depth.

Bark and cambial zone (Fig. 1)

The modifications in the bark tissues due to peatification are schematically presented in Fig. 1 in which A and B represent recent and peatified bark, respectively. From this schematic representation we can deduce that swelling of cell walls and modifications of cell inclusions are the main biodegradation characteristics. Concerning the cell inclusions, two types of decomposition trends are observed. The first type involves the complete removal of the cell contents, although, sometimes, a preservation of vesicle-membranes of plastids is observed. In the second type, which appears more prominent in the CB40 sample, the cell contents are fragmented or highly deformed. The removal of cell contents is especially evident in phloem parenchyma elements and is already observed in the shallowest sample (CB20). This implies that the contents are highly sensitive to biodegradation and/or highly water soluble. The fact that the vesicle membranes are sometimes retained suggests that

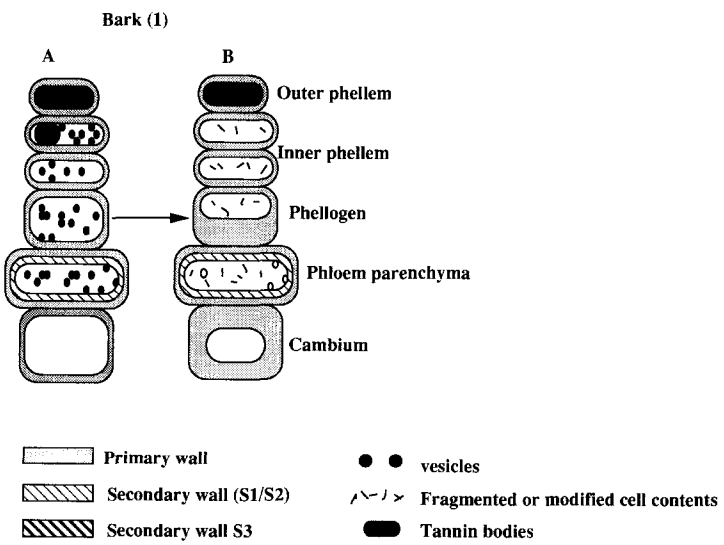

Fig. 1. Schematic representation of recent (*A*) and peatified bark (*B*)

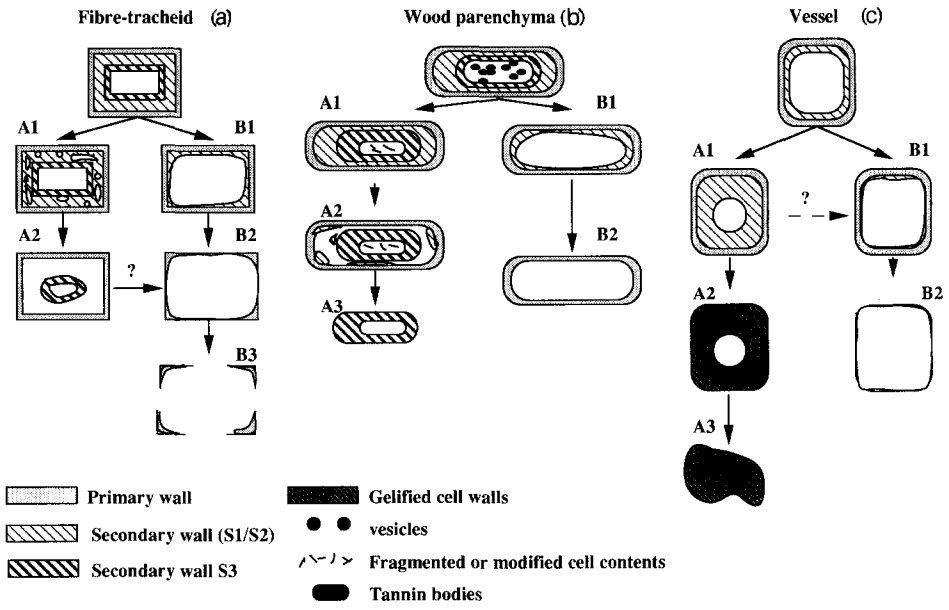

Fig. 2. Schematic representation of the biodegradation pathways such as observed in fibre-tracheids (a), wood parenchyma (b) and vessels (c). *A* and *B* represent alternative directions of decomposition (see results and discussion section).

these membranes contain highly resistant chemical constituents, or points to a kind of auto fixation by naturally occurring plant phenolics before fossilization (Niklas et al., 1985). The discrepancy in the preservation of cell contents between inner phellem and outer phellem is remarkable, because both tissues are ontogenetically related. A possible explanation for this phenomenon might be that the outer phellem cells are protected by thick suberized cell walls, thus preventing microorganisms from penetrating the cell lumen. The relatively minor presence of suberin in the inner phellem and phellogen reduces the protective potential and might explain the quick removal of the cell contents from these tissues.

