Annals of Botany **86**: 575–587, 2000
doi:10.1006/anbo.2000.1219, available online at http://www.idealibrary.com on IDEAL®

Early Lignotuber Formation in *Banksia*—Investigations into the Anatomy of the Cotyledonary Node of Two *Banksia* (Proteaceae) Species

RAELENE MIBUS and MARGARET SEDGLEY*

Department of Horticulture, Viticulture and Oenology, Waite Agricultural Research Institute, The University of Adelaide, Glen Osmond, South Australia 5064 Australia

Received: 31 January 2000 Returned for revision: 3 April 2000 Accepted: 24 May 2000 Published electronically: 21 July 2000

Anatomical descriptions are made of the cotyledonary node region of seedlings of *B. menziesii* R. Brown (1830) and *B. serrata* Linnaeus f. (1782), including information on node anatomy, bud type, location and number. Seedlings were sampled at 8, 15 and 26 weeks after seed sowing and observations made of serial transverse sections through the node region. In *B. menziesii*, fused auricles in combination with bases of the cotyledons form a thick sheath of parenchymatous tissue surrounding the base of the seedling stem. Three types of buds are present: (1) an exogenous axillary bud in the axil of each cotyledon and leaf; (2) endogenous accessory buds in the cortical tissue at the base of each cotyledonary axillary bud; and (3) exogenous adventitious buds generated serially along the line of fusion of opposing auricles which occur either side of the stem. Additional adventitious buds occur singly on the adaxial wall of the sheath. At 26 weeks, phenolic compounds such as tannins accumulate in the sheath, which later develops into a lignified protective encasement in *B. menziesii*. Early cotyledonary node anatomy of *B. serrata* differs from that of *B. menziesii*, the fusion line of the auricles curving away and downwards from the stem base, thus precluding the formation of a sheath. Also, accessory buds are absent from the basal tissue of the cotyledonary axillary buds. Only two types of buds are present: (1) an exogenous axillary bud in the cotyledon and leaf axils and (2) exogenous adventitious buds generated along the line of fusion of opposing pairs of auricles, as well as from tissues next to each cotyledonary axillary bud. Knowledge of the number, types and location of buds present in the cotyledonary node will establish a base for further comparative anatomical studies and research on clonal cultivar development of *Banksia*.

Key words: Proteaceae, *Banksia menziesii*, *Banksia serrata*, cotyledonary node, lignotuber, burl, anatomy, axillary buds, accessory buds, adventitious buds, clonal propagation.

INTRODUCTION

The term ‘lignotuber’, first coined to describe the swellings at the base of stems of many Australian eucalypts and angophoras (Kerr, 1925), is now generally used to describe any conspicuous woody swelling of the stem base at or below ground-level which bears dormant buds (Debenham, 1970). In other parts of the world such structures are known as burls (James, 1984). Lignotubers serve a dual, interrelated function as a regenerative and storage organ. The removal of apical dominance through defoliation, or damage caused usually by fire or pruning, stimulates buds on the upper surface of the lignotuber to sprout. This enables rapid re-establishment of the plant’s aerial shoot system (Chattaway, 1958; Blake and Carrodus, 1970; James, 1984; Pate and Beard, 1984). This may occur several times in early seedling development, continuing until starch reserves in the lignotuber are depleted. The ecological function and occurrence of lignotubers in fire-prone Mediterranean-type plant communities worldwide is well documented (James, 1984; Pate and Beard, 1984; Bradstock and Myerscough, 1988; Bradstock, 1990, 1991; Pate *et al.*, 1990; Enright and Lamont, 1992; Lamont and Markey, 1995). However, little is known about the early anatomical development of bud structures in lignotubers of genera in which they are known to occur. *Banksia*, an Australian genus of the family Proteaceae, is the focus of this descriptive anatomical study. At least half of the taxa within the genus are lignotuberous (George, 1981; Thiele, 1993), 36 species having fire tolerant trunks or lignotubers (George, 1987). Because of the increasing horticultural importance of *Banksia* for the production of cut flowers and foliage (Johnson and Burchett, 1996), an understanding of the biology of existing bud structures may prove fundamental to further developing the new clonal cultivars. Lignotuber buds or shoots are a likely source of clonal propagules because they are derived from tissues of young ontogenetic age at the base of the stem. It is well known that such juvenile basal shoots from woody perennials can be propagated with greater success (Hartmann *et al.*, 1990). Investigations of early development of lignotuber buds in *Banksia* may also help to distinguish the different types of lignotuberous structures formed in mature plants of this genus. Furthermore, it will shed light on the anatomical similarities to other lignotuberous bud structures observed in other taxa (Graham *et al.*, 1998).

In mature plants of *Banksia* species known to be lignotuberous, two distinct types of bud morphology are apparent (Mibus, 1998); however, published descriptions do not yet exist for either type. *B. menziesii* exemplifies one type of lignotuber bud morphology typical of more fire prone, xeric climates, where the buds are concealed beneath the bark of the cotyledonary node region. It is this type of

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* For correspondence. E-mail margaret.sedgley@adelaide.edu.au

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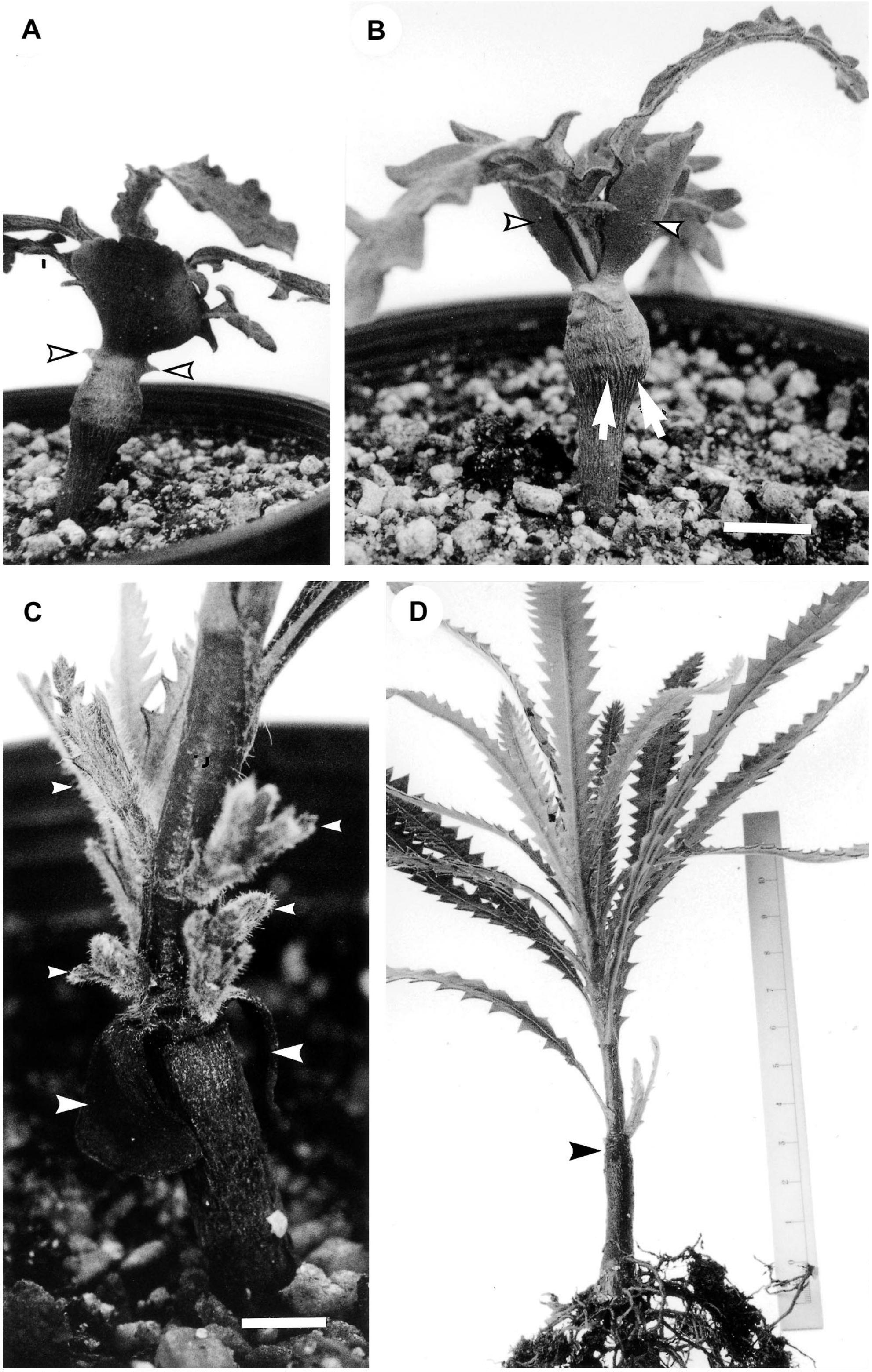


