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THE DEVELOPMENTAL ANATOMY OF CRYPTOGEAL GERMINATION IN BUNYA PINE (ARAUCARIA BIDWILLII)

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Bunya pine (*Araucaria bidwillii* Hooker) produces large seeds that consist of a massive megagametophyte that surrounds an embryo 25–30 mm long and 3–5 mm in diameter. The seeds germinate on the soil surface, but elongation of the cotyledonary tube pushes the hypocotyl, with its associated plumule and radicle, into the soil (cryptogeal germination). The hypocotyl subsequently develops into a large parenchymatous tuber that is supplied with carbohydrates translocated from the megagametophyte through the cotyledonary tube. Later the epicotyl grows upward through the overlying soil to commence photosynthesis. Elongation of the cotyledonary tube from 3 to 9 cm in less than 7 d results entirely from cell elongation, principally in the proximal regions of the tube. Cell elongation is also important in the tenfold elongation of the hypocotyl in less than 2 wk, but a substantial number of anticlinal divisions followed by cell elongation also occur. The sevenfold increase in hypocotyl diameter involves both increase in cell diameter and periclinal cell divisions. When elongation of the cotyledonary tube ceases, the distal portion that remains embedded in the megagametophyte remains structurally unaltered for several weeks. In contrast, the tube outside the seed quickly develops tannin deposits in the surface layers, and between weeks 2–4 extensive collapse of the ground parenchyma cells occurs, leaving the vascular bundles suspended within a framework of collapsed cells. Unlike other cryptogeal species, bunya pine forms an abscission zone at the base of the cotyledonary tube, and thus an organized detachment of the tube from the tuber can occur. The initial divisions leading to abscission zone formation commence between weeks 2–3, and by week 4 the zone extends across the ground tissues but not the vascular bundles. The vascular system of the tuber consists of four to six pairs of vascular bundles that run parallel to each other, without anastomoses, for most of the length of the hypocotyl. At week 0 the vascular bundles were completely undifferentiated, but at week 1 they had typical collateral bundle tissue distribution and proportions, although abutting the abaxial side of the primary phloem was an unusual group of relatively large-diameter (25–50 μm) cells that never accumulated starch grains and elongated to over 2,500 μm in length. After several months the cambia in each pair of vascular bundles differentiate through the intervening parenchyma toward each other, eventually forming four to six small cambial rings. This results in some highly unusual patterns of secondary growth as several cylinders of secondary vascular tissues begin to develop within the storage parenchyma of the tuber.

Introduction

The 19 or 20 species of *Araucaria* have an uneven geographic distribution throughout the Southern Hemisphere and an uneven taxonomic distribution into the four sections of the genus. Australia and Papua New Guinea each have an endemic species (*A. bidwillii* and *A. hunsteinii,* respectively) and a common one (*A. cunninghamii*); ca. 13 species are endemic to New Caledonia, one species is endemic to Norfolk Island, and there are two species (*A. angustifolia* and *A. araucana*) in South America (Stockey and Ko 1986).

Araucaria angustifolia and *A. araucana* are placed in the section *Columbea* and *A. bidwillii* is in *Bunya,* while the remainder are in *Eutacta,* except for *A. hunsteinii,* which is in *Intermedia* (Haines 1983*a,* 1983*b;* Stockey and Ko 1986). *Araucaria bidwillii, A. angustifolia,* and *A. araucana* produce large seeds that have an unusual cryptogeal germination sequence that leads to the production of tuberous seedlings, while the other species have smaller seeds and a typical epigeal germination (Wilde and Eames 1948, 1952; Haines 1983*a;* Burrows et al. 1992). The differences in germination and several other contrasting characteristics indicate that *Bunya* and *Columbea* are closely related and are also relatively distant from *Eutacta* and *Intermedia* (Haines 1983*a*). While the unusual cryptogeal germination has been frequently described morphologically (Burrows et al. 1992), anatomical studies are fewer and have been largely based on limited material from a single developmental stage. The aim of this study was to undertake a developmental anatomical study of the germination of bunya pine, complementary to the morphological study of Burrows et al. (1992).

Material and methods

Bunya pine cones were collected in late summer on the day they fell from specimen trees growing at Wagga Wagga, N.S.W., and the mature seeds were extracted. The seeds were planted in sterile potting mix in 15-cm-diameter pots. Two seeds per pot were planted 1 cm below the surface, with the long axis positioned horizontally and the radicle end of the seed pointing toward the middle of the pot to permit unimpeded pseudoradicle

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emergence and subsequent hypocotyl enlargement. All pots were maintained in a shade house, under prevailing weather conditions.

Representative tissues from the megagametophyte, cotyledonary tube, hypocotyl/tuber, and root were collected on the day imbibition commenced and then weekly for a further 6–8 wk. Six seedlings were lifted each week and representative tissues were excised. Isolated collections were made from seedlings aged 12 wks to 1 yr. Tissues were fixed in 50% FAA, dehydrated in a graded tertiary-butyl alcohol series, embedded in paraffin wax, sectioned at 10 μm in a transverse or radial longitudinal plane, and then stained in either safranin–fast green or toluidine blue O.

In descriptions of the change from cotyledonary tube to hypocotyl anatomy the cross section through the extreme base of the cotyledonary tube is designated as 0 μm. Proceeding in a basipetal direction, from the 0 μm reference point, a minus sign is prefixed to distances to denote the position of a particular section. Five seedlings, two at week 2 and three at week 6, were serially sectioned, at 10 μm per section, from immediately below the abscission zone into the upper hypocotyl for a distance of 3–4 mm.

When assessing cell dimensions (tables 1 and 2), at least 30 cells in the specified region were measured and the mean and standard errors of the mean calculated.

Results

Morphology

Bunya pine produces large seeds that germinate on the soil surface, but elongation of the cotyledonary tube pushes the hypocotyl, with its associated plumule and radicle, into the soil (fig. 1*a*). The hypocotyl subsequently develops into a large parenchymatous tuber that is supplied with carbohydrates translocated from the megagametophyte through the cotyledonary tube (fig. 1*a*). Later the cotyledonary tube detaches from the tuber at an abscission zone and the epicotyl grows upward through the overlying soil to commence photosynthesis.

Embryo

Mature embryos consisted of three main components: the hypocotyl and the associated root and shoot meristems, the cotyledonary tube, and the "root cap" or calyptroperiblem (fig. 1*b*). The embryo was surrounded by, but not attached to, a massive megagametophyte that was composed of small epidermal cells and large (up to 200 μm diameter) parenchyma cells packed with starch grains (fig. 2). After several weeks of seedling development, these cells were devoid of starch (fig. 3), thus showing the complete utilization of the stored carbohydrate. Like the surrounding megagametophyte, almost all cells of the embryo were densely packed with starch grains (figs. 1*b*, 4).

The hypocotyl was 3–4 mm in length and 3 mm in diameter, excluding the overlying cells of the root cap. The bulk of the hypocotyl consisted of a parenchymatous ground tissue in which were embedded three concentric rings of resin canals and a ring of vascular bundles (fig. 23). Most commonly there were 10 bundles in five groups of two; less commonly, eight or 12 bundles, also in pairs. For most of the length of the hypocotyl the bundles were parallel to each other; i.e., there were no anastomoses. All cells of the bundles were nucleated, densely cytoplasmic, and largely undifferentiated, although some differences in cell size and wall thickening were apparent (fig. 27). The inner ring of 15–25 resin canals was associated with the vascular bundles, the outer ring of 50–95 canals was situated near the boundary between the hypocotyl and the root cap, and there was an intermediate ring of 20–30 canals (fig. 23). At the distal end of the hypocotyl was a small, densely staining, domed apical meristem that lacked leaf primordia (figs. 1*b*, 13). A plug of fibrillar or mucilaginous material was located above the apical dome, extending up into the cavity of the cotyledonary tube (fig. 1*b*).

The cotyledonary tube was 25–30 mm long, with the tip divided into small lobes or projections. Externally the tube was usually elliptical in cross section (2.5–3.5 × 5.0–6.0 mm), while the inner hollow was narrow oblong (0.1 × 2.5–3.5 mm) for most of its length (fig. 4). Near its base the hollow rapidly widened to become a conical cavity above the shoot apex. The inner and outer epidermal layers of the tube were composed of small, isodiametric cells that had minimal or no cuticular development, and both layers possessed well-developed sunken stomata (fig. 5). While the number of resin canals became progressively smaller (60–75 in total) toward the distal end of the cotyledonary tube, the number of vascular bundles increased from 12–15 at the base to 20–40 by the irregular splitting of a single bundle into two or three bundles. While the cells of the vascular bundles in the cotyledonary tube were largely undifferentiated, they were more differentiated than those in the hypocotyl, and they became progressively more differentiated toward the distal end of the cotyledonary tube (figs. 4, 6).

The massive root cap extended 4–5 mm from the root initial zone to the apex. Its overall length was doubled as it tapered up the sides of the hypocotyl and several millimeters of the cotyledonary tube (figs. 1*b*, 4, 7, 23). Most cells of the root cap had abundant starch deposits, although those of the column and the outermost layers had fewer grains. These latter cells often had tannin deposits that gave the root cap a slightly darker

Table 1

MEAN (± SE) LENGTHS (μm) OF PARENCHYMA CELLS FROM THE DISTAL, MIDDLE, AND PROXIMAL REGIONS OF THE COTYLEDONARY TUBE OF BUNYA PINE EMBRYOS AND SEEDLINGS OF VARIOUS AGES

	Distal	Middle	Proximal
Week 0 . .	46.5 (± 3.0)	42.5 (± 2.6)	40.3 (± 2.8)
Week 1 . .	83.3 (± 4.7)	128.5 (± 5.0)	168.5 (± 6.8)
Week 2 . .	92.8 (± 3.4)	149.8 (± 8.1)	168.0 (± 8.0)
Week 3 . .	85.3 (± 4.1)	151.3 (± 8.0)	159.8 (± 7.6)

appearance. The root cap appeared to be covered by the same mucilaginous material that formed the plug above the shoot apex.

COTYLEDONARY TUBE

WEEK 1. Between weeks 0–1 the cotyledonary tube elongated from 2.5–3.0 to 8.5–9.5 cm, which is more than 90% of its total elongation. In embryos from ungerminated seeds average lengths for parenchyma cells from the proximal, middle, and distal regions of the cotyledonary tube were 46.5, 42.5, and 40.3 μm, respectively (table 1). At week 2 the corresponding dimensions were 92.8, 149.8, and 168.0 μm (table 1), which represent elongations of × 2.0, × 3.5, and × 4.2, respectively. The cotyledonary tube of a mature embryo averages 2.5–3.0 cm in length. Assuming the distal third of the tube elongates by × 2.0, the middle third by × 3.5, and the proximal third by × 4.2, then the rapid elongation of the cotyledonary tube to 9 cm can be accounted for purely by cell elongation; cell division is not required. In cross sections of the proximal and middle regions of the tube at week 1 or 2, it appeared that there had been a rapid utilization of the starch (fig. 8). Longitudinal sections revealed that the starch grains had accumulated at the lower end of the 110–210-μm long cells, and thus a 10-μm thick cross section intercepted relatively little starch.