An important degradation characteristic, not shown in the scheme, is discolouration of cell walls. Many authors have observed discolouration in cell walls submitted to peatification and early coalification (e.g. Stout et al., 1989; Stout and Spackman, 1989). The chemical changes involved are not clear. The process might be caused by the production of melanoidin or humic acid-like materials within the cell walls, by oxidation of phenolic compounds (Stout et al., 1989; Stout and Spackman, 1989) or by the removal of white cellulose and the retention of brown lignin (Stout et al., 1989). Other possibilities, such as adsorption of cytosolic flavonoid pigments on the cell walls, must be born in mind (Spackman and Barghoorn, 1966). The occurrence of brown

precipitates in both phloem parenchyma and adjacent wood cells, such as observed in the CB20 sample, points to the presence of water soluble compounds migrating around in the wood and bark. The absence of these brown precipitates in adjacent periderm cells points to the fact that suberized cell walls act as an excellent barrier against these migrating compounds.

Conspicuous swelling was especially observed in the cell walls of the cambium. It is not clear yet why these thin-walled cells are preserved, although we believe that their chemical composition is responsible for this phenomenon. According to Thornber and Northcote (1961), the amount of pectic polysaccharides is much higher in the cambial zone than in the xylem, while the amount of lignin is much lower. Pectin might therefore play an important role in the resistance of the cambium cell walls against biodegradation. Preservation of thin-walled parenchymatous cells were also observed in coalified xylem parenchyma studied by Spackman and Barghoorn (1966), although they did not observe swollen cell walls.

Vessels (Fig. 2c)

Two types of biodegradation pathways are observed in the vessels. The first type (A) involves swelling and gelification of cell walls, while the second type (B) involves gradual thinning of the cell wall. Although most prominent in the vessels, gelification and swelling is also observed in the phellogen, the cambium and the S_3 layers of wood parenchyma and fibre-tracheids. Gelification involves the formation of a homogenized gel from cell wall material. Usually the cell wall passes through a plastic phase and swells to varying degrees (Stout, 1985; Stout and Spackman, 1987). The chemical or ultrastructural background of gelification is still unknown, although a loss of cellulose crystallinity may play an important role. It is not clear whether gelification is triggered by specific chemical properties of the cell walls or is caused by specific biological, chemical or physical environmental conditions. The tissue specificity of this process points to a chemical background, whereas the rare occurrence of gelification in early springwood fibre-tracheids, such as observed in the CB20 sample (Plate III, 5), points to a random process invoked by specific environmental conditions. The fusion of primary and secondary cell walls was also observed by Scott and Jones (1991) in charred fossil wood cell walls.

An important feature observed in the vessels is the occurrence of structured and non-structured cell inclusions, which are thought to originate from decomposed native cell walls and cell inclusions. Interestingly, we observe that even though fibre-tracheids were often much more degraded than vessels, precipitates are more frequently found in vessels than in fibre-tracheids. These observations are in contrast with those of Stout and Spackman (1987) who observed, in some of their wood samples, an opposite pattern. Probably fibre-

tracheid derived material is easily solubilised and does not accumulate, whereas material generated from the vessels is less soluble and accumulates much more easily. On the other hand, migration of tracheid derived material into the vessels must be kept in mind. Due to the fact that the distal ends of the vessels are open, migration of decomposition products is probably much easier in vessel elements than in fibre-tracheids. This is confirmed by the observation of precipitates in non-degraded vessels, which supports the idea that migration of decomposition products through the conducting system of the xylem is a common feature. Migration of decomposition products in vessels is also mentioned by Cohen et al. (1987). The origin of tyloses in the degraded vessels is still obscure as it does not occur in recent xylem. We postulate that the generation of these structures starts before peat incorporation, and that their formation is induced by fungal infection or mechanical injury (Fahn, 1982). After incorporation in the peat, the tylose structures are well preserved. The chemistry of these structures is still unknown, although we suspect that it chemically resembles the CML, because tyloses are usually generated from the primary walls of wood parenchyma (Fahn, 1982).

