BANKSIA FLORICULTURE EXPORT MARKETING AND VEGETATIVE BIOLOGY FUNDAMENTAL TO CLONAL PROPAGATION

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Summary

A survey of retailers and conjoint analysis (card-sorting task), normally applied to general merchandise products, was applied to the floricultural product, *Banksia*, in the German market. The main market access points for *Banksia* (international wholesalers, dried flower importers, regional wholesalers and Dutch auctions), separate market channels and end-uses for dried and fresh *Banksia* were identified. Low levels of customer satisfaction for quality attributes (flower size to stem length, grading uniformity and the number of blooms packed per carton) and a lack of promotional information exits. Research showed a negative linear relationship between price and preference, a greater utility for lime and red blooms, and higher utilities for shorter stem lengths, (eg. 30 cm). The criticism of this lack of uniformity supports further research on the vegetative biology underlying clonal propagation.

Quantitative assessment of CSI (current season's internode) and PSI (previous season's internode) of *Banksia* stems using image analysis identified interspecific and seasonal differences in anatomy. Significant differences in parameters such as the percentage of cortex and the distance from the cambium to the stem surface were found between the CSI and PSI of stems collected in autumn, whereas fewer significant differences in tissues were found in spring. Microscopic observations of the CSI and PSI sections were useful in identifying anatomical features that may influence successful vegetative propagation. Structures observed in *Banksia* which are likely to impair vegetative propagation are the presence of cork and cell occlusions in aging stem internodes, and the fibrous nature of young wood: pericyclic phloem fibres and leaf traces surrounded by fibrous zones in the cortex.

Self-, intra- and interspecific whip grafts between five species of *Banksia* from across the genus were conducted using *B. serrata* and *B. spinulosa*, var. *cunninghamii* as rootstocks, and *B. coccinea, B. ericifolia* and *B. menziesii* as scions. Histological sections of graft unions of two, four and 12 weeks post-graft were examined to assess the key events occurring in unions of *Banksia*.

Serial sectioning through cotyledonary nodes of post-emergent seedlings of non-lignotuberous *B. serrata* and lignotuberous *B. menziesii* was undertaken. In *B. serrata* sampled at 15 weeks exogenous axillary buds are present in the cotyledon and leaf axils, the base of the cotyledons were not fused, and accessory and adventitious buds were not observed. In *B. menziesii* sampled at eight and 26 weeks the fused base of the cotyledons forms a thick sheath of parenchymatous tissue around the stem, creating a narrow encircling lumen between the protective sheath and the stem in which three types of buds arise. Exogenous axillary buds arise in the axils of the each cotyledon and first true leaves. Endogenous accessory buds arise in the cortical tissue at either side of the axillary buds. Exogenous adventitious buds develop on the adaxial surface of the protective sheath, along the fusion line of the bases of the cotyledons at either side of the stem axis. In addition, adventitious buds occur singly on the adaxial wall of the sheath.

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Raelene Mibus

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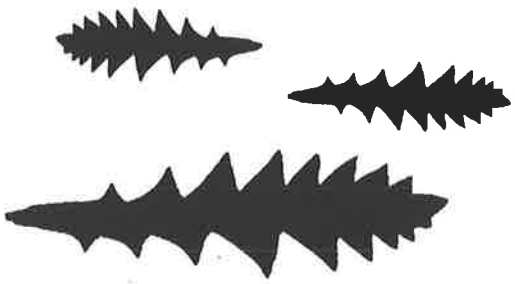

Chapter 1

General Introduction

Introduction

The Australian wild flower industry is a new industry broadening to encompass the commercialisation of an ever-increasing number of wild flower species for the international and domestic cut flower and amenity industries. The newness of the industry is indicated by the recent statistic that 32% of wildflower growers have been producing for less than five years, 23% for less than ten years (Karingal Consultants 1994). Currently the industry (domestic and export) is valued at greater than A$25 million *per annum* and is expected to reach A$100 million by the year 2000. Members of the *Banksia* genus have received much attention for several decades as cut-flower species and have now grown to be the major focal flower export species for use in fresh and dried arrangements. *Banksia* (family Proteaceae) constitutes 10% of all floricultural exports from Australia in terms of the total Australian export volume (1992/93), accounting for 13% of the total *Banksia* production capacity and earning Australia annually over $1 million. A recent review of the wildflower industry (Karingal Consultants 1994) identified the main weaknesses and threats it faces, which are typified in *Banksia* production. As for many native species, the main difficulties facing *Banksia* production occur at either end of the production chain. The above mentioned report identified these as follows:

1. "inadequate market intelligence and market data feedback to growers and poor grower market understanding" and
2. "the shortage of high quality clonal selections"

These two areas are currently hindering further commercial development of the *Banksia* genus and the research presented in this thesis addresses these two areas. Export market research is particularly necessary because local markets are relatively small and already at saturation point; also because of the size of international markets and their interest in obtaining novel flower forms, and the increasing trend towards market oriented production. Clonal propagation is currently not commercially feasible for *Banksia*, but it is mandatory if the industry is to move on to uniform production from superior vegetatively multiplied cultivars. These two points, together with a brief background to *Banksia* production are elaborated upon in the next section.

Banksia commercialisation and development

Banksia flowers are among the most popular and well known of the Australian flora. Although often confused with the South African *Protea*, they stand very much as an Australian icon in the floral world. Even when compared to the large, stunning focal flower, the waratah (one of four species in the endemic genus, *Telopea*) (Nixon 1987), the *Banksia* genus (78 species) offers greater diversity in flower and leaf forms, habit and growth conditions. Consequently, they have long been picked from wild stands in Western Australia. Since the early 1970s bush harvesting, together with production from commercial plantations has increased to supply domestic and overseas markets. Governmental and conservation agencies now aim to prohibit bush harvesting due to the detrimental effects on habitats and genetic resources (Morgan & Fuss 1994; Wills & Robinson 1994; Lamont *et al.* 1995; Witkowski & Lamont 1995). Yet harvesting from bush on private land will continue.

The most commonly cultivated cut-flower species, *B. baxteri, B. burdettii, B. coccinea, B. prionotes, B. hookeriana, B. speciosa,* and *B. praemorsa* (foliage), are all endemic to Western Australia. The continued interest in *Banksia* both locally and abroad has lead to greater plantation production, derived predominantly from seedlings. Plantation production now occurs across southern Australia (West Australia, 190 Ha (Pegrum 1989); South Australia 56 Ha (Windle *et al.* 1990); Victoria, ca. 30 Ha[1]; New South Wales, 2 Ha (Kloosterboer *et al.* 1992)). However, the fact that production is largely seedling based has inherent disadvantages. Seed used for plantation establishment is often sourced from quite different provenances; consequently, plantations can differ

[1] Estimate based on personal communication with six *Banksia* growers in Victoria, 1992

widely in production (Fuss & Sedgley 1991b), flower colour (Bickford & Sedgley 1994) and size, leaf form and their resistance to soil borne disease – a major problem in the industry (Dixon *et al.* 1984; McCredie *et al.* 1985b, c; Tynan *et al.* 1995). Even within plantations from the same seed source there can be high levels of variability. This is due to the predominance of outcrossing in *Banksia,* producing an overall lack of crop uniformity, and differing management practices and standards that are undesirable when servicing international markets with high expectations.

The effects of the relatively haphazard manner in which *Banksia* domestication has occurred are being felt by the industry today as it endeavours to keep abreast of increases in production, agronomic problems such as soil diseases, the lack of clonal production and market demands for consistent and new products in large predictable volumes.

Market oriented production

A wealth of knowledge on cultivation and post-harvest practices (Dubois & Joyce 1989, 1990; Jones & Faragher 1991; Jones & Moody 1993; Joyce *et al.* 1993) for *Banksia* is available, from farm notes (Curtis 1986; Maughan & Merriman 1986; Barth 1988; Webb 1991; Wood *et al.* 1996) to government extension reports (Windle *et al.* 1990), the floriculture newsletter (Burton *et al.* 1994) and specialist literature (Salinger 1985; Handreck 1991; Sedgley & Fuss 1992; Sedgley 1996). However information on how *Banksia* blooms are channelled in the key international markets and how they are used is unknown or inaccessible. Germany is the largest consumer worldwide of flowers and the major export destination of Australian *Banksia* (refer to Appendix 3, p 2 & 3). The future success of the local production industry lies in understanding this marketplace.

Clonal propagation

Clonal propagation is a crucial step in the development of new and improved cultivars. The practical and theoretical aspects of clonal (or vegetative) propagation of woody angiosperms as opposed to sexual propagation by seed, is well documented due to their wide use in ornamental and amenity horticulture, forestry, fruit and nut production (Hartmann *et al.* 1990). Consequently, a wide range of techniques has been developed for a large number of diverse plant types. Over the last 200 years, since settlement by Europeans, vegetative propagation of native Australian flora has been pursued. The pursuit of successful vegetative propagation methods for an increasing number of native plants has occurred in recent decades. This is due to their unique appearance, the

increasing commercial interest both locally and abroad, and due to their increasing worldwide usage as ornamental and amenity species. Propagation by cutting, together with grafting and budding are the most widely used methods of vegetative propagation. Success has been achieved with members of the South African and Australian Proteaceae, notably *Macadamia* in Australia.

Vegetative propagation of many native plants, particularly sclerophyllous species, has been fraught with many unexpected difficulties. This is due to several reasons: our unfamiliarity with the fundamental biology of much of the Australian flora compared to commonly cultivated non-indigenous plants, the lack of selection, the relatively short period of domestication, if at all, of many native species and the limited agronomic knowledge needed for successful cultivation. Commercial exploitation of native species, such as *Banksia*, requires research aimed at overcoming the difficulties of vegetative propagation. For *Banksia* this knowledge is slowly becoming available, and this is reflected in the increased number of publications on the biology, ecology and horticulture of this genus over the past two decades (Cavanagh 1997).

The development of improved cultivars for release to industry is a broad area, drawing from a gamut of different techniques. In the *Banksia* genus it involves working with undomesticated selections from wild plants or elite individuals identified in seedling based plantations. Such selections do exist and have been registered (Sedgley 1991, 1995). However, methods for bulking-up selections or the products of interspecific or intraspecific hybridisation (Fuss & Sedgley 1991a; Sedgley *et al.* 1994, 1996) have not been reliable and are currently holding back industry's progress towards production from improved, clonal plantations to supply the specialist needs of the market. Industry would benefit greatly from the selection criteria chosen to correct weaknesses presently holding back production, such as disease resistance, higher and earlier yielding varieties, longer flowering windows and the introduction of new floral colours and forms.

Clonal propagation is the linchpin for the *Banksia* cut-flower industry. It is needed to match specific market demands, to capitalise on the wealth of genetic diversity in flower forms, colours and leaf forms which exist in the genus, and to open up the possibilities of the development of new ones. The main reasons for clonal propagation are uniformity in production and the development of improved varieties with distinct marketable qualities and production features. Currently, the seedling-based production is variable or limited with respect to these factors.

Selection of specific genotypes, breeding and cross-hybridisation is required in the development of improved forms. Successes in the selection area have resulted in the registration of three new cut-flower cultivars (cvs. 'Waite Orange', 'Waite Flame' and 'Waite Crimson') (Sedgley, 1991, 1995a). However the release of improved selections or hybrids to industry necessitates the clonal bulking up of superior individuals, and it is this step which is rate-limiting in the adoption of selections and hybrids for planting by industry.

Taxonomy and background of species studied

Comparison of *Macadamia* is included in this work on *Banksia*. These two genera are from the class *Angiospermae*, order *Proteales* and are members of the moderately large Family *Proteaceae*, which is primarily a southern hemisphere family (Morley & Toelken 1983). Based on information from the recently completed Flora of Australia, the Proteaceae is estimated to consist of 80 genera worldwide, containing ca. 1768 species. Sixty four and a half percent (64.5%) of these species (1142 species) are found in Australia in 46 genera (57.5%) and 98% are endemic (George 1996).

The Proteaceae is split into seven subfamilies, Macadamia and Banksia belonging to subfamily Grevilleoideae, Tribe Macadamieae and Banksieae (Douglas 1995). The former of these tribes is divided into six subtribes, and *Macadamia* is one of three very similar genera placed in subtribe Macadamiinae. *Macadamia integrifolia*, one of six species of the *Macadamia* genus, is endemic to Australia. Other extra-Australian species of *Macadamia* exist. The *Banksia* genus is placed into one of the two subtribes of the Tribe Banksieae. Seventy-eight (78) species of *Banksia* occur throughout Australia and southern Papua New Guinea and adjacent islands. Eighteen species are spread along the eastern coast, and one species throughout the northern tropics (*B. dentata*). The south-western botanical province in Western Australia is the centre of greatest species diversity with about 56 species, this state having the greatest number of species (Larson 1995). There is no overlap in distribution of any one species between east and west.

Over the past decade the accepted taxonomic classification of the genus *Banksia* is based on George (1988). However, a recent reclassification places *B. coccinea* into a monotypic section, Section *Coccineae*, which is more closely allied to Section *Oncostylis* (Maguire *et al.* 1996). Genus *Banksia* L. f. is divided into two Subgenera: *Banksia* and *Isostylis*. The former is divided into three Sections, *Banksia*, *Oncostylis*

and *Coccineae*. Section *Banksia* is divided into 9 Series, three of which are investigated in this study: *Banksia*, *Crocinae* and *Cyrtostylis*. Section *Oncostylis* is divided into three Series of which one, *Spicigerae*, is investigated, as is the single species in Section *Coccineae, B. coccinea*. An overview of the phylogenetic relationship of the species used is given in Table 1.1.

Early indigenous usage of *Banksia* and *Macadamia* was as bush food plants (nuts and nectar) and medicine (Larson 1995). Since the first records of *Banksia* observations by Europeans in the eighteenth century many *Banksia* species have been singled out for cultivation due to their brightly coloured and spectacular inflorescences, the long lasting qualities of blooms and foliage. They are also used for other economic purposes, such as in timber and honey production (Lazarides & Hince 1993).

Macadamia integrifolia has been included in this study as a comparison. This species has been successfully domesticated and is subject to continued improvement and development. *M. integrifolia* is one of six species in the genus occurring in the subtropical forests of southern Queensland and northern New South Wales. The nuts of *M. integrifolia* are edible, unlike those of some other species, in which the accumulation of cyanogenic glycosides renders them unsuitable for consumption as raw nuts. *Macadamia integrifolia*, first taken off shore for development, now constitutes a productive and growing industry in southern Queensland and northern NSW. Nut bearing plantings now exceed 2,400 Ha (1987) and in 1987 Australian production was 14% of world production, expected to rise to 20% in 5 to 10 years (Coombs 1995; Larson 1995). Nut orchards today are based on grafted rootstocks. As recently as the late 1970s commercial grafting of *macadamia* was unheard of, although strived for. Similar development would be desirable in *Banksia* production.

This study

The research presented in this work provides information fundamental to the "alpha" and "omega" of the *Banksia* production chain, namely: the development of improved cultivars for release to industry and an understanding of the structure and needs of the major *Banksia* export market, Germany. The first experimental chapter delves into the commercial end of the production continuum, examining the German cut-flower market, the destination of the largest proportion of the Australian *Banksia* harvest. The following three biological chapters provide information fundamental to improving clonal propagation within the genus through understanding three aspects of vegetative

biology: anatomy of the stem material used in vegetative propagation, intra- and interspecific grafting, and lignotuber ontogeny. The literature relevant to each of these topics is reviewed at the beginning of each of the respective chapters. How the outcomes of each area have application to the native cut-flower industry, in particular *Banksia* production, is drawn together in the final chapter.

Table 1.1 Taxonomy of the species studied (George 1981, 1988; Maguire *et al.* 1996)

	Wood study Chapter 2	Grafting study Chapter 3	Lignotuber study Chapter 4	Market research Chapter 5	*PC* status[1]	Range
GENUS *Banksia*						
Subgenus *Banksia*						
Section *Banksia*						
Series *Banksia*						
B. serrata	**	Stock	•		RH	E
B. menziesii	**	Scion	•		SH	W
B. speciosa	*				SH	W
B. baxteri	*			•	SH	W
Series *Crocinae*						
B. prionotes	*				SH	W
B. hookeriana	*				SH	W
B. burdettii	*				SH	W
Series *Cyrtostylis*						
B. ashbyi				•	SH	W
Section *Coccineae*						
B. coccinea	**	Scion		•	SM/SH	W
Section *Oncostylis*						
Series *Spicigerae*						
B. spinulosa var. *spinulosa*	*		•		RH	E
B. spinulosa var. *cunninghamii*	**	Stock	•		RH	E
B. ericifolia	**	Scion			RH	E
GENUS *Macadamia*						
M. integrifolia	*					E

1. based on Cho (1983); McCredie *et al.* (1985b); ** = histology and image analysis; * = histological analysis only; PC = *Phytophthora cinamomi,* status: RH = highly resistant; SH = highly susceptible; SM = moderately susceptible; E = eastern Australia; W = western Australia

Chapter 2

Retail Survey and Conjoint Analysis of *Banksia* in the German Market

Quantitative methods and critique of the

case study:

Germany - a major export market for *Banksia*

Abstract

Methods used to collect primary data using two quantitative market research techniques applied to the retail level of the German cutflower industry are described. These are (i) the questionnaire and (ii) the conjoint analysis. The conjoint analysis, normally applied to general merchandise products, was applied to the floricultural product, *Banksia*. This is one of the first reports applying this method to a floricultural product. The combination of research methods proved successful in (i) identifying customer needs and satisfaction levels, (ii) measuring the importance of product attributes, and (iii) understanding market structure, product channelling and cultural differences related to product end-uses.

The major outcomes show:

- four main market access points for *Banksia*, including large international wholesalers, dried flower importers, regional wholesalers and Dutch auctions; separate market channels and end-uses exist for dried and fresh *Banksia*
- cultural differences related to end-uses of *Banksia*: grave floristry is the main market segment, and specialised cemetery nursery florists and days of commemoration to the dead are important in the end-use of *Banksia*
- low levels of "customer" satisfaction with important quality attributes, such as flower size to stem length, grading uniformity and the number of blooms packed per carton, as well as the lack of promotional information

Preliminary experimentation with conjoint analysis on preferred stem length, colour and price combinations generally showed a negative linear relationship between price and preference, a greater utility for lime and red blooms over orange flowers, and higher utilities for shorter stem lengths, (eg. 30 cm). This latter feature shows a negative linear relationship with preference.

Introduction

Adjusting local production to fit the requirements of specific export markets is pertinent to Australian floricultural export activities. This is especially so considering Australia's long history of exporting to western Europe, the close proximity to Asia's large markets, the relatively small domestic market, extensive production capacity (tropical to temperate regions, encompassing different climates and soils) for a diverse range of horticultural products and the uniqueness of the Australian flora. However, understanding the 'wants' of overseas customers located in marketplaces with distinctly different cultural backgrounds is imperative to successfully satisfying overseas customer's needs.

A selection of market research methods can be used to identify customer needs, measure importance of the particular attributes of a floricultural product and assess cultural differences in their usage, as well as understand market access and structure. Such knowledge will allow growers and exporters to supply quality products best suited to their intended end-use. The case study, *Germany – a major export market for Australian wildflower – Banksia* (Mibus 1996, refer to Appendix 3), illustrates how a mixture of qualitative and quantitative market research methods can achieve this. This chapter

describes the methods used to collect primary data and critiques the results of quantitative data derived from two market research techniques applied to the retail level of the German cut flower industry: (i) the questionnaire and (ii) the conjoint analysis.

Marketing and horticulture

Although the discipline of marketing has existed for over fifty years, there is still confusion about what it really involves. It encompasses more than selling and sales, or the production, advertising and promotion of a good or customer service. These are all involved and influence the overall process of marketing a product, but they do not cover the full breadth of the marketing concept, which has been defined as "a business's coordinated and integrated efforts aimed at satisfying its customers at a profit". Or more generally, "the matching of a company's capabilities and the wants of customers in order to achieve the objective of both parties", i.e. profit for the business and satisfaction for the customer (McDonald 1989). In the floricultural sector a "business" or "company" may be an individual flower growing enterprise, a grower-exporter, an exporter or a government marketing agency (*Australian Horticultural Corporation*) or corporation (RSA, *Interspan*). A combination the "four Ps": product, price, placement and promotion, are used as focus points in matching a grower's or exporter's capability to the needs of the customer in the overseas marketplace. It is the best mixture of these variables which makes up the "marketing mix", the marketing term for the mixture of the "four Ps" used to satisfy a particular target group. The marketing plan is a strategic statement identifying the target market segment and which combination of the product, price, placement and promotion, i.e. which marketing mix, will be used to satisfy them. It furthermore embodies the time-related details of how this will be executed (McDonald 1989).

The development of a marketing mix for a certain native plant product, such as dried or fresh-cut flowers, potted plants or nursery plug stock, necessitates making decisions based on information on what is "wanted" in the marketplace. Market research is this information gathering process. It entails: (i) defining the problem, (ii) analysing the situation, (iii) getting problem specific information, (iv) interpreting the data and finally (v) solving the problem by relating the information obtained back to production and delivery of the product (McCarthy *et al.* 1994). Through this process it is possible to match the capabilities of a grower or exporter with the needs of an overseas target market. A two way communication process, involving information flow in both

directions between the producer or exporter and the customer, or customers throughout the market channel (grower to exporter, to overseas importer-wholesaler, to regional wholesaler, to florists and finally consumers), enables the marketing mix to be adjusted to suit the requirements of all market channel members. Thus, the aim is to know the requirements of customers at each level within the distribution chain and to satisfy them as closely as possible.

Marketing principles and market research practices applied routinely in the general merchandise sectors are not widely used in horticulture, and even less so in floriculture. Few research methods have been adapted, tested and applied to the specific requirements of floricultural products. A single report using conjoint analysis and value-pricing methods for assessing value-adding of Australian floricultural product in the Japanese market exists (Alford 1994).

Market research methodology

Numerous methods are available for conducting market research to obtain primary data and generally involve questioning or observations. Questioning can be carried out either qualitatively or quantitatively. Qualitative questioning uses focus or discussion groups, or interviews to obtain in-depth information through open-ended questions and involves a degree of judgement to summarise outcomes (Metcalfe 1994). Yet it provides information from which ideas and hypotheses can be built by further quantitative questioning. Furthermore, the value of observations and visits to customers or members of the market channel made in the marketplace should not be underestimated in the contribution to qualitative information and general cultural understanding. These less intrusive methods are important, as they provide contextual information to support other qualitative and quantitative data.

Quantitative questioning is a structured means of generating objective results with greater rapidity, which can be analysed with relative ease. This type of questioning involves surveys or questionnaires, which are most commonly and conveniently done by mail-outs. Telephone surveys are fast and effective; surveys can also be carried out in person. The type of questions and how they are asked is influenced by the method of questioning chosen to collect the primary data. Question formulation involves closed, usually multiple choice questions and rating scales are often applied to quantitatively measure attitude or preferences. Some commonly used market research approaches will be briefly outlined.

Preliminary market research entails desk research, surveying a wide cross-section of secondary literature from diverse sources to understand the key issues to be addressed and the problems to be solved. On the basis of this situational analysis, research objectives are formulated and the methods best suited to collecting this information selected.

Market research methodology commonly includes questionnaires. These can be powerful tools, if careful consideration is given to the information to be gained and the accurate formulation of questions, both of which are critical for questionnaires to produce meaningful data which can be interpreted and provide useful quantifying measures for statistical analysis. Furthermore, export market research involving a foreign language requires the translation of the questionnaire. To avoid changes in meaning resulting from translation, it is routinely translated back into the original language and compared. Once this standard procedure is complete, the questionnaire is usually pre-tested on a small population sample to check its effectiveness.

Analysis of quantitative primary data has been made more manageable with the advent of statistical computer software, such as *SPSS*. Cross-tabulation is frequently used to analyse and interpret the primary data and determine relationships between variables. In order to ensure that final results can be interpreted accurately the sample size and type must be suitable. However, non-random, or convenience sampling is often used in industrial research due to the high cost otherwise involved and the difficulty of obtaining a truly random sample. Convenience sampling is typically used in exploratory research, where the sample population is small and less variable, as in the context of the conjoint undertaken in this study.

Conjoint analysis (Bretton-Clark 1992; Hair *et al.* 1995) is a unique multivariate method used to understand choice judgements and preferences of consumers and buyers. This can be a powerful technique used to assess how customers make decisions about products (or services) that consist of several features. This method approach often involves a card-sorting task where the buyer (or consumer) is asked to sort cards displaying different combinations of product features, or attributes, for example: price, size or colour. They are then asked to decide which feature combinations they would *purchase* or *not purchase*, and order them from the *most* to the *least* preferred. The critical point is that purchase decisions can be affected by a relatively small number of features. The method assumes the utility (preference) of a product is the sum of the

utilities of each of its features. Features describe the particular choices under consideration. *Conjoint Analyzer* software provides a quantitative estimate of how different features influence the purchase decision by preforming metric conjoint analysis using least squares (OLS) techniques and estimating *vector, ideal point* and *part-worth models*. These three utility models generate estimates of how much a feature impacts on the respondent's evaluation, but in different ways. So the most appropriate model can be selected for each product feature. The resulting utility measure is a relative function measuring the impact of each feature relative to the impact of the other features studied and contains quantitative estimates for each feature. Abstract units, *utiles*, are used to make the measurements of the rating or ranking points - the higher the *utiles*, the greater the preference. Features can be either quantitative or categorical. Price and stem length are quantitative features, whilst colour is categorical (or qualitative).