At week 1 the cells of the outer epidermal and subepidermal layers, in particular, those outside the seed, began to accumulate tannins or phenolic compounds. In the distal and middle sections of the tube the outer epidermal cells were evenly arranged and appeared to be covered by a thin cuticle, while in the more proximal regions the surface layers were not evenly arranged and were probably remnants of the root cap.

While at week 0 the vascular bundles were slightly less differentiated in the proximal regions (fig. 7) of the tube compared with the distal regions (fig. 6), this difference was largely lost at week 1 because of rapid differentiation of the vascular system. At week 1 the vascular bundles in the proximal regions of the tube consisted of (i) an arc of weakly lignified tracheids, two to three cells wide, (ii) a zone of thin-walled, angular, vacuolated cells that will differentiate as metaxylem, (iii) a poorly defined, incipient fascicular cambium, (iv) a thin band of primary phloem, and (v) a group of thin-walled, nucleated but mostly vacuolated cells that made up 30%–40% of the cross-sectional area of a vascular bundle (fig. 8). The number of functional tracheids increased markedly in the 7 d, as previously each bundle had only five to eight tracheids split into two or three groups. Progressing distally: (i) the incipient cambium became better defined, (ii) the phloem was usually thicker walled, and (iii) the group of cells on the abaxial side of the phloem was reduced in extent.

WEEK 2. Little further anatomical change had occurred except for additional accumulation of tannins, especially toward the base of the tube (fig. 8). In the vascular bundles the cambium was more defined, and primary xylem differentiation had proceeded to within one to two cells of the cambium. The annular secondary wall thickenings of the initial protoxylem tracheids were now widely separated, helical to scalariform thickenings had formed on the maturing tracheids, and a few tracheids in the later differentiated areas had circular bordered pits.

WEEK 3. Between weeks 2 and 3, a 30%–40% reduction in the diameter of the proximal region of the cotyledonary tube occurred (figs. 8, 9, 20, 21), brought about by two forms of cell collapse. First, some of the larger parenchyma cells near the vascular bundles lost turgor and separated from the majority of their neighbors (fig. 9). When

Table 2

MEAN (± SE) DIAMETER × LENGTHS (μm) OF PARENCHYMA CELLS FROM THE UPPER, UPPER-MIDDLE, AND MIDDLE REGIONS OF THE HYPOCOTYL/TUBER OF BUNYA PINE EMBRYOS AND SEEDLINGS OF VARIOUS AGES

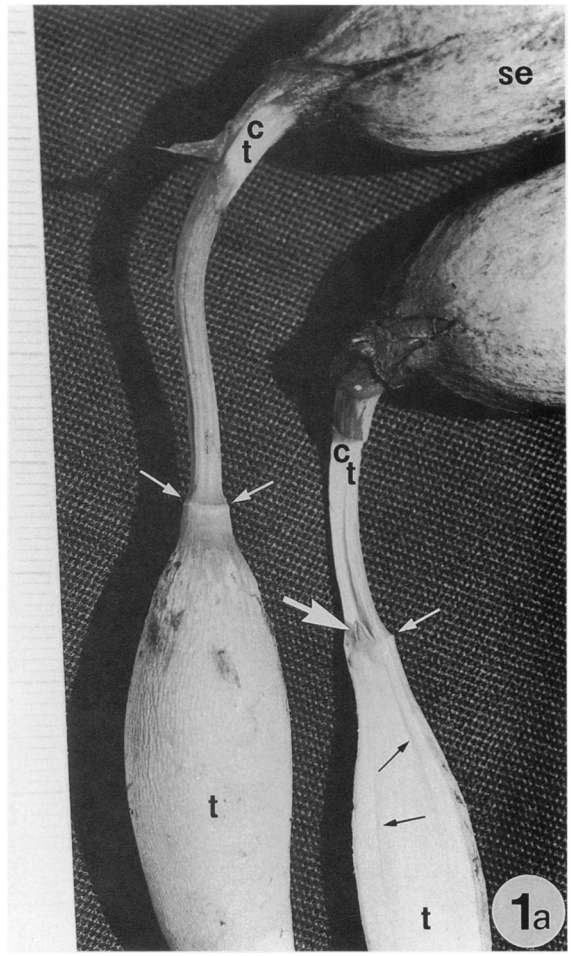
	Upper	Upper-middle	Middle
Week 0 . .	21.3 (± .5) × 38.5 (± 2.3)	21.3 (± .5) × 38.5 (± 2.3)	21.3 (± .5) × 38.5 (± 2.3)
Week 1 . .	25.3 (± .6) × 185.0 (± 7.6)	25.3 (± 1.0) × 114.8 (± 4.4)	26.8 (± .9) × 94.0 (± 5.0)
Week 2 . .	25.3 (± .8) × 176.0 (± 8.0)	48.3 (± 1.6) × 109.8 (± 7.2)	50.3 (± 1.5) × 116.0 (± 6.1)
Week 3 . .	51.5 (± 1.6) × 153.3 (± 8.3)	89.8 (± 5.7) × 95.0 (± 3.3)	88.0 (± 3.8) × 94.0 (± 3.7)
Week 4 . .	52.3 (± 1.7) × 139.8 (± 5.7)	79.8 (± 3.4) × 103.5 (± 3.7)	93.3 (± 4.7) × 106.0 (± 4.7)

this occurred in several cells in close proximity to each other, a large intercellular space was formed. Second, the majority of the smaller cells located near the outer and inner epidermises collapsed, except for the tannin and resin canal epithelial cells, but without any cell wall separation (figs. 9, 10). The numerous intercellular air spaces between these cells maintained their original shape. Thus, in cross section, this tissue appeared to be composed of numerous small, thick-walled triangular cells that are actually intercellular air spaces surrounded by the walls of collapsed cells (figs. 10, 11).

WEEK 4. Cell collapse and lacunar development was now more extensive in the lower cotyledonary tube and had started to progress acropetally through the tube. The surface ridges and furrows that formed as the tube's diameter decreased were not correlated with the internal anatomy; i.e., each ridge was not associated with a vascular bundle.

WEEKS 5–13. At week 5 breakdown of the ground parenchyma in the cotyledonary tube outside the seed was so extensive that the vascular bundles, and an encircling sheath of intact parenchyma cells, were suspended in a network of air canals (fig. 11). In contrast, the cotyledonary tube that remained surrounded by the megagametophyte showed little anatomical change from weeks 1–5 (fig. 12). At week 5 there was some breakdown of the parenchyma cells between the tube cavity and the vascular bundles (fig. 12), and at week 13 the breakdown was more extensive but still largely restricted to this area. Between weeks 5–6 some parenchyma cells abutting the xylem and some cells located between the vascular bundles in the distal tube differentiated as tracheids with a reticulate pattern of secondary wall material or, less often, as tracheids with bordered pits. At week 13 a ring of these tracheids linked the vascular bundles.

At week 6 the cambium in the proximal tube was well defined, but relatively little cell division had occurred, and most of this was for phloem, not xylem, production. The thin-walled cells to the outside of the phloem began to distort by week 4, and by weeks 6–8 these cells and the primary phloem had been flattened, resulting in a distinct layer of crushed cells (fig. 11).



SHOOT APEX

At week 0 the shoot apex was a rounded dome with no leaf primordia (figs. 1, 13). At week 1 a marked widening of the basal region of the shoot apex occurred that was associated with the initiation of several leaf primordia on the lower

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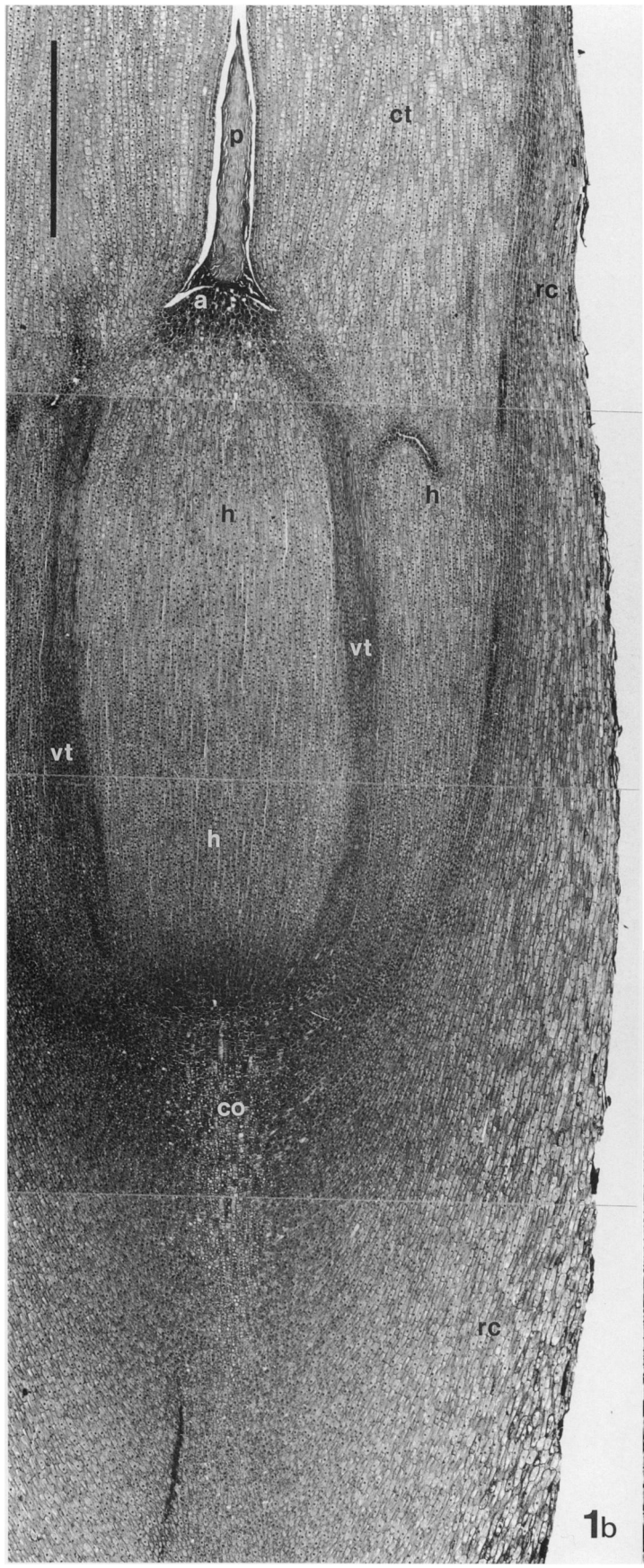
Figs. 1–5 Sections of bunya pine seed tissues. Fig. 1*a*, Bunya pine seedlings, ca. 4 wk of age. The right-hand-side seedling has been sectioned longitudinally through the tuber (*t*) and proximal cotyledonary tube (*ct*). Note that the distal cotyledonary tube remains in the seed (*se*), where it is surrounded by the megagametophyte. Note also the ridged nature of the proximal cotyledonary tube, the hollow in the cotyledonary tube of the right-hand-side seedling, the abscission zones (small white arrows), the shoot apex (large white arrow), and the developing periderm and vascular tissues (black arrows) of the tuber. Scale in mm. Fig. 1*b*, Radial, near-median, longitudinal section of a mature embryo. *a*, shoot apex; *co*, column; *ct*, cotyledonary tube; *h*, hypocotyl; *p*, plug; *rc*, root cap; *vt*, vascular tissue. Bar = 1,000 μm. Fig. 2, Cross section of megagametophyte at week 0. Bar = 250 μm. Fig. 3, Cross section of megagametophyte at week 13. Note the almost complete mobilization of the starch grains that were present at week 0. Bar = 250 μm. Fig. 4, Cross section of proximal cotyledonary tube at week 0. Note the thin slit in the center of the tube, the resin canals (small arrows), the ring of vascular bundles (large arrow), and the overlying cells of the root cap. Bar = 750 μm. Fig. 5, Cross section of stoma from inner epidermis of cotyledonary tube at week 0. Bar = 30 μm.