Fibre-tracheids and wood parenchyma (Fig. 2a, b)

Two types of degradation pathways are distinguished in both peatified fibre-tracheids and wood parenchyma. The main decomposition pathway (Fig. 2a, A and 2b, A) is a selective removal of S_1 and S_2 layers and a preservation of S_3 layers and compound middle lamella. It is a dominant decomposition process in the Holocene (MB) and deeper sub-recent (CB40) samples. The second degradation pathway (Fig. 2a, B and 2b, B), which is more prominent in the fibre-tracheids and wood parenchyma of the shallower CB20 sample, involves a gradual thinning of the secondary cell wall, beginning at the lumen and progressing outward to the middle lamella.

The loss of structural integrity, including removal of cell wall material and inclusions, is commonly observed in tissues submitted to biodegradation (Hedges et al., 1985; Stout, 1985; Stout and Spackman, 1987; Hoffmann and Jones, 1990). According to our observations in *Calluna*, the decomposition pathways seen in fibre-tracheids and wood parenchyma are mainly governed by fungal attack in the early aerobic stages of peatification, as the different morphological patterns observed tend to correlate closely with taxonomic groupings of wood inhabiting fungi. The first biodegradation pathway (A) is probably caused by soft rot fungi, which create helically oriented cavities in the S_2 layer, while the second type (B) is usually attributed to white rot fungi, which remove the cell walls completely, leaving the middle lamella unmodified (Wilcox, 1970; Blanchette et al., 1990). A similar decomposition pathway was observed by Cohen and Spackman (1980) in sclereid cell walls of

Nymphaea from Mangrove peat deposits. This suggests that identical types of microorganisms act in different types of peat deposits.

Many fibre-tracheids of the sub-recent wood samples show unaffected secondary cell walls, which points to a better xylem preservation compared with the Holocene (MB) samples. A high variability in the state of preservation of the fibre-tracheids was observed in all samples investigated, which points to a large variation in microbial activity in the xylary tissues. The preference of microorganisms for different cell types has been observed by Stout (1985) and Spackman and Barghoorn (1966) and is usually attributed to differences in cell wall chemistry. This also applies to the variability in decomposition characteristics within the cell wall system. An extensive knowledge of the chemical architecture of different cell wall entities and cell tissues is therefore necessary in order to explain decomposition characteristics such as described in this paper. In case of the fibre-tracheids, it is known that the middle lamella, as well as the primary wall, mainly consists of pectic polysaccharides (rhamnogalacturonan), cellulose and xyloglucan (Meier, 1985; Bacic et al., 1988; Fry, 1988; Northcote et al., 1989). According to Fukushima (1990), the lignin incorporated in the primary wall is very condensed. The secondary wall mainly consists of cellulose and xylans (Fry, 1988; Northcote et al., 1989), while the lignin units are less condensed (Fukushima, 1990). Although little is known about the susceptibility of different types of polysaccharides and lignins to biodegradation, it is often assumed that xylans are more easily degraded than cellulose and pectin (Hedges et al., 1985; Hedges, 1990). The higher concentration of xylans in the S_1/S_2 layers of the fibre-tracheids might therefore be an explanation for the fact that these cell wall layers are first removed during peatification. Furthermore, the presence of highly condensed lignin may play an important role in the protection of the compound middle lamella from microbial attack. The preservation of S_3 layers is peculiar, because chemically it appears to be not very different from the S_1 and S_2 layers of the secondary wall (Meier, 1985; Fukushima, 1990). Perhaps the S_3 layer, being adjacent to the lumen, receives a coating of enzyme inhibiting material carried in the water flowing through these tissues.

