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Partial shoot reiteration in *Wollemia nobilis* (Araucariaceae) does not arise from 'axillary meristems'

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• *Background and Aims* Conifers are characterized by the paucity of axillary buds which in dicotyledonous trees usually occur at every node. To compensate, conifers also produce 'axillary meristems', which may be stimulated to late development. In juvenile material of *Wollemia nobilis* (Araucariaceae: Massart's model) first-order (plagiotropic) branches lack both axillary buds and, seemingly, axillary meristems. This contrasts with orthotropic (trunk) axes, which produce branches, either within the terminal bud or as reiterated orthotropic axes originating from axillary meristems. However, plagiotropic axes do produce branches if they are decapitated. This study investigated how this can occur if axillary meristems are not the source.
• *Methods* The terminal buds of a series of plagiotropic branches on juvenile trees were decapitated in order to generate axillary shoots. Shoots were culled at about weekly intervals to obtain stages in lateral shoot development. Serial sections were cut with a sliding microtome from the distal end of each sample and scanned sequentially for evidence of axillary meristems and early bud development.
• *Key Results* Anatomical search produced no clear evidence of pre-existing axillary meristems but did reveal stages of bud initiation. Buds were initiated in a group of small starch-rich cortical cells. Further development involved de-differentiation of these small cells and the development of contrasting outer and inner regions. The outer part becomes meristematic and organizes the apex of the new branch. The inner part develops a callus-like tissue of vacuolated cells within which vascular cambia are developed. This kind of insertion of a branch on the parent axis seems not to have been described before.
• *Conclusions* Axillary meristems in *Wollemia* characterize the leaf axils of trunk axes so that the origin of reiterated shoots is clear. Plagiotropic axes seemingly lack axillary meristems but still produce axillary branches by distinctive developmental processes. These observations demonstrate limited understanding of branch initiation in trees generally.

Key words: Araucariaceae, axillary branching, bud morphology, conifers, plagiotropy, reiteration, shoot morphology, *Wollemia nobilis*.

INTRODUCTION

Conifers and woody angiosperms differ in the morphological expression of branch development from axillary buds. This was pointed out by Henry (1846) in his study of axillary bud composition in a diversity of woody plants. He commented on the fact that in those few conifers he studied axillary buds are only produced at selected nodes. This is a general feature of conifers, and these normally form the framework of the deterministic architecture of the tree. Angiosperms usually produce one or more buds at every node, these having varying developmental potential (Romberger, 1963). They may either grow out in the deterministic expression of tree form or remain suppressed, but as 'reserve buds' that subsequently can develop opportunistically in the process of reiteration. Garrison (1949) draws attention to the paucity of information about late stages of bud development in seed plants. Nevertheless, this combination of deterministic and opportunistic components determines in most trees the mechanism of forest canopy construction (Hallé *et al.*, 1978).

Conifers also show contrasting deterministic and opportunistic aspects of branch development, although the former may be said to dominate, producing a more consistent crown form, but with limited architectural expression. Reiteration still remains an essential component, and is most familiar in trimmed plants used for hedges or in topiary (e.g. *Podocarpus macrophyllus* and *Taxus baccata*). However, in the absence of reserve buds, the question is raised as to where these reiterated shoots originate.

The question was answered by Fink (1984) who demonstrated the existence of a meristem complex in the leaf axils of conifers, but not differentiated as a visible axillary bud. These he termed 'detached meristems' because they were considered to represent a residuum of cells derived directly from the shoot apex. They can persist for long periods within outer tissues of the stem but become organized into a visible shoot under appropriate stimuli (e.g. decapitation of the parent shoot). Burrows (1989) confirmed these observations in his work on Araucariaceae but preferred the term 'axillary meristem' (as used here) because they could be observed

without reference to their mode of origin. This term itself can be ambiguous, as elaborated in the Discussion. Subsequently, Burrows demonstrated their presence in shoots in *Wollemia* (Burrows, 1989, 1990). His work primarily focused on juvenile trunk portions in relation to propagative techniques and led him to the suggestion that axillary meristems could exist in all leaf axils, with the exception of those that produced a visible bud or branch. In extreme instances, meristem complexes in angiosperms can also be long-lived and become important in the ecological response of trees to disturbance (e.g. Waters *et al.*, 2010). Despite this important component of canopy construction in forest trees, there has been little attempt to study the phenomenon further.

This topic is developed in the present article by exploring the process of partial reiteration in the recently discovered *Wollemia nobilis* (Jones *et al.*, 1995), which has very precise architecture, exactly conforming to the definition of Massart's model in the tree architectural systems of Hallé *et al.* (1978), but differing in important features from other Araucariaceae (Veillon, 1978; Tomlinson, 2009). Propagation of this rare conifer has been possible because of its reiterative properties so that juvenile material has become freely available for scientific study. Plants cultivated in the shade houses of the Montgomery Botanical Center, Coral Gables, Florida were used.