Part-worth models are the simplest for determining the utility of a given feature level by constraining their sum to zero. This means some feature utilities must be positive, some negative, and they can be rated against one another: the utility of a part-worth feature is one point higher for product X compared to product Y. Alternatively, the difference between two utilities can be compared. For a categorical feature, such as colour, part-worth estimates for each level of the feature are used to generate the utility function.

Vector models on the other hand have only one parameter, the vector coefficient, and are used to model all the levels of non-categorical features with linear utilities. The utility for $level_i$ of a 'vector' feature is equal to the product of the vector coefficient and the value of $level_i$:

$$\textbf{Utility}_i = \textbf{Vector Coefficient x Value}_i$$

The ideal point model is more complex than the vector model, having an extra ideal point term. The utility of an 'ideal point' feature is calculated as:

$$\textbf{Utility}_i = [\textbf{Vector Coefficient x Value}_i] + [\textbf{Ideal Point Coefficient x Value}_i \textbf{ x Value}_i]$$

If the ideal point coefficient equals zero, then the ideal point model generates the same outcome as the vector model. It is recommended to analyse quantitative features firstly using the vector model, which 'cleans' the data, then re-analyse using the ideal point model and compare the results. Generally, the effects of quantitative features are studied using either vector or ideal point models, the latter used for features with non-linear utilities.

Rationale

Germany is the largest consumer of flowers world-wide and receives the largest proportion of Australian *Banksia* exports. Currently, little information exists or is easily accessible on this marketplace or the end uses of *Banksia* product in it. To obtain this information several market research methods used normally for general merchandise products, were applied to the floricultural product, *Banksia*, in the German cut-flower market. The combination of research methods proved successful in providing detail on market structure, distribution channels, customer needs and satisfaction, and information on product end-uses.

Materials and methods

A mixture of qualitative and quantitative methods was chosen to obtain specific information on the German cut flower marketplace. These methods were used to identify: (i) the points of access for *Banksia* into the German market, (ii) the market channels peculiar to the structure of the market and through which *Banksia* move, (iii) the needs of the different "customers" throughout market channels, (iv) the trade-off between important product attributes such as stem length, flower form and price, and (v) the cultural context.

Qualitative research methods

The qualitative research was conducted after preliminary desk research in Australia and in Germany. The qualitative methods used in this study consisted of (i) interviewing key industry people, including importer-wholesalers, governmental representatives from different organisations and representatives of industry associations; (ii) visits to and discussions with different bodies from each industry level: wholesale, retail, education & training, promotion, research, extension and policy and trade.

Desk research

Initial desk research in Australia was undertaken to survey previous literature on the cut flower export industry, which was already available, although not readily accessible. This provided background on the industry, shedding light on the current situation and level of knowledge amongst industry members in Australia on which market research objectives could be formulated.

Existing research results and information on the German cut flower and associated industries was collected during a two week visit to the *Institute of Horticultural Economics*, at the University of Hanover. Many reports and analyses of data on the horticultural sector were available because of the continual monitoring of the industry and the organised infrastructure to sustain this long-term monitoring. Information on other industry organisations visited also provided detailed information, especially primary literature, such as industry associations, ministry or departmental reports.

Interviews

Pre-arranged qualitative market research was conducted by interviewing key people in the German floriculture industry. This method was chosen to obtain an in depth understanding of the main issues relevant to this market research and provide

contextual information not available through other sources. To the 80 letters sent to a broad cross-section of industry representatives, 23 replies (29%) were received. Taped interviews were conducted with eight respondents, including several large international importer-wholesalers (3); government agency representatives (3 including representatives from the German Federal Ministry for Agriculture and a State Chamber of Agriculture, and Austrade in Frankfurt), non-governmental industry trade associations (1) and a retail chain (*Interflora-Fleurop*) (1). Detailed notes were taken during the remaining interviews and discussions.

Visits and observations

Visits to regional flower wholesale markets (traditional (11) and non-traditional (2) BGM) throughout Germany allowed informative discussions to be held with managers and regional importers (13). Additionally, visits were made to people involved in research, education and training (11), workshops and seminars (3), auction houses (2), other governmental organisation representatives (3) and retailers (18).

Many representatives of the ornamental flower industry from Germany and other European countries were present at key industry events, such as the IPM (Germany), NTV (Holland) and the Flower Forum held at the MIFLOR (Italy). These events were ideal locations at which to make contact with most of the key industry bodies and personalities, as well as observe the most recent industry developments and fashion trends.

Projected outcomes from qualitative research

The expected outcomes from the qualitative methods were to gain information on as broad a cross section of the German cut-flower industry as possible. To this end specific market research objectives were established at the beginning of the project. Although many of these areas were incorporated into discussions and interviews where appropriate, these objectives were also used as a basis for the quantitative methods employed. The expected outcomes of the qualitative research methods were: (i) to ascertain detailed information on the structure and operation of the German cut flower market, (ii) to understand the present distribution channels for banksias, both fresh and dried, dyed, and general mark-ups along these channels, (iii) to determine the nature and degree of the demand for banksias, (iv) to determine the current usage and perceptions of fresh and dried banksias, (v) to ascertain which aspects are of importance for the

packaging and transport of banksias and (vi) to explore the areas of product competition, substitution and branding.

Quantitative research methods - retail intercept survey

The retail intercept survey (RIS) conducted in Germany, was used to collect quantitative primary data from German flower retailers on the current issues and perception regarding *Banksia*. This survey of retail florists comprised two tasks: (i) questionnaire and (ii) card sorting task (for conjoint analysis). These methodologies are presented below in sections A (Questionnaire) and B (Conjoint) following general information on how and where the intercept survey was conducted.

Location

The survey was conducted at the International Plant Trade Fair (IPM), in Essen. With between 10,000 and 15,000 florists attending, it was an appropriate venue at which to survey florists. The IMP has over 40,000 visitors over the three days and is the largest European horticultural and floristry fair. It has over 75,000 m^2 and over 160 florist exhibits alone, in three of the exhibition halls. These three halls were targeted as having the greatest likelihood of finding suitable participants. The following problems were encountered.

Firstly, the visitors on each day of the IPM were there for different reasons. For example, most of the trading and placement of orders and re/establishing business contacts occurred on the Friday. On Saturday people were mainly interested in the fair's special exhibits and on Sunday the majority were general interest visitors.

Secondly, a major aspect not envisaged was the psychological attitude of visitors. Generally speaking people attend trade fairs to receive information about their specific branch or are there to fulfil a specific task, such as make purchase orders for their business or visit a possible client or business contact. They are switched into a mode of receiving information, not imparting information as required in a survey.

Thirdly, finding the appropriate location in the vast complex of stands and activities also proved a difficult operation.

Finally, the location which gave the best results was the site of the National Bouquet Competition, the theme of which was "a bouquet for a special occasion", where the floral bouquets of 113 entrants were displayed for the duration of the trade fair. Generally, florists coming to view this display had a casual, relaxed attitude, and were

more open to an invitation to participate in the survey. This location was, however, only found on the eve of the last day of the fair.

In order to increase the number of surveys completed, floristry businesses were visited on an individual basis after the IPM and invited to participate. Four surveys were sent by post to florists with whom contact had previously been made.

Interception method

The general aim was for respondents to sort cards and answer survey questions. Due to organisational and financial limitations of the project, a mobile method of intercepting respondents was adopted, rather than a stationary method where the surveyor would be seated at a table with the option of surveying one or several respondents concurrently. This would have incurred site fees and additional organisation and assistance. Using the chosen method, the surveyor addressed possible respondents with an introductory preamble, inviting them to participate[2]. They were then handed a hard-backed presentation folder, which they could hold comfortably while standing and enter their responses in the questionnaire. The card sorting task could also be done in the standing position, using the folder as a support for sorting the nine cards. The initial approach to individuals was crucial in obtaining participation. The introduction including: greeting, name, where the interviewer was from and the reason for and how long the task would take was used with success.

Participation encouragement

To encourage people to participate in the RIS, they were offered the possibility of winning one of three gift packs of Australian red wine, kindly donated by *BRL Hardy*. The draw was made during the last week in Germany after all questionnaires were completed. The prizes were then posted to the winners. In addition, a glossy colour postcard of a mixed banksia bouquet was presented to all those invited to undertake the RIS (Report frontispiece).

[2] Guten Tag, mein Name ist Raelene Mibus. Ich komme aus Australien und mache eine wichtige Befragung über australische Wildblumen in Deutschland. Ich möchte Sie einladen, Ihre Meinung oder Erfahrung darüber mitzuteilen. (Show wildflower postcard in presentation folder.). Dies bedeutet ein paar Fotos auszusortieren und einen Fragebogen auszufüllen.Wenn Sie mitmachen, haben sie die Möglichkeit eine Geschenkpackung australischen Rotweins zu gewinnen.

RIS compilation

In the final compilation of the RIS, four other pages were fixed permanently into the presentation folder (refer to Appendix 2 of the appended Report). These consisted of (i) an invitation and prize information, (ii) instructions on card sorting and questionnaire, (iii) thanks for participation, instruction for the entering the prize draw and (iv) name and address details for the draw. The individual cards for sorting and the questionnaire were inserted into the folder for each new respondent.

A. Questionnaire - methodology and sample

Quantitative intercept survey methods were used to gain accurate, current information on the knowledge and use of *Banksia* by retail florists in Germany.

The sample population – retail intercept survey

The overall number of questionnaires completed was 24. Ten of these (42%) were completed by visitors (florists) at a professional trade fair, the *Internationale Pflanzen Messe* (IPM). The majority of people (14 respondents) completing the questionnaire were florists, usually the managers, senior staff or owners of their own business, who were approached in person at their business premises. Four questionnaires (16%) were posted to florists. Twenty-two questionnaires were completed in conjunction with a card-sorting task, which was to be conducted as an integral part of the survey.

Although not truly a random sample, the respondents who participated represent one of two types; either a convenience sample of those who are interested in acquiring information about the floristry branch (trade show attendants), or those actively employed as florists in major city centres (Hanover, Berlin, Bonn, and Stuttgart regions) selling directly to end consumers.

Questionnaire design process

A draft questionnaire was compiled based on the preliminary assumption that *Banksia* blooms were relatively unknown to German retail florists. In the preparation of this questionnaire guides were used as norms of good practice *(Question Instruments*, Chapter 5 from (Metcalfe 1994).

The interviews and visits to wholesale markets and retailers in the initial two week period in Germany gave a more detailed insight into the main issues regarding *Banksia* blooms. Through the qualitative information received from these wholesalers and retailers a realistic perspective of the marketplace was formed based on current

information. The initial assumption that *Banksia* would be little known in Germany was overturned because most German flower retailers, mostly florists or florist apprentices working in a florist retail business, specialist florist shops or cemetery nursery florists, were familiar with *Banksia* blooms and had used them in the previous 12 months. Senior staff from the Department of Market Research, Institute for Horticultural Economics, Hanover, were consulted on details of question design and target group. The draft questionnaire based on predefined market research objectives, compiled in Australia in English, was used as a basis for these discussions. The decision was made to target the retail level of industry, specifically retailers visiting the IMP trade fare in Essen, where the survey was to be conducted. The questionnaire was then translated in to German. During the final design period one retailer (a cemetery nursery florist) was approached regarding the general concept of *the questionnaire* and the questions to be used. Although this cannot be considered normal pre-testing, it together with the extensive experience accessed within the department were useful in ensuring the effectiveness of the questions generated.

The questionnaire and data types

The questionnaire consisted of 21 questions, primarily *Likert scale* questions. Nine questions produced data of the lower[3], nominal (categorical) data type (Table 2.1); seven questions produced interval data and three ratio data. Only two questions required written, descriptive answers, question 10 and 11, on the perceived negative and positive attributes of *Banksia*. The last five questions pertained to the demographics of the business that the respondent was associated with: city size, location within the city, number of employees, turnover and type of florist. Code numbering in the top right-hand corner of each questionnaire was used to identify respondents and ensure anonymity. The topic addressed by the remainder of the questions and the type of data collected is summarised in Table 2.1.

Statistical analysis

The analysis of the questionnaire was done using computer software packages, *SPSS* (made available by the University of Adelaide, Graduate School of Business

[3] The data forms listed from highest to lowest: ratio > interval > nominal

Management) and *Microsoft Excel.* The post-codes of respondents were obtained from the information filled in by respondents for the prize draw. These gave an indication of the geographical range of respondents from within Germany (refer to Appendix 4 of the appended Report).

B. Card sorting experiment – Conjoint analysis

The aim of this experiment was to determine the main predictor variables (factors) and their respective values used in the determination of buyer preferences. The buyer was assumed to be German flower retailers (mostly florists or florist apprentices employed in florist retail businesses, specialist florist shops or cemetery nursery florists). Also, based on information gathered whilst in Germany, it was assumed that most florists know what *Banksia* flowers are and have worked with them in the previous 12 months.

Methodology

The method involved using computer software packages (*Conjoint Design* and *Conjoint Analyzer)* (Bretton-Clark 1992) to design the feature and level combinations and then analyse the data collected from the card sorting tasks. The card sorting was completed by 30 florists and was done before the questionnaire in 70% of cases. The remaining 30% were completed as separate card-sorting tasks, where the respondent's time was limited. Of the thirty respondents, 13 were visitors at the IPM trade show, whilst the others were approached at their florist premises.

Conjoint design process

Initially, a description of all attributes (features) of *Banksia,* which provide value and utility to this product, were recorded. These were: stem length, percentage anthesis, colour, size of bloom, foliage (form and amount), price, country of origin, availability, production method (bush harvested, plantation or "clean green"), bunching, packaging and labelling. The *Conjoint Designer* software (made available by the University of SA, School of Marketing) applied a method of special experimental design (orthogonal arrays) to automatically generate the smallest number of cards to sort, given a specific set of features.

The features used in the conjoint were kept to a minimum of three, allowing for the fact that the other features could be addressed in the questionnaire. Three of the most important features involved in purchase decisions were elected - colour, stem length and price - each feature having three levels (Table 2.2). Once features and levels were

Table 2.1 A key to questions and the type of data collect from each and the location of the results in the appended Report

QUESTION NO.	DATA TYPE	SUBJECT	LOCATION OF RESULTS
1	Interval	Familiarity with three *Banksia* (*B. coccinea, B. baxteri, B. ashbyi*)	Table 4
2	Nominal	Source of fresh *Banksia*	Table 4
3	Interval	Quality	Report, Fig.18, p.51
4	Nominal	Last used	Table 4
5	Nominal	Colours and seasonal usage	Report, Tables, 16, 17 & Fig.20, p.57
6	Interval	Information availability	Report, Fig.19, p.53
7	Nominal	Source of dried/dyed	Table 4
8	Ratio	Proportion used (fresh, dried, dyed)	Table 4, Report Fig. 16, p.41
9	Interval	Confidence in arranging them for different end uses	Table 4
10	Descriptive	Positive and negative attributes of *Banksia*	Report, Table 15, p.49.
11	Descriptive	Open question - other factors considered when purchasing	-
12	Nominal	Importance of origin: cultivated or bush picked	Report, Table 18, p.59
13	Interval	Probability of using the different *Banksia* value-added products	Table 4
14	Interval	Important criteria in the decision to buy a new flower type	Table 4
15	Interval	Preferred source for buying a small weekly delivery of Australian natives	Table 4
16	Ratio	Percentage of turnover towards four major purchase segment	Report, Fig.12, p.24
DEMOGRAPHICS			
17	Nominal	City size of business location	Report, Appendix 4
18	Nominal	Location in the city	"
19	Ratio	Number of full time and part time employees	"
20	Nominal	Turnover	"
21	Nominal	Type of florist	"

Table 2.2 Features and levels for each, used in the conjoint analysis

FEATURE	LEVEL
Flower colour	Lime
	Red
	Orange
Stem length	30 cm
	50 cm
	70 cm
Price per stem	DM 4
	DM 8
	DM 15

Table 2.3 Photo-cards used in the sorting tasks, showing the different level defined for each of the three features, colour (or flower type: small or long), stem length and price

Card identification no.	COMBINATION OF LEVELS		
	Colour (species)	Stem length cm.	PriceDM
1	Lime (*B. baxteri*)*	70	4
2	Red (*B. coccinea*)*	70	15
3	Lime (*B. baxteri*)	50	15
4	Orange (*B. ashbyi*)*	30	15
5	Orange (*B. ashbyi*)	50	4
6	Red (*B. coccinea*)	50	8
7	Red (*B. coccinea*)	30	4
8	Orange (*B. ashbyi*)	70	8
9	Lime (*B. baxteri*)	30	8

* shown in Plate 2.1

entered, the *Conjoint Designer* produced a random, minimum number of combinations of features and levels to be used on a valid minimum number of cards. So, although not all twenty-seven (27) possible combinations are used, it calculates the smallest design of cards to achieve the most statistically efficient results for a main effect only model. The *Conjoint Designer* produced nine combinations, the features and levels of which are shown in Table 2.3.

Previous trials using the *Conjoint Designer* software produced designs with 16 possible combinations (cards). These preliminary designs included an additional one or two features ((i) plantation grown or bush harvested and (ii) small or large sized flowers) in addition to those features used in the final selection. However, these designs were

Plate 2.1 Three examples of photo-cards

Examples of three of the nine photo-cards (10 cm x 15 cm) used in the card-sorting exercise. The three *Banksia* species: *B. baxteri, B. coccinea,* and *B. ashbyi* represent different flower colours (lime, red and orange), as well as different forms (small and long). The same photos were used for the other card, only the label details varied, as listed in Table 2.3.

Translation of photo-card labels:

(a) Lime, small *Banksia* (*B. baxteri*), 70 stem length, wholesale price of DM 4 (life size)

(b) Red, small *Banksia* (*B. coccinea*), 70 stem length, wholesale price of DM 15 (life size)

(c) Orange, long *Banksia* (*B. ashbyi*), 30 stem length, wholesale price of DM 15 (¾ life size)

grüngelb, kleine Banksien

70 cm. langer Stiel

4,00 DM Großhandelspreis

Lebensgröße

rot, kleine Banksien

70 cm. langer Stiel

15,00 DM Großhandelspreis

Lebensgröße

orange, lange Banksien

30 cm. langer Stiel

15,00 DM Großhandelspreis

¾ Lebensgröße

Plate 2.2 German and Italian cemeteries and end-uses of *Banksia*

(a) Italian cemetery, 50 km south of Milan, depicting the different cultural application of tradition and synthetic flowers used to decorate graves

(b) Dried-dyed *B. coccinea* used as a focal flower, together with other fillers in a dried arrangement for personal use in the home on sale in an Italian retail florist-garden centre near Milan, Italy

(c) *B. coccinea* dried and "coloured" in bunches of five on sale in an Italian retail florist-garden centre on the outskirts of Milan, Italy. 40,000 Lira

(d) A typical German cemetery, Kornwestheim, with "forest-like" appearance, wide paths, large established conifers and deciduous trees. Source: James (1997)

(e) *B. baxteri* - dried and dyed – as a focal flower in a typical grave decoration in a German cemetery, Stuttgart, 1996


a


b


c


d


e
DEUSCHLE
ERNST
MAGDALENE

discarded due to the unrealistic number of cards to sort. Once the card design was finalised, the photo cards were produced, labelling each photo with the specific colour, stem length and price, and a scale to indicate the actual size of the flower represented in the photo-card. The photo-cards were numbered on the reverse side from one to nine for the purpose of recording the order of respondents' preferences. Plate 2.1 shows examples of three of the final photo-cards used and the labelling in German. The task that followed involved respondents rank ordering the cards, sorting them from "most" to "least" preferred. Lower scores indicate a higher preference in the ranking task.

The task

Florists were presented with nine cards and asked first to sort the cards into two groups: those they *would buy* and those they *would not buy*. They were then requested to order each group from most preferred to least preferred. The two groups of cards were put together with the "would buy" group on top. The resulting order of the cards showed the respondents response from most preferred to least preferred. The card order was recorded and they were re-shuffled for the next respondent to sort.

Conjoint analysis

Conjoint Analyzer software (University of South Australia, School of Marketing) was used to analyse the data collected from the card sorting task. This included model selection, comparison of alternative models using individual and group statistics and the distribution of preferred levels. Market simulation was not conducted. Ranking of the data was non-metric. Microsoft *Excel* was used for the frequency analysis of the data.

Results

The qualitative research methods were chosen to give in depth understanding to the key issues, as well as a broad spread of information on each industry level. Importers and wholesalers were targeted for detailed qualitative information, whilst preliminary quantitative results were obtained from the retail level of the industry. However, all the analysed questionnaire data have been integrated with qualitative data throughout the Final RIRDC Project Report (Appendix 3); a key to where the data from each question are located in the Report is presented in Table 2.1. The next section (section A) is a summary of the main findings of the qualitative interviews and visits, together with the main outcomes of the questionnaire, nine questions of which are summarised in Table 2.4. Results of the experimental card-sorting task and conjoint analysis are presented in section B.

A. Summary of main outcomes of the questionnaire and qualitative research

Market structure

The information on market structure obtained included: market access points, separate market channels for dried and fresh Banksia, as well as user segmentation based on the reason for making flower purchases (Report, pp. 6 – 31).

Although the structure of Germany's cut flower industry is complex, it can be simplified to a six tiered structure (Report, Fig. 6). Large international importer-wholesalers are positioned at the apex, followed by the German auction houses, the importers of artificial and dried flowers, the wholesale level, with this all founded on a large consumer base.

There are four main access points of Banksia into the German market. These are the large international importer-wholesalers, several very large companies dealing with the importation of dried/artificial flowers, the Dutch auctions (sourced mainly from Israel) with their door-to-door van distribution network, and to a lesser extent, importers at the flower wholesale markets, who act as regional points of entry for Australian products.

Segmentation of the German market based on the reason why people purchase flowers generally gives these four segments: gifts (64%), personal use (16%), grave decoration, (10%) and institutional (10%) (Report, Fig. 23). The Grave decoration segment is, however, the main segment in which *Banksia* is used Plate 2.2e. Compared to other

flowers, the market segmentation of *Banksia* purchases alone is quite different, with the grave/cemetery segment dominating (Report, p 26 & 46).

Customer needs

As the product moves through the market channel it goes through the hands of the various channel members. The needs of each of these "customers" must be satisfied. The case study, however, showed low levels of customer satisfaction. Qualitative data received from interviews of wholesale-importers supported the results of the retail questionnaire that needs were not being satisfactorily met. One of the market research objectives was to obtain information on exactly by whom and why dissatisfaction was occurring, so that the areas of product quality and delivery requiring improvement could be identified. Some of the prominent areas identified in this study were (i) the lack of promotional information, and (ii) unsatisfactory quality for the production criteria: grading uniformity, flower size to stem length, and the number of blooms packed per carton. These product criteria were not suited to the end-uses within the marketplace. Furthermore, these all reflect on the product's quality image within the marketplace and are discussed further below.

(i) Information

Florist retailers were asked about how satisfied they were with the information available on *Banksia* (Report, Fig. 19). Examples of four information criteria judged were: the assortment of *Banksia* species which were available, times of the year they could be obtained, methods of care and storage, and complementary material which could be used successfully in arrangements with banksia. Florists were asked to indicate their level of satisfaction for each of these criteria, using a six point scale ranging from very good to unsatisfactory. Satisfaction research shows that a minimum of 80% of respondents should give responses that fall into the good and very good end of the scale for the product to be creating adequate levels of customer satisfaction (Dickson 1997).

However, results showed low levels of satisfaction for all the information criteria surveyed and that there is room for improvement in the area of information and promotion. Furthermore, throughout other industry levels (wholesale and importers) the perception exists that little information is available on assortment, care and storage and times of availability. Yet with a unified, market driven industry these promotional issues can be addressed for the benefit of all.

(ii) Quality

German flower retailers were also surveyed on how satisfied they were with the quality of *Banksia* blooms they used (Report, Fig. 18). The quality criteria assessed by them included: uniformity of grading, the ratio of stem length to flower size, freedom from blemish for flowers and leaves and if flowers were at their optimal stage of flowering. Inadequate levels of satisfaction were recorded for all quality criteria assessed, which were well below the acceptable 80% level (i.e. less than 50%), ranging from very good to sufficient or poor. All these criteria are controllable production variables, which are within producers' and exporters' power to optimise and thus increase the degree of customer satisfaction by providing product suited to its end-use and which fulfils the needs of the channel members who handle it.