FIG. 1. A, Cotyledonary node morphology in *B. menziesii* at 5 months from sowing showing the auricles (arrows) and pronounced swelling of the upper hypocotyl. B, Side aspect of A, depicting both cotyledons (outlined arrows) fused at the base to form the auricle, the first leaves subtended perpendicular to the cotyledons and the enlarged bumps on the surface of the upper hypocotyl. The average diameter of the hypocotyl for seedlings of this age was 3·9 mm. Bar = 1 cm. C, *B. serrata* (14 months) showing recurved senesced cotyledons (large arrows) and basal shoots (small arrows) developing from cotyledonary axillary buds and axillary buds from the first pair of leaf scars. The average hypocotyl diameter of seedlings of this age was 6 mm. Bar = 0·5 cm. D, *B. serrata* (15 months) with the cotyledonary node (arrow) and thicker hypocotyl compared to the main stem; note the region of shorter internodes (at 6–8 cm on the scale) above the cotyledonary node. Scale in cm.

morphology that is the focus of this investigation. In the other type of bud morphology observed in *Banksia*, masses of superficially visible buds are located at the cotyledonary and occasionally also at the lower leaf nodes. This bud morphology is exemplified by *B. robur* and *B. spinulosa*, varieties *spinulosa*, *collina* and *neoanglica*, species from mesic climates receiving higher rainfall.

Whilst *B. menziesii* is clearly lignotuberous (George, 1987; Lamont *et al.*, 1994; Thiele and Ladiges, 1996) with concealed lignotuber buds, confusion exists in the literature as to whether *B. serrata* is lignotuberous. *B. serrata* is characteristically arborescent, with a single stem (Thiele, 1993; Bradstock and Myerscough, 1988; Bradstock, 1990). In his taxonomic survey of quantitative characters, Thiele (1993) was uncertain about the presence or absence of lignotubers in seedlings of *B. serrata*. George (1987) does not describe *B. serrata* as lignotuberous, stating that this species is fire tolerant, sprouting from epicormic shoots. In ecological field studies, mature plants of *B. serrata* are reported as resprouting from epicormic buds above the plant base, or a basal lignotuber (basal resprouter) (Taylor and Hopper, 1988; Bradstock and Myerscough, 1988). This latter usage of the term 'lignotuber' describes the pronounced woody thickening which is often observed at the base of the stem in *B. serrata*, even from a very early stage (Mibus, 1998). This, however, is unlike the extensive subterranean lignotubers of *B. oblongifolia* (Zammit, 1988), or the lignotuberous trait observed in *B. menziesii*. This suggests that the term 'lignotuber' is loosely used to describe a combination of macro features (woody swellings at the plant base; latent buds; resprouting) that may be morphologically different and substantially different from one another with respect to their ontological origins.

As far as we are aware, the only other anatomical studies so far conducted on lignotuber development and structure referred to the genus *Eucalyptus* (Myrtaceae) (Kerr, 1925; Carrodus and Blake, 1970; Bamber and Mullette, 1978; Mullette, 1978; Carr *et al.*, 1984*a*,*b*). The view currently held is that lignotuberous eucalypts must possess double sets of concealed buds on either side of the naked axillary buds found in leaf axils, each leaf axil occurring in opposite pairs on the stem (Chattaway, 1958; Graham *et al.*, 1998). It is the presence of this type of bud structure that is thought to predispose *Eucalyptus* species to the formation of a lignotuber and the continual generation of buds in the expanding woody structure. Most of the concealed buds develop exogenously in the cortex of very young lignotubers; in older lignotubers some also develop endogenously from the phloem and remain surrounded by tissue beneath the surface. Preliminary observations of the lignotubers in *Banksia* seedling displaying either type of bud morphology mentioned above indicate that very young lignotubers differ radically in mode of development from those of *Eucalyptus* (Graham *et al.*, 1998).

This anatomical investigation of early cotyledonary node development in two closely related species *Banksia menziesii* and *B. serrata*, series *Banksia*, aims to identify the numbers, types and location of buds present. This will establish a base for further comparative anatomical studies and research on clonal propagation of members of the *Banksia* genus.

MATERIALS AND METHODS

Plant material

Seeds of *Banksia serrata* and *B. menziesii*, series *Banksia*, both fire resistant resprouters, purchased from Nindethana Seed Services (RMB 939, Woogenillup, WA 6324, Australia), were sown in autumn directly onto tamped no. 3–4 vermiculite in deep seed trays (300 mm × 400 mm × 120 mm) and covered with a 10 mm layer of vermiculite. A systemic fungicide, Previcur (Shering-Höchst, active constituent: 600 g l^{-1} propamocarb), was applied at 2 l m^{-2} (at 1·5 ml l^{-1} concentration) to control damping-off. Trays were placed in full sunlight in a well-ventilated glasshouse at 18°C and hand-watered daily. Emergence occurred 30 d after sowing; germination success was 67 and 100 % for *B. serrata* and *B. menziesii*, respectively. Seedlings were pricked out and planted into 10 cm (0·5 l) pots in a medium of equal parts of peat (Eurotorf), perlite and fine sand. Pots were placed on raised benches in an evaporatively cooled glasshouse at 18°C and hand-watered three times per week.

Destructive sampling and embedding for microscopy

Three seedlings of each study species were destructively sampled at 8, 15 and 26 weeks after sowing. The cotyledonary node was dissected including the base portion of the stem, the upper hypocotyl and the basal region of the cotyledons. The outside of the base of each cotyledon was scored with a scalpel to assist fixative penetration. Tissue was fixed in 3 % glutaraldehyde in 0·025 M phosphate buffer, pH 7 at 4°C for 24 h, dehydrated through an alcohol series and embedded in 2-hydroxyethyl-methacrylate (Feder and O'Brien, 1968). Serial transverse sections, 4 μm in thickness, were collected on glass slides and stained with Periodic acid-Schiff's reagent and Toluidine Blue O. Sections were viewed and photographed using a transmitted light microscope (Zeiss, Axiophot Pol Photomicroscope).

