

DEVELOPMENT OF INCLUDED PHLOEM IN THE STEM OF *COMBRETUM NIGRICANS* (COMBRETACEAE)

by

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SUMMARY

The development of diffuse included phloem strands in *Combretum nigricans* stems is described. During a short period of time, a small phloem strand is cut off locally in an inward direction by an otherwise normal bidirectional vascular cambium. This contrasts with previous descriptions and interpretations because these strands are not formed after redifferentiation of secondary xylem parenchyma. A complementary cambium formed at the inner border of the young strand somewhat enlarges the strand and, during a relatively long period, produces secondary phloem outwards. Finally this complementary cambium stops functioning as a cambium and merges with the secondary phloem it has produced. Radial rows of cells are present within the included phloem strands which continue into the later-formed secondary xylem; rays transverse the strands. Crushing of the phloem takes place near the outer border of the strand, forming cap-like tissues of disorganized cells.

The strands form a three-dimensional secondary phloem network. Because sieve tubes are rare in the external secondary phloem, and internal phloem at the periphery of the pith only temporarily functions, the functional assimilate transporting system of older *C. nigricans* stems consists of this network of included phloem.

Key words: *Combretum*, anomalous secondary growth, diffuse included phloem, phloem network.

INTRODUCTION

Scattered, isolated strands of secondary phloem embedded in secondary xylem should be named diffuse included phloem and not interxylary phloem, or included phloem of the foraminated or island type (IAWA Committee 1989). The definition of diffuse included phloem is based on the appearance of the wood and does not have developmental inferences. Only where it is clearly demonstrated that the secondary phloem has been produced inwards by a single vascular cambium, can or should the term interxylary be used instead of diffuse included phloem (Mikesell & Popham 1976; Carlquist 1988). Bonnemain (1969) and Philipson (1990) distinguished three ways

diffuse included phloem strands originate, viz. the *Combretum*-, *Strychnos*- and *Azima*-subtype. In all three, a single permanent cambium continues to function throughout the life of the stem. In the *Combretum*-subtype (similar to the *Mucuna*-type of Solereder 1908) inward derivatives of small parts of the cambium differentiate for a short time into phloem elements instead of xylem (Philipson et al. 1971: 99–100). In the *Strychnos*-subtype, xylem production inwards is retarded over small arcs of the cambium. The original circular outline of the vascular cambium cylinder is restored as a complementary cambium, which is formed in the outer border of the future phloem strand, and joins the vascular cambium leaving a strand of phloem in the xylem (Philipson 1990; Van Veenendaal & Den Outer 1993). In a third subtype found in *Leptadenia reticulata* and *L. spartium* (Singh 1943), *Azima* (Den Outer & Van Veenendaal 1981) and some members of the Brassicaceae and Onagraceae, certain groups of parenchyma cells of the secondary xylem divide (dedifferentiate) and (re)differentiate into strands of phloem at some distance from the cambium.

Mauseth (1988) states that it is important to emphasize that the cambia in the three subtypes mentioned above are considered to be bidirectional, producing secondary xylem inwards and secondary phloem outwards, and never produce phloem inwards. So in his view the *Combretum*-subtype, to which *Thunbergia mysorensis*, *T. grandiflora* (Mullenders 1947) and *Aquilaria agallocha* (Rao & Dayal 1992) also belong, where the interxylary phloem is said to be produced on the inner face of the cambium directly from cambial derivatives cut off towards the xylem, does not occur and should be classified within the third (*Azima*-)subtype. Solereder (1908: 404–406) points out that the *Combretum*-subtype and the *Azima*-subtype are difficult to distinguish since only the rate of differentiation into phloem of the thin-walled (parenchymatous) tissues formed inwards by the cambium, determines the difference.

Diffuse included phloem occurs in a restricted number of genera in the Combretaceae (Metcalf & Chalk 1950: 612–620). In *Combretum* diffuse included phloem was only found in African species, in half of the about 40 African species investigated by Van Vliet (1979). Furthermore, its occurrence is largely restricted to erect species and absent from the lianas, except for one climbing species, viz. *C. edwardsii*. Included phloem of the concentric type is incidentally present in *Combretum*. Also internal phloem at the periphery of the pith is recorded in species of *Combretum*.

Since there is still controversy about the precise sequence of ontogenetic events by which diffuse included phloem is produced (Metcalf 1989), the present study was performed. *Combretum nigricans* was used because suitable material was available.