Cultural differences

Three areas of cultural difference important in this study were (i) grave floristry, which is the main market segment for *Banksia* (Plate 2.2d and also refer to Report, Plate 3, p. 39) and, associated with this, (ii) specialised "cemetery nursery florists" located near cemeteries with a florist retail outlet - the main users of dried, dyed and fresh *Banksia* - and which are quite distinct from anything in Australia and (iii) the days of commemoration to the dead: All Saints Day (1 Nov.), All Souls Day (2 Nov.), Commemorative days for war dead (Nov.). A knowledge of these cultural differences is crucial to successfully supplying this or any market.

Table 2.4 Summary of frequency analysis of the results of questionnaire conducted in February 1996. Note: Cum. % = Cumulative %

Subject	Question.	Response	
Familiarity with three types of *Banksia*	1	Cum. %[4]	
B. ashbyi		65	
B. coccinea		57	
B. baxteri		23	
Confidence in usage	9	Cum. %[5]	
Grave arrangements & decorations		91	
Wreaths		67	
Room arrangements		42	
Table bouquets		37	
Dried arrangements		33	
Primary source of fresh *Banksia*	2	Valid %	Count
Flower wholesale market		52	2
German importer-wholesaler		9	7
Door-to-door wholesale vans		30	1
Dutch auction		4	1
Australian exporter		4	1
Primary source of dried *Banksia*	7	Valid %	Count
Flower wholesale market		43	9
German importer-wholesaler		48	10
Australian Exporter		5	1
Florist accessory supplier		5	1
Last used	4	Valid %	Count
Since beginning of 1996		17	4
Since mid 1995		61	14
Before 1995		22	5
Volumes of fresh, dried and dried-dyed used[6]	8	Valid %	Count
Fresh *Banksia*			
< 30%		83	20
30% to 60%		4	1
> 60%		13	3
Dried-dyed *Banksia*			
< 30%		63	15
30% to 60%		25	6
> 60%		13	3
Dried, natural *Banksia*			
< 30%		38	8
30% to 60%		38	8
> 60%		24	5

[4] cumulative percent of familiarity levels 1 & 2

[5] cumulative percent of confidence levels 1 & 2

[6] as percentage of the total volume of *Banksia* purchased

Table 2.4 continued

Subject	Question	Response	
Product range – value added *Banksia*	13	Cum. %[7]	
Fresh foliage		73	
Large dried flowers		57	
Dried foliage		52	
Dried dyed flowers		46	
Small dried flowers		41	
Small dried dyed flowers		38	
Small cones		36	
"candles" (Immature inflorescence)		36	
Dried "candles"		29	
Large cones		27	
Hypothetical: most desirable direct source	15	Cum. %[8]	
From the producer		68	
German-importer wholesaler		57	
Australian Exporter		55	
German auction house		7	
Main source of information for retailers	14	Cum. %[9]	
Trade magazines		58	
Trade exhibitions		55	
Wholesalers recommendations		42	
Auction trends		40	
Other retailers		29	
Importers		26	
Purchase segmentation	16	Valid %	Count
Gifts			
< 30%		58	14
30% to 60%		33	8
> 60%		8	2
Cemetery decorations/wreaths			
< 30%		75	18
30% to 60%		21	5
> 60%		4	1
Commercial/ special event			
< 30%		83	20
30% to 60%		17	4
> 60%		-	0
Personal use			
< 30%		88	21
30% to 60%		13	3
> 60%		-	0

[7] cumulative percent of probability scale levels 1 & 2

[8] cumulative percent of preference scale levels 1 & 2

[9] cumulative percent of importance scale levels 1 & 2

B. Card sorting – conjoint analysis

The sample population

The people who undertook the card sorting task consisted of visitors to the professional trade fair (43%) and others present at their place of work, usually the managers (or senior staff) or owners of florist shops. Although not truly a random sample, the respondents who participated represent either a convenience sample of those who are interested in acquiring information about the floristry branch and the latest industry trends or those employed actively as florists in major city centres (Hanover, Berlin, Bonn, Stuttgart and Munich).

Model selection and analysis

Three different utility models are used in *Conjoint Analyzer* to estimate how much the features and levels have influence on the respondent choices. The choice of model is dependent on whether the feature is quantitative, qualitative or categorical. The results of these models must be interpreted in a relative manner, because the influence of a particular feature and level is measured relative to the influence of all the other feature-level combinations in the study.

The part-worth model is best suited to the colour feature, whereas vector (linear) or ideal point (curvilinear) models are best suited to the two other quantitative features: stem length and price. The software allows only vector features to be logically ordered in the analysis.

Once data has been screened for reversals [10] and bad data, it is recommended that the data are re-analysed using several models (Bretton-Clark 1992).

Feature Utilities

Figure 2.1 shows the utilities of the features price, colour and stem length for the top three models. The utility, or preference, is measured by the abstract units of *utiles*. Only part-worth models are presented in the graphs with standard errors, because *Conjoint Analyzer* only generates these for part-worth models. Generally, for this study, price is a

[10] Reversals are data of logically ordered features which had been violated, ie. the ordering assumption for the feature involved has not been upheld eg. price is logically ordered because rational consumers prefer lower prices compared to higher prices. Also in floriculture and floristry, it is assumed that longer stems are superior to shorter stems. However, depending on the specific end use of the flower, this may not always hold true (refer to discussion)

non-linear feature best suited to ideal point analysis, lower prices having higher utility. However, model three suggests that for some florists there may be increased utility for flowers from higher price categories, blooms of higher prices being of better quality. Colour is a categorical feature; part-worth modelling is best suited to this feature. The part-worth analysis of flower colour in Models 1 and 4 produces results only nominally different from one another (the lines over-lapping in the graph). There seems to be an equal preference for lime and red flowers over orange blooms. Stem length is best suited to vector model analysis, shorter stems having greater utility than longer stems.

Comparison of alternative models

Using the 'comparison of alternative models' feature in the *Conjoint Analyzer* program, the models were compared to assess which has the strongest predictive accuracy (Table 2.5) indicated by the adjusted Rsquare values of each model. Table 2.5 below shows five model combinations tested and how well each fits the data (average adjusted Rsquares). Of the model combinations tested, model 1 and 3 are tied, as both having adjusted Rsquares of 0.716. This is followed by model 4 (Adj. Rsquare = 0.672). These three models are best at fitting the data.

The feature of stem length was analysed first by using the vector model (model 4), followed by analysis using the ideal point model (model 5). The analysis using the ideal point model resulted in an ideal point co-efficient of zero, which means this model is equivalent to the vector model. This indicated that stem length is a vector feature, which has a linear utility function. The use of the vector model for the feature, stem length, is supported by the 'group relative importance' and 'individual relative importance', which are less than one standard error away from one another in model 4.

Price is usually considered to be a vector feature, but in this study it tends towards being more curvilinear (ideal point) than linear.

Group statistics

Considering the three models with the highest predictive accuracies (models 1, 3 and 4) in Table 2.5, it can be generalised that *price* has the highest relative importance of the three features tested. However, care should be taken when ascribing a percentage to the importance of *price*, (eg. 72%, model 4), as this depends on the range of levels of all features used in the study.

Figure 2.1 Utilities of price, colour and stem length for the top three models

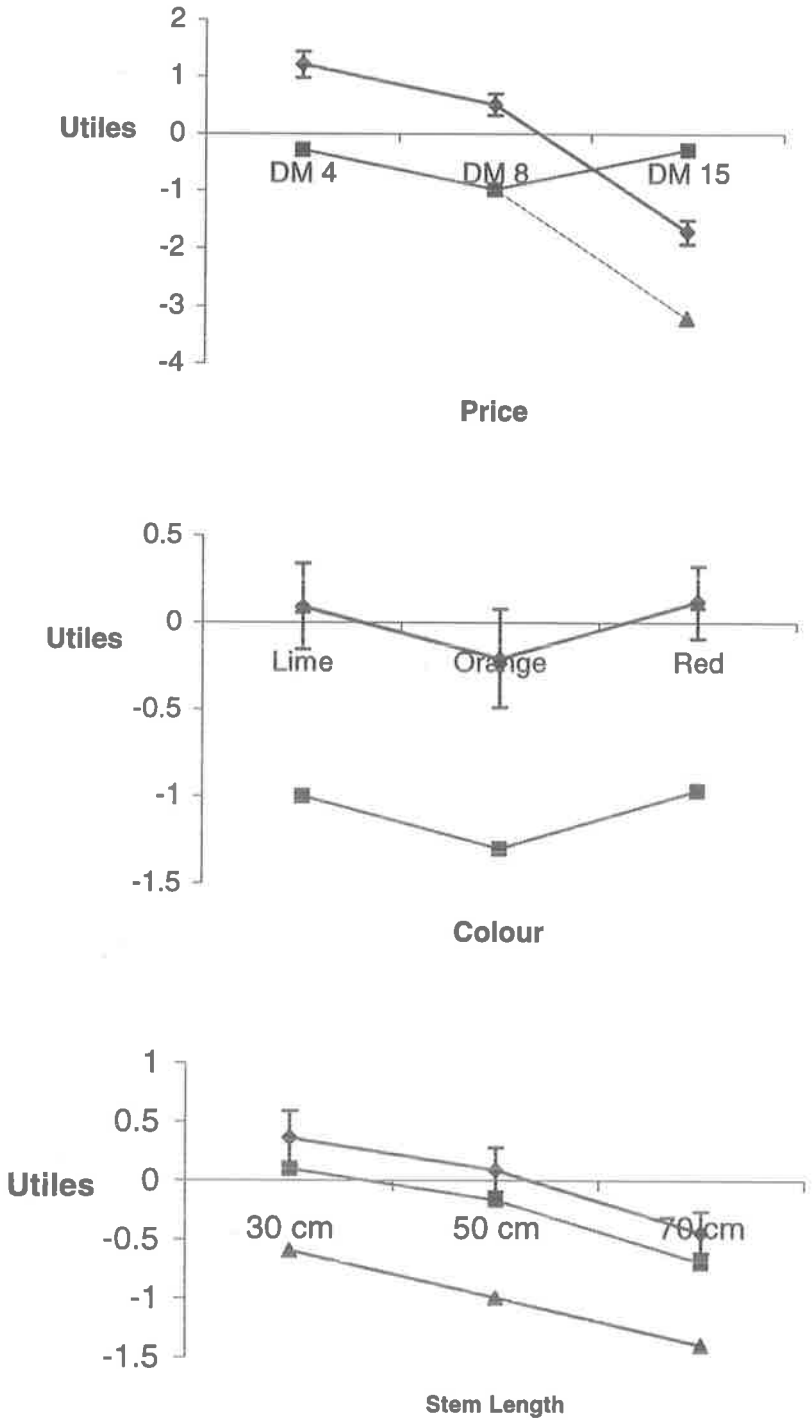

Table 2.5 A comparison of five alternative models resulting from the analysed data for the card sorting exercise

Model no.	Feature	Utility model applied[11]	Adjusted Rsquare 'best fit'[12]		Relative Importance, RI %			no. of standard errors distance between 'group' and 'individual' RI
					Individual	(SE)	Group	
			%	(SE)				
1	Price	Part worth	**0.716**	(0.091)	42.01	(4.06)	72.13	7
	Colour	Part worth			31.53	(3.33)	8.20	7
	Stem length	Part worth			26.46	(3.04)	19.67	2
2	Price	Vector	0.613	(0.090)	39.99	(4.49)	72.57	7
	Colour	Part worth			35.79	(3.60)	8.07	8
	Stem length	Vector			24.22	(3.25)	19.36	1
3	Price	Ideal point	**0.716**	(0.091)	42.17	(3.96)	72.13	8
	Colour	Ideal point			31.33	(3.30)	8.20	7
	Stem length	Ideal point			26.50	(3.05)	19.67	2
4	Price	Ideal point	**0.672**	(0.086)	45.20	(4.15)	72.13	6
	Colour	Part worth			33.24	(3.48)	8.20	7
	Stem length	Vector			21.57	(2.80)	19.67	< 1
5	Price	Vector	0.649	(0.093)	37.34	(4.35)	72.57	8
	Colour	Part worth			33.10	(3.40)	8.07	7
	Stem length	Ideal point			29.56	(3.48)	19.36	3

[11] Definitions for the different models are given in the market research methodology section of the introduction

[12] Rsquare measures how much of the variance in the original data is captured by the utility function, but it is a bias measure with typically few degrees of freedom. This is however corrected by the use of an adjusted Rsquare (Bretton-Clark 1992).

Sample heterogeneity

A comparison of the relative importance (RI) for individual respondents with the "average" or group RI (Table 2.5) indicates that respondents are heterogenous across all of the resulting models. Therefore the group utility function can be considered as misleading and will be ignored here. Furthermore, as group data differ widely from individual data and accordingly will lead to aggregation errors, only the individual level results will be considered for the features of *price* and *colour*.

Distribution of preferred levels

The distribution of preferred levels for the top three models are shown in Table 2.6. This shows the percentage of respondents that preferred each level for each feature. In this case, 'preference' means the respondents had greater utility for this level, compared to the other feature levels. So in model 3, 63% of respondents had a greater utility for low price (DM 4), 33% for medium price (DM 8) and 3% for highest price (DM 15). This is consistent with the importance of price.

For the colour feature, 42% preferred lime (*B. baxteri*) over orange (*B. ashbyi*) (35%) or the red banksia (*B. coccinea*) (23%). For the three stem lengths, 30 cm, 50 cm and 70 cm, the utility varied. The greatest number of respondents (57%) preferred short stems (30 cm), whilst 25% preferred 50 cm stems and 18% preferred 70 cm stems lengths.

In contrast to model 3, where stem length was analysed using the ideal point utility model, model 4 showed markedly different results for the feature of stem length (Table 2.6). That is, the preferences for stem length were distributed between two levels only: 30 cm, which received 67% of preferences and 70 cm, which received 33% of preferences

Frequency analysis

A summary of the frequency analysis of the card sorting responses is presented in Table 2.7. (Raw data together with frequency and cumulative percent tables are presented in Appendix 2). The frequency summaries indicated that the lime, 30 cm banksia, priced at DM 8 received the highest number (27%) of placements as the first card in the sorting task; the lime, 70 cm banksia, priced DM 4 scored second, and the red, 30 cm banksia, priced DM 4 was the third most popular choice as first card.

The most preferred second card was the red, 30 cm stemmed banksia, priced at DM 4 (33%), followed by the orange, 50 cm, DM 4 (23%) and the lime, 70 cm, priced at DM 4 (13%).

For those cards placed third in the sorting task, the red *B. coccinea* with 30 cm stems, priced at DM 4 and *B. ashbyi* with 50 cm stems at DM 4 were preferred equally (20% of preferences), followed by *B. ashbyi* (orange) with 70 cm stems, at DM 8 (17%).

The least preferred of all nine cards were those at the DM 15 level, regardless of flower colour or stem length. All three cards labelled DM 15, i.e. lime, 50 cm, DM 15; orange, 30 cm, DM 15 and red, 70 cm, DM 15, were the three cards with the highest frequencies at place seven, eight and nine in the sorting exercise.

For the smaller type *Banksia* (*B. coccinea* and *B. ashbyi*), 30 cm stem lengths received greater preference scores, whereas the larger flowered *Banksia* (*B. ashbyi*) required longer stem of 50 cm or 70 cm. Large flowers with 30 cm stem length were least preferred.

Table 2.6 Summary of the distribution of 'preferred levels' for the three models with the highest predictive accuracy

FEATURE	LEVEL	MODEL 1 (pw.pw.pw) %	MODEL 3 (ip.ip.ip) %	MODEL 4 (ip.pw.v) %
Price	DM 4	60	63	60
	DM 8	36	33	37
	DM 15	4	3	3
Colour	Lime	42	42	42
	Orange	35	35	35
	Red	23	23	23
Stem length	30 cm	54	57	67
	50 cm	26	25	0
	70 cm	19	18	33

Note: pw (part-worth), ip (ideal point) and v (vector) are the combinations of utility functions used in each model.

Table 2.7 Summary of frequency analysis of the preferences for each card in the sorting task[13].

Colour	species	Stem Length cm.	Price DM	Card no.	No. of placements of each photo-card for each preference level								
					Preferences								
					1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Lime	*B. baxteri*	30	8	6	1(27%)	8	4	2	6	2	5	8	7
Lime	*B. baxteri*	50	15	3	6	5	7	4	8	5	2	2	3
Lime	*B. baxteri*	70	4	9	2(20%)	3(13%)	5	8	7	3	6	9	4
Red	*B. coccinea*	30	4	5	3(17%)	1(33%)	1(20%)	6	9	7	7	7	9
Red	*B. coccinea*	50	8	2	5	6	6	1	1	1	9	5	8
Red	*B. coccinea*	70	15	8	9	9	9	9	4	8	3	1	1
Orange	*B. ashbyi*	30	15	4	7	7	8	5	5	6	1	3	2
Orange	*B. ashbyi*	50	4	1	4	2(23%)	1(20%)	7	3	9	4	4	6
Orange	*B. ashbyi*	70	8	7	8	4	3(17%)	3	2	4	8	6	5

[13] Refer to Appendix 2 for comeplete raw data and cum. % tables for each preference leve.

Discussion

This study confirms that a mix of market research methods is appropriate when the situation is largely unknown, as in the case of *Banksia* exported to Germany. Qualitative methods such as discussions, interviews and observations provide a broad insight into the marketplace, allowing the key issues to be identified. The areas requiring more detail can be identified and quantitative methods applied to gather primary data. This proved a most appropriate approach in the case study described here, where little starting material specific to *Banksia* was available. A choice of only qualitative methods would give a broad view of the marketplace without detail. On the other hand, applying quantitative methods without a broad understanding of the industry and the main issues regarding the product may run the risk of producing ineffectual, inconclusive outcomes which require further research. The preliminary results gained from quantitative questioning have supplemented information translated from existing German statistics and market reports. Important information over and above the expected outcomes was provided from the qualitative questioning. This included experts' opinions on the current situation regarding consumption and local production, competition between importers, and a historical perspective on the development of use of *Banksia* in Germany over the past two decades. Generally a context was provided into which quantitative information could be viewed. Moreover, the qualitative questioning helped finalise the best approach for the conjoint analysis, in particular what were deemed to be reasonable stem length and price levels, and which level of industry to approach, namely the retail level.

The market segmentation for *Banksia* based on the reason for purchase shows *Banksia* is predominantly used in funeral floristics, contrasting with the largest segment of general flowers purchases which is as gifts or for personal use. This difference in market segmentation was confirmed by the use of quantitative questioning.

Information on market structure generated by interview and desk research has provided a framework on which the market channels and points of access of *Banksia* into the German market can be superimposed. This knowledge facilitates more effective market penetration. Furthermore, qualification of customer satisfaction in the areas of *Banksia* quality and the availability of information provide benchmarks which can be used to assess the effectiveness of future efforts to improve the image of *Banksia* in the German market. It also indicates to producers the current less than satisfactory perception of

Banksia, and that improvement is necessary to maintain and increase competitiveness in this marketplace.

An understanding of the cultural differences peculiar to the German market and how this influences the primary end-use has been gained through visits and observations, and this has been further substantiated through quantitative outcomes. Florists were most at ease using *Banksia* in grave arrangements. Product specifications must be oriented towards satisfying the florist requirements, such as smaller, dried *Banksia* blooms with stem lengths of 10 cm, called 'picks', in a range of suitable colours. However, promotional activities to change the perception of *Banksia* as a grave flower would need to focus on other positive features, such as their longevity as fresh flowers and their nostalgic association with warm, exotic, distant countries.

Conjoint analyses provide exploratory data because results obtained are directly related to the assumptions made on the model forms and the variables Hair *et al.* 1995. Also, heterogeneity found in most conjoint analysis studies is due to market variability. For heterogenous markets or samples consisting of different segments, each with different utility functions, it is not possible to average across these segments, because this leads to biased results. Moreover, market simulations are the best way to summarise the findings. This would require further research based on the current conjoint findings. In this conjoint analysis large differences of more than six standard errors between the group and individual Relative Importance values of *colour* and *price* were observed. This indicates that different florist retailers have very different preferences for *colours* and *price*. The significant degree of heterogeneity for *price* indicates florists differ in their degree of price sensitivity. This supports other findings refer to Report, Fig. 5 showing a polarisation of the market with the share of medium value product decreasing in favour of the high and low value product.

The outcomes of the quantitative research presented here are useful because they support other general information on the German cut flower industry and show trends on how *Banksia* fits into this marketplace. However, the quantitative data should be viewed as preliminary only, and in combination with other qualitative information from translated reports. The low sample numbers and the non-representative convenience sampling place inherent limitations on how the data can be interpreted. Yet it is interesting to note that despite the convenience sampling it is still possible to obtain outcomes which provide enough detail to show segmentation within the sample

population as described in the previous paragraph. Cross-tabulation of the primary data from the questionnaire was ineffectual due to the small sample size. A larger data set would be required to draw meaningful conclusions on the intersection of different subsets of data. However, the trends arising from this work can be used to conduct more detailed quantitative market research on a particular market segment where cross-tabulation could be effectively carried out.

The rating scales were effective in measuring perception and attitudes towards banksias. More thorough pre-testing may have shown weaker areas in the questionnaire design and how it could possibly be shortened, although respondents did not show irritation or impatience at the length of the questioning. The question validity was generally high, questions giving useful data on the areas required. This was due mainly to the prior involvement of and scrutiny by senior market research staff in Hanover and Adelaide, and the design of the questionnaire based on question formats routinely used in horticultural market research. The task realism of the card sorting exercise was also high, and although the application of the technique was novel to floral products, respondents quickly understood the task at hand and were more than willing to participate. One card was, however, pointed out on several occasions by respondents, lime flower *B. baxteri* with a long stem length 70 cm, priced at DM 4 Card no. 1, Table 2.3 - long stem length is normally regarded as a product feature for which premium prices are paid. However, different segments exist for *Banksia* depending on its end-use. For example, some florists are prepared to pay higher prices for stem lengths of 30 cm to be used in round table bouquets; less work is involved trimming each stem to the ususal 30 cm length for such arrangements, so some florists are willing to expend more for shorter stem lengths. Another segment of florists are prepared to pay a premium for long stemmed *Banksia* blooms 70 cm to be used in tall arrangements for commercial or corporate foyers and reception areas. These trends are reflected in Figure 2.1, where a curvilinear response to price is observed for model 3 – a slightly greater utility in paying a higher price. The graph for stem length in the same figure showed that shorter stem lengths have greater utility compared to long stem lengths.

So it is the analyst's responsibility to choose the utility model best suited to each feature and resulting in the highest predictive accuracy. Of the three models which best fit the data, Model 4 which is based on a combination of price, colour and stem length features, probably best represents buyer's purchase behaviour for banksia flowers. Even though

this model has a slightly lower predictive accuracy Rsquare compared to Models 1 and 3, the individual utility models applied are better suited to the features studied, that is ideal point for price, part-worth for colour and the vector model for stem length. All models show a large heterogeneity between the group and individual relative importance, but Model 4 gives the least amount of difference between these, having the lowest number of standard errors between the group and individual relative importance. However, to understand the exact relationship between these variables, analyses based on larger sample sizes would be needed to draw firmer conclusions.

In export market research, a fluent command of the language of the marketplace is crucial. During interviews, questions would be worded and ideas developed to suit the level of understanding and participation of the respondent in the industry. Although the outcomes involve the subjective interpretation and judgements of the interviewer, this would not be possible without a high degree of fluency. This is particularly so when interpreting nuances, which are often difficult to identify and are closely associated with cultural differences.

The development of the different cut-flower marketplaces within Western Europe is also worth mentioning. Although Germany is the largest cut-flower market and consumer of *Banksia* overall, blooms are also exported in large volumes to other western European countries, such as Italy and the other German speaking countries, Austria and Switzerland Report, Fig. 3. However, it cannot be assumed that the end-uses will be at all like that in Germany. For instance, in Italy the cemetery and funeral tradition is distinctly different Plate 2.2a and dried *Banksia* is predominantly used for personal use in the home in dried arrangements Plate 2.2b & c. Traditional or synthetic flowers are preferred for use in Italian cemeteries, which are a "culture apart" from Germany's forest like "Friedhöfe" Plate 2.2d & e. On the other hand, flower consumption in the UK is one of the lowest *per capita* in Europe Pullar *et al.* 1993. This distinct difference in the cultural and sociological role of flowers in the UK can be traced back to iconoclasm of the reformation era Goody 1994, the events of which have had a particularly long-lasting impact on British floral usage.

The implications for industry of the research presented has been thoroughly dealt with in the appended Report. This chapter reports the novel application of the conjoint analysis to a floricultural product. However, optimising production to deliver the most suitable product for a specific end-use within the overseas markets must be founded on

information gained from a mixture of qualitative and quantitative market research methods as described. Visits, interviews, questionnaires and card sorting tasks can provide detailed information to direct decisions on the most appropriate production, post-harvest and promotional measures needed for horticultural products in a specific market.