By stimulating axillary branch formation in decapitated shoots, we obtained stages in their initiation which indicated that branches do not originate from axillary meristems in the manner described by Fink (1984), Burrows (2009) and Burrows *et al.* (2003). Instead they are initiated by cell dedifferentiation from a group of cells at a precise axillary location. Subsequent development leads directly to the formation of an apical meristem and the outgrowth of a shoot with basal bud scales, but without any resting period. Furthermore, there is an elaborated centripetal tissue formation within the rather complex cortical tissues of the parent axis and within which cambial formation begins, ultimately forming a precise connection to the parent vascular system. None of these features appears to have been reported previously for any tree.

MATERIALS AND METHODS

Materials

Two approx. 1·5-m tall pot-grown saplings of *Wollemia nobilis* in a lightly shaded greenhouse were sampled: one was exactly model conforming (Fig. 1A) and the other had produced several reiterated trunks (Fig. 1B). Buds were cut off with a single-edge razor blade and cut shoots with about 4–5 internodes (Fig. 1C) were later sampled at about weekly intervals up to a total of 6 weeks. Segments were immediately fixed in FAA (40 % formaldehyde, five parts; glacial acetic acid, ten parts; 70 % ethanol, 85 parts). The experiments were conducted in May–July 2009 and repeated in November–December 2009. A total of 20 shoots were treated. Some samples at a later date were also collected when it became clear that removal of the first sample continued to induce buds behind the third cut. These were pooled with the first collections and showed equivalent developmental features. In all, a total of 64 cut ends were examined.

Sectioning

Subsequently freehand (unembedded) serial sections of the fixed material were cut with a sliding microtome from the distal portion of each sample at thicknesses of 60–90 μm, a thickness that minimizes labour but gave clear histological details. Sections were mounted in serial sequence in 50 % glycerol/water and initially examined unstained. Analysis proceeded backwards from older to younger samples so that the initial location of bud formation could be determined. A total of about 2000 sections were thus initially surveyed. We surveyed sequential sections using methods of Huggett and Tomlinson (2010) in which microphotographs were converted into movie sequences allowing easy viewing and a clear understanding of vascular topography. Additional information was obtained from the same series of paraffin sections used in Tomlinson and Murch (2009).

Staining

Once the approximate position of the bud initial was located, selected appropriate sections were either photographed unstained or subjected to various histochemical treatments. Starch was identified by Lugol's iodine (iodine–potassium iodide (I_2KI), e.g. Fig. 2C, D). De-starching was done with concentrated HCl, complemented in some treatments with 95 % alcoholic phloroglucinol as a lignin stain (e.g. Fig. 3F–H); other sections were bleached in commercial sodium hypochlorite. Bleached sections were stained in 0·1–0·01 % aqueous toluidine blue. A few bleached sections were stained in safranin/alcian blue, dehydrated and made permanent (e.g. Figs 2A and 3A–E). All accumulated sections have been stored in 70 % ethanol.

Illustrations

Imaging was done with a Coolpix 4500 digital camera (Nikon, Japan) using selected stages and stains. Images were manipulated only by autocorrection (brightness and contrast) in Microsoft Image.

RESULTS

Anatomy

As has been reported in some detail, orthotropic (trunk) and plagiotropic (branch) axes are sharply contrasted in both morphology and histology (Tomlinson and Murch, 2009). Orthotropic (trunk) axes grow erect (Fig. 1A), retain only scale leaves with at most a reduced blade, and branch from lateral meristems pre-formed within the terminal bud to produce plagiotropic axes in a process that is intrinsically sylleptic (branch and parent axes are contemporaneous in their extension). Phyllotaxis on trunk axes is irregularly spiral, the resting bud protected by numerous overlapping bud scales. In contrast, branch axes are ±horizontal (Fig. 1A, B), with strict decussate phyllotaxis, the leaf insertions extended and twisted during shoot elongation in a process of induced dorsiventrality but still retaining the primary four leaf orthostichies (Fig. 1C–G). In normal development, no kind of further branch is produced on these shoots, which show rhythmic growth, alternating between periods of extension and rest, the resting buds protected by numerous enclosing narrow