Legends to Figures 1–10:

Light microscopic photographs (1–9) and CSLM-photograph (10) of stems of *Combretum nigricans* var. *elliottii*. In TS (transverse section) of the secondary xylem, the vascular cambium is towards the top of the photograph. — Scale bar = 50 μm . — ca = vascular cambial zone; cr = large crystal-containing idioblast; ex = external secondary phloem; li = libriform fibres; RLS = radial longitudinal section; v = vessel in secondary xylem.

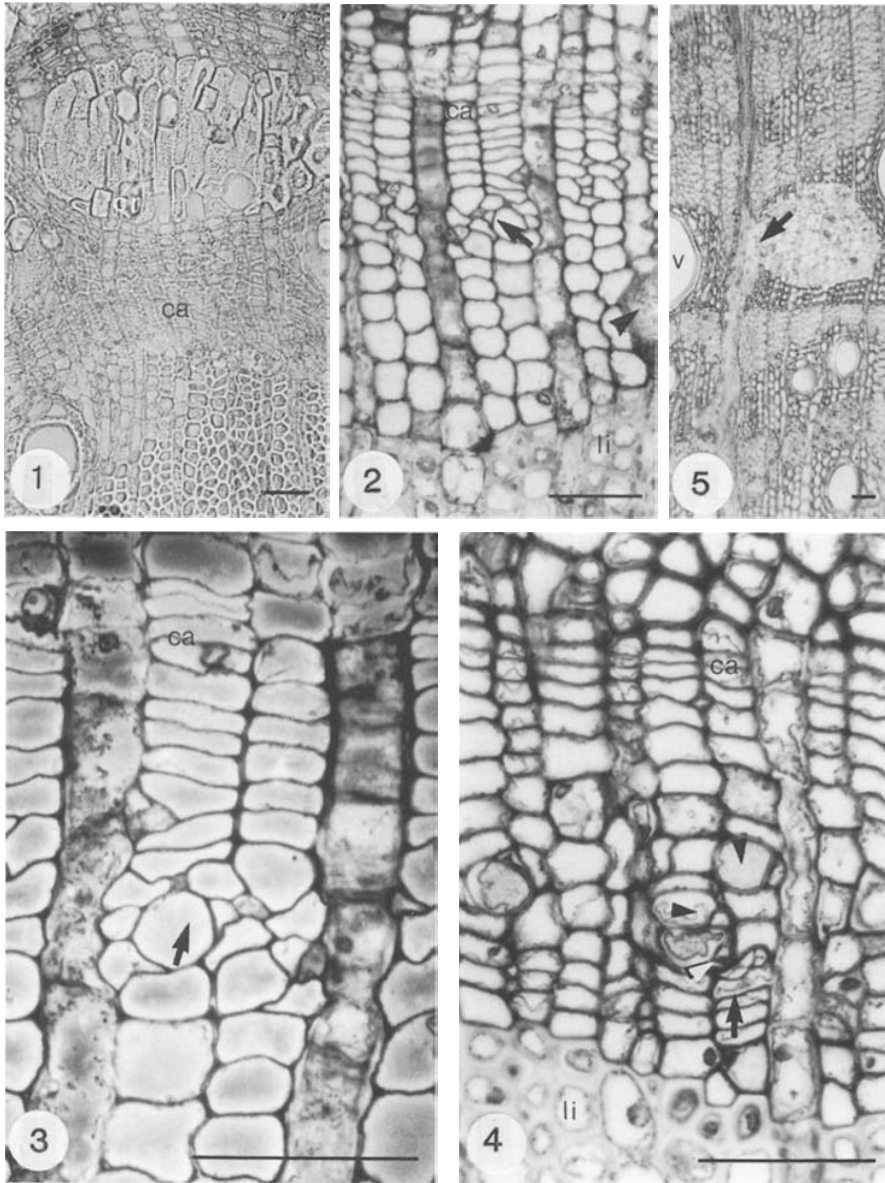


Fig. 1–5. All TS. – 1: Large stone cell centres accompanied by idioblasts with solitary crystals (cr) in the external secondary phloem close to the vascular cambium (ca). – 2: An included phloem strand (arrow) has just been produced inwards by the vascular cambium (ca). Note the large wood ray parenchyma cell differentiating into a crystal-containing idioblast (arrowhead). – 3: Enlargement of part of Fig. 2 with the included phloem strand (arrow) close to the vascular cambium (ca). – 4: Formation of a complementary cambium (arrow) has started, at the adaxial side of a small included phloem strand, at a distance of c. 100 μm from the vascular cambium. Arrowheads: sieve tubes and companion cells. – 5: A longitudinal included phloem strand in connection (arrow) with a horizontal phloem strand in a wood ray, c. 1450 μm from the vascular cambium.