Chapter 3

Banksia Stem Anatomy

Abstract

The stem anatomy of twelve commercially important *Banksia* taxa: *B. baxteri, B. burdettii, B. coccinea, B. ericifolia, B. hookeriana, B. menziesii, B. prionotes, B. serrata, B. speciosa, B. spinulosa,* varieties *spinulosa, collina* and *cunninghamii*, was examined to assess differences in wood structure and changes in composition due to season and age. *Macadamia integrifolia* was used as a comparison. Material collected was that commonly taken for vegetative propagation, comprising current and previous season's internodes (CSI and PSI respectively). CSI and PSI were collected from field grown trees, sectioned and stained with PAS-TBO or Sudan Black B to locate various anatomical features. Image analysis was used to quantify the gross morphological tissue types and microanalysis of vascular bundles was conducted to quantify the tissue components of the vascular bundles.

Quantitative assessment of CSI and PSI of *Banksia* stems using image analysis identified interspecific and seasonal differences in anatomy. Significant differences in parameters such as the percentage of cortex and the distance from the cambium to the stem surface were found between the CSI and PSI of stems collected in autumn, whereas fewer significant differences in tissues were found in spring. The percentage of cortex accounts for the largest proportion of the cross-sectional area of the stem, ranging

from 23% in *B. spinulosa* var. *cunninghamii* to 50% in *B. coccinea (CSI)*, or 20% and 44% respectively for the PSI. The distance from the cambium to the stem surface was shortest in *B. ericifolia* (161 µm) and greatest in *B. coccinea* (660 µm) for the CSI, and *B. menziesii* (899 µm) and *B. serrata* (876 µm) for the PSI. Cork was completely absent in some species in the CSI or PSI, however, it was found in the CSI of *Macadamia integrifolia* and *B. spinulosa* and in the PSI of *B. ericifolia* and *B. menziesii*. No cork was observed in *B. serrata* or *B. coccinea* in either internode.

Microscopic observations of the CSI and PSI sections were useful in identifying anatomical features that may influence successful vegetative propagation. Structures observed in *Banksia* which are likely to impair vegetative propagation are the presence of cork and cell occlusions in aging stem internodes, and the fibrous nature of young wood: pericyclic phloem fibres and leaf traces surrounded by fibrous zones in the cortex. Frequently, cortical cells at the distal end of the interfascicular rays occlude, forming a continuous non-living barrier with the adjacent phloem fibres which separates living phloem and interfascicular ray cells on the inside from the cortex on the outer margin.

Introduction

This investigation of the comparative anatomy of *Banksia* and the allied species *Macadamia integrifolia* uses microscopy and image analysis techniques to explain how season and age influence stem anatomy and composition in these species. This information will help develop improved methods of vegetative propagation crucial to realising the full commercial potential of members of the *Banksia* genus.

This introduction briefly contrasts wood and stem anatomy commonly found in woody dicotyledonous angiosperms with that found in the Proteaceae. Particular reference is made to the unique wood anatomy found in the two genera, *Banksia* and *Dryandra*. This is then put in a horticultural perspective by considering how stem structure and composition may impact on vegetative propagation in *Banksia*.

Wood anatomy

Dicotyledonous angiosperm wood

The wood structure of angiosperms is complex. Woody dicotyledonous plants, usually perennials, differ from annual monocotyledons due to the extensive secondary growth, which gives them their longevity. Secondary growth is the process in which additional cells are produced from a lateral meristem - the vascular cambium - as opposed to the apical meristem, which forms the primary plant body. This commonly cylindrical vascular cambium it not usually present in monocotyledons. As secondary growth progresses producing secondary phloem and xylem, the primary phloem is obliterated and the outer epidermal and cortical tissues are crushed and eventually sloughed off.

The fascicular cambium of the vascular bundles is originally located between the primary xylem and primary phloem of the vascular bundles. The vascular bundles separate the regions of the cortex on the outside and the pith to the inside. All these tissues are alive in the primary plant body before secondary growth commences, with the exception of the primary xylem. As the secondary vasculature begins to be deposited through the activity of the vascular cambium, adjacent parenchyma cells in the interfascicular rays are stimulated to divide. These dividing initials form the interfascicular cambium, which links with the fascicular cambium to form a continuous cylinder of vascular cambium. When secondary growth commences the vascular cambium generates secondary phloem to the outside and secondary xylem to the inside, pushing away the initially formed primary tissues towards the cortex and the pith, respectively. The seasonal activity of these cells produces secondary xylem, causing an increase in stem diameter. Tissues external to the xylem, like the epidermis and cortex, respond by cell division and limited cell expansion. After several seasons a cork layer (phelloderm) is generated from a new meristem, the cork cambium (or phellogen) arising in the cortex beneath the cuticle. The cork cambium produces regular rows of cuboidal cells without intercellular spaces, which are rendered impenetrable to water through impregnation of the cell walls with suberin. In a recurrent process the cork and outer tissue forming the bark die and are sloughed off. A thin layer of living phloem remains. New cork cambia arise, generating a new, either entire or fissured phelloderm, and the cycle continues.

As the stem ages the primary and oldest, inner, secondary xylem tissues become non-conductive due to embolisms (tyloses) in the vessels and tracheids. These tissues, together with the central pith are dead and constitute the heartwood. Sapwood is the remaining secondary xylem formed in the past few seasons. This is functional and contains living parenchyma cells, tracheids and vessels. Sapwood cells retain their shape and size after differentiation and ray and axial parenchyma stay alive for some time after differentiation, unlike specialised vessels and tracheid, which die soon after differentiation and cell wall thickening. As these living cells die, the transition from sapwood to heartwood begins and is completed with the deposition of tannins, polyphenolics and resin, giving the heartwood a characteristic dark appearance. In a living tree the sapwood serves three main functions (Bamber 1964):

(i) mechanical support in hardwoods is provided by fibres which are the most abundant wood cells, constituting up to 95% of the total wood volume.

(ii) conduction of water and solutes, which is confined to the outer sapwood; in hardwoods this occurs via xylem vessels.

(iii) temporary storage of photosynthate which is translocated down through the phloem and inward via the ray parenchyma, where it is converted to starch granules and deposited in the axial and ray parenchyma. These temporary reserves are readily mobilised in times of growth and can be completely resorbed during regrowth after a fire.

Radial transport is through radially elongate ray cells produced by the interfascicular cambium; these form the primary and eventually the secondary medullary rays. In the stems of younger, rapidly growing trees the bulk of the secondary xylem is sapwood.

Seasonal differences are observed in wood due to annual fluctuations in cambial activity. Wood produced in spring in temperate regions is distinguished by the larger tracheids or the different frequency of vessels compared to those formed in the previous autumn. These fluctuations in activity produce characteristic annular rings clearly visible at the ends of cut tree trunks.

Generally, cells of protoxylem still undergoing extension must have mechanical support and be capable of extension. Bands of lignin thickening reinforce the cellulose fibrils in the cell walls forming rigid rings (annular tracheids) or continuous spiral lignified bands (spiral tracheids). In tracheids produced in wood after the completion of extension growth, longitudinal and tangential bands are deposited and scalariform tracheids are formed; if the lignin is deposited almost continuously, leaving small areas of unthickened cell wall, characteristic pitted tracheids result. In angiosperms very large, continuous xylem vessels form when the end walls of individual xylem elements are resorbed, resulting in a continuous, lignified tube (Gill & Vear 1980).

In Mediterranean climates, where a mild spring is followed by a hot summer, large vessels tend to dominate the first layers of spring wood. This results in a large size differential between vessels produced in spring and summer wood. This type of wood is known as *ring porous* due to obvious bands of large vessels. In *diffuse porous* wood, on the other hand, vessels of similar size occur uniformly throughout the annular ring. Genera with diffuse porous wood, as found in *Banksia* (Record 1936; Pate *et al.* 1995),

are considered to be more primitive than ring porous species, and relatively few exist. As well as being smaller in diameter, the vessels of diffuse porous wood are also shorter. The overall effect is that these species have a much lower rate of water conduction compared to ring porous species with their larger, longer vessels (Mauseth 1988).

The evolutionary development of vessels from tracheids is extensively preserved across a wide range of dicotyledonous angiosperm families (Foster & Gifford 1959). As the Proteaceae is an ancient family displaying primitive wood features (Johnson & Briggs 1963) it would be useful to compare the stem anatomy with another angiosperm of similar phylogenetic age. *Magnolia* is also a primitive genus (Metcalfe 1987a) and correspondingly displays primitive wood anatomy (Fisk & Millington 1959). In the woody stems of one year old *Magnolia*, primary and secondary vascular tissue can be observed in collaterally organised vascular bundles separated by medullary rays (eustele). Vascular rays can be seen in the xylem. The primary xylem is endarc (i.e. develops with older xylem closer to the centre of the stem axis). The cork cambium originates in the cortex and produces a continuous peridermal layer. Groups of secondary thickened fibre cells cap each end of the vascular bundles at the cortex and pith ends. At three years annular rings of xylem are visible, and medullary and vascular rays are present, the latter more obvious and frequent than in one year old wood. Sclereids, fibres and fibre tracheids are also present. Primitive vessel elements are long with annular thickenings, and scalariform bordered pits and scalariform perforation plates are present; rays are heterogenous.

Proteaceous wood anatomy

Historically, the comparative study of angiosperm wood anatomy has received enormous attention and is relatively complete (Foster & Gifford 1959), although an almost endless variation in different types exists. The structure of mature wood (xylem) in many species has been well documented for the timber industry, due to the many commercial uses for which it is employed (Dadswell & Record 1936; Ilic 1991). The features of interest in these reports pertain to the ground mass wood, which consists of non-living xylem. These wood characteristics are used to categorises woody plant families (Record 1936). Furthermore, wood characteristics have been used to elucidate the taxonomic relationship between different woody taxa of the family Proteaceae (Chattaway 1948b; Johnson & Briggs 1975; Behnke 1995).

Although this study is on immature terminal and sub-terminal wood of new growth in species of *Banksia*, a general overview of the features of mature proteaceous wood will be presented.

Mature proteaceous timber is characterised by large conspicuous rays with pores (xylem vessels) typically arranged in tangential festoons between the rays. It is pale brown to dark red in colour and rather soft to very hard, with pores of variable size. Dadswell and Record (1936) identify rays of two sizes: uniseriate low, heterogenous rays and larger high, conspicuous and rather homogenous rays. Several other main characters of proteaceous wood are the intercellular canals (in *Banksia*, *Cardwellia* and *Grevillea*) and the spiral thickening on the inside of the secondary vessel walls (*Dryandra, Grevillea, Helicia* and *Persoonia*), which are without conspicuous bordered pits. From the lists of family classifications produced by Record (1936), it is possible to describe Proteaceous wood as follows: it is not ring porous, has no rippled marks or storied structure, no included phloem or raphides (sharp needle like crystals), is not characterised by vessels with simple or scalariform perforation plates, nor wood fibres with conspicuous bordered pits; furthermore it has no spiral thickening in fibre tracheids.

Carlquist (1985) recorded that vasicentric tracheids occur widely in genera that constitute major proportions of the flora of Mediterranean-type areas throughout the world, such as *Banksia* and *Dryandra*. Generally, vasicentric tracheids are not common in dicotyledons, but are present in many drought tolerant, evergreen species. In the Proteaceae and Myrtaceae they are very similar to the fibre tracheids in the same wood, adding to the difficulty of identifying them. Vasicentric, vascular and true tracheids are alike in that they are imperforate with relatively large bordered pits, similar in density to those on the lateral walls of vessel elements. Unlike true tracheids, which are the only type of tracheids in the wood in which they occur, or vascular tracheids, which only occur in late wood at the end of the growth ring (where they grade into narrower vessel elements), vasicentric tracheids occur throughout the growth ring or in species with diffuse porous wood. Their presence ensures water conduction throughout the growth ring and appears to be a drought survival mechanism in xerophytic, woody, evergreen species from arid regions.

Detailed transmission electron microscopy studies of sieve-element characteristics of the phloem in the Proteaceae and Elaeagnaceae have been used to clarify the

classification of these two families of the same order. *Banksia baxteri*, *B. serrata* and *Macadamia ternifolia* were included in this study of 34 proteaceous species (Behnke 1995). The unique character of proteaceous wood can be traced to an ultrastructural level.

Unique wood anatomy of Banksia

Banksia woods are light hardwoods of a primitive type. Compared to other proteaceous genera, such as *Hakea* (46-60 lb/cu.ft), *Macadamia* (44 lb/cu.ft.) and *Telopea* (39-46 lb/cu.ft.), *Banksia* wood is lighter (29-50 lb/cu.ft.). *Dryandra* wood is structurally indistinguishable from that of some smaller *Banksia* species (Chattaway 1948b). In the study of 26 proteaceous genera by Chattaway (1948a), radially oriented vasculature in the ray tissue of two closely allied, highly xerophytic genera - *Banksia* and *Dryandra* – was observed. The occurrence of radial vascular tissue in these two genera is thought to be a mechanism for the radial movement of water, acting as an "accessory conducting system" similar to ray tracheids found in *Pinaceae*. Vasculature of this nature had not previously been described and there was no reference to similar structures in the literature. However, similar protuberances have been reported in *Populus euramericana*, *Combretum* and in the Annonaceae, but from a different cause (Larson 1994). In *Banksia* radial vascular tissue has been observed in *B. serrata, B. aemula* (syn. *B. serratifolia*), *B. ilicifolia,* and *B. grandis*. In *B. marginata* it is extremely rare.

In *Banksia* and *Dryandra* the radially aligned vascular tissue is restricted to the interfascicular rays and has been observed to penetrate into the cortex, particularly in *B. serrata*, where strands are extremely numerous and large enough to see with the naked eye. In *B. aemula* spiky projections result which can be seen if the bark is removed from fresh stem material (Chattaway 1948a). Interfascicular cambial initials previously producing parenchymatic ray cells concurrently switch production to radial vascular tissue throughout the stem, as shown in sections of rays where newly formed vascular strands occur at the same distance from the pith. The nature of this switching mechanism is not clearly understood. The radial vasculature is always connected to the vertical vascular tissue and consists of short vessels and tracheids, which become shorter and irregularly shaped with alternate bordered pits as the vascular tissue passes through the ray adjacent to the phloem. The cambium is distorted by radial elongation of the vascular tissue as it extends outwards. Radial vascular strands are not present in root rays.

Banksia wood is diffuse porous; the mean length of vessel in the trunk, lateral roots and sinker roots of *B. prionotes* has been recorded as 4 cm, 75 cm and 150 cm, respectively (Pate *et al.* 1995). Other adaptations present in the *Banksia* genus are the dimorphic root structure. The proteoid roots and parent laterals supplying limited nutrients, such as phosphorus and nitrogen, are responsible for seasonal movement of nutrients to shoots (Pate & Jeschke 1993), whereas sinker roots tap the water table at over 1.8 m depth. *Banksia prionotes* and *B. ilicifolia* have narrower vessels and a higher proportion of area occupied by non-conducting tissues in lateral roots compared to corresponding sinker roots. A five to 20 fold increase in hydraulic conductivity from the top to the base of the sinker is associated with increased mean radii of the conducting elements.

The peculiar wood features found in *Banksia* may explain the difficulties encountered during vegetative propagation, such as grafting and budding. Other proteaceous genera that do not have radial vasculature in ray tissues, such as *Grevillea*, *Hakea*, *Macadamia*, *Protea*, (Chattaway 1948b) can be readily grafted ((Burke 1983a, b), (McKenzie, D. 1994), (Stephenson 1980), (Moffatt & Turnbull 1993)). A thorough understanding of *Banksia* wood anatomy and seasonal responses may help overcome the difficulties of vegetative propagation. The next section highlights the main features of wood anatomy and composition, which are relevant to the vegetative propagation of *Banksia*.

Aspects of wood anatomy and composition which influence propagation

The vegetative propagation of woody angiosperms is a wide field, encompassing the two most commonly used methods: rooting cuttings and grafting, the former the main vegetative method used for propagating *Banksia*. Wood anatomy and composition influence the grafting and rooting of stem cuttings. The main aspects of how anatomy and composition influence vegetative propagation by cutting will be reviewed here, with particular reference to the Proteaceae and *Banksia*. The grafting method is dealt with more fully in the next chapter.

There are inherent differences between woody and non-woody angiosperms that influence vegetative propagation by cutting or grafting. It is possible, for instance, to propagate some herbaceous dicotyledons from leaf cutting as they have maintained a residual meristematic potential. The use of leaf cuttings has not been reported in woody flowering species (Metcalfe & Chalk 1979), although the capacity to form adventitious roots from stem cuttings of numerous flowering woody species is widely exploited and

well documented (Beakbane 1961, 1969; Garner & Beakbane 1968; Hartmann *et al.* 1990). In woody plants the outer tissues of the stem, the stem being the most commonly used vegetative propagule, must retain residual meristematic capacity, either to form root initials, as in the case of cuttings, or callus and specialised vascular cambium, as in the case of grafting. During rhizogenesis in cuttings, root initials must form and undergo enlargement and differentiation. The formation of initials and their growth will depend on wood structure and the balance of endogenous substances in the internal milieu of the outer living tissues in the cutting. The initiation of adventitious roots in woody plants takes place adjacent to, or outside the central core of vascular tissue, usually in the secondary phloem in association with a ray. At the distal end, the ray has contact with living cells by means of cytoplasmic strands, which pass through the cell walls (Beakbane 1969).

Several features of wood anatomy and composition have been recorded as hindering root development (Beakbane 1961, 1969), including the development of a sclerenchymatic sheath from the primary phloem. This is the most frequent anatomical feature responsible for low rootability in many fruit plants and numerous difficult to root species from around the world. The sclerotic sheath may provide a physiological block to root initiation or a physical barrier to root emergence. When the phloem rays abut cells without living contents, such as sclereids, fibres or other elements, root initiation in the secondary phloem does not occur. Similar interference of root initiation is caused by several other events:

(i) rapid senescence of the phloem causes a fibrous sheath of elements without living contents to form early on, close to the shoot tip; where this occurs the species are very difficult to propagate (*Cinnamom, Acacia, Erica, Fagus, Hevea, Quercus, Mahonia*).

(ii) lignification of the cell wall of the primary phloem before cell senescence; although the cells are still alive, root initial formation is very low (*Acacia mearnsii*).

(iii) cork produced by phellogen has a similar effect of blocking ray ends with tissues without living cell contents. In species in which this occurs, it is important to propagate from young tips where the cork has not yet developed (*Erica*).

(iv) the formation of secretory canals in the primary phloem as it senescences; this has an effect similar to the presence of a fibre sheath (*Pittosporum*)

The extent to which these tissues are formed is subject to variation related to climatic conditions. Terminal and sub-terminal soft and semi-hard wood has a greater rooting potential than older tissues with a higher degree of sclerification and lignification. Highly differentiated, lignified tissues found in the vasculature are least likely to produce callus initials for rhizogenesis.

There may also be developmental changes in anatomy as the stem matures, which need to be clearly understood for best practice in propagation to be developed, as is the case in the grafting of pepper (*Piper* spp.) (Garner & Beakbane 1968). Pepper is unusual amongst dicotyledons, having a stem structure similar to monocotyledons. Also, unlike dicotyledons it forms an endodermis; and mucilage canals occur in the ground tissues of some species. In young *Piper* stems vascular bundles are scattered, but as secondary thickening occurs an outer ring of vasculature consisting of collateral bundles separated by wide medullary rays develops, although the medullary bundles remain in the inner core of the stem. At a later stage fibre groups form at the distal end of the rays; sclerification proceeds until a band of periphloic fibres is formed and flanges of fibres penetrate deeply into the medullary rays, breaking the vascular cambium continuum. These flanges are a conspicuous feature in older stems of *Piper nigrum*. In the case of Chinese chestnut (*Castanea* spp.), a knowledge of its peculiar wood anatomy, particularly the location of the phloem fibre bundles, also greatly improves the success rate of grafting. For successful grafting the scion buds must be inserted between the phloem fibre bundles, instead of directly over the area where the fibre bundles lie (Huang *et al.* 1994).

Ontogenetic age also plays an important role in vegetative propagation. It is a common horticultural practice amongst plant propagators to maintain mother stock plants in a juvenile state (Kester 1976; Hackett 1985) by various practices, such as pruning and hedging (Hartmann *et al.* 1990). A balance of genetic, physiological and biochemical factors is responsible for maintaining juvenile and mature phases. Generally, just prior to the transition from the juvenile to the mature phase is the time of greatest rooting potential (Hartmann *et al.* 1990). In *Prunus avium* this transition from juvenile to mature phase has been identified using two dimensional protein separation and *in vitro* translation methods (Besford *et al.* 1996). Juvenile cuttings have been shown to have a

greater concentration of auxins and growth promoters (Hackett 1988). Consequently, cuttings taken from woody stock plants maintained in juvenile phase will have a greater propensity to form roots.

Pre-treatment of propagules by addition of growth hormones in liquid, powder or gel form, especially auxins which are essential for adventitious rooting (Hackett 1988), or by wounding, is commonly practiced to induce rooting in cuttings (Levey 1973; Hartmann *et al.* 1990). An acid or base pre-treatment before the application of rooting hormone can be beneficial, an acid pre-treatment increasing rooting in plants native to neutral to alkaline soils, a base pre-treatment for plants from acid soils. Such pre-treatment helps some plant cuttings maintain turgor, whilst in woody species which take more than six weeks for root initiation, it helps reduce the loss of foliage (Lee *et al.* 1976).

The use of mycorrhizal fungi has facilitated the propagation of several native plants (Donnelly 1995; McLean 1995). However, species of the Proteaceae do not normally form mycorrhizal associations (Mabberley 1987), so it is unlikely that this method would be beneficial. Developmental and seasonal changes in plant physiology will also impact on success of vegetative propagation. The level of carbohydrate, endogenous hormones, polyphenols, tannins, cyanogenic compounds and regulatory enzymes will vary according to the (ontogenetic) age of the plant material and the season in which it is collected.

The balance of compounds in softwood and hardwood tissues can act in a promotive or inhibitory manner. Flavonoids present in softwood cuttings have a promotive effect, whereas phenolics in hardwood cuttings decrease the success of rooting of waxflower cuttings (*Chamaelaucium uncinatum*) (Curir *et al.* 1993). Both the accumulation of phenolics and the deposition of lignin increase with wood age. Lignification and sclereid development can be retarded for up to three months by the exclusion of light during early shoot development in woody angiosperms, resulting in improved rooting potential (Hartmann *et al.* 1990; Maynard & Bassuk 1996).

The water relations of the stock plant (Davis *et al.* 1988) and its nutritional status (Hartmann *et al.* 1990) are crucial to the end success; turgid plant material gives best results (Loach 1988b), however the physiological basis of how this improves rootability is not understood.

Wood vasculature will also relate to how rapidly a propagule desiccates once excised from the stock plant. Consequently, determining the optimal conditions for maintaining turgor in a propagule from a particular species once in the propagation system is paramount to successful vegetative propagation. Influential environmental factors (Loach 1988a) are: the type of enclosure; ambient temperature; root zone temperature, maintained by bottom heating mats at optima depending on the species varying from 22°C – 30°C; light quality and quantity (Ellyard 1976; van Lieburg & Kendrick 1996); water quality (Middleton 1978; Loach 1988b), humidity levels (Hall 1981), and substrates (Handreck & Black 1989).

Even at the post-harvest level wood structure influences the treatment ("So werden sie richtig" 1995) and handling necessary for the best storage and vase-life of fresh plant products (Van Doorn 1995). This is particularly so for woody cutflower species from the Proteaceae, many of which have been tested (Jones & Faragher 1991; Criley & Parvin 1993; Offord & Campbell 1994; Beal *et al.* 1996; Joyce *et al.* 1996). Generally, proteaceous species have a long vase-life. It is possible that this may be attributed to the vasculature, which has contributed to the physiological plasticity of the genus, enabling its members to survive in a wide range of dry, inhospitable environments (Blake & Hill 1996).

From this knowledge it may be possible to define or suggest the reason for the difficulties in propagation of banksias and how this may be overcome. Semi-hardwood stem tissues of various ages is used most commonly in cutting and grafting propagation of proteaceous plants, including *Banksia*, although micro-propagation from other tissues has been attempted (Niccol *et al.* 1994; Sedgley 1995b).