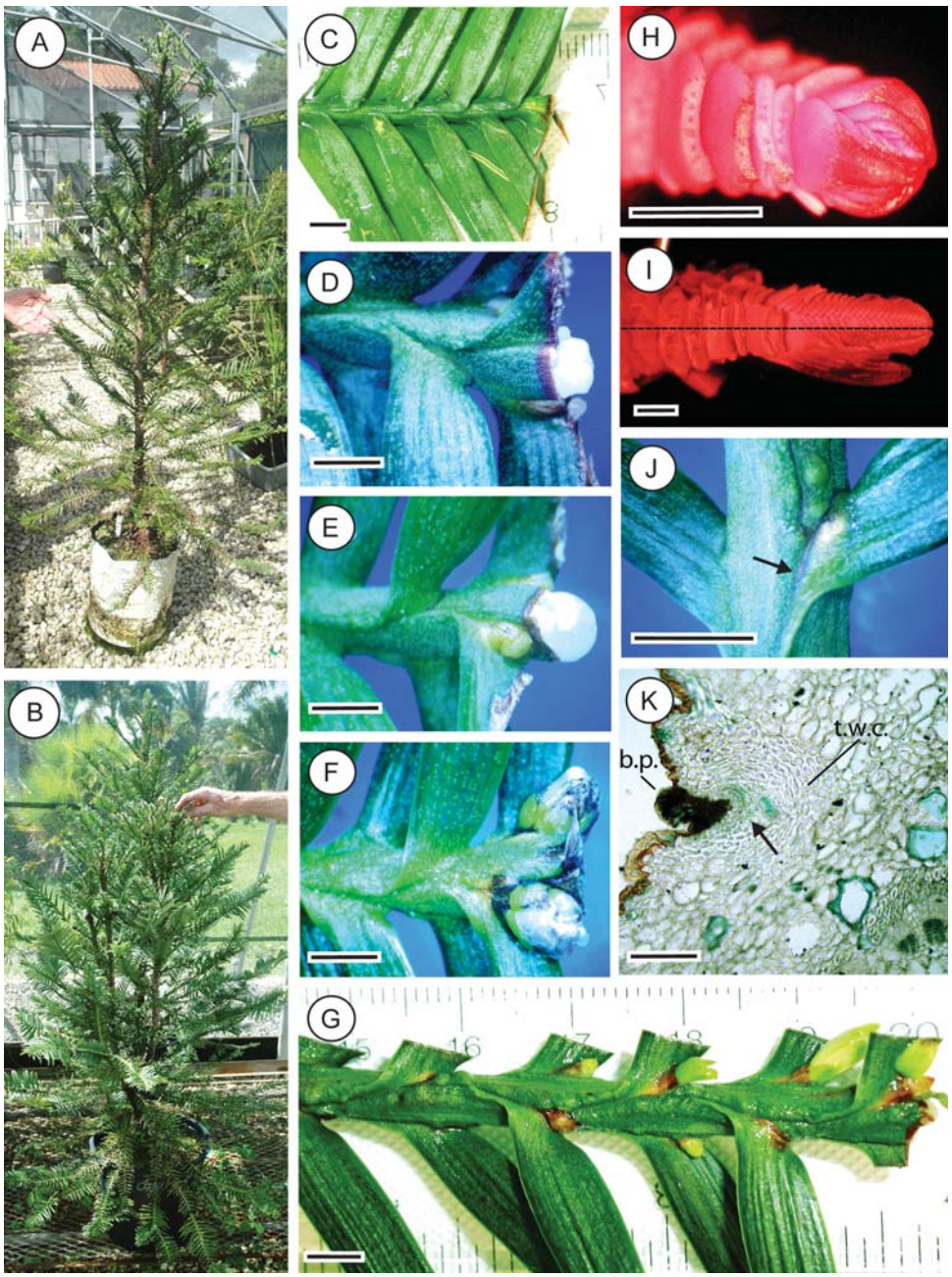

FIG. 1. *Wollemia nobilis*. Habit of juvenile specimens, as used in this study, and details of plagiotropic axes. (A) A strictly model-conforming tree. (B) Several trunk axes developed by reiteration of the tree's total architecture are included. (C) Distal portion of a plagiotropic branch sampled after decapitation. (D) Tip of s decapitated axis 2 weeks after decapitation; the resin cap is a standard wound response. (E) An axillary bud evident in the lower leaf axil, and a possible early bud in the upper leaf axil. (F) Early extension of an equivalent pair of buds. (G) A decapitated shoot with a sequence of solitary or paired buds showing the acropetal prominence of distal buds. (H, I) Epi-illumination images stained in acid fuchsin. (H) A distal portion of a shoot within a bud, with proximal leaves removed; their insertion is ± transaxial. (I) A dissected mature bud, scale leaves and proximal foliage leaves removed, with one orthostichy indicated by a dotted line. (J) One node with a pair of opposite leaves showing oblique insertion, the bark patch (arrow), included in the groove, but no evidence of axillary meristems; the axillary blisters represent enlarged surface resin canals, not buds. (K) Transverse section of the axis in the region of a superficial necrotic bark patch (b.p.), including internally an exaggerated complex of cells with thick unlignified walls (t.w.c.); the arrow indicates the region within which a bud can arise. The section was bleached to remove starch, and stained with Sudan IV and dilute toluidine blue. Scale bars: (C, D–G, J) = 5 mm; (H, I) = 1 mm; (K) = 200 μm.

bud scales. Occasionally a branch is produced, but always associated with the abortion of the terminal bud; this kind of development was the basis for our experimental manipulation.