RESULTS

The gross morphology of the stem and cotyledonary node region of seedlings of *B. menziesii* and *B. serrata* is shown in Fig. 1. Seedlings of *B. menziesii* at 5 months have a

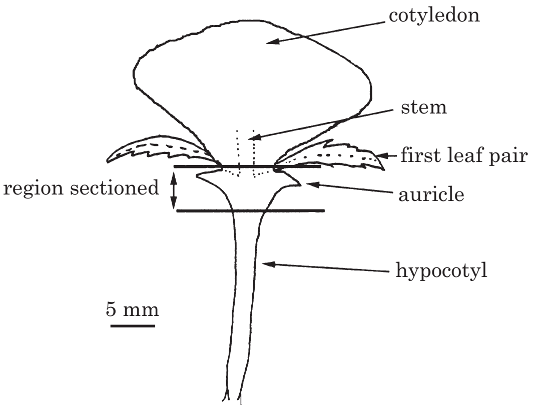


FIG. 2. Intact seedling showing the general morphology and location of the cotyledonary node region whose anatomy is described in detail in Figs 3 and 4.

TABLE 1. *Bud location and numbers in three replicates of* B. menziesii *(lignotuberous) and* B. serrata *(epicormic) at 8, 15 and 26 weeks after seed sowing*

Bud type	Bud location and origin		Bud count																	
			Week 8						Week 15						Week 26					
			B. menziesii			*B. serrata*			*B. menziesii*			*B. serrata*			*B. menziesii*			*B. serrata*		
		Replicates	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Axillary	Cotyledon axil (exogenous)		2	2	2	2	2	2	2	2	2	1 (1)	2	2	2	2	2	2	2	2
Accessory	Cotyledon axil—lower (endogenous)		2	2	5+	0	0	0	0	2	5	0	0	0	6	2	6	0	0	0
	Cotyledon axil—upper (endogenous)		2	2+	5+	0	0	0	0	2	3	–	0	0	5	2	5	0	0	0
Adventitious	Fusion line of lower auricle (exogenous)		2	4, M	3, M	0	0	1	1, M	2, M	16	2	M	M	12, M	4, M	14	7	12	17
	Fusion line of upper auricle (exogenous)		1	4, M	–	0	0	1	1, M	1, M	20	–	M	0	12, M	4, M	8	7	12	17
	Other locations (exogenous)		0	0	4	0	0	0	4	17	6	–	0	0	4	4	4	†	4, †	†
Axillary	Leaf axil (exogenous)	Node 1		2	2	–	–	–	–	2	2	–	–	2	2	2	2	–	2	2
		Node 2		2	2										2					
		Node 3		A	2															
					A															
Total			9	18+	25+	2	2	4	8+	28+	48+	4	2	4	45+	20+	41+	16	32+	38+

A, Apical meristem; M, meristematic tissue at the line of fusion of opposing auricles; † meristematic tissue extending from each cotyledonary axillary bud into the groove on the adaxial wall of the cotyledon bases and on the stem base in *B. serrata*; +, plus the presence of developing buds; –, tissue area not sectioned; ( ), numbers in parentheses indicate expected counts due to absence of these tissues in the mounted block.

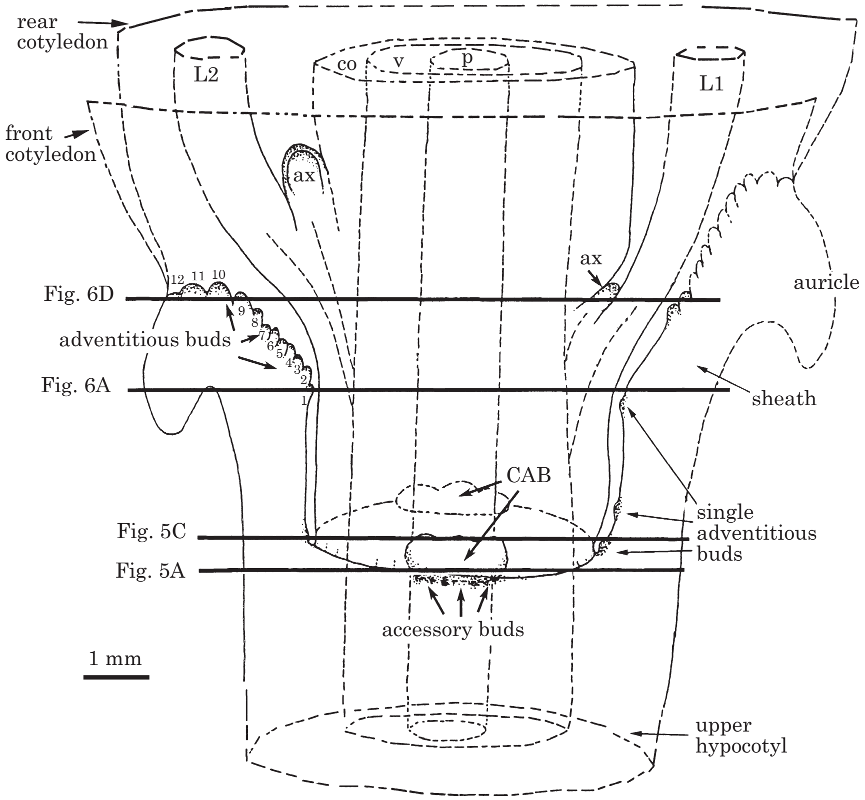


FIG. 3. Median longitudinal reconstruction from transverse sections of cotyledonary node of *B. menziesii* (26 weeks) showing the location of the low power photomicrographs of transverse sections (horizontal solid lines) depicted in Figs 5 and 6. CAB, Cotyledonary axillary bud; co, cortex; v, vascular tissue; p, pith; L1, first leaf base; L2, second leaf base; ax, leaf axillary bud. Adventitious buds generated from the fusion line (denoted 'M' in Table 1) of the auricles on the LHS are numbered 1–12 and single adventitious buds are present on the RHS in the lumen on the adaxial wall of the sheath.