One attempt to propagate *Banksia* by cutting (Bennell & Barth 1987) recommends that semi-hard wood cuttings from *B. coccinea* be subjected to 8,000 to 12,000 p.p.m IBA after stem wounding. Cuttings were sourced from mature field plants, given infrequent watering and maintained with minimal permanent moisture in the soil. Other research reports success with *B. coccinea* using semi-hard wood collected from glasshouse cultivated stock plants between May and August (Sedgley 1995b). Cuttings were wounded with two 1.5 cm longitudinal cuts and dipped for five seconds in 5,000 p.p.m. IBA. A strike rate of 46% was achieved using this method. Field grown *B. coccinea* and *B. menziesii* yielded comparatively poor results when trialed under the same conditions. This was thought to be due to inadequate water relations and fertiliser regimes of field

plants, compared to glasshouse grown plants. Similar methods for propagating *Banksia* by cuttings are described by Elliot and Jones (1982), George (1987) and Wrigley and Fagg (1989). Numerous informal reports indicate the use of soft tip material or semi-hardwood cutting propagules for a range of Australian proteaceous species, including the waratah (*Telopea speciosissima*) (Offord & Campbell 1994), and particularly *Grevillea* (Levey 1973; Ellyard 1976; Bunker 1981; McCormack & O'Neill 1993; Thomas 1993) which has also been cultured *in vitro* (Ben-Jaacov & Dax 1981). Similarly, numerous reports exist on propagating South African Proteaceae (McKenzie 1973; Gibson 1974; Wood 1978; Asper 1984; Brits 1986; Perry 1987).

In vitro regeneration of plants from apical shoot explants from *B. serrata* and *B. oblongifolia* seedlings at first leaf stage has been reported using half strength Murashige and Skoog media and 0.25 µM benzyladenine for multiple shoot induction (Niccol *et al.* 1994). Another report used liquid media for the initiation of explants of *B. attenuata*, *B. hookeriana* and *B. menziesii*, which were transferred onto solid media; adventitious shoot formation from cotyledon explants was achieved (Bunn 1992).

The biochemical role played by cyanoglycosides, carbohydrates or phenolics in vegetative propagation in *Banksia* has not been examined.

Node structure in Macadamia and Banksia

In *Macadamia* grown on the Hawaiian Islands vegetative flushing occurs throughout the year, predominantly during autumn, coinciding with nut maturation (Nagao *et al.* 1994). New shoots emerge from apical meristems or axillary buds. Each leaf axil bears up to three buds, which lie directly above one another. During apical shoot extension the top bud flushes first; it is possible for nine or 12 shoots to be produced from one node. Branches forming from these axillary buds grow at increasingly wider angles to the main stem, rendering the tree susceptible to splitting (Storey *et al.* 1961). *B. serrata* has a trilacunar node structure where the leaf petiole adjoins the stem (Metcalfe & Chalk 1979).

Taxonomy of the study species

Of the twelve taxa studied the majority come from western Australia: *B. menziesii, B. baxteri, B. speciosa, B. burdettii, B. hookeriana, B. prionotes* and *B. coccinea*. The eastern species used were *B. serrata, B. ericifolia*, three varieties of *B. spinulosa: cunninghamii, spinulosa* and *collina,* and *Macadamia integrifolia.* Table 3.1 shows the

phylogenetic relationship of the species studies. Although species from Subgenus *Isostylis* have commercial potential, none were included in this study. Investigation into their wood anatomy may, however, help identify taxonomic relationships to other members of the genus, as well as provide information useful to their vegetative propagation.

Study rationale

Through the increased cultivation of native flora for ornamental horticultural purposes and the development of domestic and international cultivation and consumption of native Australian flowers, the need for solutions to production and cultivation problems has arisen, particularly for new cultivars which can be clonally mass-produced. The development of new clonal cultivars is especially a problem in *Banksia* species, which are difficult to clonally propagate on a commercial scale. Currently, either hardened new season's growth or subterminal internodes of semi-hardwood are used in cutting propagation; scion wood used in grafting is subterminal semihardwood, the new soft growth is discarded.

Literature on the Proteaceae, particularly *Banksia*, highlights the fact that this ancient southern family has peculiar wood characteristics, e.g. vasicentric tracheids (xylem fibres) (Carlquist 1985), radial vasculature in medullary rays (Chattaway 1948a) and phloem fibres, which are specialised adaptations facilitating survival in highly xerophytic habitats. Commercially important *Banksia* species are widely regarded as being difficult to vegetatively propagate. So the question arises: do features of the unique wood anatomy observed in *Banksia* impact on the vegetative propagation of members of this genus, either by grafting or cuttings?

In response to this question the following study of the wood anatomy of *Banksia* was undertaken. Samples of current season's internode (CSI) and previous season's internodal growth (PSI) were studied. Microscopy methods together with quantitative image analysis have been used to identify interspecific and seasonal difference in wood structure and composition. These findings may help clarify the difficulties associated with the vegetative propagation of *Banksia* by grafting, budding or by cuttings. More importantly, it may shed light on how propagation may be improved to produce clonal elite cut-flower and foliage genotypes.

Table 3.1 The taxonomy of species studied, based on George (1981, 1988) and Maguire *et al.* (1996)

SPECIES STUDIED				
Genus	**Section**	**Series**		**Species**
Banksia L. f	*Banksia*	*Banksia*	*	*B. serrata*
Subgenus *Banksia*			*	*B. menziesii*
				B. baxteri
				B. speciosa
		Crocinae		*B. burdettii*
				B. hookeriana
				B. prionotes
	Coccineae		*	*B. coccinea*
	Oncostylis	*Spicigerae*		*B. spinulosa* var. *spinulosa*
			*	*B. spinulosa* var. *cunninghamii*
			*	*B. spinulosa* var. *collina*
			*	*B. ericifolia*
Macadamia			*	*Macadamia integrifolia*

* species used for image analysis studies, others sectioned for light microscopy

Materials and Methodology

Source and sampling of stem material

The majority of *Banksia* trees used for sampling was sourced from either Happy Valley Reservoir (latitude 35° 04' S., longitude 138°34' E.), the property of Mr Geoff Watton, Glen Osmond, Adelaide, or the bush-house at the Waite Institute (Table 3.2). For each of the species *B. baxteri, B. burdettii, B. coccinea, B. ericifolia, B. hookeriana, B. menziesii, B. prionotes, B. speciosa* two trees were designated, with the exception of *B. ericifolia,* for which only one field tree was available. Bush-house grown plants of *B. ericifolia* and *B. serrata* were also used. The trees at Happy Valley Reservoir were planted in the 1970s and grown untended without any agronomic in-puts. Some pruning was carried out to encourage growth in May 1994. Single trees of *B. serrata, B. spinulosa,* varieties *spinulosa* and *cunninghamii,* were sampled from the garden of Mr Geoff Watton. *Macadamia* was included as an inter-genus comparison. This was not only because of its close relationship to the *Banksia* genus, but also because of its commercial value as a nut producer for which a successful grafting technique has been developed. Wood from *Macadamia integrifolia* was collected from managed trees at the Alverstoke orchard, Waite Campus, University of Adelaide (latitude 34°58' S., longitude 138°38' E., altitude 120m, winter rainfall of 625 mm).

Three branches consisting of a CSI and a PSI were harvested at the end of spring 1994 (11.12.94), in autumn 1995 (30.05.95) and in spring 1995 (25.10.95) when the new flush growth was fully extended and starting to harden. Meteorological data (rainfall, mean temperature, incident light) for this period are presented in Figure 3.1. Branches were transported in water to the laboratory where they were stored overnight at 4°C. Five millimetre lengths from the mid-point of the CSI and PSI of each branch were dissected in the fume-hood and fixed.

Stem microscopy

The histological methods used in this study of *Banksia* wood follow those described by Feder and O'Brien (1968); the details of which are given below.

Table 3.2 Species and planting codes of trees used for image analyses: Part I, Gross tissue types of six species, Part II, Gross tissue types of *B. coccinea* and *B. menziesii*, Part III, Vascular bundle microanalysis; GW = Geoff Watton; A = Alverstoke, Waite Campus orchard; other bushes from Happy Valley Reservoir plantings

IMAGE ANALYSIS	SPECIES	SEASON	
		Spring 1994	Autumn 1995
Part I	*B. coccinea*	#296	#296
	B. ericifolia	#292	#292
	B. menziesii	#226	#226
	B. serrata	GW	GW
	B. spinulosa var. *cunninghamii*	GW	GW
	Macadamia integrifolia	A	A
Part II		Spring 1994	Autumn 1995
	B. coccinea	#296	#296
		#43	#42
	B. menziesii	#226	#226
		#229	#125
Part III			Spring 1995
	B. coccinea		#296
	B. ericifolia		#292
	B. menziesii		#125
	B. serrata		Bush-house
	B. spinulosa var. *cunninghamii*		GW
	Macadamia integrifolia		A

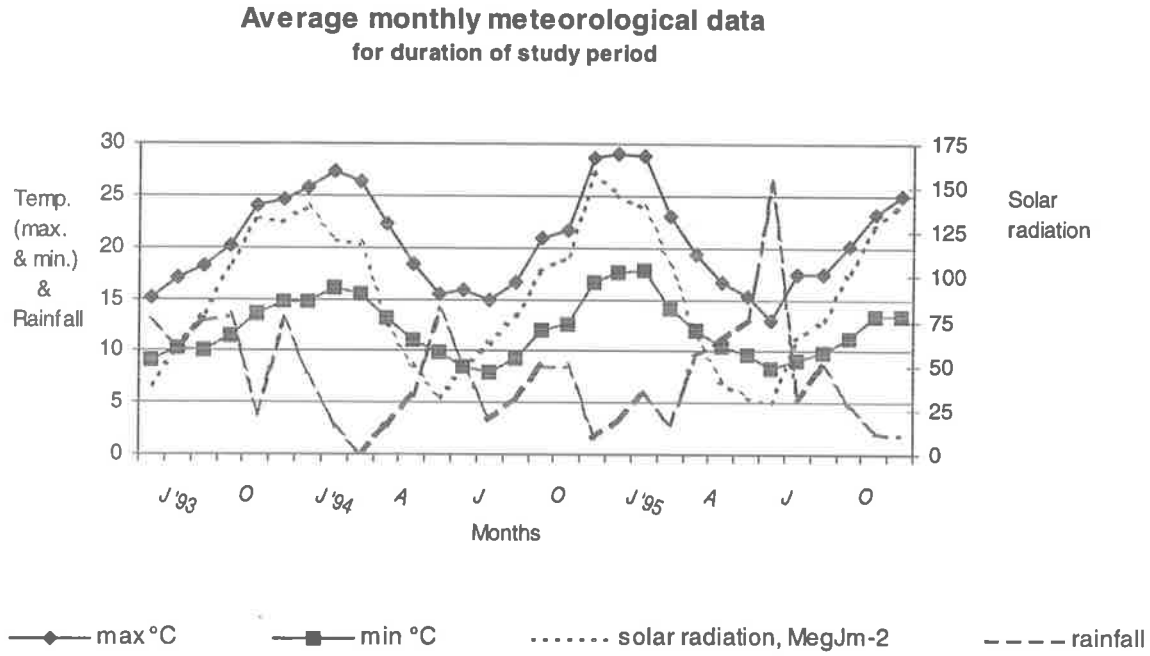

Figure 3.1 Meteorological data for the duration of study period. Source: Waite Institute (latitude 34°58' S., longitude 138°38' E.), continuous meteorological records

Glycol-methacrylate embedding and sectioning

Wood samples were dissected and fixed in 3% glutaraldehyde (Unilab) in 0.025M phosphate buffer pH 7.0 for a minimum of 24 hours in glass capped vials or 4 x 5 welled cell culture trays. After dehydration through an alcohol series: methoxy ethanol, ethanol, propanol and butanol, samples were infiltrated for two hours in 1:1 butanol:glycol methacrylate (GMA) (2-hydroxyethyl methacrylate, Sigma H-8633)[14], followed by two successive changes of 100% GMA each 48 hours, then embedding in gelatine capsules (size: no. 2 or no. 00, Park-Davis, Sydney) in GMA. The embedding plastic, GMA, was polymerized at 60°C over 48 hours.

GMA blocks were filed back to remove excess plastic and serial transverse (TS) and longitudinal (LS) (radial and tangential) sections, 4 µm thick, were made on a Reichert Jung *2050 Supercut* Microtome using glass knives. Sections were collected with forceps and needles, and floated in a water droplet on a microscope slide and dried overnight at 40°C to 60°C.

PAS-TBO Staining

Sections were routinely stained with periodic acid-Schiff's reagent (PAS) and counterstained with Toluidine Blue O (TBO) as described by McCully & O'Brien (1981). This combination is a good general stain for plant tissues, counterstaining polysaccharides, polysulphates, polycarboxylates and pectic acid red or pink, while polyphenols and lignin are stained green, blue or an aqua colour. After slides were submerged in a saturated solution of 2,4-dinitrophenylhydrazide[15] for 30 minutes and rinsed in running water (one hour), they were placed for 30 minutes in 1% periodic acid (BDH laboratory supplies) then rinsed again for five minutes. Commercial Schiff's reagent (BDH laboratory supplies) was used to stain (one hour) tissue components bearing aldehydes. The slides were submerged in three successive changes of metabisulphite solution[16], each for two to three minutes. Following a brief rinse in water (2 – 3 minutes), 0.05% Toluidine Blue O (Aldrich) in benzoate buffer pH 4.5[17] was used to stain the sections (5 minutes). Excess TBO was removed by rinsing in running

[14] GMA monomer was prepared by mixing 93 ml GMA with 7 ml polyethylene glycol 400 and 0.6g benzoyl peroxide at room temperature for two hours.

[15] 0.5 g in 100 ml 15% acetic acid mixed 1 hour and filtered

[16] 5 ml 10% sodium metabisulphite solution, 5ml 1N HCl and 90 ml water

[17] 0.29 g sodium benzoate plus 0.25 g benzoic acid (Sigma, B-3250) in 200 ml water is mixed for 30 minutes, left to stand overnight, then filtered.

water until the surrounding plastic was clear. Dried slides were mounted using *Micromount* mounting medium (Surgipath) and a glass cover slip.

Sections were viewed using a transmitted light microscope (Zeiss, *Axiophot Pol* Photomicroscope) and photographed using Ilford Delta Professional black and white film (ISO 400/27 or 100/21) and colour slide film (Kodak Ektachrome 400X Professional). The plates presented are photocopies of black and white prints, with the exception of Plate 3.1, which is a *VideoPro 32* image, traced and labelled with *Photoshop* (Adobe).

Periodic acid-Schiff's and Sudan Black B staining

Differential Periodic acid-Schiff's/Sudan black B (PAS/SBB) counter-staining of stem sections of *Banksia* species and *Macadamia integrifolia* was used to identify the presence of cork (Bronner 1975; McCully & O'Brien 1981). Periodic acid-Schiff's reagent reacts with plant tissue components such as starch and some complex polysaccharides, especially the compound mid-lamella of the cell wall (Feder & O'Brein 1968), which stains bright red or magenta. Callose and cellulose are not stained and some phenolics stain red (McCully & O'Brien 1981). Additional counterstaining with Sudan Black B renders the cuticle and suberised cell walls black, the other cell walls and starch remaining red.

A fresh saturated solution of 0.3% SBB was made by placing 0.05 g of SBB (Sigma, no. S-2380) in 15 ml 70% ethanol, incubated overnight covered at 60°C, filtered (Whatman filter, no. 1, 110 ∅) and incubated at 60°C for 30 minutes.

PAS stained slides were submerged in 70% ethanol for one to two minutes, then stained with the fresh SBB solution for one hour at 60°C, rinsed in 70% ethanol followed by water, then mounted in 60% glycerol.

Image analysis using Video Pro 32

For the quantitative descriptions of component wood tissues, five *Banksia* taxa: *B. coccinea, B. ericifolia, B. menziesii, B. spinulosa* var. *spinulosa* and *B. spinulosa* var. *cunninghamii*, and *Macadamia integrifolia* were used. A high performance image processing and analysis facility, *Video Pro 32* (Leading Edge Pty. Ltd., Marion, South Australia) was used for this purpose.

Experimental design

The experimental design involved using monochromic images of sections prepared as described above. Five sections (or, as in the case of vascular bundle microanalysis, five

vascular bundles from one transverse section) from current and previous season's internodal wood taken from three branches of each species in two seasons were used (i.e. 5x2x3x6x2).

Image capture

Section images were captured using a *Nikon* Diaphot 300 inverted microscope, together with *BioRad* confocal microscope operating software (*CoMOS*, Version 6.05.9), which was used due to the large diameter of the stem sections. It stored pixilated images (PIC files) made with 4x, 10x, 20x or 40x optical objectives with an additional electronic zoom, ranging from 1.5x to 2.0x, onto magneto-optical discs (128 MB, 90mm discs, *SONY* EDM-128M). The *Confocal Assistant* software (version 3.10, copyright 1994/95 Todd Clark Brelje) was used to convert PIC files to BMP files for image analysis using *Video Pro 32* (version 3.29, copyright Leading Edge Pty. Ltd.).

Programming

Set-up and measurement programs (refer to Appendix 1 for full program descriptions) were written for both the gross tissue type and the vascular bundle microanalysis. Set-up programs arrange the data file, in which information generated by the measurement program is saved and which contains information on calibration (scale factor), image identification, arrangement of raw data and formulae columns. The last line in the set-up program runs the measurement program.

The measurement program guides the process of creating and saving the outline overlay (*.vbo file), which was made from tracing over the image on the screen using a computer mouse, generating information to be stored in the data file. Specifically, entries of image identification and scale factor are entered directly from the command line for each of the images, then the binary overlay process (tracing) is carried out and temporarily stored (image store load). This is followed by several specified measure area and count functions, the outcomes of which are recorded and saved in the data file in the allocated columns. The last line re-runs the measurement program for the next image[18].

Data generated from further measurement sessions was added to existing files or stored in new data files, usually made for different species. The final data files are viewed in *Video Pro 32* or edited using *MS Windows, Notepad*. Conversion (using *Video Pro 32*

[18] Frequent soft re-booting of the computer after analysis of ca. five images clears RAM and avoids loss of data due to computer failure.

Convert) to other formats (*MS Works* or *Excel*) is necessary for further detailed statistical analysis.

Image analysis technique development

Creating an overlay by tracing the gross tissue types was done firstly by drawing two transects from the centre of the pith out to the outer edge of the cuticle, placing them between vascular bundles and taking care not to intersect leaf traces (Plate 3.1a). Thus, 90° to 180° tissue segments were measured. Transects were joined by tracing the outer cuticle periphery. Individual vascular bundles were outlined and joined via a line along the interfascicular cambium. This line sufficed to delineate the pith from the cortex. The pith area measurements included the interfascicular cells up to the line of the interfascicular cambium. Leaf traces and cork, if present in the cortex, were outlined and measured. A leaf trace was defined as a vascular bundle located in the cortex, away from the central vasculature and the cambium, with often noticeably different vessel orientation (obliquely cut) and a rounded, rather than elongate, shape. Measurement of cork area included the cuticle. Measurements of the thickness of cork and cuticle were made separately (Plate 3.1a, lines 7 & 8). To achieve consistent measurement of the distance from the cambium to the cuticle base a line was drawn perpendicular to the cambium so that it would intersect the centre of the pith. Individual vascular bundles were widely spaced in current season's wood, especially in *B. coccinea*, making it difficult to delineate the interfascicular area in these sections (Plate 3.1b).

The tracing used for the vascular bundle microanalysis is presented in Plate 3.1b. Each of the five vascular bundles were measured together with the tissues between it and the neighbouring vascular bundle. The vascular bundle tissue was split into pith fibres, xylem, cambium, phloem and phloem fibres. The interfascicular tissue was split into *interfascicular* and *non-interfascicular* areas, the latter (Plate 3.1b, two areas labelled "6") consisted of pith and cortex.

Tears and folds in tissue sections were compensated for in the tracing procedure. This together with the hand tracing process and the thickness of the line will have introduced a more or less constant degree of error in the data collected.

Measurements recorded in the data file as pixel units were used for percentage area calculations. A scale factor was necessary for determination of length in microns (μ). This scale factor entry was made at the beginning of each run of the measurement program and was recorded in the second column of each line in the data file where it was used in length calculation formulae.

Tissue parameters

The percentage area of each tissue type was determined in the image analysis of gross tissue types. The parameters (i.e. output variables) included percentage area of pith, vascular bundles, cortex, leaf traces and cork. Counts of leaf traces and the distance from the cambium to the inner edge of cork or cuticle (if present), together with the total distance from cambium to surface and the thickness of cork and cuticle were made.

The output variables measured in the microanalysis of the vascular bundles were the percentage area of pith fibre, xylem, cambium, phloem, phloem fibre, interfascicular cells, together with the number (count) and length of seriate rays in each vascular bundle.

Statistical Analysis

Multivariate analysis was conducted on the measurements made on the CSI and PSI. The data in which autumn was the current season were analysed separately from the data where spring was the current season. This was the case for both gross tissue type analyses. The data for the vascular bundle analysis was taken from wood collected in one season, spring 1995. Within each data set, each output variable was analysed using repeated measures analysis of variance, with "season" and "species" as grouping factors and a "within branch" factor for sections or vascular bundles (1 to 5 in number - Plate 3.1b). A compound symmetry error structure was assumed for each analysis. A pair-wise difference of means was conducted using appropriate estimated standard errors. Program *5V* from the BMDP statistical software package (UCLA, 1993, ed. W. Dixon) was used for the analysis. Pair-wise comparisons of means used a critical z *value* ≥ 3.

Results

Descriptive microscopy of *Banksia* stems

The structural and anatomical features observed in transverse sections of CSI and PSI of ten *Banksia* species are presented: *B. serrata* (Plates 3.2 & 3.25d), *B. menziesii* (Plates 3.4, 3.5, 3.26c & 3.27a), *B. baxteri* (Plate 3.8), *B. speciosa* (Plates 3.9 & 3.10), *B. burdettii* (Plate 3.11), *B. hookeriana* (Plate 3.13), *B. prionotes* (Plate 3.14), *B. coccinea* (Plates 3.15, 3.16, 3.25a & b), *B. spinulosa,* vars. *spinulosa* (Plate 3.19), *cunninghamii* (Plates 3.20 & 3.27b), and *collina* (Plate 3.21), *B. ericifolia* (Plates 3.22, 3.23 & 3.26a) and *Macadamia integrifolia* (Plates 3.24 & 3.27c). Radial and tangential longitudinal sections of the CSI and PSI of four species are included: *B. serrata* (Plate 3.3), *B. menziesii* (Plates 3.6 & 3.7), *B. burdettii* (Plate 3.12), *B. coccinea* (Plates 3.17, 3.18, 3.25c & 3.26b).

Summary of early wood anatomy in Banksia

***Banksia* stem of 12 – 18 months age**[19] has primary and secondary tissues present in CSI (Plate 3.9c) forming a monostelic eustele comprising collateral bundles (Plates 3.2b, 3.5a, & 3.16), endarch primary xylem (Plate 3.5b), interfascicular rays (Plate 3.15d) and is of the diffuse porous type (Plate 3.2b). **Xylem** consists of primitive long vessel elements with annular secondary wall formation (Plate 3.26b), circular inconspicuous bordered intervascular (Plates 3.7, 3.10c 3.17a & 3.26b) and vessel-ray pits (Plate 3.18c), vascular tracheids (Plate 3.10c) and abundant fibre tracheids (Plates 3.8e & 3.10c). **Cambium** is non-stratified (Plate 3.7), forming secondary xylem and phloem and vascular rays (Plates 3.5b, c & 3.8e). **Interfascicular cambium** is comprised of two or more cells adjacent to fascicular cambium (Plates 3.2e, 3.5d & 3.8e). **Interfascicular rays** are multiseriate (Plates 3.5a & 3.9c) and heterocellular (Plate 3.6a, b & 3.12c), while **vascular rays** are uniseriate and phenolic rich (Plates 3.5a, 3.8b, 3.10c & 3.11d). **Cortex** has numerous large intercellular spaces (Plates 3.7, 3.9d, 3.15d & 3.17b), heterogenous cell contents (Plate 3.6a & c) and phenolic rich cells beneath cuticle or cork (Plates 3.6c & 3.12b). The outer cortical cells are elongate (parallel to main axis), mid and inner cortical cells isodiametric (Plate 3.18d). **Leaf traces,** present in the cortex of all *Banksia* species (Plates 3.9d, 3.13e, 3.15a, b, 3.21a,

[19] Terms used as in Metcalfe & Chalk (1979) and Metcalfe (1987b)

3.24a, 3.26a, 3.29a & c) but not in internodes of *Macadamia integrifolia* (Plate 3.24), have an endarc vascular arrangement similar to the vascular bundles (Plate 3.4c). Occasionally the vascular arrangement is amphicribral (Plate 3.14c) – the central core of xylem surrounded by phloem – and the outer **fibre zone** may encircle the leaf trace. Non-xylary **phloem fibres** form a pericycle fibre zone between primary phloem and cortex (Plate 3.11a & c). **Pith fibre strands** are present at the pith margin in contact with primary xylem (Plates 3.9c & 3.11a). **Pith** is active, phenolic rich, and starch is stored four or more granules per cell (Plates 3.2b, 3.7 & 3.28b). **Cork (phellem)** is superficial, generated from phellogen at the cortex margin beneath the cuticle and forming a periderm (Plates 3.6, 3.7, 3.14d, 3.19 & 3.27); in CSI of some species the cork periderm is entire and four or more cells thick (Plates 3.20 & 3.24) and in other species no cork is observed in PSI (Plates 3.15 & 3.2). **Cuticle** is present in all species and is lipid based (Plate 3.26c). **Trichomes** are unbranched, long and unicellular when present (Plates 3.3a & 3.5a). **Cellular occlusions** were observed in all species, mostly in cortex adjacent to distal end of interfascicular rays (Plates 3.2b, 3.5d, 3.10b, 3.11f, 3.13d, 3.17b, 3.19f, 3.24d & 3.24d) or in mid cortex region (Plates 3.2b, 3.6, 3.7, 3.9, 3.16b, 3.21b, 3.29b, d & 3.26c), but also occasionally in the interfascicular rays (Plate 3.25c) and pith (Plates 3.6b, 3.7, 3.29a). The incidence of cell occlusions increased in cortex of PSI. **Starch grains** were present in the pith, interfascicular parenchyma and cortex close to vasculature and accumulation increased in PSI (Plates 3.14c, d, 3.15, 3.26c & 3.28b).