Vascular anatomy, correlated with phyllotaxis, is different in the two kinds of axis, but a distinctive character shared by all Araucariaceae is that the single vascular trace to every kind of leaf (a feature of all conifers) divides progressively in its extended course across the stem cortex so as to produce up to nine leaf traces that enter the leaf base (Fig. 2A). Consequently, the cortex can include traces to at least two

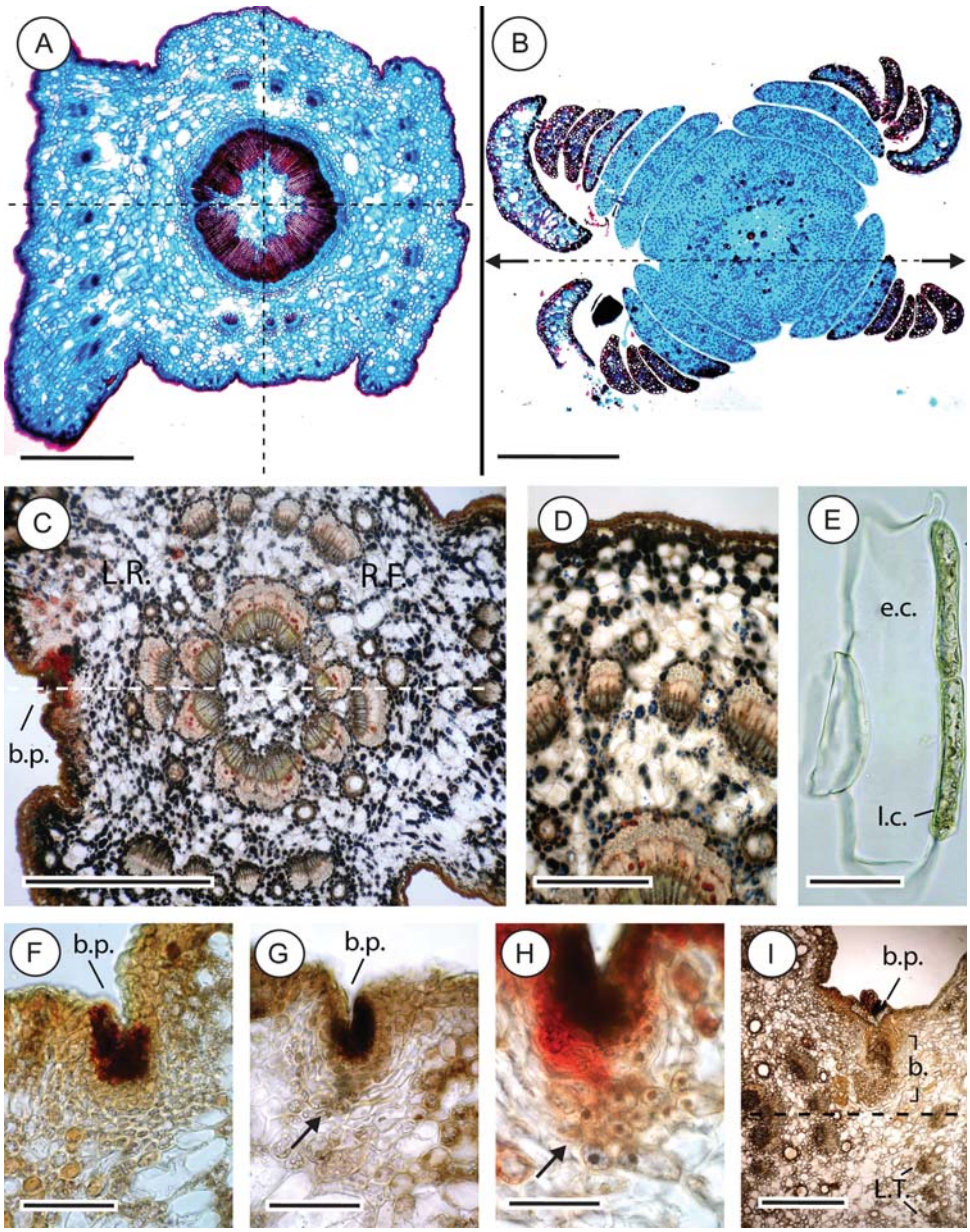

FIG. 2. *Wollemia nobilis*. Histology of a plagiotropic branch (A–E) and bark patch region (F–I); all transverse sections except E. (A) Transverse section of the stem bleached and stained with safranin/alcian blue. Co-ordinates (dotted lines) indicate the plane of insertion of four leaf ranks; right- and left-hand leaf traces correspond to a leaf inserted immediately above; top and bottom leaf traces in the mid cortex belong to a pair of leaves inserted at the next node above. (B) Transverse section of the base of a bud; paraffin section stained with safranin/alcian blue. Bud scales and foliage leaves, initially inserted ± transaxially showing progressive twisting of older, outer leaves that results in an intrinsic dorsiventral plane (double-headed arrow). (C) Transverse section of the axis stained with I_2KI. The location of the bark patch (b.p.) corresponds to the medium plane in which leaf traces are inserted (dotted line). The leaf to the left (L.R.) is not fully detached and the leaf to the right (R.F.) still not separated at this level. (D) Transverse section of the cortical details, stained with I_2KI, showing the reticulum of starch- and tannin-containing cells dispersed among empty cortical cells. (E) Macerated cortical cells; large empty cells (e.c.) contrasted with narrow elongated living cells (l.c.). (F–I) Transverse sections: bleached or destarched, unstained sections; bark patch (b.p.) uppermost. (F) A section without any evidence of bud inception. (G) Early possible cell complex as differentiated starch-rich cells (arrow). (H) Possible early bud inception (arrow), starch removed. (I) Low-power view of a distinct bud initial (b.), its long axis oblique to the plane of the leaf trace insertion (dotted line); part of the leaf trace (L.T.) system to its subtending leaf to the right and still within the cortex. Scale bars: (A–C, I) = 1 mm; (D) = 500 μm; (E) = 125 μm; (F, G) = 400 μm; (H) = 200 μm.

successive nodes in branch axes and many sequential nodes in the radially symmetrical trunk axes. Other histological features include the system of interconnected resin canals, but a well-developed series of sclereids is present only in trunk axes (Tomlinson and Murch, 2009). In the present description we refer only to branch axes.

Cortex anatomy

In branch axes, apart from the reduced number of leaf trace systems because each only passes through two internodes, the cortex is complicated histologically by a network of narrow, elongated living cells forming a reticulum within a