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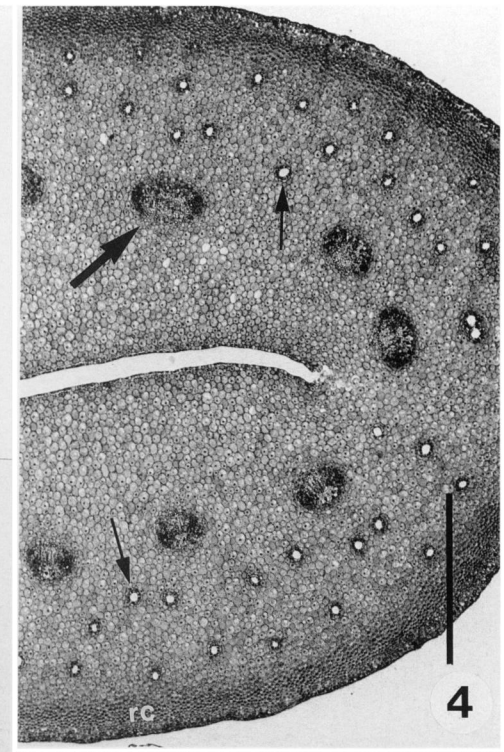

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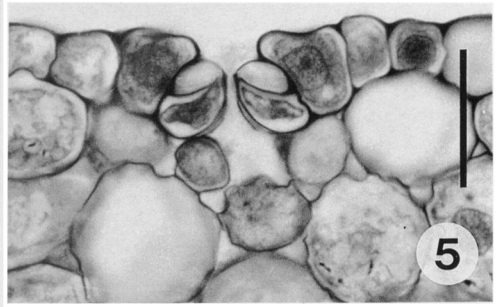

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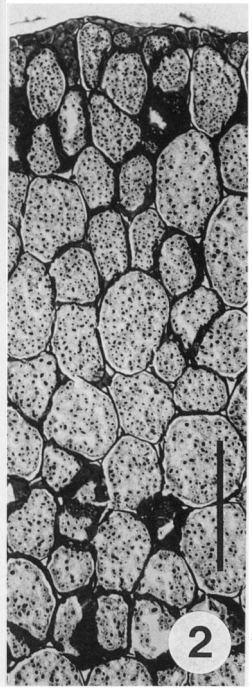

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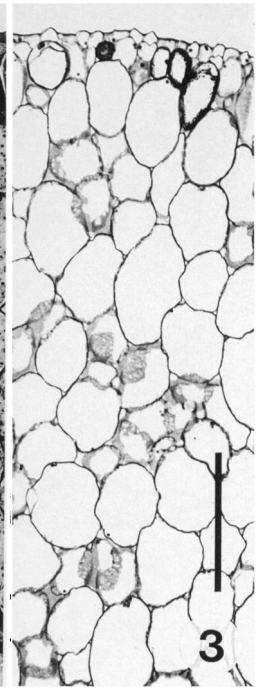

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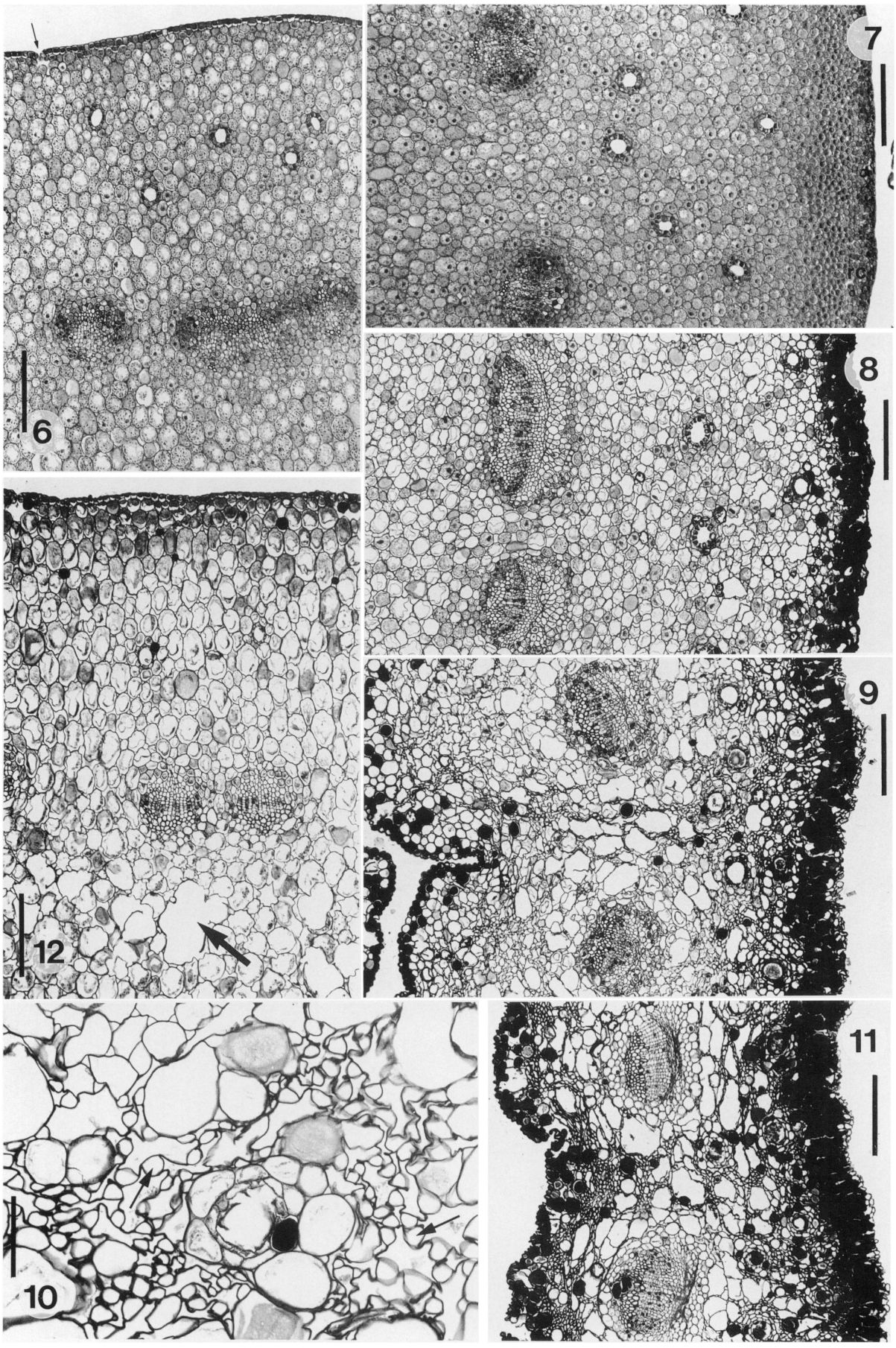
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flanks of the dome (fig. 14). By week 2 a marked increase in the height of the shoot apex had occurred, and the larger leaf primordia were starting to elongate up into the cotyledonary tube hollow (figs. 15, 16). Sectioning leaf axils from the developing shoots revealed the presence of axillary meristems. Subsequent shoot development provided the force necessary to detach the cotyledonary tube from the tuber at the abscission zone (fig. 1*a*). Initially the shoot had reduced leaf expansion, no chlorophyll development, and short internodes as it elongated through the overlying potting mix. Once above ground level, normal shoot morphology was established.

Integration of the cotyledonary tube and hypocotyl vascular systems

In the specimens examined, 13–16 vascular bundles were present in the lower part of the cotyledonary tube, and they were arranged in pairs or groups of three bundles (fig. 16). These bundles continued into the hypocotyl and maintained the same shape and relative positions to at least −500 μm. Between −700 and −800 μm the bundles within each group began to merge, gradually resulting in five or six blocks of vascular tissue (fig. 17). At −600 μm five or six areas of provascular tissue had differentiated from the peripheral meristem of the shoot apex. These areas were ca. 400 μm in length, as seen in cross section, and gave the vascular tissue a pentagonal or hexagonal appearance (fig. 17). Between −800 and −1,200 μm the groups of vascular tissue from the cotyledonary tube began to align radially with the gaps in the shoot apex vascular system (fig. 17).

At ca. −1,000 μm the previously oblong segments of provascular tissue of the shoot apex supply began to arc, with the tips pointing outward. At ca. −1,500 μm fusion of the cotyledonary tube vascular bundles into five or six units was largely complete, and their cambia curved inward to join with the tips of the incipient cambia of the arcs of the shoot apex vascular system. This produced, between −1,600 and −1,800 μm, a continuous ring of vascular tissue with the appearance of a cog wheel (fig. 18). Throughout the hypocotyl to cotyledonary tube junction the ring of cotyledonary tube vascular bundles maintained a relatively constant diameter of 2.4–2.7 mm. The epicotyl vascular system, when first discernible, had a diameter of 1.1 mm and gradually increased to 1.9–2.1 mm, at which stage the segments of the two rings were close enough for fusion to occur.