Developmental differences between CSI and PSI

The secondary growth in *Banksia* is characterised by the generation of secondary phloem and secondary xylem, which was visible in the CSI (Plate 3.9c). This is accompanied by a corresponding extension of the interfascicular rays. In addition, secondary growth of the PSI usually includes the generation of phellem (cork) (Plates 3.8a, b, 3.14a, b, d & 3.24d), an accumulation of secondary metabolites, such as polyphenolics, starch in the pith and interfascicular rays (Plates 3.26c & 3.28b), and deposition of secondary metabolites producing cell occlusions (refer to above section).

Quantitative image analysis

Image analysis was used to quantify species, age and seasonal differences in early wood characteristics in the CSI and PSI of *Banksia* (Table 3.2). This was done in three parts. Part I involved analysing the gross tissues types (Plate 3.1a) of single bushes from five

Banksia species and *Macadamia integrifolia* collected from two seasons, spring 1994 and autumn 1995. Part II involved the same analyses as Part I, using replicated bushes for two species, *B. coccinea* and *B. menziesii* for the same two seasons. In Part III a microanalysis of vascular bundle components (Plate 3.1b) of the six species used in Part I was undertaken from stems collected in spring 1995.

PART I Gross tissue types of six species

Image analysis of five *Banksia* species and *M. integrifolia* was used to quantify the age and species differences in nine stem characteristics of *Banksia* over spring 1994 and the following autumn, 1995. Significant differences exist for all nine parameters measured for the CSI and PSI from all species (Table 3.3), yet seasonal effects were not significant, with the exception of the parameter cuticle thickness in the PSI. There were significant complex interactions for species and seasons for the CSI for four tissue parameters: percentage vascular bundles, distance from the cambium to the cuticle (or cork if present), distance from the cambium to the surface and cuticle thickness. There were species-seasons interactions in the PSI for three parameters: percentages of cortex and cork, and cork thickness.

Mean values for each parameter of the CSI and PSI for individual species are recorded in Table 3.4. The percentage pith varied from 22% in *B. ericifolia* to 36% in *B. serrata* for the CSI and the same trend was apparent for the PSI, but values were 20% to 50% lower. The percentage vascular bundles for the CSI was highest in spring in *Macadamia integrifolia* (50%) and lowest in *B. coccinea* (15%) and similarly in the PSI (58% and 27% respectively). The cortex accounts for the largest proportion of the cross-sectional area of stem tissue, ranging from 23% in *B. spinulosa* var. *cunninghamii* to 50% in *B. coccinea*, and similarly 20% and 44% for the PSI. A seasonal difference exists for the percentage cortex in the PSI of *B. ericifolia*: winter (18.5%) and summer (33.2%). Internodes of *Macadamia* had no leaf traces and the percentage leaf traces was not significantly different between *Banksia* species, accounting for a small percentage of the total cross-sectional area: 4.3% (*B. coccinea*) to 6.1% (*B. spinulosa*) for the CSI and 1.7% (*B. menziesii*) to 7.4% (*B. serrata*) for the PSI. Due to the complete absence of cork in some species in the CSI or PSI, irregularities have arisen in the data making it difficult to interpret unambiguously. Generally, cork was found in the CSI of *Macadamia integrifolia* and *B. spinulosa* and in the PSI of *B. ericifolia* and *B. menziesii*. No cork was observed in *B. serrata* or *B. coccinea* in either internode. The distance from the cambium to the stem surface for the CSI was shortest in *B. ericifolia*

Table 3.3 Summary of the main effects in gross tissue type analysis of six species (** = significant at $P \leq 0.01$, nsd = no significant different)

	I GROSS TISSUE TYPES ANALYSIS FOR SIX SPECIES					
Parameter	Current season's internode (CSI) spring vs. autumn			Previous season's internode (PSI) winter vs. summer		
	species	season	interaction	species	season	interaction
% pith	**	nsd	nsd	**	nsd	nsd
% vascular bundle	**	nsd	**	**	nsd	nsd
% cortex	**	nsd	nsd	**	nsd	**
% leaf traces	**	nsd	nsd	**	nsd	nsd
% cork	**	nsd	nsd	**	nsd	**
Cambium to cork or cuticle, µm	**	nsd	**	**	nsd	nsd
Cork thickness, µm	**	nsd	nsd	**	nsd	**
Cuticle thickness, µm	**	nsd	**	**	**	nsd
Cambium to surface, µm	**	nsd	**	**	nsd	nsd

Table 3.4 Image analysis of gross tissue types of six species' branches collected in spring 1994 and autumn 1995. Where species show a seasonal difference between the spring and autumn, seasonal means (± s. e.) are presented; otherwise new *estimated means* (± s. e.) from both the spring and autumn means are given. CSI and PSI were analysed separately. Adopting Bonferoni-like principles for multiple comparisons, a critical z value ≥ 3 was chosen to correspond to an approximate significance level ($P \leq 0.01$); means followed by the same letter are not significantly different; (-) indicates insufficient variation in the data to return a meaningful result; n = 15

I GROSS TISSUE TYPE ANALYSIS OF SIX SPECIES																
Species	% pith		% vascular bundles		% cortex		% leaf trace		% cork		Cambium to cuticle or cork, µm		Cork thickness µm		Cuticle thickness µm		Cambium to surface µm	
CURRENT SEASON 'S INTERNODE, CSI # est. mean ± est. *s.e.m.*																
B. coccinea	30.3bcd	*1.4*	14.9a	*1.3*	50.4c	*1.5*	4.3b	*0.7*	0a	*1.1*	612.1b	*20.0*	0a	*5.0*	47.6c	*2.6*	659.7b	*21.4*
B. menziesii	34.7b	*1.4*	Sp 17.7 Au 26.8	*1.8* *1.8*	37.0a	*1.5*	6.0b	*0.7*	0a	*1.1*	566.6b	*20.0*	0a	*5.0*	47.8c	*2.6*	614.4b	*21.4*
B. serrata (n = 10)	35.9ad	*1.5*	22.2b	*1.9*	36.9b	*1.7*	4.3b	*0.8*	0a	*1.2*	569.2c	*22.1*	0a	*5.4*	Sp 54.2 Au 35.9	*2.9* *3.7*	613.9c	*23.6*
B. ericifolia	22.4a	*1.4*	25.0b	*1.3*	47.9c	*1.5*	4.7b	*0.7*	0a	*1.1*	133.7a	*20.0*	0a	*5.0*	27.7b	*2.6*	161.4a	*21.4*
B. spinulosa var. *cunninghamii*	25.6b	*1.4*	31.5c	*1.3*	23.3a	*1.5*	6.1b	*0.7*	13.4b	*1.1*	223.9b	*20.0*	52.2b	*5.0*	23.97ab	*2.6*	300.0b	*21.4*
Macadamia integrifolia	28.2ac	*1.4*	Sp49.9 Au40.9	*1.8* *1.8*	25.3b	*1.5*	0a	*0.7*	1.1a	*1.1*	293.5c	*20.0*	6.2a	*5.0*	16.8a	*2.6*	316.5c	*21.4*
PREVIOUS SEASON 'S INTERNODE, PSI * Est. mean ± est. *s.e.m.*																
B. coccinea	25.4b	*1.72*	26.9a	*1.97*	44.1c	*1.5*	3.6bc	*0.63*	-	-	618.3c	*29.1*	-	-	Win 70.5 Sum 63.9	*2.4*	685.5c	*31.1*
B. menziesii	16.9a	*1.72*	37.7c	*1.97*	35.7b	*1.5*	1.7ab	*0.63*	Win 4.2 Sum 11.9	*1.3* *1.3*	775.7d	*29.1*	Win 45.1 Sum 11.9	13.7 13.7	Win 51.4 Sum 44.8	*2.4*	898.6d	*31.1*
B. serrata (n = 10)	18.7ab	*1.72*	34.5b	*1.97*	37.8b	*1.5*	7.4d	*0.63*	-	-	798.3d	*29.1*	25.7a	9.7	Win 54.9 Sum 48.3	*2.4*	875.5d	*31.1*
B. ericifolia	18.6a	*1.72*	46.7b	*1.97*	Win18.5 Sum 33.2	*2.1* *2.1*	2.1ac	*0.63*	Win 12.6 Sum 0.8	*1.3* *1.3*	206.3a	*29.1*	-	-	Win 36.7 Sum 30.1	*2.4*	260.8a	*31.1*
B. spinulosa var. *cunninghamii*	15.0a	*1.72*	46.3ac	*1.97*	19.7a	*1.5*	2.7bc	*0.63*	16.4b	*0.9*	260.8ab	*29.1*	71.7b	9.7	Win 26.6 Sum 20.0	*2.4*	355.9ab	*31.1*
Macadamia integrifolia	12.3a	*1.72*	58.0d	*1.97*	19.3a	*1.5*	0a	*0.63*	10.4a	*0.9*	346.8b	*29.1*	56.9ab	9.7	Win 23.8 Sum 17.2	*2.4*	424.3b	*31.1*

CSI were collected in either spring or autumn; individual spring (Sp) or autumn (Au) means are presented where significant differences exist.

* Note the growth of PSI collected in spring has been influenced by the preceding winter; conversely, the growth of PSI collected in autumn has been influenced by the preceding summer. Hence for the PSI the seasonal means are nominated as *sum* (summer) and *win* (winter).

Table 3.5 Summary of main effects in gross tissue type analysis of *B. coccinea* and *B. menziesii* (** = significant at P ≤ 0.01; *** = significant at P ≤ 0.001; nsd = not significantly different)

II GROSS TISSUE TYPE ANALYSIS OF *B. COCCINEA* AND *B. MENZIESII*						
	Spring 1994			Autumn 1995		
	Species	Age	Interaction spec·age	Species	Age	Interaction spec·age
% pith	nsd	***	nsd	nsd	***	***
% vascular bundle	nsd	***	nsd	***	***	nsd
% cortex	**	nsd	nsd	***	***	nsd
% leaf traces	nsd	**	nsd	nsd	nsd	nsd
% cork	nsd	nsd	nsd	***	***	***
Cambium to cork or cuticle, µm	**	nsd	nsd	***	***	**
Cork thickness, µm	nsd	nsd	nsd	**	**	**
Cuticle thickness, µm	nsd	***	nsd	***	nsd	**
Cambium to surface, µm	nsd	nsd	nsd	***	***	***

Table 3.6 Gross tissue type analysis of *B. coccinea* and *B. menziesii*; CSI and PSI analysed separately; means followed by the same letters are not significantly different; species pair-wise comparison is indicated by a, b, c, d; age (CSI or PSI) pair-wise comparison is indicated by I, II, III IV; $n = 15$; critical z value ≥ 3; $P \leq 0.01$

II GROSS TISSUE TYPE ANALYSIS OF *B. COCCINEA* AND *B. MENZIESII*																			
			% pith		% vascular bundles		% cortex		% leaf trace		% cork		Cambium to cuticle or cork µm		Cork thickness µm		Cuticle thickness µm		Cambium to surface µm	
SPRING 1994			Estimated mean ± estimated *standard error*																	
B. coccinea	(est. mean)	CSI	34.0	*0.7*	15.3	*0.4*	46.4	*1.0*	4.3	*0.3*	0	-	535.7	*22.5*	0	-	50.3	*2.6*	586.0	*21.2*
		PSI	22.2	*0.6*	32.7	*1.1*	41.5	0.9	3.6	*0.2*	0	-	578.6	*17.6*	0	-	67.6	*2.3*	646.2	*18.6*
B. menziesii	(est. mean)	CSI	34.2	*1.3*	21.8	*1.1*	39.1	0.9	4.9	*0.5*	0	-	571.8	*13.6*	0	-	48.7	*2.5*	620.6	*13.4*
		PSI	19.8	*1.4*	63.4	*1.1*	36.5	0.9	2.4	*0.4*	4.9	*1.0*	850.7	*100.4*	43.5	10.7	57.0	*2.2*	951.3	*105.5*
	Overall combined means*		34.1 I	*1.5*	18.6 I	*1.6*	44.0 a	*1.4*	3.8	*0.3*	1.2	*0.7*	634.2	*38.6*	10.9	6.6	49.5 I	*2.6*	701.0	*44.4*
			21.0 II	*1.5*	34.6 II	*1.6*	37.8 b	1.4									62.31 II	*2.6*		
AUTUMN 1995			est. mean ± est. *s.e.*																	
B. coccinea	(est. mean)	CSI	30.2cI	*1.8*	17.3aI	*1.5*	46.5aI	*1.0*	3.7	*0.2*	0	-	511.1aI	*37.1*	0	-	59.0aI	*2.7*	618.42aI	*37.4*
		PSI	26.0bI	*1.8*	30.2cII	*1.5*	42.7cI	*1.0*	3.3	*0.3*	0	-	643.3cII	*37.1*	0	-	62.4aI	*2.7*	657.3aI	*37.4*
B. menziesii	(est. mean)	CSI	34.6cII	*1.8*	25.7bIII	*1.5*	38.2bII	*1.0*	3.7	*0.5*	0	-	698.6bIII	*37.1*	0	-	56.6bII	*2.7*	706.8bII	*37.4*
		PSI	15.4aIII	*1.8*	38.5dIV	*1.5*	34.4dII	*1.0*	1.6	*0.2*	7.8	*1.0*	830.7dII	*37.1*	81.5	*12.4*	45.4 bIII	*2.7*	1006.0bIII	*37.4*
	Overall combined means*								3.1	*0.4*	2.0	*0.9*			20.4	*9.5*				

* overall combined means presented only for non-significant species or age effects

Table 3.7 Summary of vascular bundle analysis of the CSI and PSI collected in spring, 1995 (nsd = not significantly different, **: $P \leq 0.01$, ***: $P \leq 0.001$)

III VASCULAR BUNDLE ANALYSIS			
SPRING 1995			
Variable	Bush	Age	Interaction Bush·Age
% vascular bundles	***	**	nsd
% pith fibre	nsd	nsd	nsd
% xylem	nsd	***	***
% cambium	nsd	***	**
% phloem	***	***	***
% phloem fibre	***	nsd	***
% total fibre	***	nsd	***
% interfascicular cells	**	***	***

Table 3.8 Vascular bundle analysis of six species (25.10.95), CSI and PSI from the same branch. Means (± s. e.) of different species followed by the same letters are not significantly different (CSI: a, b, c, d and PSI: v, w, x, y, z); underlined means indicate a significant difference between the means from the CSI and PSI in that species (age effect). (n = 15; critical *z* value ≥ 3; P ≤ 0.01)

III VASCULAR BUNDLE ANALYSIS, SPRING 1995 Est. mean ± est. *s.e.*																	
Species	age	% vascular bundles		% pith fibre*		% xylem		% cambium		% phloem		% phloem fibre		% total fibre (phloem + pith)		% interfascicular cells	
B. coccinea	*CSI*	68.5a	*2.1*	2.7	*0.4*	29.5ab	*3.3*	5.4ac	*0.7*	15.6a	*1.7*	13.6b	*1.7*	16.2b	*1.9*	4.1ac	*1.3*
	PSI	73.8 v	*2.1*	1.6	*0.2*	54.0 v	*3.3*	4.1 v	*0.7*	10.7 v	*1.7*	5.1 v	*1.7*	6.8 v	*1.9*	6.4 v	*1.3*
B. ericifolia	*CSI*	80.1c	*2.1*	2.8	*0.3*	36.2ad	*3.3*	6.0bcd	*0.7*	19.4a	*1.7*	17.1b	*1.7*	19.9b	*1.9*	2.1a	*1.3*
	PSI	85.3 x	*2.1*	1.8	*0.2*	42.5 v	*3.3*	4.0 v	*0.7*	21.0 wxy	*1.7*	14.4 wxy	*1.7*	16.3 wxyz	*1.9*	2.4 v	*1.3*
B. menziesii	*CSI*	63.8a	*2.1*	2.0	*0.8*	22.9a	*3.3*	5.8ad	*0.7*	26.5b	*1.7*	3.3a	*1.7*	5.3a	*1.9*	2.3a	*1.3*
	PSI	69.0 v	*2.1*	2.0	*0.3*	43.8 v	*3.3*	4.7 v	*0.7*	15.4 vx	*1.7*	6.4 v	*1.7*	8.4 vw	*1.9*	13.2 w	*1.3*
B. spinulosa var. *cunninghamii*	*CSI*	71.3ab	*2.1*	5.5	*1.8*	32.7ac	*3.3*	2.7a	*0.7*	16.3a	*1.7*	14.2b	*1.7*	19.7b	*1.9*	8.1bcd	*1.3*
	PSI	76.5 vw	*2.1*	2.1	*0.3*	44.6 v	*3.3*	2.5 v	*0.7*	17.8 wxy	*1.7*	9.3 vy	*1.7*	11.4 vy	*1.9*	6.1 v	*1.3*
B. serrata (n = 10)	*CSI*	64.3a	*3.4*	0	*0*	20.5a	*5.7*	7.5bcd	*1.3*	37.0c	2.9	0.0a	*3.0*	0.0a	*3.3*	2.0ad	*2.3*
	PSI	69.6 v	*3.4*	2.8	*0.7*	40.7 v	*5.7*	2.9 v	*1.3*	13.4 vw	*2.9*	9.0 vx	*3.0*	11.8 vz	3.3	14.5 w	*2.3*
Macadamia integrifolia	*CSI*	79.6bc	*2.1*	4.9	*0.8*	42.1bcd	*3.3*	5.1ab	*0.7*	17.6a	*1.7*	12.9b	*1.7*	17.8b	*1.9*	3.5ab	*1.3*
	PSI	84.8 wx	*2.1*	3.1	*0.5*	52.2 v	*3.3*	2.1 v	*0.7*	16.3 vy	*1.7*	8.2 vw	*1.7*	11.3 vx	*1.9*	4.5 v	*1.3*
				2.7*	*0.4*												

* percentage of pith showed no significant species or age effects, hence individual species means have been presented together with the overall combined mean.

(161 μm) and greatest in *B. coccinea* (660 μm); a similar trend exists for the distance from the cambium to cuticle or cork (if present) in the CSI. The largest distance to the surface in the PSI was recorded in *B. menziesii* and *B. serrata* (899 μm and 875 μm, respectively). *Macadamia integrifolia* has the thinnest cuticle in the CSI (17 μm), whereas in *B. serrata*, the only species to show a seasonal difference for cuticle thickness, the cuticle was thickest, 54 μm in spring. The PSI produced in winter by all species showed a significant increase in cuticle thickness (6 μm) over that produced in summer (Table 3.4).

PART II Gross tissue types of B. menziesii and B. coccinea

In spring 1994 there were fewer significant differences in stem tissues compared to the following autumn 1995 (Table 3.5). Mean values for each parameter for the CSI and PSI from each season for *B. coccinea* and *B. menziesii* are recorded in Table 3.6. In spring significant differences between the CSI and PSI regardless of species (i.e. an age effect) were recorded for the percentages of pith and vascular bundles, and cuticle thickness. The percentage cortex was significantly different for *B. coccinea* and *B. menziesii* (species effect), and as there was no significant difference between the CSI and PSI from within each species (no age effect), the combined means of the CSI and PSI for each species are given, 44% and 38% respectively. Cuticle thickness displayed an age effect only, stem from the PSI having a significantly thicker cuticle (62 μm) than that from the CSI (49 μm). Similarly, percentage vascular bundles and the percentage pith showed significant age, but no species effects. The percentage vascular bundles increased from the CSI (19%) to the PSI (35%), whereas the percentage pith decreased (34% to 21%). In stems collected in autumn there was a greater number of significant species and age effects with complex interactions, which were not present in material collected in spring. Significant age and species differences were recorded for the parameters: distance of the cambium to the surface or to the cuticle, cuticle thickness, percentages of cortex, pith and vascular bundles. The species and age means are presented for each in Table 3.6. As cork was only observed in the PSI of *B. menziesii*, means of the cork data are dominated by "outliers" and should be viewed accordingly.

PART III Microanalysis of vascular bundles

The vascular bundle analysis of stems collected in spring 1995 from six species was undertaken to quantify the components of vascular bundles (Plate 3.1b). This analysis took into account the effect of the PSI on the production of the CSI, the CSI and PSI data from each branch being linked and analysed together.

From the summary of main effects presented in Table 3.7, the percentage of vascular bundles displayed a significant species and age main-effect, as did the percentages of phloem and interfascicular cells. The remaining parameters: percentages xylem, cambium, phloem, phloem fibre and total fibre displayed mixed age and species effects, together with complex interactions. The exception was the percentage of pith fibre, which did not show an age or species effect, consequently an estimated mean value of 2.7% was generated from the combined data of this variable (Table 3.8). There was a 5% increase in vascular bundles across all species from the CSI to the PSI (Table 3.8). The percentage of xylem in vascular bundles from CSI and PSI of *B. coccinea* (30% and 54%) and *B. menziesii* (23% and 44%) showed significantly different estimated (underlined) means. The percentage of xylem in the CSI of the remaining species varied from 20.5% in *B. serrata* to 42% in *Macadamia*, compared to 41% for *B. serrata* and 54% in *B. coccinea* for the PSI. Only *Macadamia* showed a significant difference in the estimated mean of the percentage of cambium for the CSI (5%) and PSI (2%). *Banksia* species did not show significant differences, although there was a trend for the CSI to have a greater percentage of cambium, varying from 2.7% in *B. spinulosa* var. *cunninghamii* to 7.5% in *B. serrata* for the CSI. In the PSI it varied from 2.5% in *B. spinulosa* var. *cunninghamii* to 4.7% in *B. menziesii*. *B. menziesii* and *B. serrata* showed significantly different estimated means for the percentage of phloem in the CSI and PSI, and there is a general trend for the percentage of phloem to be slightly higher in the CSI (with the exception of *B. ericifolia*). The others varied from 16% (*B. coccinea*) to 19% (*B. ericifolia*) in the CSI and from 10% (*B. coccinea*) to 21% (*B. ericifolia*) for the PSI. *B. coccinea* showed significantly different means for both percentages of phloem fibre (current 13% and previous 5%) and total fibre (16% and 7%); whereas the CSI and PSI of *B. spinulosa* var. *cunninghamii* showed significantly different means only for the parameter total fibre (20% and 11% respectively).

The percentages of interfascicular cells in the CSI and PSI were statistically different for *B. menziesii* (2% and 13%) and *B. serrata* (2% and 14%). The other species ranged from 2%

(*B. ericifolia*) to 8% (*B. spinulosa* var. *cunninghamii*) for the CSI and from 2.4% (*B. ericifolia*) to 6% (*B. coccinea*) in the PSI.

Generally, vascular bundles in the CSI produced in spring had fewer and shorter vascular rays compared to wood produced at other times (Table 3.9). Vascular rays were significantly shorter in the CSI than the PSI for all species, with the exception of *B. ericifolia*. There were almost twice as many rays in autumn stems compared to those harvested in spring for all species, except in *B. spinulosa* where the ray count in spring and autumn was almost equal. Vascular rays were mostly longer in autumn compared to spring except for *B. serrata, B. spinulosa* and *Macadamia*.

Table 3.9 Vascular ray mean length (μm) and count for CSI and PSI collected in spring 1994 and the following autumn, 1995. Means of different species followed by the same letter are not significantly different (CSI: a, b, c, d; PSI: w, x, y, z); * = means of CSI and PSI of *B. ericifolia* in spring are not significantly different; all other species have significantly different means for the CSI and PSI for spring and autumn (critical z value ≥ 3, $P \leq 0.05$).