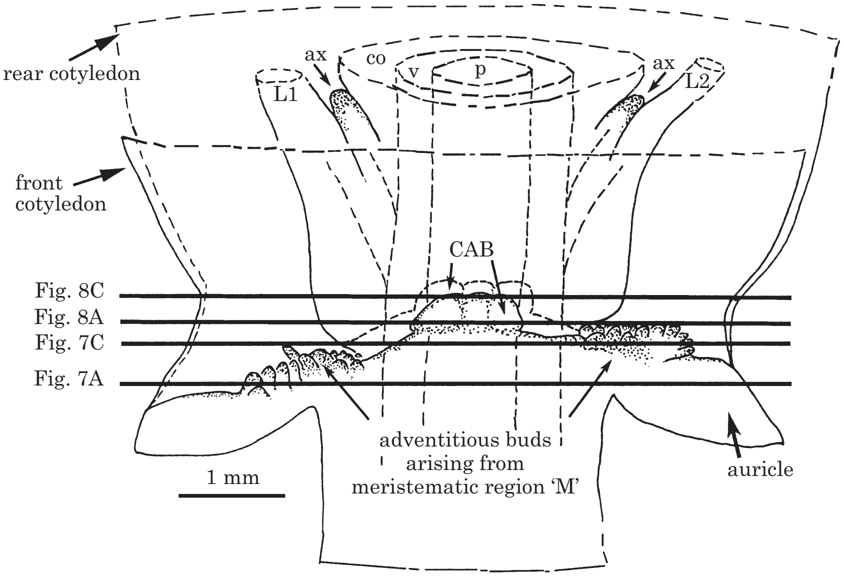
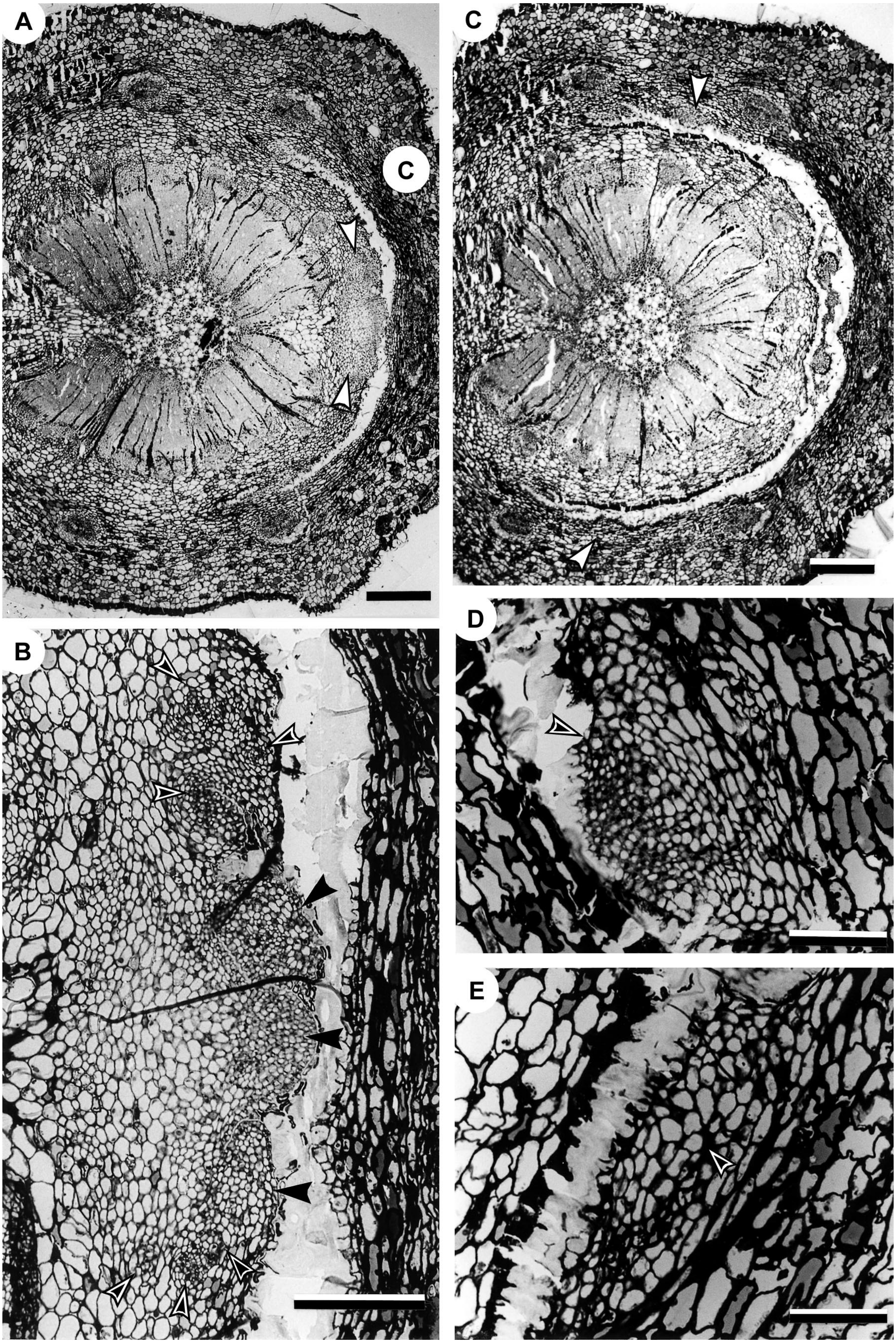


FIG. 4. Median longitudinal reconstruction from transverse sections of cotyledonary node of *B. serrata* seedling (26 weeks) showing the location of the low power photomicrographs of transverse sections (horizontal solid lines) depicted in Figs 7 and 8. CAB, Cotyledonary axillary bud; co, cortex; v, vascular tissue; p, pith; L1, first leaf base; L2, second leaf base; ax, leaf axillary bud.



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thickened upper hypocotyl with thick green flabellate cotyledons; the first two pairs of leaves have short internodes (Fig. 1A and B). In *B. serrata* seedlings at 14 months, the upper hypocotyl region and lateral shoots from the first and second leaf nodes above senesced cotyledons can be observed (Fig. 1C and D).