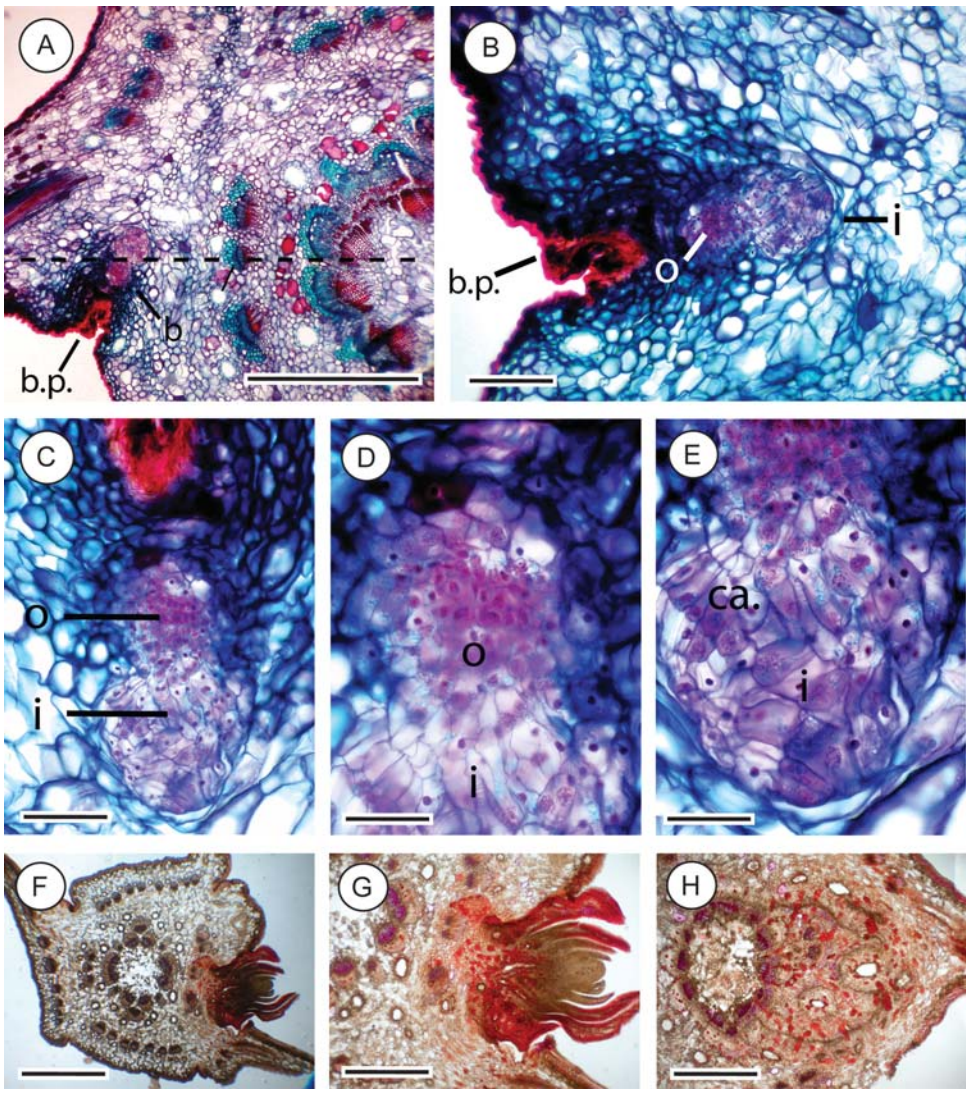

FIG. 3. *Wollemia nobilis*. Bud development, all transverse sections. (A–E) Early stages, bleached and stained in safranin/alcian blue. (F–H) Late bud stages, stained in phloroglucinol and concentrated HCl. (A) Low-power view of a bud (b.) oblique to the plane of insertion of leaf traces (dotted line). (B) Detail of the same bipolar bud initial within the bark patch (b.p.). (C) Detail of a bud initial with a contrasted outer (centrifugal) future shoot region (o) and inner (centripetal) future cortical region (i). (D) Meristematic cells of a future shoot region (o). (E) Vacuolated inner cells (i) of a future cortical complex; there are many cells with apparent prophase nuclei; and evidence of cambial inception (ca.) to the left. (F) Transverse section of a plagiotropic bud axis; to the right developing a protruding branch axis seen in approximate longitudinal sectio (G) Detail of the same shoot bud with its apex initiating numerous scale leaves; the cortical surface is ruptured. (H) Detail of the same bud at a lower (proximal) level. The massive cortical complex includes several series of active cambial initials, the outermost making contact with the cambium of the parent stele. Scale bars: (A, G, H) = 1 mm; (B) = 400 μm; (C) = 200 μm; (D, E) = 100 μm; (F) = 2 mm.

complementary set of enlarged cells completely devoid of cell contents, as is most obvious in macerated material (Fig. 2E). The living cells are uniformly starch rich (Fig. 2C, D) and this radial discontinuity of cell files has important consequences for branch insertion, as described later.

Branch morphology and decapitation

Terminal buds of branch axes were cut off in order to stimulate reiteration of renewal buds (Fig. 1D). An immediate response is the exudation of resin, which forms a white cap (Fig. 1E, F). On examination of treated axes at progressively longer intervals, necrotic changes were conspicuous immediately below the cut. These included extensive development of tannin, formation of an irregular phellogen and periderm, and extensive cell proliferation from the living cells of the cortex, but these details are not illustrated because they bear no relation to development of buds, which occurs somewhat proximal to the necrotic tissue. Our attention was devoted to the unmodified tissues immediately below the cut, with emphasis on the insertion of the uppermost leaf pairs.