SPECIES	AGE	SPRING 1994				AUTUMN 1995			
		Mean length, μm		*s. e.*	Count	Mean length, μm		*s. e.*	Count
B. coccinea	CSI	0		-	0	139.5	b	*11.9*	13
	PSI	179.8	x	*0*	1	322.5	w	*31.2*	14
B. menziesii	CSI	147.7	bc	*0*	1	228.2	c	*20.4*	29
	PSI	175.2	x	*25.0*	34	489.3	z	*38.2*	39
B. serrata	CSI	271.9	d	*21.0*	15	140.0	b	*10.6*	37
	PSI	415.3	wx	*66.5*	22	448.5	y	*48.8*	38
B. ericifolia	CSI	* 57.7	a	*5.9*	11	68.5	a	*3.0*	15
	PSI	* 127.3	w	*55.1*	11	129.4	w	*11.8*	37
B spinulosa var. *cunninghamii*	CSI	192.2	c	*16.6*	38	121.3	b	*6.3*	45
	PSI	255.3	x	*24.2*	44	197.6	w	*15.9*	36
Macadamia integrifolia	CSI	150.6	bc	*10.4*	6	130.3	b	*9.3*	28
	PSI	310.3	wx	*35.4*	19	334.1	w	*27.7*	36

Plate 3.1 Delineation of tissues measured in image analysis

(a) Gross tissue type measurement showing the order of measurement and delineation of the tissues. Image of a section of the CSI of *B. coccinea*. Note that no cork is present in this sample. Bar = 500 μm

1 = pith area
2 = vascular bundles area
3 = cortex area
4 = leaf trace area
5 = cork area (if present)
6 = distance - cambium to cuticle
7 = cork thickness (if present)
8 = cuticle thickness

(b) Vascular bundle measurement showing the delineation of the tissues of five vascular bundles (large numbers at the top) and the adjacent tissues; the order of measurements shown on the LHS bundle are as follows. Bar = 100 μm

1 = pith fibres
2 = xylem
3 = cambium
4 = phloem
5 = phloem fibres
6 = non-interfascicular area (pith + cortex)
7 = interfascicular area

Note: (a) and (b) are *VideoPro 32* images, traced and labelled with *Photoshop* (Adobe)

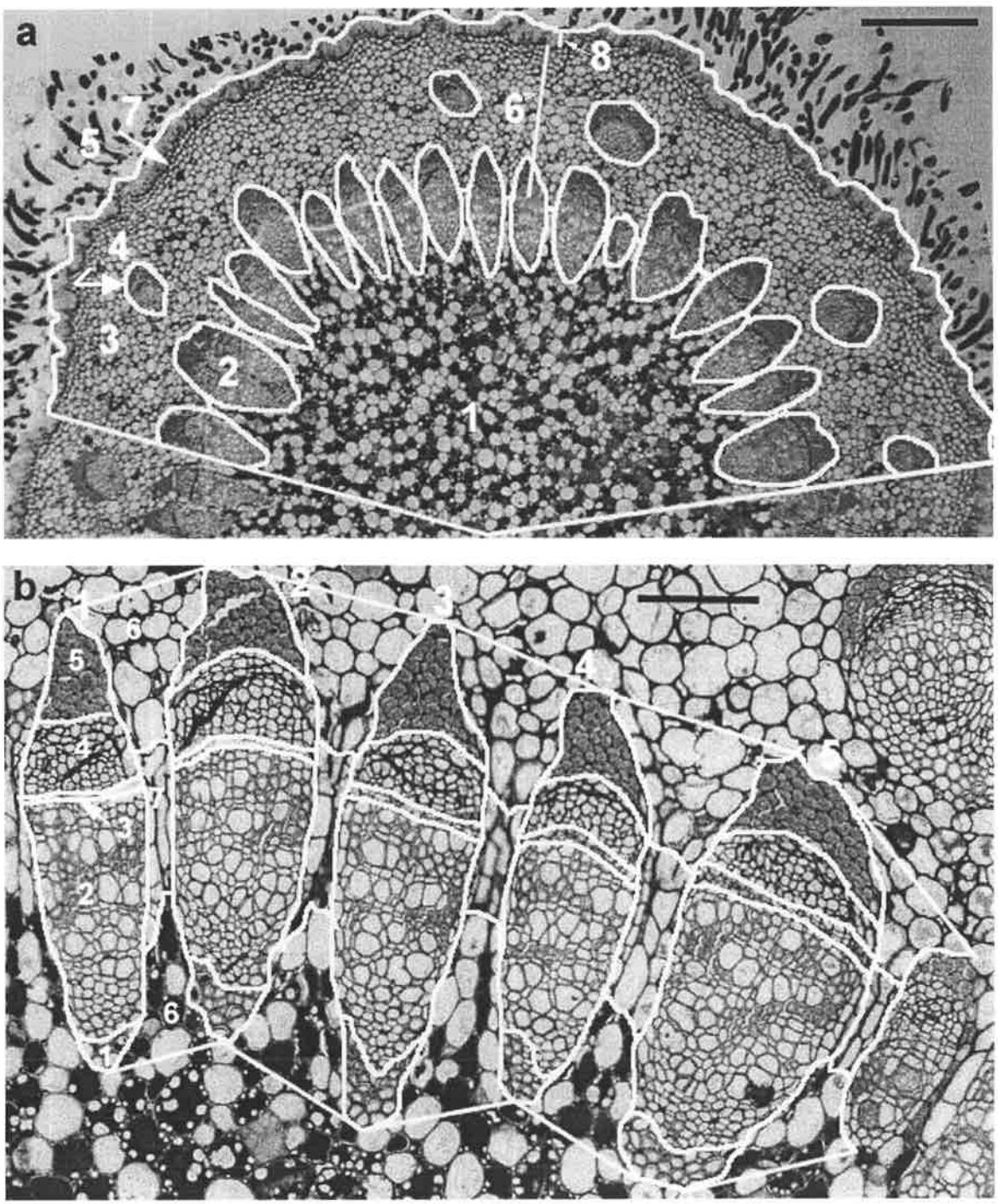
a
8
7
6
5
4
3
2
1
b
6
5
4
3
2
1
2
3
4
5
6
1

Plate 3.2 *B. serrata* CSI and PSI, TS

(a) CSI collected in spring, 1994 showing phloem fibres and pith fibres at either end of the vascular bundles and the loosely packed heavily stained cortex. Bar = 100 μm

(b) PSI collected in spring, 1994 showing phloem and pith fibre caps at either end of the vascular bundles which occur side by side (collateral); fibre caps have increased in size due to the generation of alternating rows of fibre cells and vessels of the secondary xylem (diffuse porous wood type). Note the starch grains in the pith and the frequently occluded cortex cells between the phloem fibres and the interfascicular cambium (arrows). Bar = 100 μm

(c) Leaf trace from CSI collected in spring, 1994. Bar = 100 μm

(d) Occluded cortical cells (centre) in close proximity to phloem fibres (arrow) in CSI collected in spring, 1994. Note the large intercellular spaces between the cortical cells. Bar = 50 μm

(e) Early growth of bush-house grown plants collected in autumn, 1994 showing uniseriate vascular rays (arrow) containing phenolics, the fascicular cambium and adjacent interfascicular cambium and parenchyma cells, and darkly stained pits in the cell walls of larger xylem vessels. Bar = 100 μm

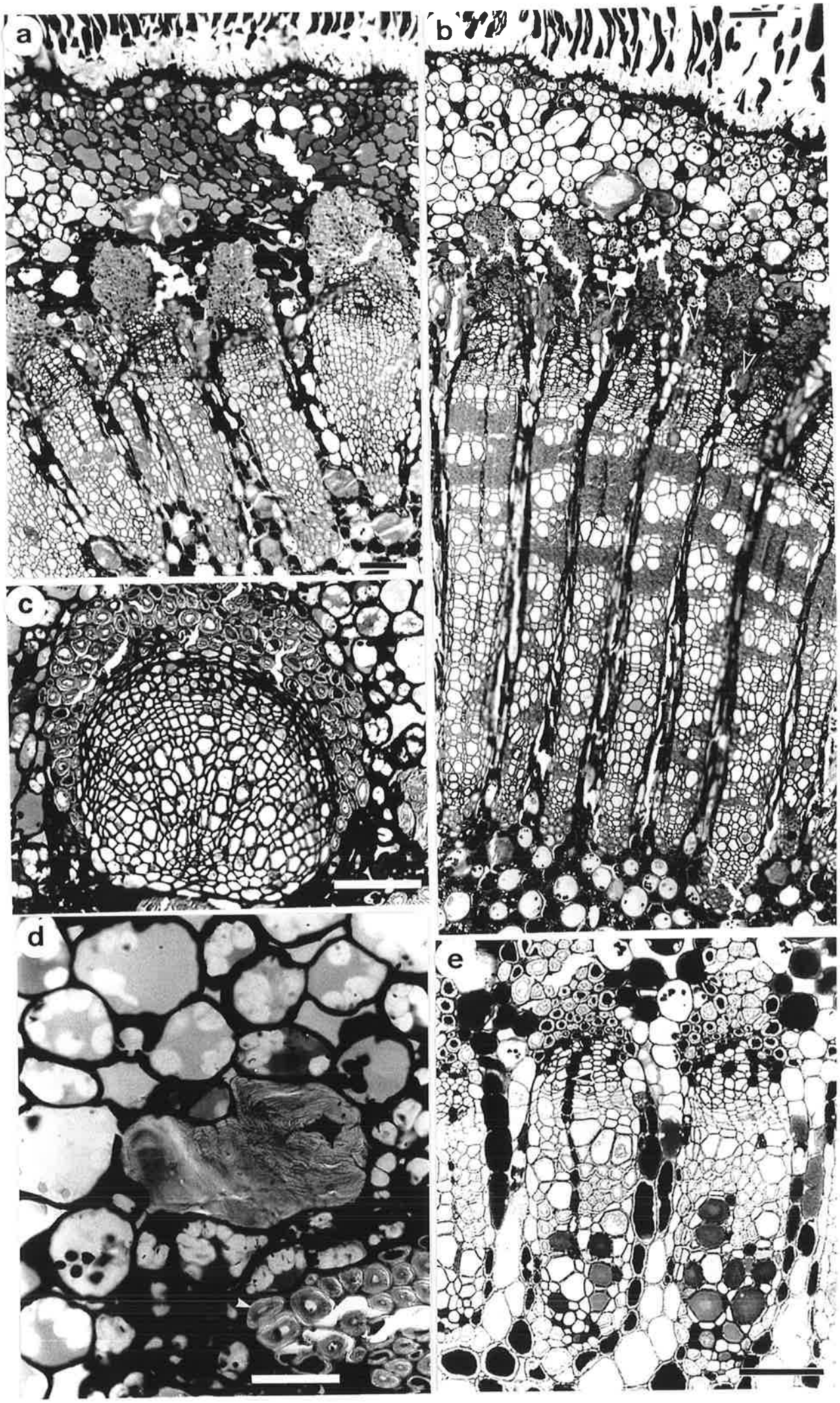
a
b
c
d
e

Plate 3.3 *B. serrata* collected in spring 1994, tangential LS

(a) Distribution of surface trichomes appearing as black dots in TS (arrows), and showing the cuticle (cu) and phenolic rich cortical cells (c) just beneath the surface. Bar = 500 μm

(b) Cortical cells beneath the cuticle (either side of the photomicrograph) with scattered semi- and completely occluded cells and phloem fibres of a leaf trace in the centre. Bar = 500 μm

(c) Vascular bundles containing vessels and occasionally fibres, alternating with interfascicular parenchyma. Bar = 500 μm

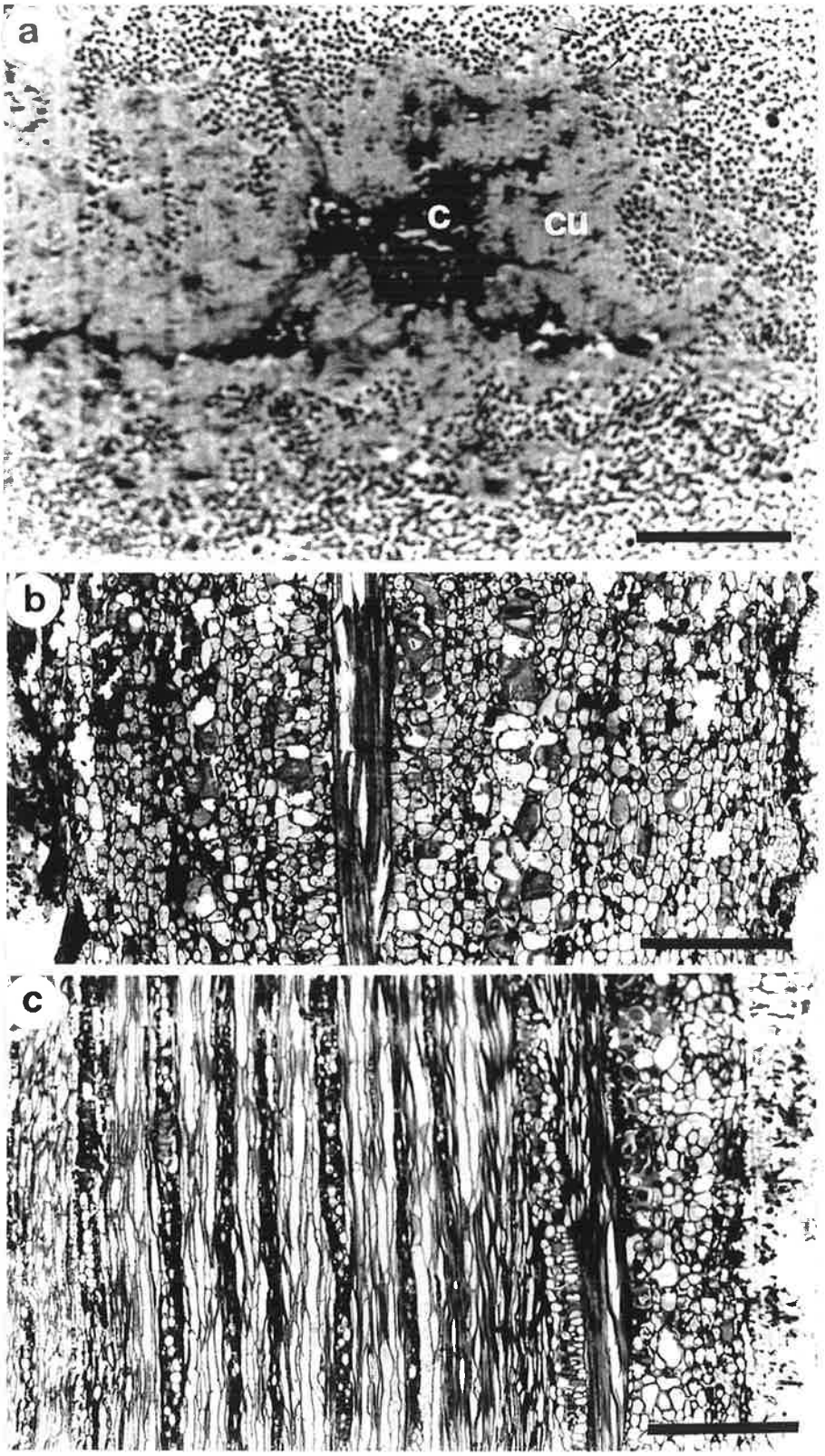
a
c
cu
b
c

Plate 3.4 *B. menziesii* CSI collected in autumn 1994, TS

(a) Vascular bundles and leaf trace of early stem with phloem fibres capping the phloem in the vascular bundles and the leaf trace (top right hand corner). Note the long vessel like interfascicular cell (arrow) shown at higher magnification in (b). Bar = 100 μm

(b) Higher magnification of the long vessel-like interfascicular cell (arrow) seen in (a). Bar = 50 μm

(c) Higher magnification of a leaf trace almost surrounded by secondary thickened fibres (f); the phloem (p) is separated from the xylem vessels (x) by several rows of cambium (ca). Bar = 50 μm

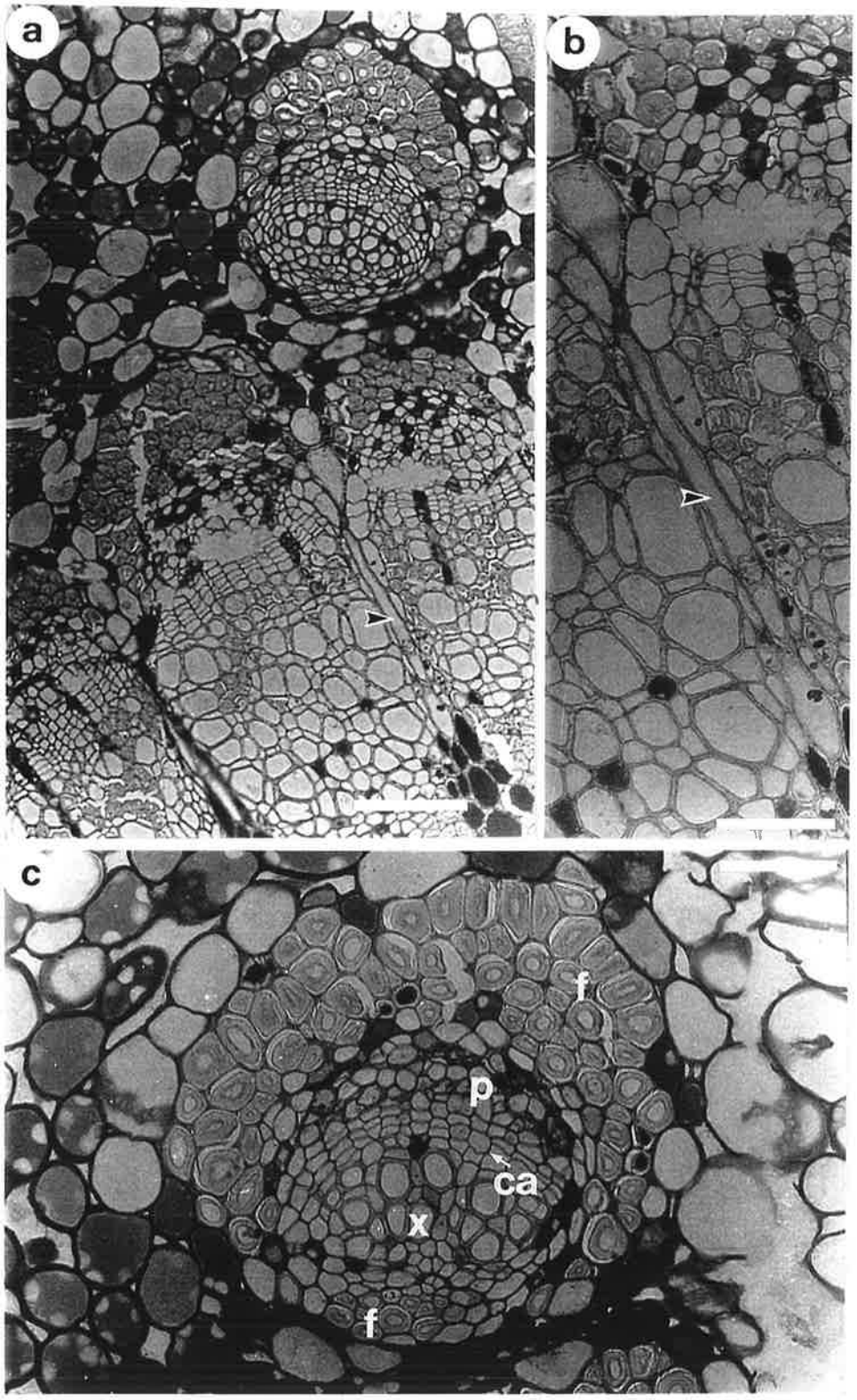
a
b
c
f
p
ca
x
f

Plate 3.5 *B. menziesii* PSI collected in autumn 1994, TS

(a) Gross tissue anatomy displaying the surface trichomes in this species, the thick cuticle, cortex, collateral vascular bundles with uniseriate vascular rays (arrows), multiseriate interfascicular rays and pith. Occluded cells are visible in the cortex and pith. Bar = 500 μm

(b) Outer region of the stem of PSI depicting the living cortical cells (c), interfascicular cambium and active fascicular cambium (ca) generating phloem (p) towards the outside, xylem (x) consisting of xylem vessels and fibres towards the inside (endarc) and uniseriate vascular rays (r) extending into the phloem and xylem. Note the numerous starch grains in the interfascicular cells (lower edge of photomicrograph), the occluded cells in the cortical regions between the vascular bundles, and the leaf trace in the cortex. Bar = 100 μm

(c) Higher magnification of (a) showing the leaf trace and detail of phloem, cambium, xylem, vascular rays and occluded cortical cells. Bar = 50 μm

(d) High magnification of cortical cells adjacent to the fascicular (small arrow) and interfascicular cambium (large arrows) in progressive stages of occlusion. Bar = 100 μm

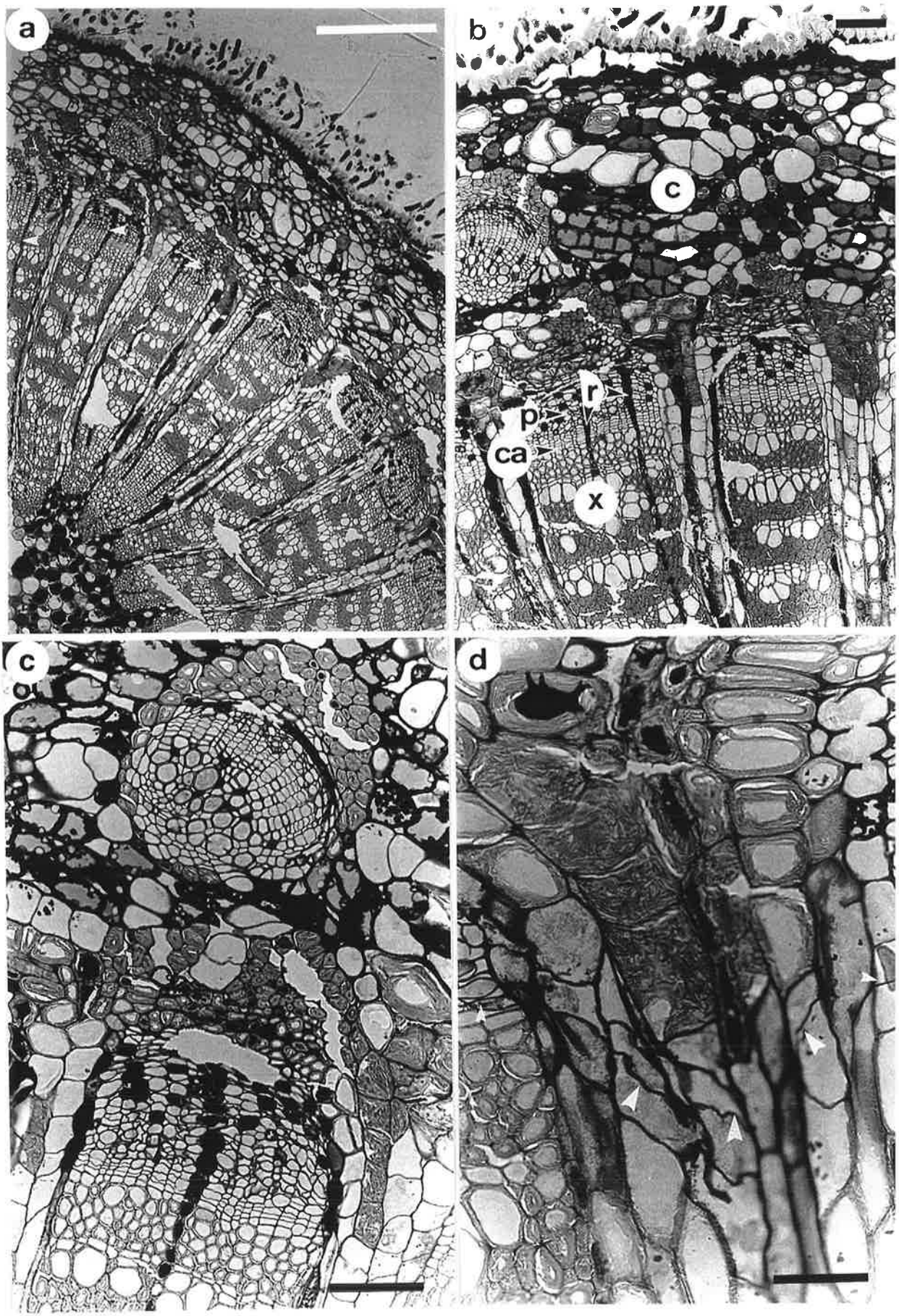
a
b
c
r
p
ca
x
c
d

Plate 3.6 *B. menziesii* PSI collected in autumn 1995, LS

(a) Radial section through the cortex, vasculature and pith of a PSI. Note the position of the horizontal interfascicular rays relative to the vertically aligned xylem elements and also compared to (b), a few sections further on. Interfascicular parenchyma is heterocellular. Note the occluded cells in the cortex (arrows). Bar = 500 μm

(b) Radial perspective a few sections further on from (a). Note the numerous occluded cells in the cortex and in the pith (small arrows), the numerous starch grains seen as black dots in the horizontal interfascicular ray parenchyma and pith cells, and the parts of the xylem running obliquely to other xylem tissues (large arrows). Details of this section at higher magnification are shown in Plate 3.7. Bar = 500 μm