From ca. −2,000 μm the ring began to divide into segments. In some seedlings the ring first divided in the shoot apex vascular tissues, with each half integrating with the adjacent segment of cotyledonary tube vascular tissue (fig. 19). These large arcs of vascular tissue then divided to give the 10–12 strands of vascular tissue that extended most of the length of the tuber. In other seedlings, the ring first divided in the middle of the cotyledonary tube vascular tissues and later in the shoot apex tissues. From these observations it is possible to discern which bundles constitute a pair, as their xylem groups face each other. Thus, between −600 and −1,500 μm there were two concentric rings of vascular tissue of different function, origin, degree of differentiation, and cell composition, and below −3,000 μm there was a single ring of separate vascular bundles. Only between −1,600 and −2,500 μm was there a single continuous ring of vascular tissue.

Abscission zone of the cotyledonary tube

Week 3. Between weeks 0 and 2 there was no anatomical evidence of an abscission zone, and hence it was not a predetermined structure laid down in the embryo. Commencement of abscission zone formation began just before week 3 with a series of anticlinal divisions in the elongated parenchyma cells near the bottom of the cotyledonary tube (fig. 20). The abscission zone formed perpendicular to the cotyledonary tube surface, ca. 600 μm above the base of the shoot apex. At this early stage a uniform formation of the abscission zone had not occurred, as some sectors were further advanced than others.

Week 4. Through further divisions the abscission zone became a relatively uniform structure that extended around the entire base of the cotyledonary tube (fig. 21). The cell layers immediately to the outer side of the phellogen-like layer began to accumulate tannins (fig. 21). Ab-

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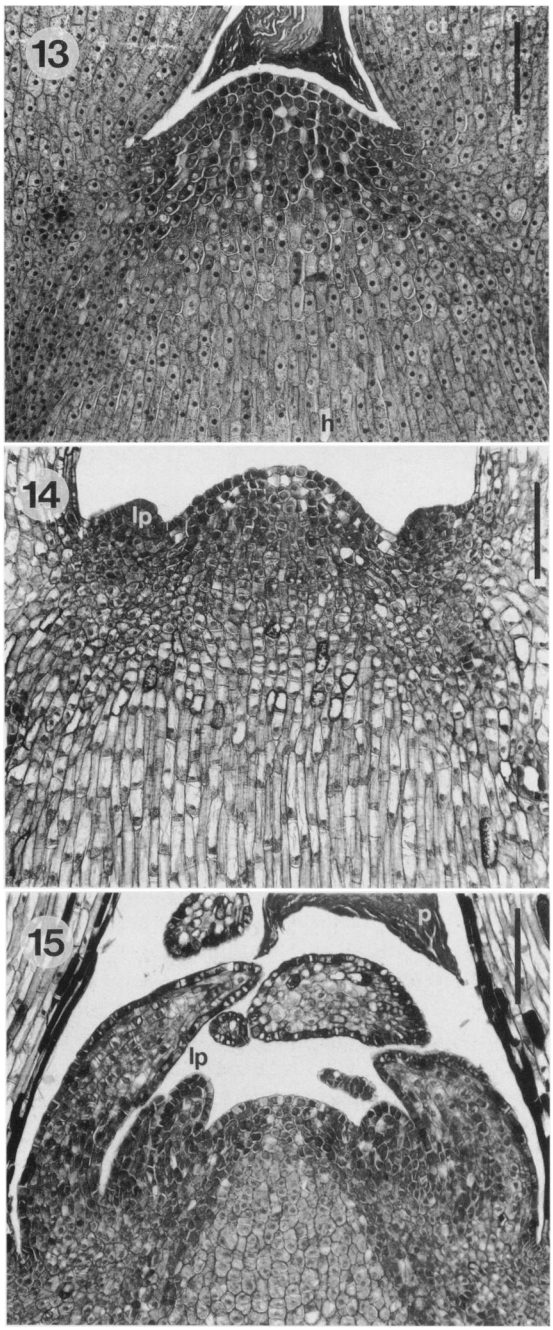
Figs. 6–12 Cross sections of the cotyledonary tube from bunya pine embryos and seedlings of various ages. Figs. 6 and 12 are of the distal part of the tube, while figs. 7–11 are of the proximal region. Bars = 200 μm for all figs. except fig. 10, where the bar = 50 μm. Fig. 6, Week 0. Note the abundant starch grains and stoma (arrowed). Fig. 7, Week 0. Note the overlying cells of the root cap. Fig. 8, Week 2. Note, compared to week 0 (fig. 7), the tannin accumulation in the epidermal and subepidermal cells, the vascular differentiation, and the apparent starch mobilization. Fig. 9, Week 3. Note the decrease in tube thickness associated with the extensive cell collapse. Fig. 10, Week 4. Note that what appear to be small triangular cells (arrowed) are the intercellular air spaces between collapsed cells. Fig. 11, Week 8. Note that the vascular bundles are surrounded by a sheath of intact cells, supported in a network of collapsed cells. Fig. 12, Week 5. Note that relatively little structural change has occurred, compared to the proximal areas; however, some limited breakdown is arrowed.



Figs. 13–15 Median radial longitudinal sections of the shoot apex of bunya pine. Bars = 200 μm. Fig. 13, Week 0. Note the absence of leaf primordia and the plug of material in the hollow of the cotyledonary tube. Fig. 14, Week 1. Note the leaf primordia (*lp*) on the lower flanks of the apical meristem and the marked elongation of the pith cells below the apex. Fig. 15, Week 2. Note the leaf primordia elongating up into the cotyledonary tube cavity and the widening of the shoot base.

scission zone divisions were restricted to the ground parenchyma, and the vascular tissues that traversed the zone remained intact and apparently functional (fig. 21). As the parenchymatous tissues of the proximal cotyledonary tube collapsed (figs. 21, 22), the position of the abscission zone became externally obvious when it formed a raised ring between the tube and tuber (fig. 1a). At its perimeter the abscission zone curved down to join with the phellogen that was developing in the tuber, thus giving continuity of protection. Through widening and lengthening of the developing bud base, the inner part of the abscission zone was displaced upward, giving the abscission zone an S-type profile in longitudinal section (fig. 21).

WEEKS 5–7. Continued tannin deposition resulted in a distinct band of tanniniferous cells three to four layers thick across the distal face of the abscission zone (fig. 22). Usually there was little tannin deposition on the proximal side of the layer, although some cell files leading from the phellogen had dark-staining contents. The first detachments occurred at weeks 5–6, during handling associated with material collection, although they would probably not occur this early under natural conditions. Separation occurred at the outside of the tannin layer and was never observed to occur at the phellogen or "separation zone."

HYPOCOTYL

Cell elongation and increase in cell diameter are required for the hypocotyl to elongate tenfold in 2 wk and increase in diameter four- to sixfold in 5 wk to develop into a tuberous structure (table 2). As with the cotyledonary tube, cell elongation is important for lengthening of the hypocotyl (compare cell lengths weeks 0–1; figs. 13, 14); however, anticlinal cell divisions also occurred. This is indicated by the progressive reduction in cell length in the upper parts of the tuber (table 2), the absence of cells ca. 400 μm in length, and the presence of division figures. These figures were most commonly observed several millimeters behind the shoot apex, and it appeared that most of the continued elongation occurred in this region from cell division followed by cell elongation.

While cell elongation was rapid between weeks 0 and 1, there was little increase in cell diameter