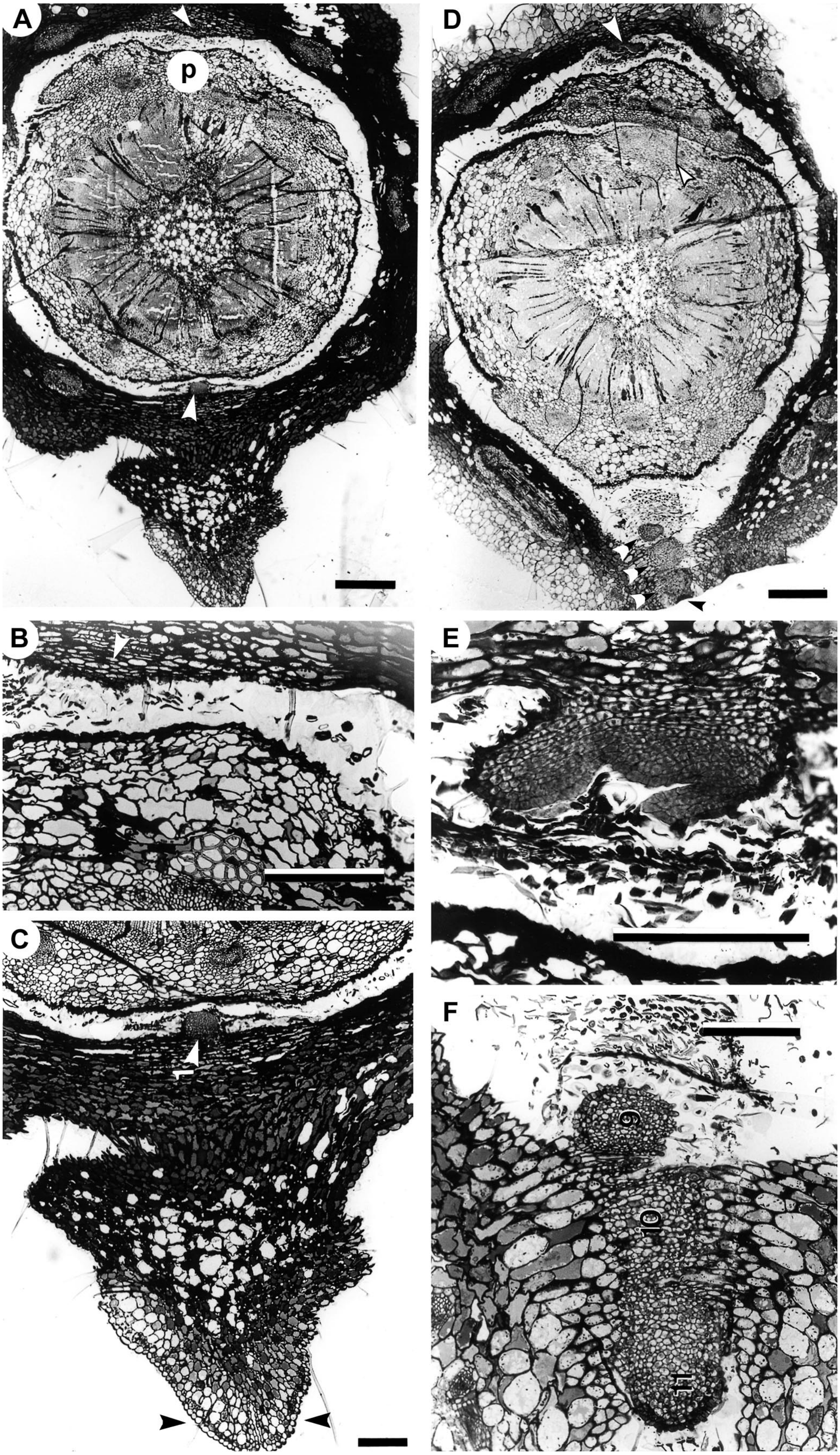
The location and number of sequentially formed buds in the cotyledonary node region of post-emergent seedlings of *B. menziesii* and *B. serrata* are summarized in Table 1 for 8, 15 and 26 weeks after seed sowing, and visualized diagrammatically for 26 weeks in Figs 3 and 4. Serial sections of *B. menziesii* show that axillary buds derived from cortical tissues of the base of the stem (Fig. 5A–C) are already present in the axils of cotyledons in seedlings of all ages. As early as 8 weeks after sowing, accessory buds develop in the cortical tissues at the base of the cotyledonary axillary buds (CAB); five to six accessory buds are present in two of the three individuals observed at 26 weeks after sowing (Table 1 and Fig. 5B). The accessory buds (endogenous) are deeply seated compared with the axillary bud, which originates from superficial tissues (exogenous).

On either side of the base of the cotyledons, the opposing auricles of each cotyledon become fused together for the most part (Fig. 6A and C) to form a thick sheath of tissue surrounding the base of the stem (Figs 2, 5A, B and D). Numerous adventitious buds are then formed at different developmental stages along the line of fusion of opposing auricles (Fig. 6B, C, E and F). The fusion line thus acts as a long seam of meristematic tissue (denoted ‘M’ in Table 1; shown in Figs 3 and 6A–E) which runs down along the inner surface of the sheath adjacent to the auricles into the space between the sheath and stem base. Such adventitious buds are clearly produced exogenously and occur in numbers ranging from one to 20; these buds are not associated with vascular traces. Rudimentary bud structures, comprising thin walled, nucleate cells of meristematic tissue are often present but not yet developed fully into discrete buds. These are denoted ‘M’ in Table 1. Adventitious buds may also develop from superficial tissues on the adaxial wall of the sheath other than along the line of fusion (Fig. 5C–E). These adventitious buds often lie in close proximity to the cotyledonary vascular bundles in 8-week-old seedlings, and may range in number from zero to 17 for seedlings of *B. menziesii* up to 26 weeks (Table 1). The vasculature of the upper hypocotyl, cotyledonary node and base of the stem is bilaterally symmetrical. Vascular bundles identified in the cortex of the upper hypocotyl and in the sheath supply the median and lateral veins of the cotyledons (Fig. 5A and C). At 8 weeks, the vascular trace leading to the median vein of the cotyledon subtends the trace to the cotyledon axillary bud. Vascular tissue also supplies cortical tissues between the trace to the cotyledonary axillary bud and the vascular stele of the main stem. This is where accessory buds develop at the base of the cotyledonary axillary bud at 26 weeks.

The sheath consists of a layer of thick spongy cortical parenchyma approximately as thick as the diameter of the inner stem in the 8-week-old seedling, but proportionally not as thick as the stem diameter in 26-week-old seedlings (Fig. 5A). At 26 weeks tannins and other phenolics have begun to concentrate in the enveloping sheath, which eventually becomes lignified as secondary thickening of the stem commences (Fig. 6A and D). Axillary buds are present in the axils of the first leaves (Fig. 6D). At 26 weeks, the three *B. menziesii* individuals observed had bud numbers ranging from 16 to 38; these comprised four different bud types and undifferentiated meristematic tissue was also present.

In *B. serrata* (Figs 4, 7 and 8), each auricle is fused to its opposite partner along the inner surface (Fig. 7A and C). Adventitious buds, arising exogenously from this fusion line comprising cortical tissues of opposing auricles (Figs 7A–C, 8A and B) increase from zero or one at 8 weeks to four to 14 at 26 weeks (Table 1). These adventitious buds are not associated with vascular traces. The line of fusion of the auricles curves outwards and downwards, away from the stem, so the protective sheath and deep space between the sheath and the stem observed in *B. menziesii* do not form in *B. serrata* seedlings. Meristematic tissues and rudimentary bud formations are visible at the line of fusion of the auricles (denoted ‘M’ in Table 1, shown in Fig. 7B and C). Each cotyledon of *B. serrata* subtends an axillary bud comprising an apex and two prophylls (Table 1, Figs 4, 8A, C, E and F). However, accessory buds, as observed in *B. menziesii* at the base of the cotyledonary axillary buds, are absent from *B. serrata*. All three 26-week-old seedlings of *B. serrata* examined showed meristematic tissue and rudimentary adventitious buds, often present on the adaxial wall of the cotyledons or superficial stem tissue close to the groove either side of the cotyledonary axillary bud (denoted ‘†’ in Table 1, shown in Fig. 8A–D). The first pair of leaves develops at right angles to the cotyledons, each leaf subtending a large axillary bud. The vasculature of the hypocotyl of *B. serrata* is bilaterally symmetrical. The bases of the cotyledons are continuous with the cortex of the upper hypocotyl, the vascular traces supplying the median and lateral veins of the cotyledons. The laminae of the cotyledons remain separate from one another, except for the lower edges of their opposing auricles; the point of attachment of the cotyledons to the main stem is

FIG. 5. Light micrographs of transverse sections of *B. menziesii* seedling (refer to Fig. 3) showing cotyledonary node anatomy 26 weeks after sowing. A, Base of the cotyledonary node, showing the cotyledonary axillary bud (arrows) in the axil of the first cotyledon. Note the bases of the cotyledons (c) are fused, and that a band of cortical parenchyma ensheaths the node. Bar = 500 μm. B, Higher magnifications of axillary bud region in A showing the main axillary bud (exogenous) comprising an apex and prophylls (large black arrows) and the proliferation of accessory buds (endogenous) (outlined arrows) in the cortical tissue at the base of the axillary bud. Bar = 200 μm. C, Cotyledonary node approx. 360 μm above A (refer to Fig. 3); the top of the prophylls of the lower cotyledon axillary buds are visible in the lumen (RHS of photomicrograph). Two single adventitious bud meristems present on the adaxial walls of the sheath (arrows) are shown at higher magnification in D and E. Bar = 500 μm. D, Single adventitious bud from C (upper arrow in C) at an early stage of development. Bar = 100 μm. E, Single adventitious bud from C (lower arrow in C) showing dome of meristematic tissue. Bar = 500 μm.