Foliage leaves in decussate phyllotaxis are extensively preformed within the enclosing bud scales and by the apical bud, their insertion being transaxial, i.e. at an approximate right angle to the long axis of the shoot (Fig. 1H). This preformation suggests that the full complement of foliage leaves for each shoot increment exists before bud burst (Fig. 1I). During shoot extension the transaxial orientation of leaf

insertion changes considerably so that the insertion becomes oblique, extending almost the length of the internode (Fig. 1C–G). Rotation of the leaf base is always in the same direction so that the original adaxial surface of the leaf base is directed vertically, producing the dorsiventrality of the shoot system. This rotation is never complete, so that the original four orthostichies of the decussate system remain evident (Fig. 1B–D). It should be emphasized that this dorsiventrality of the system does not result from any twisting of the stem itself. This process, common in angiosperms (E. H. Zacharias and P. B. Tomlinson, unpubl. res.), never seems to occur in conifers. Internally the four vascular orthostichies established by the leaf trace system are consequently never modified (Fig. 2A). Sections of branch buds show this reorientation of foliage leaves, although bud scales themselves do not become modified because the internode between them does not elongate. This is very obvious in the numerous persistent basal bud scales of extended branches. The assumption must be made that there is a slight but significant obliquity of the leaf insertion, which becomes exaggerated during internodal expansion. Clearly this developmental process is not well understood.

Leaf axil anatomy

There is no visible external evidence of an axillary structure within any leaf axil (Fig. 1D), so internal evidence had to be sought for bud induction by the experimental treatment. The extended axillary groove of the leaf insertion (Fig. 1J) is marked by a narrow brown streak, the manifestation of a strand of necrotic tissue that is visible as a dark mass in sections (Fig. 1K). This structure corresponds to the 'bark patch' described in trunk axes by Burrows (1989), a term we use here although it does not develop an extensive periderm. Internal to this are tannin cells and a band of somewhat thick-walled unlignified cells (Fig. 1K; t.w.c.), but details of other cells in this area are obscured in untreated sections by the abundance of starch.