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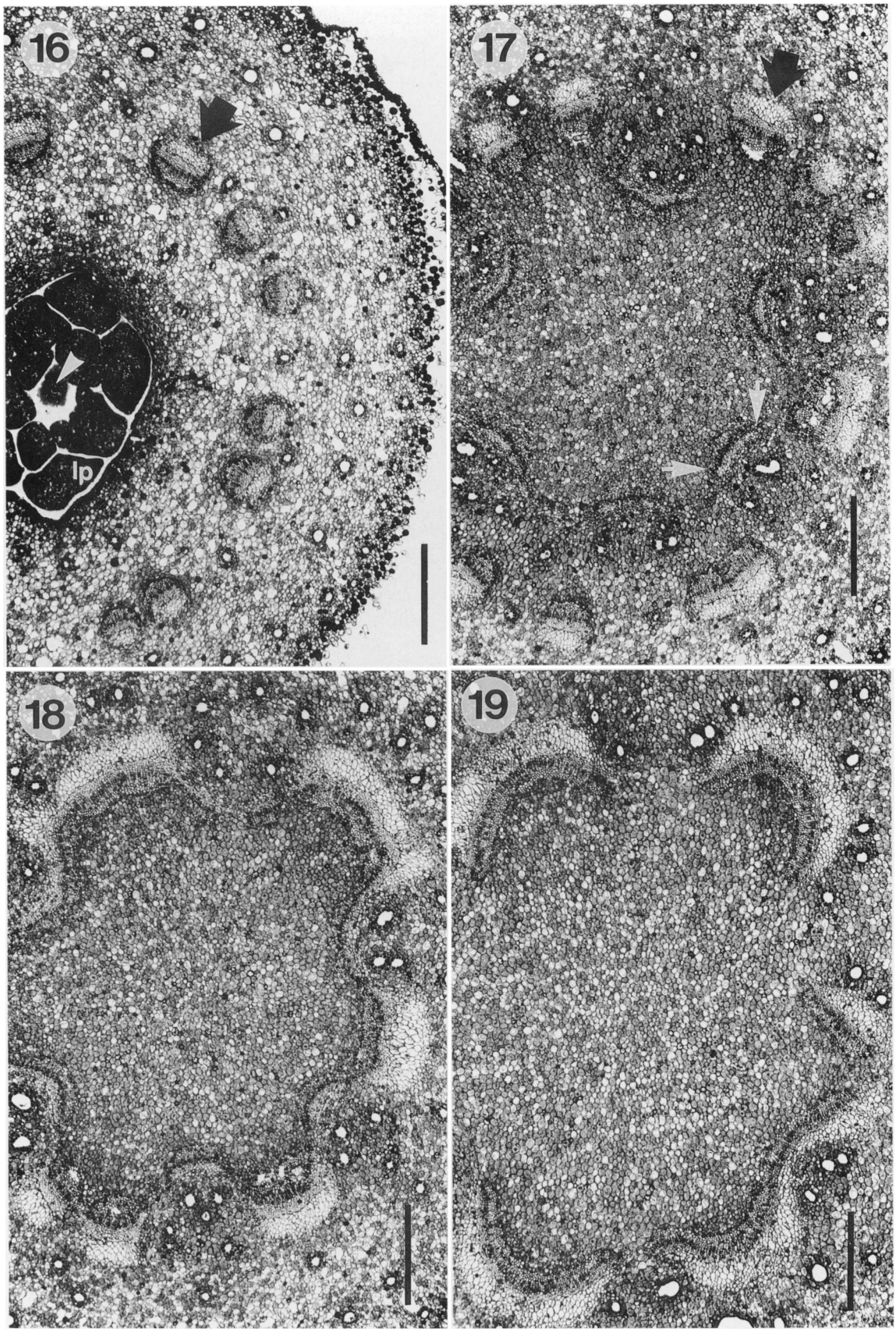
Figs. 16–19 Cross sections illustrating the vascular transition between the cotyledonary tube and the hypocotyl at week 2. Bars = 500 μm. Fig. 16, +80 μm. Note the leaf primordia in the cotyledonary tube hollow and the tip of the apical meristem (white arrow). Note also that the vascular bundles (black arrow) are in two groups of two and one group of three. Fig. 17, −1,100 μm. Note that the cotyledonary tube vascular bundles (black arrow) are in the same relative positions and six blocks of vascular tissue associated with the shoot apex have differentiated (white arrows). Fig. 18, −1,800 μm. Note that the shoot apex and cotyledonary tube vascular tissues have joined, giving the appearance of a cog wheel. Fig. 19, −2,800 μm. Note that the cog wheel has begun to divide into discrete segments, first in the shoot apex vascular tissues and later in the cotyledonary tube vascular tissues.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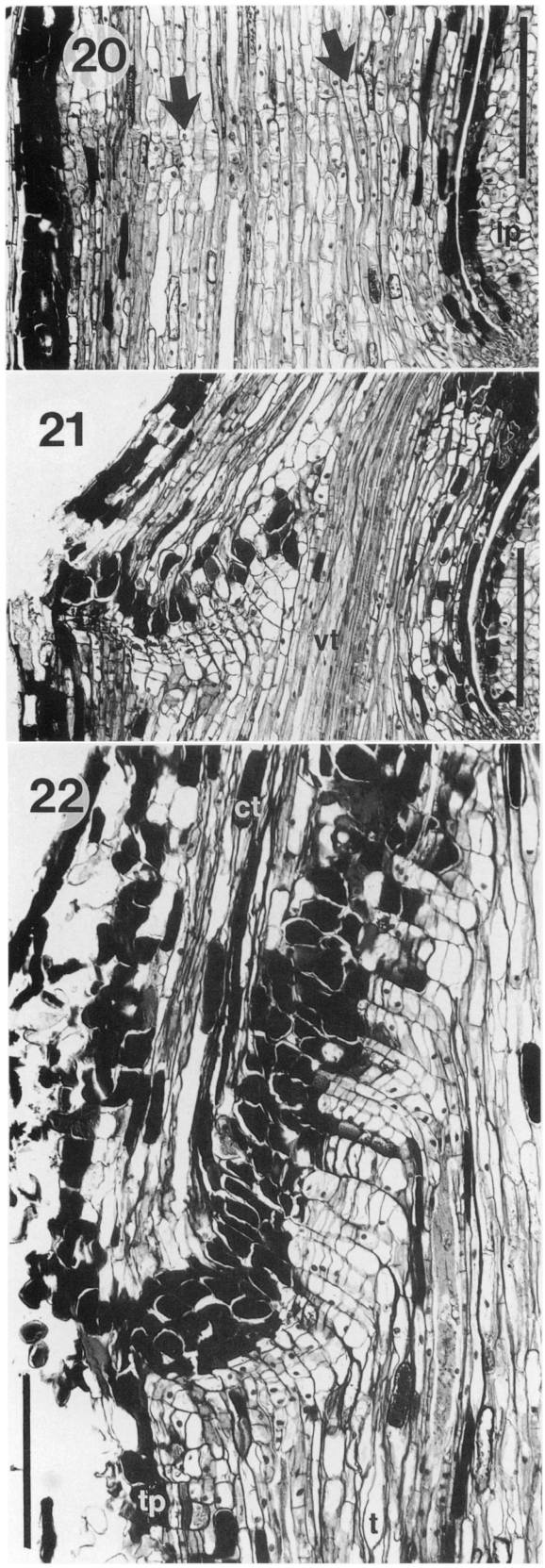

during this period (figs. 23, 24; table 2). Between weeks 1 and 3 most cells increased in diameter, on average, by two to four times (figs. 24–26; table 2), but this is probably insufficient to account for the increase in hypocotyl diameter. Comparison of the hypocotyl at weeks 2 and 3 showed that periclinal divisions began to occur in the storage parenchyma cells (figs. 25, 26). The first periclinal divisions leading to phellogen formation occurred at week 2 in cells three to six cell layers beneath the surface of the developing tuber. This, combined with the irregular nature of the surface because of its root cap origins (fig. 23), meant that at first the phellogen was quite convoluted, as viewed in cross section. By week 3 a well-defined periderm was present (fig. 26).

Hypocotyl/tuber vascularization

In embryos from dormant seed the trend of progressive reduction in vascular differentiation from the top to the base of the cotyledonary tube continued into the hypocotyl. Whereas in the lower cotyledonary tube the vascular bundles possessed some differentiated tracheids, and the future limits of the xylem and phloem were clearly defined (fig. 4), in the upper hypocotyl all vascular tissues were completely undifferentiated (fig. 27). Their smaller cell size and densely staining cytoplasm and the absence of starch grains distinguished them from ground tissues (figs. 23, 27).

Comparison of the hypocotyl at week 0 (fig. 27) and week 1 (fig. 28) illustrates a series of pronounced structural changes that resulted from less than 7 d of physiological activity. At week 1 the vascular bundles in the middle to upper hypocotyl were similar in tissue distribution to those in the lower cotyledonary tube but were much larger in cross sectional area. In the lower hypocotyl the bundles were still largely procambial. In the upper and middle regions they were composed, proceeding radially outward, of: (i) A band, two to three cells wide, of mainly helically thickened primary xylem tracheids. There appeared to be fewer stretched and elongated tracheids than in the cotyledonary tube. The tracheids that had differentiated by week 1 appeared to be the only primary xylem formed, and the seedling was not

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Figs. 20–22 Longitudinal sections through the abscission zone at the base of the cotyledonary tube. Fig. 20, Week 3. Note the anticlinal divisions (arrowed) through the parenchyma, just above the base of the tube. Bar = 400 μm. Fig. 21, Week 4. Note the S-shaped profile of the abscission zone and the absence of abscission zone divisions in the vascular tissues. Bar = 400 μm. Fig. 22, Week 6. Note that the phellogen of the abscission zone has joined with the phellogen of the tuber (*tp*). Note also the extensive tannin or phenolic deposits to the outside of the abscission zone and the collapse of the parenchyma in the cotyledonary tube. Bar = 300 μm.

supplemented by new tracheids until the production of secondary xylem started several weeks later. (ii) A band of undifferentiated metaxylem. (iii) An incipient fascicular cambium that began division around week 6 and initially proceeded to produce more phloem than xylem. (iv) A narrow band of primary phloem. (v) A large area (up to 40%–50% of the cross-sectional area of the vascular bundle) of cells conspicuous for their large diameter, thin walls, angular arrangement, and almost complete vacuolation. When starch grains began to form in the tuber between weeks 1 and 2 (fig. 29), these cells remained conspicuously free of starch deposits, even until week 8 or later (fig. 30). In the embryo these cells were, on average, 150–200 μm, and often up to 225 μm, in length (fig. 31), whereas the average cell length for the surrounding ground tissue was 45 μm. The end walls were usually at 45°, and in some the cell contents had collapsed into bodies similar in appearance to slime plugs as described in phloem (fig. 32). While the surrounding cells had a normal arrangement and number of pits and pit fields, these cells appeared to have no cell wall pitting (fig. 32). At week 3 many of these cells were at least 2,000–2,500 μm in length (fig. 33). Unlike the situation in the cotyledonary tube these cells remained intact until at least week 8, although some distortion, probably caused by secondary phloem production, began at week 5 (fig. 30). By year 1, if not sooner, these cells had been totally flattened (figs. 34, 35). This cell type made up less than 20% of the vascular bundle cross-sectional area in the upper cotyledonary tube, but was between 50% and 60% in the hypocotyl bundles. This tissue type was not found in aboveground stems or leaf tissue from seedlings 6–18 mo of age.

Secondary growth

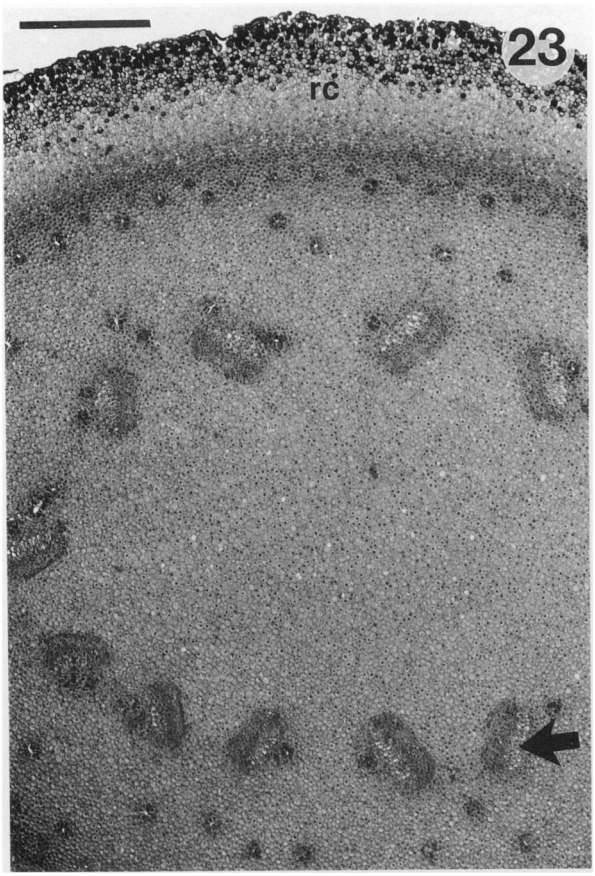
During weeks 5–7 the cambium in each vascular bundle, in the upper to middle regions of the tuber, began to extend its width by differentiating out into the adjacent ground parenchyma (fig. 30). These extensions initially curved sharply inward toward the primary xylem and then began to straighten. Over several months the outer and inner extensions of the cambia of a pair of bundles differentiated toward each other and eventually joined, usually to the outside first and then the inner, forming a convoluted ring. The period of cambial activity is shown by the relative differences in secondary xylem production (figs. 34, 35). As the bundles in each bundle pair are at different angles to each other and at different distances apart, at different levels in the hypocotyl, the cambial rings form different shapes depending on where sections are cut (figs. 34, 35). Based on morphological observations of numerous tubers sliced into 1–2-mm thick disks, it appeared that each of the four to six rings continued to increase in diameter, the ground parenchyma absorbing the associated stresses. Eventually the phloem and cambia of the cylinders of secondary growth must be forced into each other, and a form of grafting probably occurs. This results in a large outer cambial ring formed from four to six outer cambial arcs and a smaller inner ring formed from the inner arcs. The inner ring ceases activity relatively quickly, probably from the stresses associated with constricting in on itself, while the outer ring begins to function as a typical vascular cambium.