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comparatively narrow. There is a large increase in the total number of adventitious buds in *B. serrata* seedlings between 8 weeks, where two cotyledonary axillary buds and up to one adventitious bud were observed, and 26 weeks, where the number of adventitious buds ranged from 16 to 38 in the meristematic tissues between the pairs of auricles.

DISCUSSION

As far as we are aware this is the first definitive study investigating the early lignotuber anatomy of *Banksia*. Our results for *B. menziesii* seedlings show that foci for the foundation of the resprouting, lignotuberous habit observed in older plants is established in early cotyledonary node development. The thick sheath formed from the fused bases of the cotyledons in seedlings provides protection to cotyledonary axillary and accessory buds, as well as to any adventitious buds subsequently arising from the adaxial wall of the sheath, and also to axillary buds found in the axils of the first pairs of leaves. Axillary buds associated with the first pair of leaves are considered integral parts of the initial morphological complement of the cotyledonary node region in *B. menziesii* due to the short internode between them and the cotyledonary axil, and the fact that they are also protected by the cotyledonary sheath.

At the green seedling stage, the presence of the concealed type of bud structure in *B. menziesii* is noticeable due to the conspicuous swelling at the cotyledonary node region and uneven surface of the top of the hypocotyl (Fig. 1A and B). The swelling of the upper hypocotyl and cotyledonary node region in this lignotuberous species may be attributed to the number of buds and associated vascular tissue that it contains. As seedlings develop further, the hypocotyl and cotyledonary node become noticeably lignified as secondary thickening occurs, forming the woody, lignotuberous swelling bearing concealed latent buds.

Subsequent secondary growth with the progressive deposition of lignified tissues, which occurs as the lignotuber matures, may cause the obliteration of buds and generative tissues as observed in *Eucalyptus* (Graham *et al.*, 1998). Further research on older plant material must be undertaken to confirm the fate of these buds in *Banksia*, and whether they persist or are replaced by new bud initials.

It is typical of all *Banksia* species that the lower margins of the cotyledons on either side of the hypocotyl extend to form a pair of auricles (Fig. 1A and B). Auricles are a feature of most woody fruited Proteaceae (Lamont and Groom, 1998); in most species of *Banksia* they are fused (Thiele and Ladiges, 1996). The present study shows that in the two *Banksia* species studied the internal line of fusion of the auricles comprises a principal primary source of tissue capable of generating adventitious buds.

The cotyledonary sheath of *B. menziesii* forms from basal tissue of the cotyledons, which are fused together at each pair of opposing auricles, and form a wide, protective sheath surrounding the base of the stem. This sheath undoubtedly affords excellent protection to the various buds located within it at the cotyledonary node of the seedling. Due to the greater length of cotyledon fusion in *B. menziesii*, which extends from the floor of the lumen upward to where the surfaces of opposing auricles separate, there is a relatively large amount of meristematic tissue for the generation of adventitious buds in comparison to *B. serrata*. In *B. menziesii* the internal line of fusion and the adventitious buds generated from this tissue are protected, facing into the space created between the sheath and the stem. In *B. serrata* the line of fusion of the auricles is comparatively short and the generative tissues are exposed to the external environment.

Although the fused auricles of *B. serrata* provide a source of adventitious buds, and its cotyledons are of similar dimensions to those of *B. menziesii* (Thiele and Ladiges, 1996), the species lacks accessory buds in cortical tissues at the base of the cotyledonary axillary buds, as well as a sheath to protect the axillary and adventitious buds, and any other generative tissues at the cotyledonary node.

The lack of clarity on whether *B. serrata* is lignotuberous may be ascribed to observations of mature plants of this species frequently having woody, thickly swollen stem bases that are fire tolerant and resprouting from basal ‘lignotubers’ or epicormic buds above the plant base. The thick woody stem base and the buds it contains may, however, be of different ontogenetic origin to those found in species clearly classified as lignotuberous. In their first year, seedlings of *B. serrata* have a compact habit (Fig. 1D) due to the short internodes produced as stem elongation slows at the end of the first growth flush (Mibus, 1998). As the plant matures, the resulting numerous leaf scars surrounding the

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FIG. 6. Light micrographs of transverse sections of the cotyledonary node in *B. menziesii* (refer to Fig. 3) at 26 weeks after seed sowing. A, Cotyledonary node approx. 1530 μm above Fig. 5 C showing the fused, opposing auricles attached to the sheath formed from the fused cotyledon bases that encircle the stem. Two adventitious buds (arrows) can be seen adjacent to the auricle on the adaxial surface of the sheath: the least developed adventitious bud is shown at higher magnification in B; the more developed bud is shown at higher magnification in C. The petiole base (p) of the first leaf can be seen on the inner stem. Bar = 500 μm. B, Meristematic region from A showing an early bud primordium (arrow) derived by division of superficial cells of the adaxial wall of the sheath at top of micrograph. Bar = 200 μm. C, Higher magnification of A showing the fused pair of opposing lower auricles (black arrows) joined to the main axis, with an adjacent adventitious bud comprising a single meristematic dome (white arrow) on the adaxial surface of the sheath. Bar = 200 μm. D, Mid-cotyledonary node approx. 960 μm above A depicting the four adventitious buds (arrows at bottom of micrograph) at the point where the fused auricles separate, giving rise to the individual cotyledonary laminae. Another adventitious bud (arrow at top of micrograph), adjacent to the upper fused auricles is present on the inner adaxial surface of the sheath. This section was taken from immediately beneath the juncture of the upper auricle and the main axis (refer to Fig. 3). The axillary bud (outlined arrow) in the axil of the first leaf is visible on the central stem. Bar = 500 μm. E, Adventitious bud from a section 40 μm beneath that shown uppermost in D. This bud originates from the adaxial wall of the sheath and comprises an apex and two prophylls. Bar = 200 μm. F, Three adventitious buds (buds 9, 10 and 11, refer to Fig. 3) from D can be seen at higher magnification. Bar = 200 μm.