Stimulated buds are first visible externally as superficial swellings of the surface layers (Fig. 1E), but become most obvious when the surface layers are ruptured and the shoot emerges, enclosed in bud scales (Fig. 1F, G). The position of the bud is quite uniform and corresponds to an approximate median position in the leaf axil, i.e. in its midpoint, as would be expected for an axillary bud (Fig. 1G). This position is confirmed in sections, as described in detail later, which show its association with the leaf trace of the next pair of leaves on the same orthostichy, i.e. the trace system that would exit a leaf at the end of two internodes distally. This sustained precise configuration, despite the twisting of the leaf base, confirms the absence of internode twisting in the axis as a whole.

The number of buds produced by decapitated shoots varies. There may be only one, but most commonly there are two (Fig. 1G), and almost always at the uppermost node below the cut, but always proximal to the necrotic tissue induced by the cut. Where two buds develop at the same node they can differ in their degree of development (Fig. 1E, G). Exceptionally, buds may appear at more nodes; Fig. 1G shows an unusual condition because buds appear at five successive nodes, the distal ones being the most vigorous. This indicates that the potential for bud induction exists extensively, possibly at any node. Further support is provided by the observation that a second crop of buds is induced at the cut end of shoots after the first sample was taken.

Bud initiation

No evidence was found at the leaf insertions on untreated shoots for the existence of 'axillary meristems' of the type described by Burrows (1999, 2009) and Burrows *et al.* (2003). Throughout the length of one leaf axil (Fig. 1J) the anatomy of the tissues immediately internal to the bark patch is as shown in Fig. 2F. Evidence for early stages was difficult to find because of the extensive development of starch that obscures cytological details (Fig. 2C, D). In destarched sections, however, a group of cells in the appropriate axillary position could be recognized by their enlarged nuclei (Fig. 2G, H). It is assumed that these will have originated by dedifferentiation of cells in this position because no such configuration was seen in untreated shoots. This is the basis for our claim that there are no axillary meristems, derived from shoot apical tissues, in plagiotropic axes.

Subsequent stages allow a clear recognition of the topography of the induced bud. The cells proliferate and rapidly assume a distinctive orientation and cytology. The bud initial is oblique to the radial plane in which it is inserted, but always opposite the leaf trace system as described above (Fig. 2C). It therefore conforms to the intrinsic oblique orientation of its subtending leaf (Fig. 3A), and in a longitudinal plane the developed bud always projects adaxially, as in Fig. 1D–F. The further extension of the branch is always horizontal, maintaining the dorsiventrality of the branch system.

Lateral shoot development

Further enlargement of the initial cell complex (Fig. 3B, C) differentiates an outer (i.e. centrifugal, o) portion of the developing bud and an inner (i.e. centripetal, i) portion. The outer region produces cells which are at first densely cytoplasmic (Fig. 3D) in contrast to the inner portion whose dividing cells remain distinctly vacuolate (Fig. 3E). The outer portion enlarges and soon initiates an organized apical meristem (Fig. 3F) that in turn produces leaf primordia; at first those of scale leaves, subsequently those of foliage leaves (Fig. 3G). The inner portion progressively enlarges as a complex of vacuolated cells with abundant cell division, with very early tangential divisions presaging cambial development (Fig. 3E, ca.). Even in the degraded tissue resulting from de-starching, active cell division is indicated by the presence of enlarged nuclei at a prophase stage (Fig. 3E). Cells of the centrifugal region remain densely cytoplasmic with little indication of cell division stages. The overall result is the development of the bud in a bipolar direction, the outer complex forming the future shoot, and the inner complex forming the tissue that becomes responsible for vascular continuity with the stele of the parent axis.

Bud maturation

The opposed developmental direction of the two extremities of the stimulated bud shows these marked contrasts, but because the structures are oblique to the long axis they do

not appear together in the same transverse section. The centrifugal (outer) portion soon becomes recognizable as a shoot axis and breaks through enclosing outer cortical tissue (Fig. 3F, G). At this stage it becomes visible externally. The centripetal (inner) portion of the same shoot sectioned in a lower transverse plane (Fig. 3H) shows extensive cell proliferation in such a way that the original cortical tissue and especially the inflated dead cells become totally supplanted. Within this, a vascular cambium of varying configuration develops, sometimes forming a circular pattern that can enclose a proliferated resin canal or otherwise encircling the tissue overall. In only one instance did an enclosed leaf trace within this complex show proliferation of cells between xylem and phloem, i.e. a cambium-like proliferation, so this is not a normal feature. However, the significant portion of this cambial development is that which becomes contiguous with cambium of the parent stele, encircling the attendant leaf traces but without making contact with them (Fig. 3H). The variability seen relates to the variation in size of the original shoot. The most common contact made with this system of bud traces is with the cambium of the vascular bundles that frame the leaf gap next below on the same orthostichy. Stages in this linkage are illustrated in Tomlinson and Murch (2009; their fig. 5C–E).