Lower hypocotyl/tuber

At week 4, in the lower third of the developing tuber, an incomplete ring one cell wide developed to the outside of the vascular bundles (fig. 36). The ring was discernible because while its cell walls stained in a typical manner, the cell contents, including the cytoplasm and at a later date starch grains, were uniformly brownish. The ring was best developed near the vascular bundles and curved inward in this region (fig. 36). Progressing toward the roots, the ring appeared to become continuous with the endodermis of the root, and hence this ring is referred to as an endodermis, even in the middle to lower tuber region.

While the initial divisions leading to lateral root formation probably commenced around week 3, the first root primordia were sectioned at week 4 (fig. 37). They only formed in the parenchyma cells immediately to the exterior of each pair of vascular bundles, but inside the endodermis. At week 4 the root primordia consisted of a small dome of cells, without differentiated vascular connections (fig. 37). They were not externally obvious but were associated with small pustules on the tuber surface. As the vascular bundles are parallel to each other for most of the length of the hypocotyl, this explains why the lateral roots form in four to six parallel rows. The periderm was generally restricted to the surface layers of the tuber, but at each root primordium the phellogen differentiated inward to the base of the primordium, thus sealing the areas of the cortex to be exposed by root emergence. This gave the phellogen a convoluted appearance and the cut-off cells formed the pustules. At the base of the tuber the phellogen followed the endodermis and cut off all of the cortex.

Note that compared with the middle tuber region (figs. 25, 26), for each pair of vascular bundles the bundles are much closer together, the angle of one bundle to the other is greater, and an arc of xylem links the bundles together (fig. 36). This is the start of the transition from hypocotyl or tuber anatomy to typical root anato-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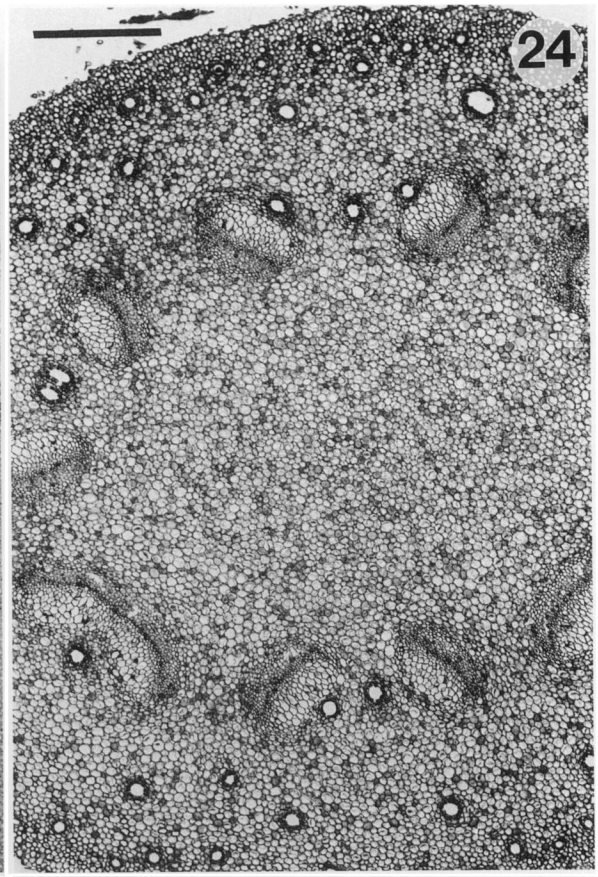
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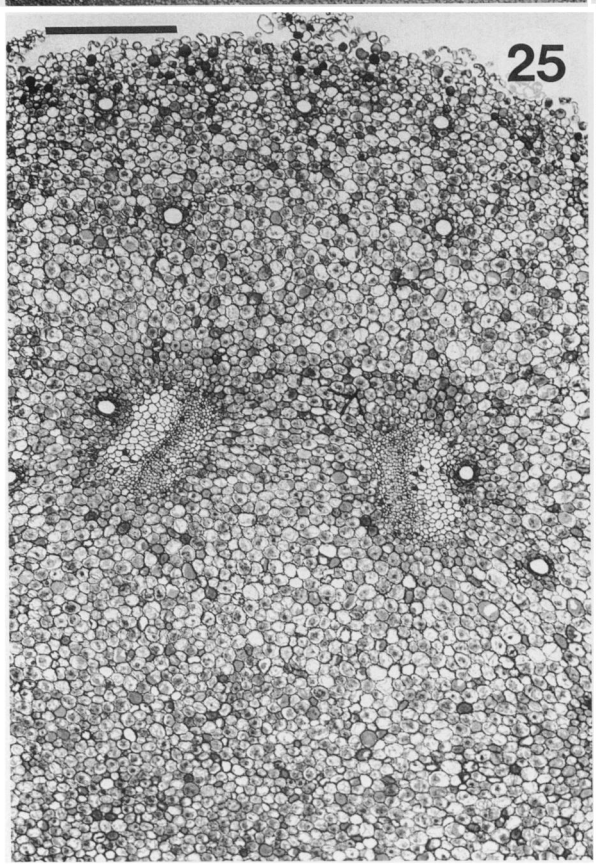
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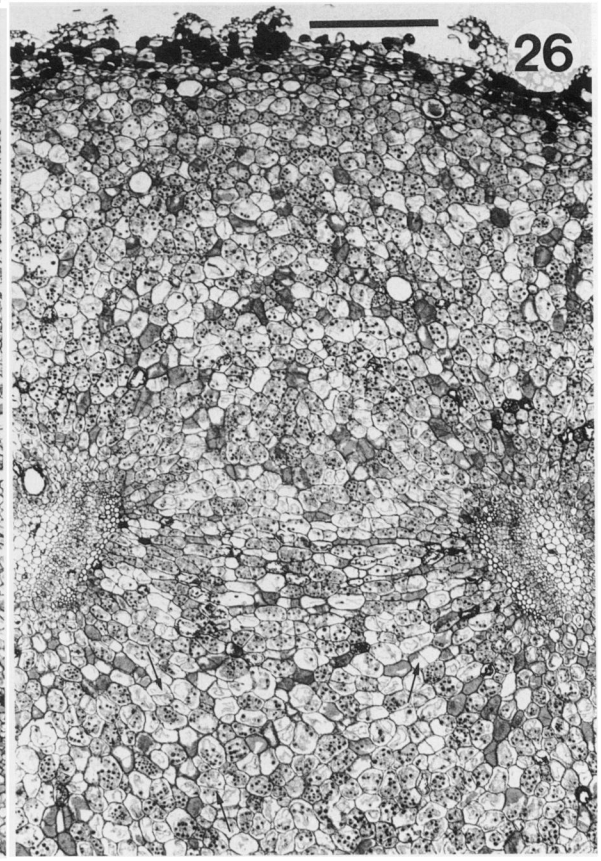
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my. The transition from cotyledonary tube to hypocotyl vascular arrangement occurred over a relatively short distance (3–4 mm), while the transition from hypocotyl/tuber vascular arrangement to a diarch root anatomy occurred gradually over several centimeters.

Discussion

Comparison with other bunya pine studies

The morphological aspects of bunya pine germination were known in the mid-nineteenth century (Dürr 1864) and have been described, at varying levels of detail, several times since (Burrows et al. 1992). In contrast, there have been few anatomical studies, and most of these have described limited material of a single age, which probably reflects problems with seed supply, storage, and viability. Haines (1983*a*) provided detailed descriptions and illustrations of the mature embryo, while Shaw (1909), Stockey and Taylor (1978), Rouane and Woltz (1979), Woltz (1986), and Stockey et al. (1990) mainly described various aspects of the tuber. None of these papers described the mode of cotyledonary tube lengthening, its subsequent tannin accumulation and eventual collapse, abscission zone formation, epicotyl growth, vascular ontogeny in the tuber, lateral root initiation, or patterns of secondary growth.

In general, the observations recorded in the above papers correspond to those made in this study. Rouane and Woltz (1979), Haines (1983*a*), and Woltz (1986) noted the increase in the number of vascular bundles from the base to the tip of the cotyledonary tube. Woltz (1986) also illustrated the eventual breakdown of the cotyledonary tube that remains surrounded by the megagametophyte and described the formation of transfusion tissue between the vascular bundles in the distal part of the tube. The concentric rings of cotyledonary tube and plumule vascular bundles at the top of the tuber have been previously illustrated and the presence of a diarch root also noted (Shaw 1909; Stockey and Taylor 1978, fig. 13). The shoot apex of the bunya pine embryo, in comparison with the South American species, has been described as "undeveloped" (Wilde and Eames 1952). This indicates that the shoot apex is only a small dome, and perhaps the apices of the South American species are larger and may possess leaf primordia.

The presence of stomata in the embryonic cotyledonary tube has previously been reported for bunya pine (Haines 1983*a*) and in the separate cotyledons of *Araucaria angustifolia* (Ferreira 1981), but not the investigated *Eutacta* species (Haines 1983*a*). As reduction in cotyledon numbers, cotyledon fusion, and cryptogeal germination are considered derived features (Haines 1983*a*), this is regarded as retention of a primitive character (Haines 1983*a*), as there are only limited opportunities for gas exchange before or after germination in either the proximal or distal regions of the tube. The tube also possesses a large number of tracheids for the minimal water conduction that would occur, and thus they may also be considered to be retention of a character that is no longer essential. Differentiated tracheids are also present in the mature embryos of *A. angustifolia* (Burlingame 1915) but not in the investigated *Eutacta* species (Haines 1983*a*). It is unusual that characters that are no longer needed are well developed in the embryo of *Bunya* and *Columbea* species, yet develop only after germination in the *Eutacta* species, where they are essential.