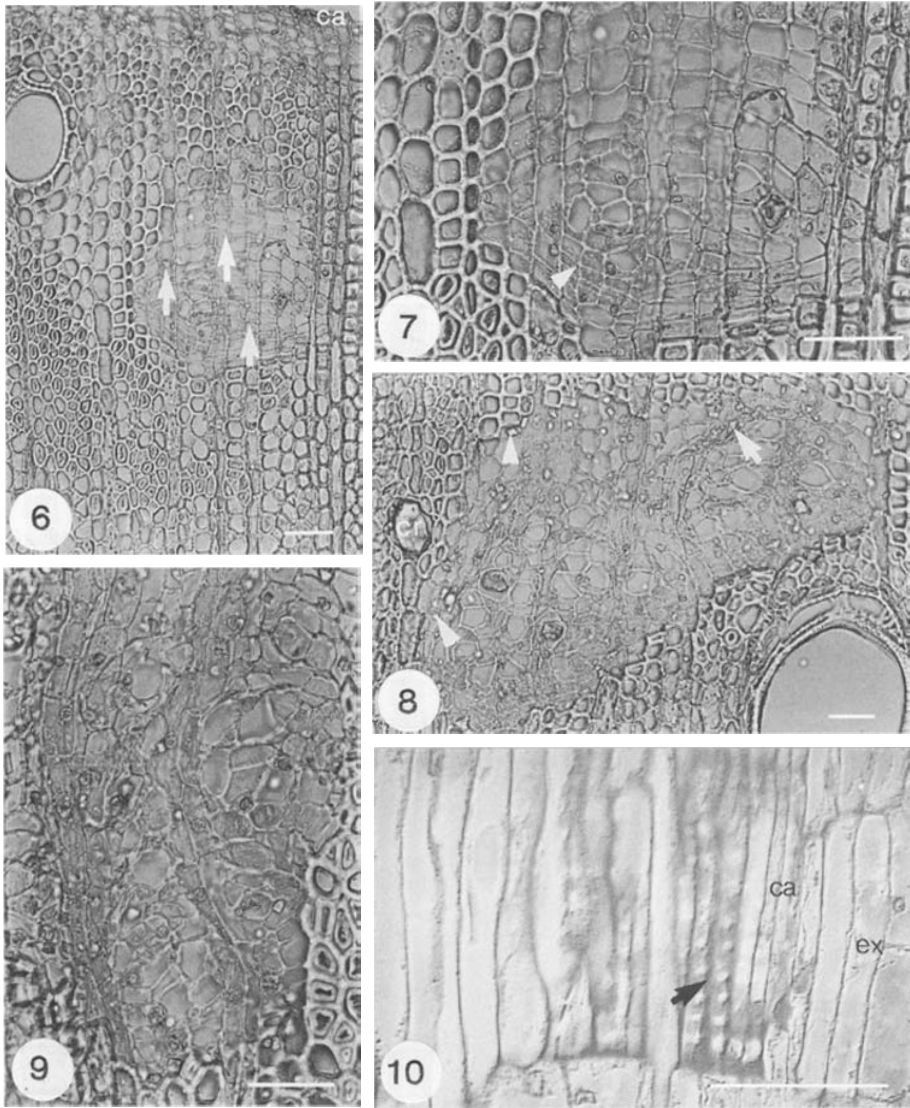


Fig. 6–9. TS; Fig. 10. RLS. – 6 & 7: Recently formed included phloem strand. Uniseriate rays (arrows) with thin-walled parenchyma cells, transverse the strand. The central part of the strand, composed of phloem parenchyma cells, sieve tubes and companion cells, is surrounded by a cylinder of parenchyma cells. A curved cambium segment (complementary cambium; arrowhead) of the strand lies a few cells from the interior border. – 7: An enlargement of a part of Fig. 6. – 8: Two almost entirely fused young included phloem strands, c. 1350 μm from the vascular cambium. The first-formed sieve elements of the strand are already obliterated (arrow). Note the idioblast with a large solitary crystal (cr) in the uniseriate wood ray, and the small crystals (arrowheads) in the parenchyma cells surrounding the strands. – 9: An included phloem strand c. 2580 μm from the vascular cambium. The complementary cambium merges into its derivatives. – 10: Young sieve-tube members (arrow) left from the vascular cambium (ca) in the secondary xylem.