Vascular union is completed by the development of secondary vascular tissues continuous from bud to parent axis, the former typically dominant because of the distal disruption of the parent stele consequent to its decapitation. The late stages are illustrated in Tomlinson and Murch (2009; their fig. 5F–K).

DISCUSSION

Summary conclusions

The tree's architecture (Massart's model) provides a marked contrast between orthotropic and plagiotropic axes in terms of phyllotaxis, symmetry and anatomy, but based on a common leaf trace configuration. Both kinds of axis can reiterate, each reproducing in lateral axes the distinctive morphology of the parent axis, i.e. orthotropic to orthotropic; plagiotropic to plagiotropic. Reiterated shoots of orthotropic axes originate from 'axillary meristems' that may be associated with most nodes. These meristems were never clearly demonstrated in leaf axils of plagiotropic shoots, but reiterated shoots could be induced, as by decapitation. The induced meristems come from a group of seemingly differentiated cells, initially by de-differentiation and subsequently by meristematic proliferation of these precursor cells. The resulting meristem shows a bipolar development contrasting the outer future shoot meristem and the inner future tissue through which a vascular connection is made. This inner tissue extensively supplants the cortical network of living and dead cells, so that vascular connection is not made by significant changes in prior cortical tissues.

Further commentary

This study should be seen as preliminary, but important in establishing the need for making a more precise cytological analysis. It demonstrates a mechanism for generating reiterated shoots in branch axes and identifies the location in which new meristems originate. The suggestion that these come from differentiated cells is certainly open to question, especially as the buds clearly have an axillary origin. Further search may reveal the presence of these elusive, but still only hypothetical, axillary meristems.

In seed plants generally, the distinction between axillary meristems that are detached remnants of the parent shoot apex (the normal condition) and those that originate *de novo* from differentiated cells, as we claim here, is not sharp. Sterling (1947) describes the early development of the axillary bud in *Pseudotsuga* incidental to the study of vascularization. However, it is significantly reported that here the buds are not evident (in those axils that produce them) until after the parent shoot begins elongation. Here the concept of a detached meristem (Fink, 1984) as originating directly from meristematic tissues of the shoot apex would be inappropriate. Garrison (1949), on the other hand, in *Syringa* clearly refers to the bud meristem as being continuous with and therefore part of the apical meristem. Thus there is ambiguity in the term 'axillary meristem' used without reference to development (Burrows, 1989, 1999) because it does not explain all circumstances. There can be a phase of partial differentiation of a normal axillary bud before axillary cells turn meristematic again. Further modification is supported by the demonstration in Veierskov *et al.* (2008) that by localizing ubiquitin they could find initial stages of axillary bud development in the axils of all needles in *Abies nordmanniana*. However, few of these initial stages achieve the status of visible buds. Nevertheless, this observation is a clear indication that axils in all leaves of conifers could retain a capacity to produce a lateral bud in appropriate circumstances, further substantiating the suggestion of Burrows (1999) and Burrows *et al.* (2003) that all leaf axils in the Araucariaceae they studied were potential sites for future bud development. However, this statement was made only with reference to orthotropic shoots. The present observation that a sequence of buds can appear along a decapitated shoot (Fig. 1C) is indicative of a similar condition in plagiotropic shoots. However, in *Wollemia*, one must always bear in mind the difference between scale leaves and foliage leaves when referring to leaf axils in a generalized way.

Research on branch expression in conifers is extensive, as indicated in the literature surveys of Rasmussen *et al.* (2005, 2010) and Veierskov *et al.* (2008), but studies on branch development in traumatic circumstances are very limited, even though they may have direct commercial significance in the production of 'greenery' [lateral branch complexes cut for Christmas decoration; Rasmussen *et al.* (2005)]. The literature on the control of shoot form in conifers has moved from the early descriptive approach to one in which the influence of phytohormones is being extensively investigated (e.g. Rasmussen *et al.*, 2010). A combination of this approach with descriptions of little-investigated reiterative responses and their anatomical consequences, as attempted here, would be very informative.

Although adventitious shoot buds can have many sources, and as root buds may not even originate from shoot tissues, their close association with leaf axils, as demonstrated here,

has important evolutionary consequences. The association of bud and leaf axil demonstrates a constancy, which could not have existed prior to the evolution of the megaphyll, which characterized modern vascular plants (Stewart and Rothwell, 1993). Could the axillary position of a branch which first appeared in Carboniferous pteridosperms (Galtier and Holmes, 1982) have originated by some such process as described here for one kind of axis in *Wollemia*? Branch meristems in leaf axils, with all their varied subsequent expression, account for much of the variability of form in modern trees and for their presumed adaptive success.

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