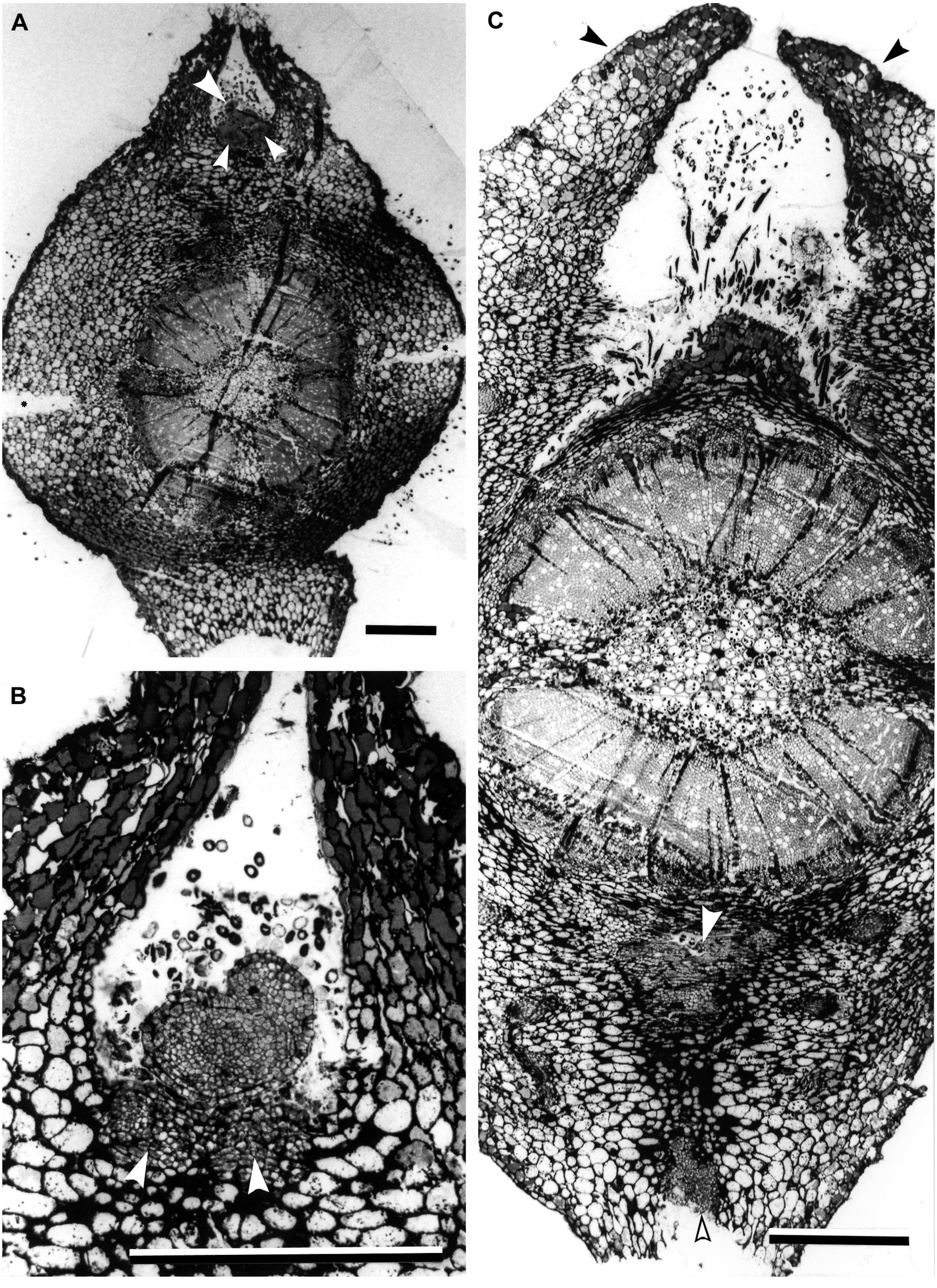

FIG. 7. Legend on facing page.

base of the stem become buried beneath the developing thick periderm, which is characteristic of *B. serrata*. Leaf scars and their axillary buds that are enveloped in this manner by the developing periderm are termed 'epicormic buds' (Chattaway, 1958; Fink, 1983). Due to the trilacunar leaf node structure in *B. serrata* (Metcalfe and Chalk, 1979), each leaf scar bears three lateral buds. So if a young *B. serrata* seedling has 12 leaves with little or no internode development and each leaf subtending three axillary buds, at the end of the first year there will be 36 epicormic buds concentrated at the base of the stem. This may explain the prolific basal sprouting observed in *B. serrata* (Mibus, 1998).

It is possible that the epicormic buds at the sites of leaf scars are present in finite numbers. The tissues generating adventitious buds observed at the line of fusion in the cotyledonary node of young *B. serrata* seedlings could possibly be a renewable source of buds, but these are less protected than those surrounded by the sheath in *B. menziesii*. Also, the early ontogeny of the cotyledonary node of these two *Banksia* species indicates that the presence of accessory buds in the basal tissue of the cotyledonary axillary bud may also be a prerequisite for the formation of lignotubers. If the presence of such accessory buds is used as an anatomical model for determining whether a species is lignotuberous, then *B. serrata* is not.

A study of lignotuberous and non-lignotuberous populations of species such as *B. marginata* is clearly needed to elucidate whether the presence of a sheath, and/or accessory buds in the basal tissues of the cotyledonary axillary bud, are essential prerequisites for lignotuber formation in *Banksia*.

The first signs of lignotuber development in *B. menziesii* are clearly distinctive from those observed in eucalypts. In the latter, lignotuberous swellings initially produced on the stem above the concealed accessory buds increase in size above the axillary shoot to eventually encircle the stem (Chattaway, 1958). In *B. menziesii* the swelling occurs more or less evenly around and localized to the circumference of the cotyledonary node, below the point of attachment of the cotyledons. In eucalypts, buds are concealed deeply in the tissues, becoming slowly enveloped as the node grows (Graham *et al*., 1998). In young *B. menziesii* seedlings the thick sheath affords protection to the buds in the lumen and axils of cotyledons and the first pair of leaves.

Lignotuber formation is thought to have evolved at several different times in different taxa (James, 1984) and under different environmental conditions, ranging from xeric, Mediterranean ecosystems to more mesic, higher rainfall climates. Although the lignotuberous trait generally serves the same ecological function of vegetative regeneration, it may have different ontogenetic histories in the different taxa in which it is known to occur. This may explain the variation observed in the different morphological structures, all of which are generically termed 'lignotubers'. In lignotubers with concealed bud morphology, which are usually found in plants from xeric environments, the cotyledonary node and upper hypocotyl increase in size and bud number, forming large, resprouting structures at ground level (e.g. *B. menziesii*), or extensive subterranean structures found in other lignotuberous taxa, such as *Banksia oblongifolia* (Zammit, 1988) and *Eucalyptus gummifera* (Mullette, 1978). In *Banksia* species with lignotubers comprising visible superficial masses of buds, usually from temperate, high rainfall climates, the bud structures are located at ground level (e.g. *B. spinulosa* var. *spinulosa*), or well above it at leaf nodes up the stem (e.g. *B. robur*) (Mibus, 1998). If these different morphological forms have distinct ontological origins, the anatomical composition and types of buds are also likely to be different.

Two varieties of *B. spinulosa*, var. *spinulosa* which is lignotuberous and var. *cunninghamii*, which is non-lignotuberous, may provide a system for investigating the physiology of lignotuber formation and identifying and isolating the genes and hormones involved. Environmental factors, such as temperature and fires, also exert considerable effects on the phenotype observed in lignotuberous plants throughout their geographic range.

Comparative anatomical studies of the structures in other lignotuberous and non-lignotuberous *Banksia* species are required. Research is also needed on the development of bud structures, how they respond to defoliation (through pruning or by fire) and whether such treatments lead to the formation of bud initials and new buds being generated in tissues of the cotyledonary node region. Also, it would be helpful to determine whether concealed accessory buds exist in the leaf nodes of known lignotuberous *Banksia* species and whether this is a predisposition to lignotuber formation and continual generation of bud initials as already observed in eucalypts (Chattaway, 1958).