We propose that the cells abutting the abaxial side of the phloem in the cotyledonary tube and the tuber may have a translocation function. This is based on the following observations: (i) these cells retain a thin peripheral layer of cytoplasm and are nucleated; i.e., they would be physiologically active; (ii) they are extremely elongated; (iii) they are in close association with the phloem; (iv) they never store starch, and this distinguishes them from the surrounding parenchyma cells; (v) they are best developed in the early stages of seedling development, when translocation from the megagametophyte to the hypocotyl is at a maximum; (vi) they appear not to be present in later-formed parts of the plant; (vii) they form a continuous system between the cotyledonary tube and the tuber; and (viii) after fixation and sectioning they sometimes possessed "slime plugs," which indicates a similarity to sieve cells. However, between weeks 5 and 8 these cells are crushed in the lower cotyledonary tube, and therefore the

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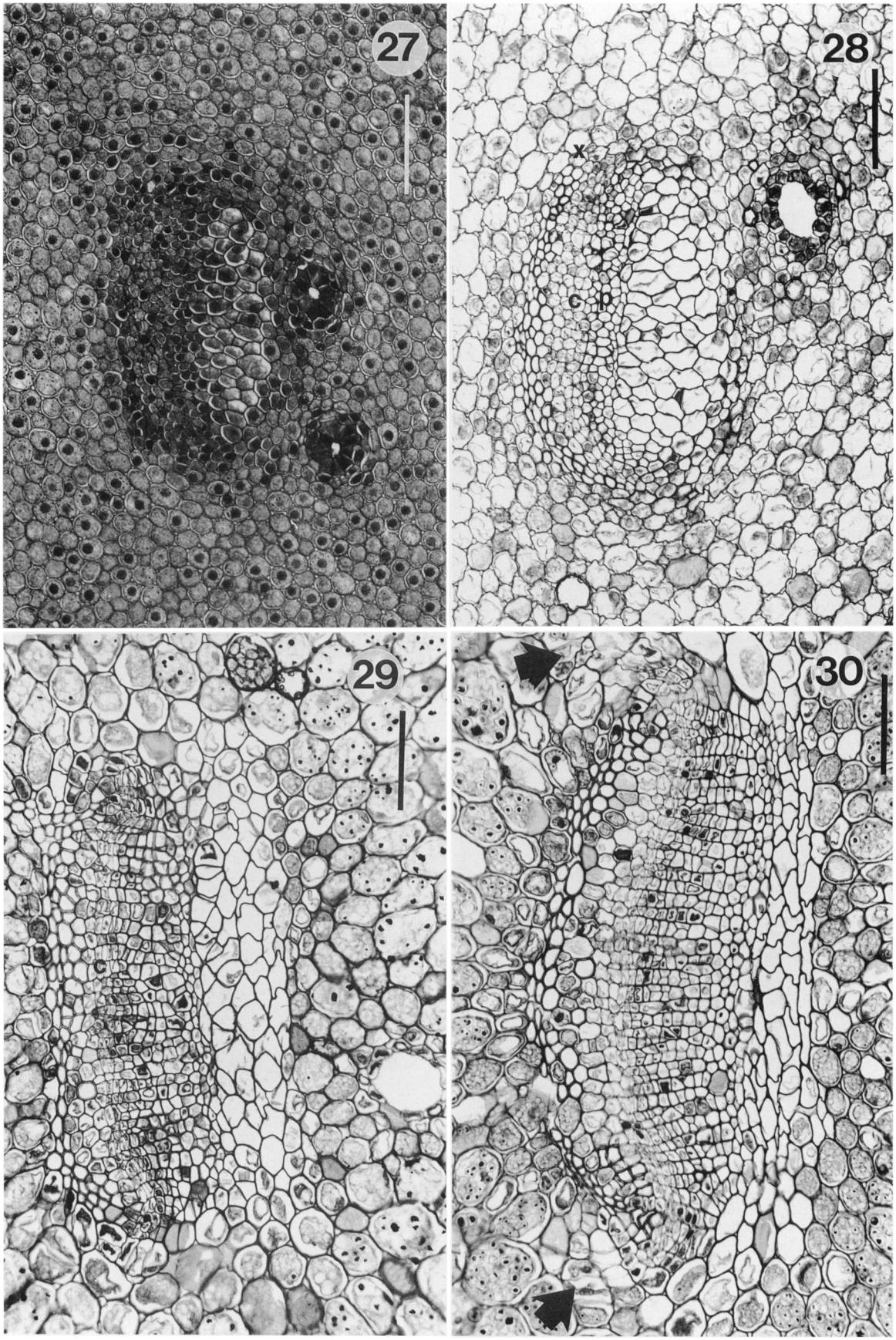
Figs. 23–26 Cross sections through the middle region of the hypocotyl/tuber of bunya pines of various ages. Bars = 500 μm. Fig. 23, Week 0. Note the ring of undifferentiated vascular bundles (arrowed) and the darkly staining interface between the hypocotyl and the root cap. Fig. 24, Week 1. Note the rapid differentiation of the vascular tissues and the rapid vacuolation of the cortical and pith parenchyma. Note also that only a slight increase in hypocotyl diameter has occurred. While evenly spaced, the vascular bundles that will form a pair have their xylem inclined toward each other. Fig. 25, Week 2. Note the rapid increase in hypocotyl diameter associated with cell division and cell diameter increase in the storage parenchyma. Fig. 26, Week 3. Note the starch accumulation, the continued increase in parenchyma cell diameter, and the newly formed cell walls within some of the parenchyma cells (arrowed). Note also the developing periderm.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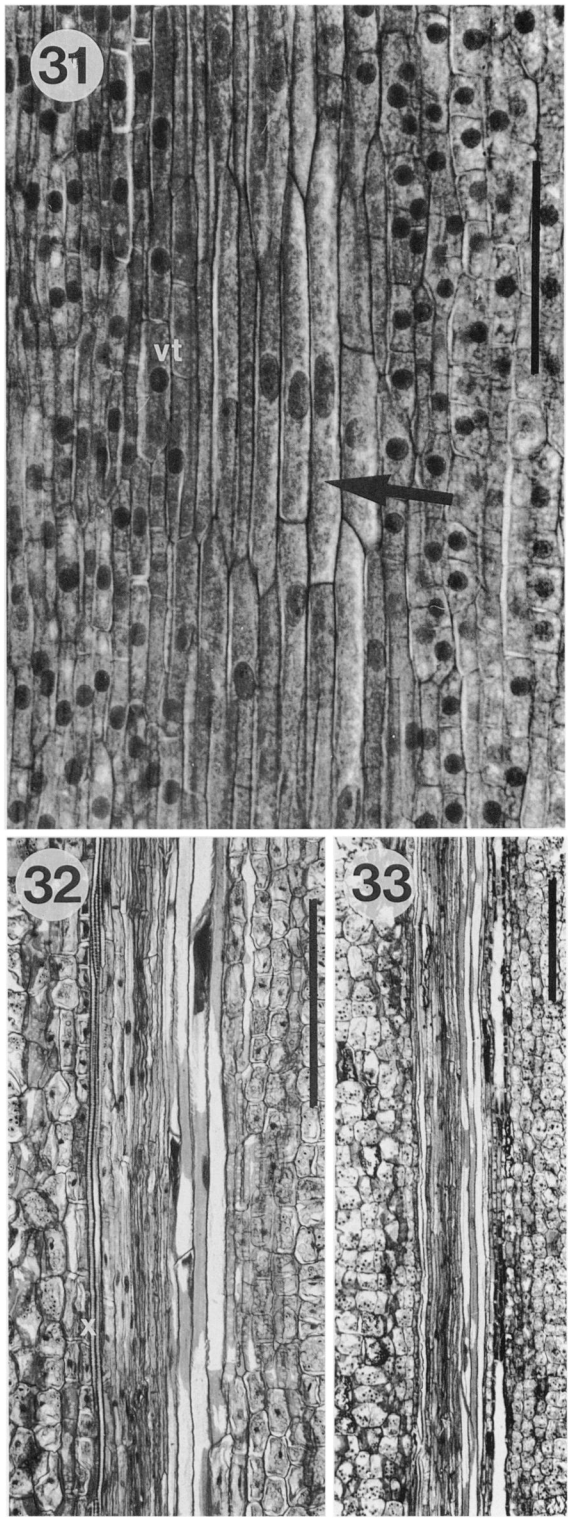
continuity of connection of this system between the megagametophyte, the cotyledonary tube, and the tuber is disrupted. The protophloic procambial cells of the dormant embryos of *Pinus lambertiana* are considerably longer and of greater diameter than the other procambial cells and are referred to as "distinctive" (Berlyn 1972). The protophloic procambial cells develop into protophloem (Berlyn 1972, fig. 40) and appear similar to the bunya pine cells described above (figs. 27–28, 31–33) in size, location, and distribution.

Vascular transition

Only Shaw (1909) had previously studied in detail the vascular transition from the cotyledonary tube to the hypocotyl in bunya pine. He examined hand-cut sections of numerous seedlings that had undergone cotyledonary tube abscission and had advanced secondary thickening. This meant that in the ring of vascular tissue at the top of the hypocotyl it was "impossible to discern the limits of the vascular tissue from either source," i.e., the cotyledonary tube vascular bundles or those associated with the plumule (Shaw 1909). Thus Shaw could not establish the relationship of the cotyledonary bundles to those in the hypocotyl. We studied younger material and the presence of the distinctive cells abutting the abaxial side of the phloem in the cotyledonary tube and hypocotyl, but not the plumule vascular bundles, allowed the different origins of the vascular tissues to be identified. In one seedling described by Shaw (1909; diagram 3, figs. 1–3), the different vascular tissues maintained their distinctness from each other, and he illustrated a sequence very similar to that described in our study.

Thus, the closely associated groups of two or three vascular bundles in the lower cotyledonary tube are almost continuous with a pair of vascular bundles in the hypocotyl, with little modification from the shoot vascular system, and this results



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Figs. 31–33 Longitudinal sections through the vascular tissues in bunya pine hypocotyls of various ages. Fig. 31, Week 0. Note the elongated cells (arrowed) on the right-hand side of the provascular tissues. Bar = 100 μm. Fig. 32, Week 3. Note the pronounced elongation of these cells, the absence of starch grains in these cells, and their lack of cell wall pitting. Bar = 400 μm. Fig. 33, As fig. 32 but showing that these cells can exceed 2,000 μm in length. Bar = 500 μm.