MATERIALS AND METHODS

Stem samples of *Combretum nigricans* Lepr. ex Guill. et Perr. used in this study were collected in the savanna (surroundings Korhogo), Ivory Coast, West Africa, in 1969 (Versteegh & Den Outer 476 and 525) and immediately fixed in formalin-acetic acid-ethanol (1 : 1 : 15). Herbarium vouchers were deposited at the Department of Plant Taxonomy, Wageningen, the Netherlands (WAGw). The small trees were 5 m high with a stem diameter of about 10 cm.

A part of the material was sectioned (sections 15 μm thick) with a sledge microtome, left unstained, and embedded in Kaiser's gelatin-glycerin. Another part was dehydrated in a graded ethanol series, impregnated in Technovit 7100 (Kulzer GmbH, Wehrheim/Ts., Germany) and sectioned (sections 6–7 μm thick) with a rotary microtome. These sections were stained with toluidine blue and embedded in eupalal. All transverse and longitudinal serial sections were used for light microscopy; for confocal scanning laser microscopy (CSLM; Bio-Rad, MRC-600) only toluidine blue stained ones were used, exposed to an argon-krypton laser.

RESULTS

Stone cell centres accompanied by idioblasts with large solitary crystals are regularly present in the secondary external phloem of *Combretum nigricans* stems (Fig. 1). Usually these centres are tangentially stretched and form, together with others, tangentially orientated longitudinal plates. These plates regularly alternate with bands of phloem parenchyma. The latest formed tangential band of stone cells, not directly differentiated from meristem derivatives, lies close to the vascular cambium at a distance of 50–300 μm . Only in the narrow phloem parenchyma zone between the stone cell band and the vascular cambium, some small functional sieve tubes and companion cells were formed.

The different tissues in the secondary xylem are also more or less arranged in tangential bands. Bands of libriform fibres alternate with bands of axial wood parenchyma, since paratracheal parenchyma is often confluent.

Diffuse included phloem strands are situated in these tangential bands of axial wood parenchyma. Only the adaxial border of the strand is regularly in contact with libriform fibres.

Development and structure of the phloem strands

After the inward production of libriform fibres, long tangential segments of the cambium start to deposit axial wood parenchyma cells, followed by some vessels and some diffuse included phloem strands. Phloem strands may also be formed almost directly after the libriform fibre production has stopped. The strands originate from phloem mother cells produced in an inward direction by the normal vascular cambium. The first sign of appearance of an included phloem strand is the formation of a number of more or less radial walls in cells on the inward side of the vascular cambial zone (Fig. 2, 3). These small groups of cells differentiate into sieve-tube members, compan-

ion cells and phloem parenchyma cells. The production of axial wood parenchyma continues for some time until libriform fibres are produced again. Relatively soon a complementary cambium segment is formed (Fig. 4) in the parenchyma cells at the inward border of the small phloem strand. This cambium segment is unidirectional and produces phloem tissue outwards. Phloem production in the median area of the developing strand is more extensive compared with that at the flanks. The phloem strand is surrounded by axial wood parenchyma (Fig. 6, 7), or is only in contact with libriform fibres at its inward border. The strand, which is oval to circular in transverse section, consists of a boundary layer of parenchyma cells enclosing a central portion composed of large sieve tubes with companion cells, phloem parenchyma, phloem rays, and an almost exclusively unidirectional cambium. Usually many small prismatic crystals are deposited in the surrounding parenchyma layer (Fig. 8). Some small crystals also develop in phloem-ray cells. Rays in the strand continue into the secondary xylem (Fig. 6). The phloem-ray parenchyma cells are thinner-walled than the wood-ray parenchyma cells and not lignified (as determined by phloroglucin-hydrochloric acid).