No relationship between state of preservation and age of wood elements has been observed. This is especially evident in the sub-recent wood samples. Some stems were characterized by well-preserved young fibre-tracheids and highly degraded old fibre-tracheids, whereas other samples showed an opposite pattern. This implies that differences in chemistry between old and young wood do not play an important role in response to microbial attack. Stout and Spackman (1989) observed a variability in preservation between summer and springwood elements, which they attributed to a variability in density of cell wall polymers. We did not observe such a variability, which implies that the decomposition process is not governed by chemical nor physical differences between both wood types.

Another important degradation characteristic, not depicted in the scheme, involves discolouration of residual cell walls. We have observed a significant inter- and intracellular variability in discolouration. In the CB20 sample we saw discolouration in bark tissues, but not in fibre-tracheids, not even in highly degraded ones. The latter observation points to the fact that discolouration is not related to microbial activities. The sub-recent (CB40) fibre-tracheids showed a dark brown pit area and light-brown secondary walls. This variability supports the idea that discolouration is closely related to chemical differences between various cell wall entities. We support the proposal of Stout et al. (1989) that duration of peatification is correlated with the increase of intrinsic colouration, because slightly decomposed fibre-tracheids (CB20) do not show any change in stain, whereas older elements, such as those observed in the Holocene (MB) samples, display a significant darkening.

Primary xylem and medullary parenchyma

We have observed an excellent preservation of the secondary cell walls of primary xylem and medullary parenchyma. A chemical preservation of central oriented tissues is also observed by Bates et al. (1991), who investigated a peatified log. They attributed this to a result of a process of microbial degradation during peatification that began at the surface of the log and progressed inward over a period of time. Because differences in the state of preservation between inner and outer tissues coincided in our samples with the boundary between primary and secondary xylem, we believe that the discrepancy in the state of preservation between primary and secondary xylem is caused by differences in chemical composition between both tissues. The chemical differences are still obscure, but are the aim of further research (Van der Heijden et al., in prep.).

MAIN CONCLUSIONS

— The decomposition characteristics among stem samples from the same peat layer are similar, which suggests that the different decomposition processes act in a wide horizontal range.
— Cell outlines of most tissues are excellently preserved, which points to the fact that significant compression has not occurred. The cell walls show signs of degradation in the very first stages of burial.
— There is a close relationship between the types of decay observed in the Dutch samples and those observed in Irish deposits.
— No differences in degree of decomposition are observed between summer- and spring-wood elements.
— In general, the decomposition processes in the *Calluna* stem wood lead to

loss of integrity of cell walls and cell inclusions, gelification and swelling of cell walls, discolouration of cell walls and deposition of structured and structureless matter in some cell lumina.
— The specific location of cell wall attack is presumably related to the chemical composition of the different cell wall layers and the dominant type of microorganism.
— The degree of preservation exhibited by different elements of the same tissue type is highly variable. This points to a high variability in catabolic activities of microorganisms within short distances in the peatified tissue.
— Discolouration is variable between cell wall entities and is probably related to chemical, physical or ultrastructural differences between these entities.
— An increase of intrinsic discolouration is related to duration of peatification.
— Within the periderm, a variability in preservation of cell contents is observed. Differences in degree of suberization may play an important role in preservation of cell contents.
— The cell walls of periderm and phloem parenchyma are anatomically preserved, although fungi are observed in the lumina. The main decomposition characteristics are discolouration and removal of cell contents. The decomposition of cell contents starts in the very first stages of burial.
— The cambial zone is well preserved. The fact that the cell walls are swollen points to a kind of chemical or physical modification.
— Two different decomposition pathways are discerned in the fibre-tracheids and wood parenchyma cells. The modifications in cell wall architecture are the result of the activity of different types of fungi.
— The vessels show two different decomposition pathways although the characteristics are different from those observed in fibre-tracheids and wood parenchyma. Swelling of cell walls and precipitation of matter in the cell lumen are most prominent in this tissue.
— Primary xylem and medullary parenchyma elements are excellently preserved in contrast with secondary xylem elements. This might be due to a variability in chemistry between both wood types.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Dutch Earth Science Foundation (AWON) for the financial support of one of the authors (Edwin van der Heijden). This work is part of the research program of Fundamental Research on Matter (FOM), with financial support from the Dutch Organisation for Scientific Research (NWO). We thank Mr. J. Dahmen (University of Amsterdam) for technical assistance and P.C. van der Molen (University of Amsterdam) for collecting some of the peat samples.

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