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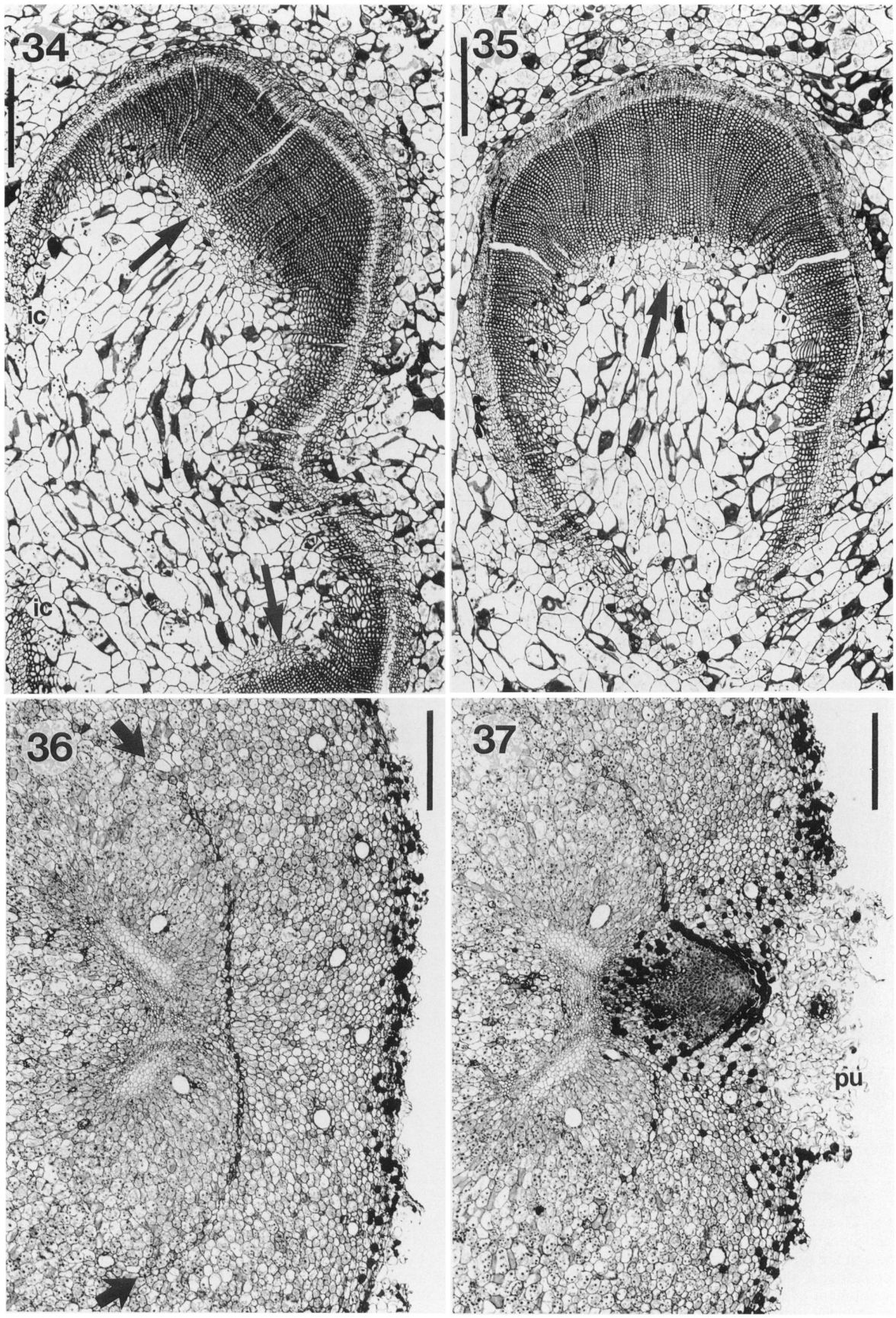
Figs. 27–30 Cross sections of vascular bundles in the middle region of the hypocotyl/tuber of bunya pine of various ages. Scale bars = 100 μm. Fig. 27, Week 0. Note the undifferentiated nature of the future vascular bundle and the abundant starch grains in the surrounding parenchyma cells. Fig. 28, Week 1. Note the rapid differentiation of the vascular tissues and apparent vacuolation of the parenchyma cells. *c*, incipient fascicular cambium; *p*, phloem; *x*, xylem. Fig. 29, Week 4. Note the greater development of secondary phloem than secondary xylem. Note also the absence of starch in the three to four layers of cells that abut the right-hand side of the phloem. Fig. 30, Week 7. Note the commencement of the crushing of the cells that abut the phloem. Note also that the cambium has begun to extend its length by differentiating out into the surrounding parenchyma (arrowed).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in a direct translocation pathway from the megagametophyte to the tuber. However, as variability occurred in the number, arrangement, and size of the vascular bundles in the lower cotyledonary tube, there was considerable seedling to seedling variation in the fine details of the vascular transition.

Comparison with A. araucana and A. angustifolia

Hill and de Fraine (1909) and Ferreira (1981) for *A. angustifolia,* Seward and Ford (1906) for *A. araucana,* and Rouane and Woltz (1979) and Woltz (1986) for both of these species did not provide a detailed sequential description of germination. As in our study, Ferriera (1981) illustrated complete mobilization of starch from the megagametophyte after 50 d. Cardemil and Reinero (1982) performed a sequential developmental light microscope and physiological study of *A. araucana* germination, but only over the first 90 h. Comparative measurements indicated that while megagametophyte cell size was similar in bunya pine and *A. araucana,* cells in the embryo of the latter are 50%–100% longer. Diarch roots were also reported in *A. angustifolia* (Hill and de Fraine 1909) and *A. araucana* (Seward and Ford 1906).

Secondary growth

This study confirms the macroscopic observations of Burrows et al. (1992) that bunya pine has an unusual mode of secondary growth. Several other studies indicate that the presence of multiple vascular cylinders would be normal for bunya pine tubers ca. 12 mo of age. Woltz (1986) illustrated that toward both ends of the tuber the vascular bundles were close together and the interfascicular cambium would probably link all the bundles into a single ring, but in the middle region of the tuber the bundles were widely spaced and could lead to the formation of separate vascular cylinders. Stockey and Taylor (1978, fig. 14) illustrated the initial stages of secondary growth as the cambia of some pairs of bundles were differentiating toward each other and thus had begun to form several circular cambia. Stockey et al. (1990, fig. 41) illustrated the numerous vascular cylinders without drawing attention to this unusual arrangement. Shaw (1909, Dia. 5, 6) illustrated what were termed "abnormal" or "anomalous" seedlings that had several cylinders of secondary xylem within the parenchymatous ground tissue of the tuber and thus are similar to normal secondary growth, as described in this study.

Araucaria angustifolia has only four vascular bundles in the middle to lower hypocotyl, and the hypocotyl does not appear to enlarge to the same extent as in bunya pine (Hill and de Fraine 1909, Dia. 11; Stockey and Taylor 1978, fig. 17; Ferreira 1981, fig. 5). As the vascular tissues are located toward the middle of the tuber and all the bundles are only 600–1,000 μm from each other (Stockey and Taylor 1978), it appears that secondary thickening could proceed in the normal manner. Little has been recorded about the hypocotyl of *A. araucana.* Rouane and Woltz (1979) and Woltz (1986) provided illustrations of the roots and cotyledons sectioned at different levels, but there was no information on the hypocotyl/tuber. Seward and Ford (1906) illustrated one seedling (fig. 16, *A–I*) that may have had typical secondary growth and another (fig. 16, *K, L*) that could have commenced secondary growth in a manner similar to that described in our study. Detailed studies of secondary growth in the South American species would provide a better understanding of relationships in the family and are essential in assessing various fossils that are considered to be possible araucarian seedlings (Stockey and Taylor 1978; Stockey et al. 1990).

Abscission zone and the cotyledonary tube

In most species the abscission zone forms during leaf ontogeny, while in others full leaf expansion is reached without development of a structurally distinct abscission zone (Webster 1973). In bunya pine the abscission zone formed in parenchyma cells indistinguishable from the surrounding cells and formation did not commence until cotyledonary tube elongation was complete; i.e., no incipient abscission zone forms. In most species detachment occurs at the thin-walled, small-diameter, closely packed cells of the separation layer, and a suberized protective layer forms on the proximal side of this layer (Addicott 1982). In conifers, the cell contents of the pro-

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Figs. 34–37 Cross sections of bunya pine tubers. Bars = 500 μm. Fig. 34, Middle region from a 1-yr-old plant. Note the primary xylem (arrowed) and the two areas of interfascicular cambium (*ic*) that have almost linked the pair of bundles together. Note also the greater amount of secondary xylem produced in the fascicular, as compared to the interfascicular, areas. Fig. 35, As fig. 34, but from lower in the tuber, demonstrating that different patterns of secondary xylem form, depending on the initial positions of the vascular bundles in a pair. Primary xylem is arrowed. Fig. 36, Lower third of tuber at week 4. Note the proximity of the vascular bundles, the arc of primary xylem that links them together, the phellogen, and the recently formed endodermis (arrowed). Fig. 37, As fig. 36, except that a root primordium has been initiated to the outside of the vascular bundles but inside the endodermis. Note that the cortical cells that have been cut off near the primordium have formed a pustule (*pu*).

tective layer are often densely staining (Montano and Proebsting 1988), and in many woody species these layers are themselves abscissed when periderm formation begins (Addicott 1982). In contrast, in bunya pine, cell wall lignification and tannin deposition occurred on the distal side of the apparent separation layer, and abscission occurred through or to the outside of the tannin layer.

Two factors should be considered when assessing these differences. First, the abscission of the cotyledonary tube from the tuber occurs surrounded by soil, where the physical stresses are markedly different compared with aboveground leaves. Second, soon after its initiation the apparent separation layer joins with the recently initiated phellogen of the tuber. Thus, in providing continuity of protection around the top of the tuber, the apparent separation layer is more phellogen in nature. Separation occurs distal to the tannin layer because there may be only a weak connection between the intact, isodiametric tannin cells and the elongated and collapsed parenchyma cells of the cotyledonary tube.

Bunya pine is the only cryptogeal species for which a predetermined separation of the storage reserve tissue from the tuber has been described (Burrows et al. 1992). It would appear that as the internal diameter of the tube is small and the tube is mechanically strong, the programmed detachment allows the epicotyl relatively unimpeded upward growth through the overlying soil. The closely related species *A. araucana* and *A. angustifolia* possess separate cotyledons (Rouane and Woltz 1979), and as physical restriction of shoot growth does not occur, abscission zones do not form.

In the other cryptogeal species described to date, the mechanism of cotyledonary tube elongation has received little attention. In *Marah* (Cucurbitaceae) most of the lengthening resulted from cell elongation, principally at the base of the cotyledons (Schlising 1969), while in *Elephantorrhiza* (Mimosaceae) van der Schijff and Snyman (1970) indicated that cell elongation was important but also suggested the presence of an intercalary meristem. In species that initially possess a short tube, some anticlinal divisions could be expected if considerable elongation occurs.

While the tuber exhibits early periderm development, the cotyledonary tube never develops a periderm. This indicates the short-term function of the tube, but tannin deposition in the outer layers of the tuber is a metabolic investment in insuring that the tube is not microbially degraded during its first 4–8 wk in moist soil. The timing and extent of abscission zone formation is a balance between allowing unimpeded shoot elongation and ensuring that translocation from the megagametophyte to the tuber is finished. While the major part of the abscission zone forms relatively early in the seedling's development, it does not extend into the vascular tissues. Thus, by the time that translocation is largely finished, continued shoot growth provides the force for separation, leaving only the vascular tissues to be sealed.

By week 4 the major proportion of the cross-sectional area of the lower cotyledonary tube consists of air space. This system may supply the oxygen demands of rapid cell division in the subterranean tuber, but this appears unlikely as there is little continuation of the air space system into the tuber. The air spaces also probably absorb stresses associated with soil expansion and contraction, and thus the vascular bundles maintain their structural and functional integrity.

Acknowledgments

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