In summary, this anatomical study of the cotyledonary node of young *Banksia* plants presents a basis for developing a more precise definition of the term 'lignotuberous' when applied to *Banksia* species which possess lignotubers with concealed bud morphology. Such a definition of 'lignotuberous' would include information on the bud composition in very early cotyledonary node development and may be useful in early identification of lignotuberous species and their capability to continually resprout from the base (e.g. fire resistance). The presence of a range of bud types present in large numbers also makes

FIG. 7. Light micrograph of transverse section of *B. serrata* seedlings (refer to Fig. 4) showing lower and mid-cotyledonary node 26 weeks after seed sowing. A, Base of cotyledonary node. The bases of the pairs of fused auricles are joined to the upper hypocotyl. Adventitious buds (arrows)—one mature bud over two developing buds—are positioned between the opposing pair of lower auricles. An asterisk denotes incisions scored into the sides of the cotyledons to assist fixative penetration. Bar = 500 μm. B, Higher magnification of a section 40 μm beneath the adventitious bud shown in A. Note the meristematic tissues of the base of the two buds positioned beneath the more developed bud. Bar = 500 μm. C, Approximately 280 μm above A showing the separated cotyledon laminae above the lower auricle (top of micrograph) (arrow); adventitious buds and the meristematic tissues can be seen at the base of the adventitious buds which have formed where opposing auricles have begun to separate (lower half of micrograph) (arrows). Bar = 500 μm.

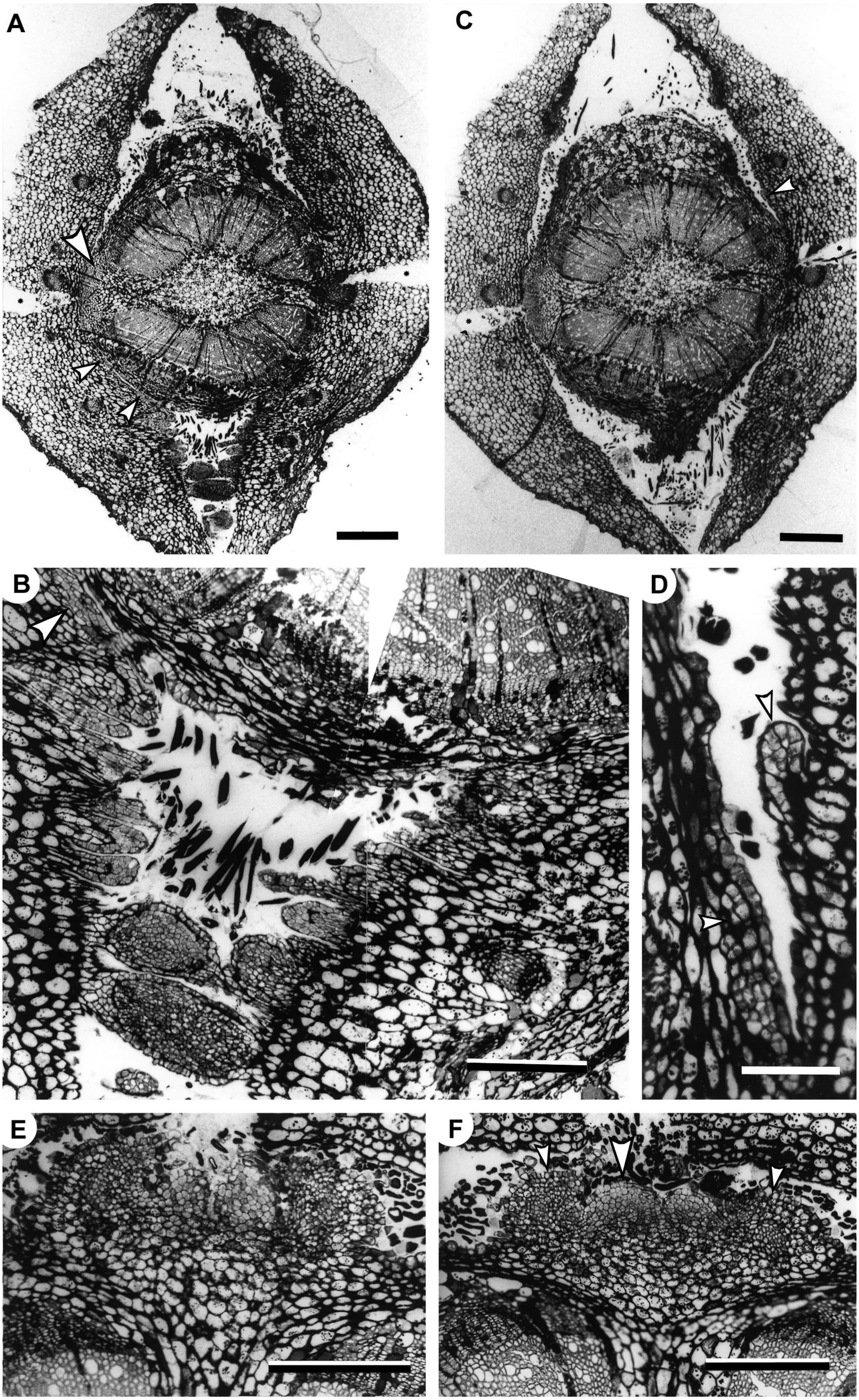

FIG. 8. Legend on facing page.

the cotyledonary node and the shoots that develop from it ideal for use in clonal propagation.

ACKNOWLEDGEMENTS

We thank the Australian Flora Foundation for supporting this research. RM was the recipient of a Postgraduate Research Scholarship, University of Adelaide.

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FIG. 8. Light micrograph of transverse section of *B. serrata* seedlings (refer to Fig. 4) showing mid and upper cotyledonary node anatomy at 26 weeks after seed sowing. A, The cotyledon base is still attached to the stem 80 µm above section in Fig. 7C. The lower cotyledonary axillary bud is visible at the LHS of the micrograph (large arrow). Adventitious buds are present between the cotyledonary laminae (bottom of micrograph) and meristematic tissues (small arrows) are located on the adaxial wall of the cotyledons and also on the stem surface near the cotyledonary axillary bud. An asterisk denotes incisions scored into the sides of the cotyledons to assist fixative penetration. Bar = 500 µm. B, Higher magnification of A showing adventitious buds at varying developmental stages between the cotyledonary laminae and meristematic tissues (arrow upper LH corner) on the adaxial surface of the cotyledonary laminae appressed to the stem. Bar = 250 µm. C, Upper cotyledonary node, 120 µm above A showing a cotyledonary axillary bud in the axil of each cotyledon. Meristematic bud generating tissues (arrow), denoted '†' in Table 1, at the base of the upper cotyledonary axillary bud are presented at higher magnification in D. Incisions into the sides of cotyledons to assist fixative penetration are denoted by an asterisk. Bar = 500 µm. D, Meristematic tissue extending from each cotyledonary axillary bud down into the groove on the adaxial wall of the cotyledon and the stem surface can be seen (arrows). Bar = 100 µm. E, Cotyledonary axillary bud 120 µm above that shown at the LHS of C showing typical exogenous axillary bud structure; endogenous accessory buds are absent. Bar = 250 µm. F, Cotyledonary axillary bud 360 µm above the RHS bud shown in C comprising a central apical dome (large arrow) with a prophyll on either side (smaller arrows). Bar = 250 µm.