The complementary unidirectional cambium continues dividing for a relatively long time. The new secondary phloem elements crush the older ones, especially the sieve tubes and companion cells. Consequently, within an older strand a layer of crushed and obliterated cells may be present near its outer border (Fig. 8). Eventually the complementary cambium activity ceases, and the cambium merges into its derivatives (Fig. 9).

Phloem network

In a tangential plane phloem strands gradually fuse with each other forming one strand, which may ramify again at another height level within the stem as seen in tangential sections (Fig. 8). In a radial plane horizontal interconnections between phloem strands are usually in wide wood rays (Fig. 5). Together these included phloem strands form a three-dimensional network.

Sieve tubes rarely were found in the external secondary phloem (Fig. 1, 2; for sieve tube and companion cells characteristics, see Den Outer & Fundter 1976). In older trees it is probable that the internal (intraxylary) phloem at the periphery of the pith does not play an assimilate transporting role, because inner layers of the wood usually have ceased to contain living cells (heartwood). Therefore, the main functional assimilate transporting system of *Combretum nigricans* consists of the three-dimensional network of included phloem strands.

DISCUSSION

Usually, only cambial derivatives produced outward become part of the phloem tissue. Deviations from this pattern are considered anomalous (Esau 1969: 212–219) or to not exist (Mauseth 1988).

Eames and MacDaniels (1947) recorded that in genera like *Combretum* and *Entada* small segments of the vascular cambium produce phloem cells toward the inside for a

brief period of time, instead of the xylem cells which are normally cut off. Also Philipson et al. (1971: 99–100) stated that in some species of *Combretum* the internal derivatives of small arcs of cambium may, for a short period of time, differentiate as phloem instead of xylem. Mauseth (1988), on the other hand, emphasized that this anomalous situation does not exist and that at no time does the cambium produce phloem to the interior. Phloem may be formed only as a result of redifferentiation of xylem parenchyma produced inward by the cambium. Our study, however, demonstrates groups of young sieve tubes cut off internally by the otherwise normal vascular cambium. These groups are differentiated before the vessels. The short distance between the actual cambium, which is often difficult to recognize with certainty, and the young included phloem strand is 3(–6) cell layers and indicates the cambial origin of the strand. A differentiation of cambial initials (as in the *Azima*-subtype) into xylem parenchyma, a subsequent dedifferentiation into cells that resume meristematic activity, and finally a redifferentiation into strands of phloem seems unlikely within these 3–6 cell layers. So the ontogeny of included phloem in the subtype *Combretum* and that in the subtype *Azima* are different from each other. In *C. nigricans* included phloem strands are directly formed inward by the vascular cambium, which is in contradistinction to the opinion of Mauseth (1988). Three different origins of included phloem should be distinguished, not two. Since in *C. nigricans* secondary phloem has been produced internally by a single vascular cambium, according to Mikesell and Popham (1976) and Carlquist (1988), the term interxylary phloem can be used instead of diffuse included phloem.

The rare sieve tubes in the external secondary phloem of *C. nigricans* lie scattered among the axial parenchyma cells and not in an orderly sequence as in the other six *Combretum* species investigated by Den Outer and Fundter (1976). These six species, all without included phloem, possess an external secondary phloem composed of different tissues arranged in tangential layers, viz. a tangential layer of crystalliferous cells (or fibres), followed by a layer of axial parenchyma cells, a layer of sieve tubes with companion cells, a layer of axial parenchyma cells, a layer of crystalliferous cells (or fibres), and so on.

In *C. nigricans*, as in *Strychnos* (Van Veenendaal & Den Outer 1993), anastomoses of axial included phloem strands in tangential directions are regularly present. Radial connections are only via phloem strands in rays. These radial strands of phloem are less frequently found than the tangential connections (see also Van Vliet 1979). Therefore, in *C. nigricans*, as in *Strychnos*, a three-dimensional network of secondary included phloem strands exists and is the main assimilate transporting system of the stem.

In *Combretum* included phloem is restricted to species with a shrub or tree habit, although not present in all of them (Van Vliet 1979), and absent from lianas (with a possibly single exception: *C. edwardsii* Exell). This is remarkable since included phloem is often recorded for lianas of other families. The irregular distribution of the included phloem strands among different systematic and ecological groups makes it difficult to speculate on the advantages of the included position of the conducting phloem (cf. Bonnemain 1969). Perhaps the duration of the functional life of the strands is longer than the normal external secondary phloem and thus would be advantageous (see also Van Veenendaal & Den Outer 1993).

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