(c) A tangential section through the cortex near the surface of a PSI. A thick layer of cork (ck) more than five cells thick has developed beneath the cuticle (cu). The cortical cells are heterogenous, incorporating living phenolic filled cells (darkly stained) particularly beneath the cork and occluded cells, which have no living contents (arrows). Bar = 500 μm

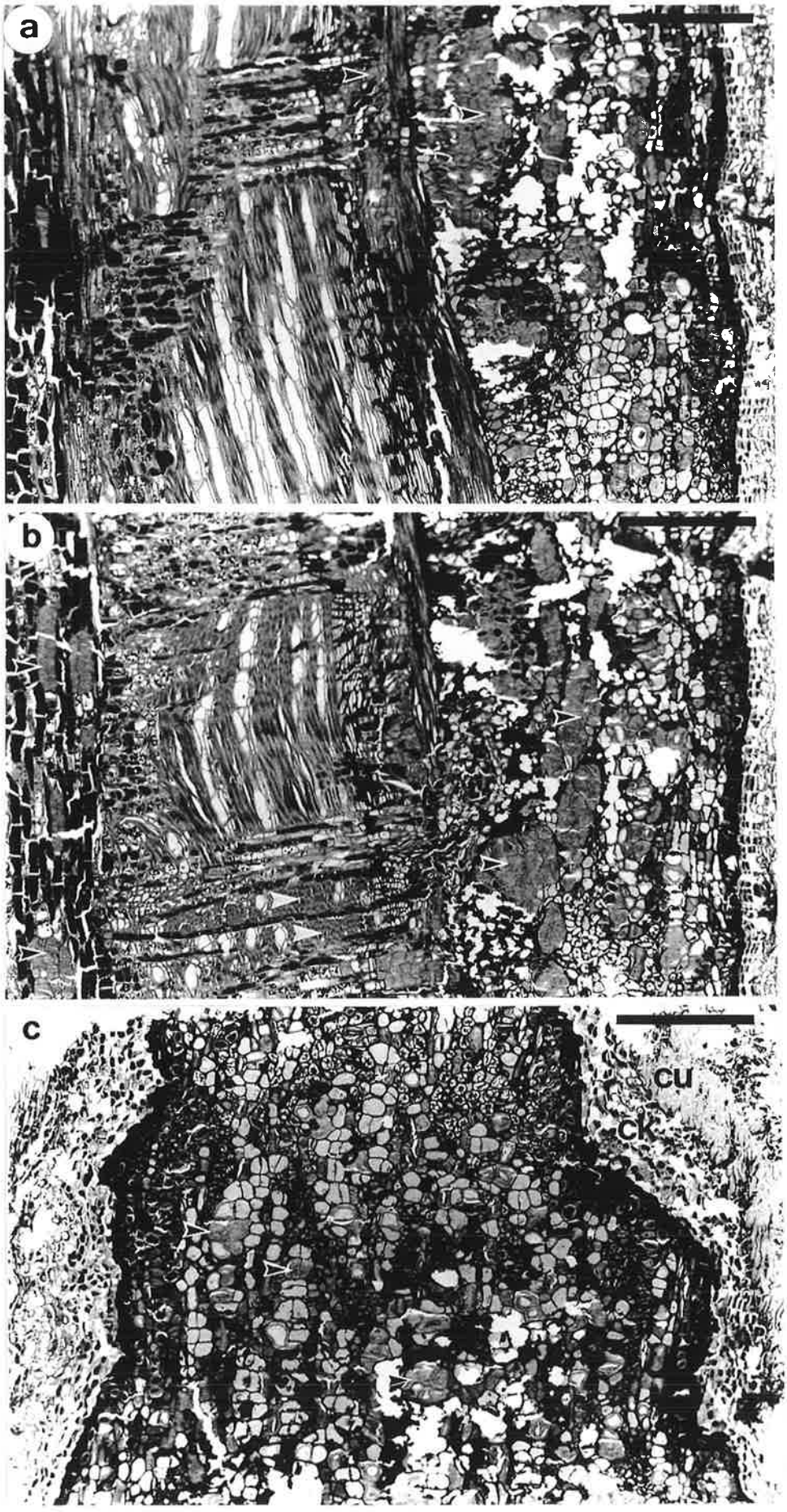
a
b
c
cu
ck

Plate 3.7 *B. menziesii* PSI collected in autumn 1995, LS

Collage showing a radial section though the PSI from the cork (top of photomicrograph) through the vasculature to the pith (bottom of photomicrograph). Note non-stratified cambial initials, the large intercellular spaces in the cortex (large arrows) and intervascular bordered pit region (small arrows). Bar = 100 μm

Plate 3.8 *B. baxteri* CSI and PSI collected in autumn 1994, TS

(a) Cortex and vascular bundles of CSI. Note the thick cuticle, the two leaf traces (large arrows) in the cortex and the single inclusion (small arrow). Bar = 100 μm

(b) Cortex and vascular bundles of PSI. In addition to the generation of xylem with secondary growth, the number of uniseriate rays increases in the vascular bundles (small arrows). Note the cork development beneath the cuticle. Bar = 100 μm

(c) CSI displaying details of the cortex, leaf trace (top centre) and two vascular bundles with phloem and pith fibres at either end. The cambium (ca) generates phloem and xylem. Note the secondarily thickened fibre tracheids (arrow) interspersed between the xylem vessels. Bar = 100 μm

(d) Leaf trace surrounded by secondarily thickened fibres, a higher magnification of (c). Bar = 50 μm

(e) Details of the fascicular (small white arrow) and interfascicular cambium (large white arrow), xylem vessels (v) interspersed with fibres tracheids (black arrows). Note the occluded cells just above the interfascicular cambium (top LH corner). Bar = 50 μm

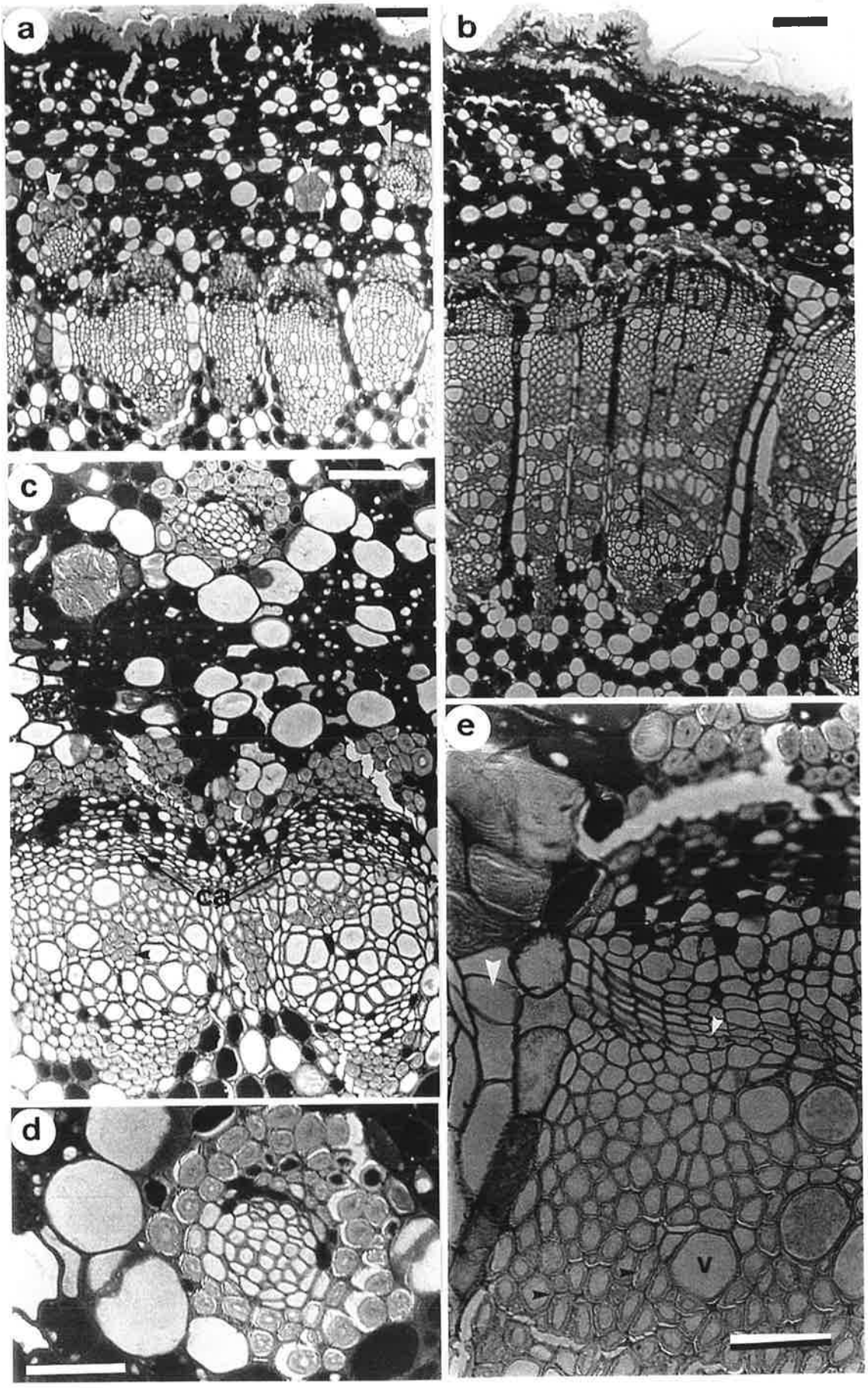
a
b
c
ca
d
e
v

Plate 3.9 *B. speciosa* CSI collected in autumn 1994, TS

(a) Anatomy from the CSI, five leaf traces ensheathed with fibres are present in the cortex, which is covered by thick cuticle and trichomes. Numerous clumps of occluded cells (darkly stained) are present in cortex and to a lesser extent in the pith. Bar = 500 μm

(b) Higher magnification of (a); details of the vascular cambium are clearly visible. Bar = 100 μm

(c) A vascular bundle displaying the primary and secondary growth in *B. speciosa*. Phloem fibres (pf) are conspicuous, yet few pith fibres (f) exist at the margin of the pith. Two cambial initials (ca) are present, on either side of which are thin-walled secondary phloem (p) and xylem (x) derivatives. Note the primary phloem (pp) and primary xylem (px) (produced by the apical meristem) are located outside the secondary tissues produced by the lateral vascular cambium. Occluded cells are present at the distal (top LHS) and proximal (lower RHS) ends of interfascicular rays (ir). Bar = 50 μm

(d) Leaf trace in the cortex of the CSI. Note the thick cuticle over loosely arranged cortical cells, numerous intercellular spaces and the two large cell occlusions between the leaf trace and the vascular bundles. Bar = 100 μm

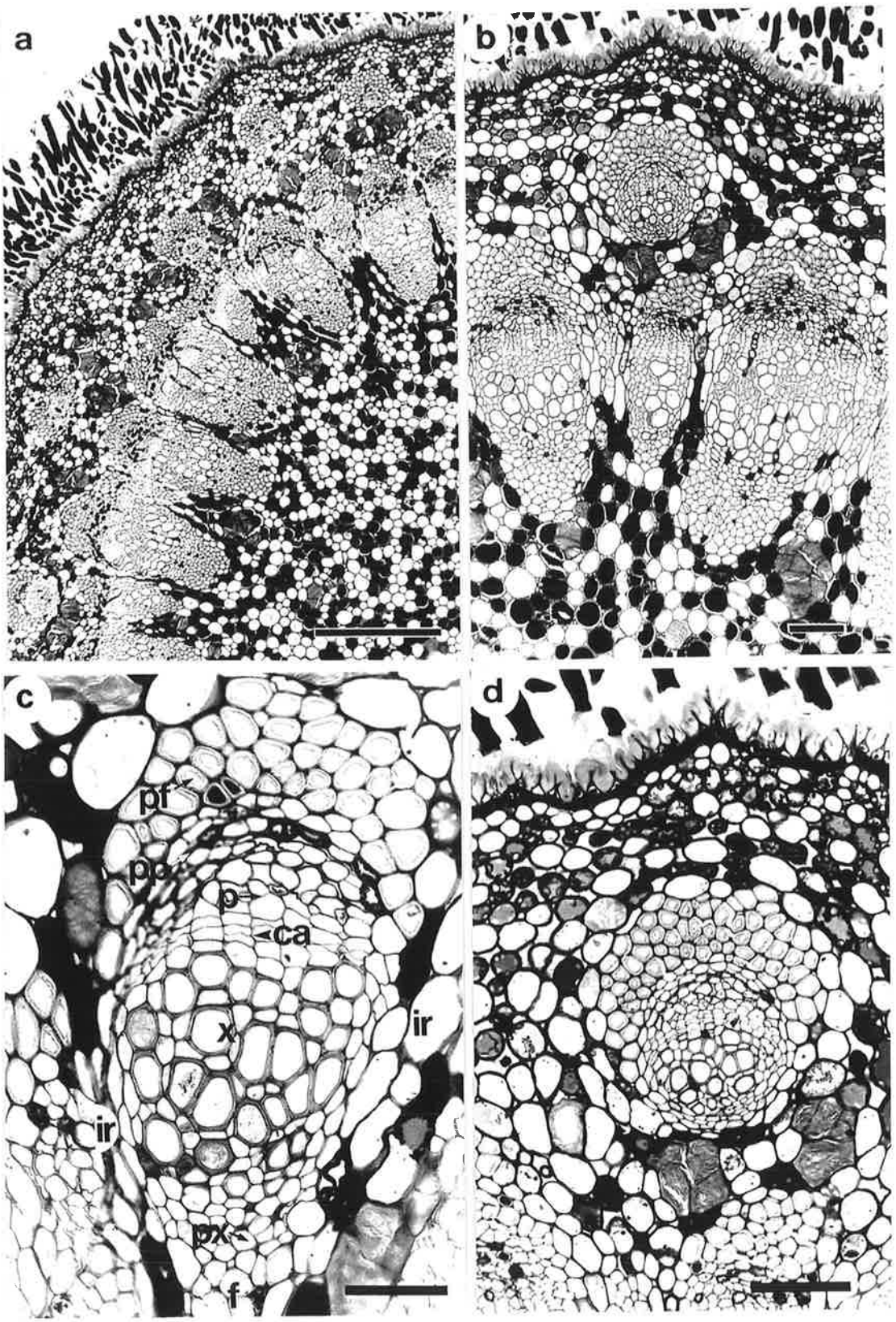
a
b
c
pf
pe
p
ca
x
ir
ir
px
f
d

Plate 3.10 *B. speciosa* PSI collected in autumn 1994, TS

(a) The cortex has two leaf traces and is covered by thick cuticle and trichomes. The lace-like festoons of vessel and fibres in the xylem become more prevalent as secondary growth occurs. Bar = 500 μm

(b) Higher magnifications of (a) showing details of the actively dividing vascular cambium consisting of four or more cells, the early development of cell occlusions in the cortex adjacent to the interfascicular cambium (arrows). Bar = 100 μm

(c) Vascular bundles of (a): inconspicuous bordered intervascular pits (small arrows) are visible in the walls of adjoining large xylem vessels. Note the clusters of fibre tracheids with thick secondarily thickened walls, the smaller vascular tracheids (large arrows) adjacent to large xylem vessels and the two uniseriate vascular rays in the lower vascular bundle. The vascular rays (r) are derived from ray initials in the cambium (ca) and are generated together with the secondary xylem and phloem. Bar = 100 μm

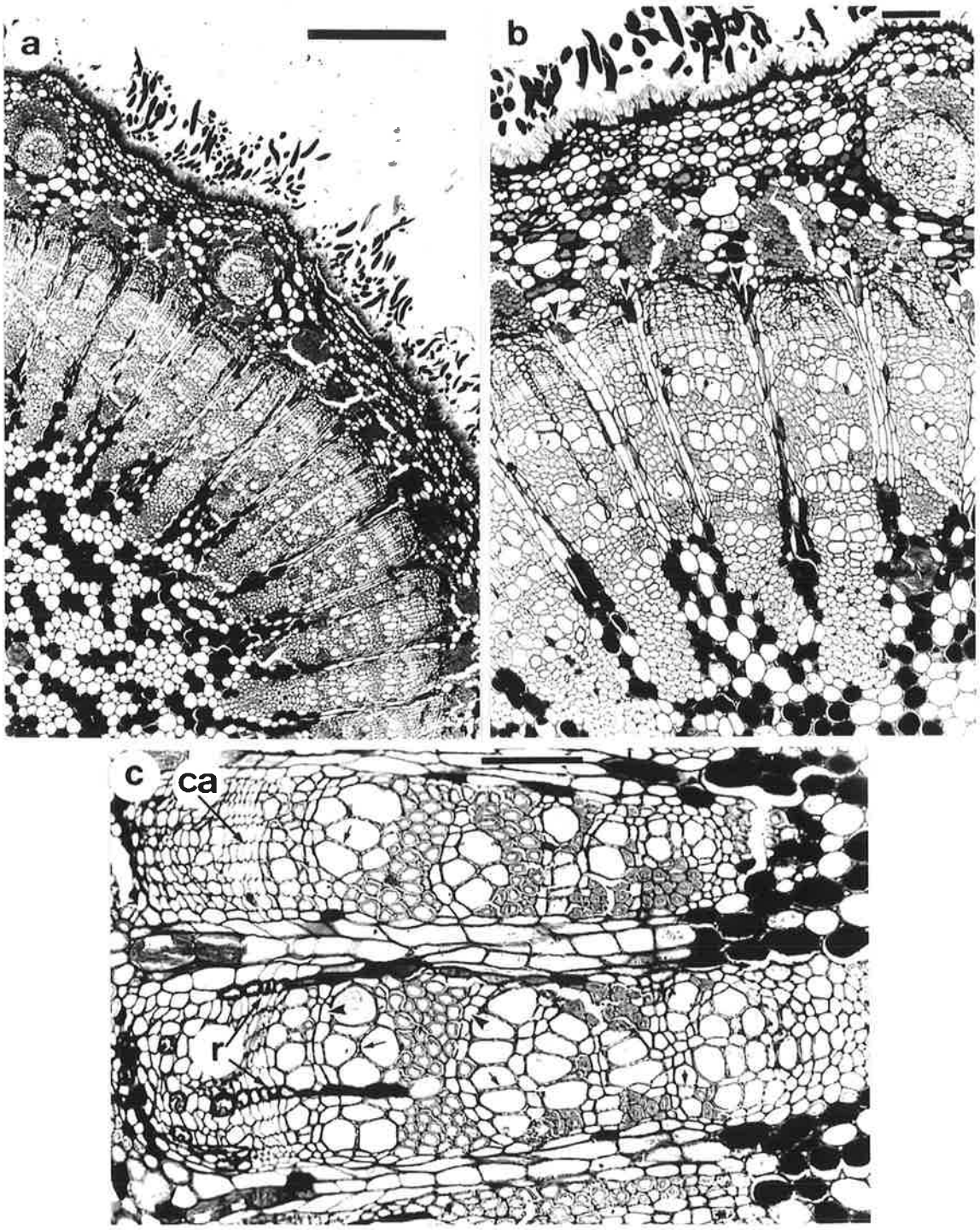
a
b
c
ca
r

Plate 3.11 *B. burdettii* CSI and PSI collected in autumn 1994, TS

CSI

(a) Cuticle and dense trichomes at the surface of the cortex in *B. burdettii*. Non-xylary phloem fibres (pf) located at the periphery of each vascular bundle form a pericyclic fibrous zone between the primary phloem and the cortex. Note the actively dividing cambium, the starch grains in the interfascicular parenchyma, pith and some cortical cells, and the non-xylary pith fibres (f) at the margin of the pith. Bar = 100 μm

(b) Overview of CSI stem segment. Bar = 500 μm

(c) Phloem fibres (pf), in contact with the primary xylem and cellular occlusions (o) in the cortex between the vascular bundles, form an almost continuous barrier of non-living cells separating the living phloem and vascular cambium on the inside and the cortex on the outside. Bar = 50 μm

PSI

(d) Xylem production during the secondary growth consists of rows of large vessels and clusters of xylem fibres. Uniseriate rays develop from ray initials (arrows) in the fascicular cambium. Bar = 100 μm

(e) Overview of secondary wood development in the PSI. Bar = 500 μm

(f) Leaf trace arising from the vascular stele between two vascular bundles. Note the abundance of occluded cells at the distal end of the interfascicular rays, positioned between the living conductive tissues of the vascular bundles and the outer cortical cells. Bar = 100 μm

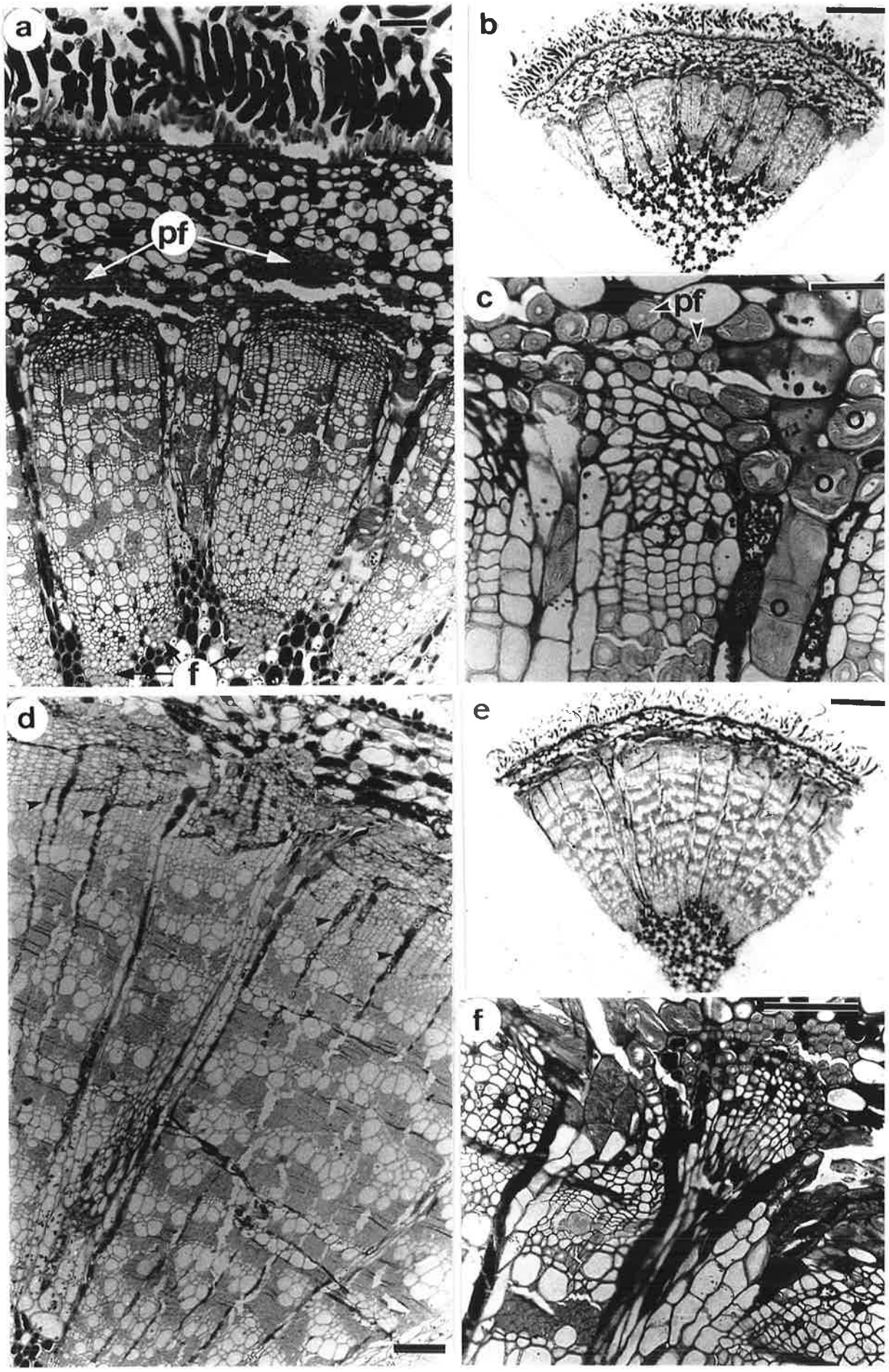
a
pf
f
b
c
pf
o
o
o
d
e
f

Plate 3.12 *B. burdettii* PSI collected in autumn 1995, LS

(a) Tangential section of the surface of the PSI showing the surface trichomes and the cuticle (grey). Bar = 100 μm

(b) Tangential section through the cortex (LHS), cuticle and dense surface trichomes (RHS) of the PSI. Note the phenolic rich cells immediately beneath the cuticle. Bar = 100 μm

(c) Tangential section through the xylem of several vascular bundles and the separating interfascicular ray parenchyma in which starch grains can be seen (arrows). Xylem vessels and fibres cross over from one vascular bundle to another, compartmentalising the heterocellular interfascicular parenchyma. Bar = 100 μm

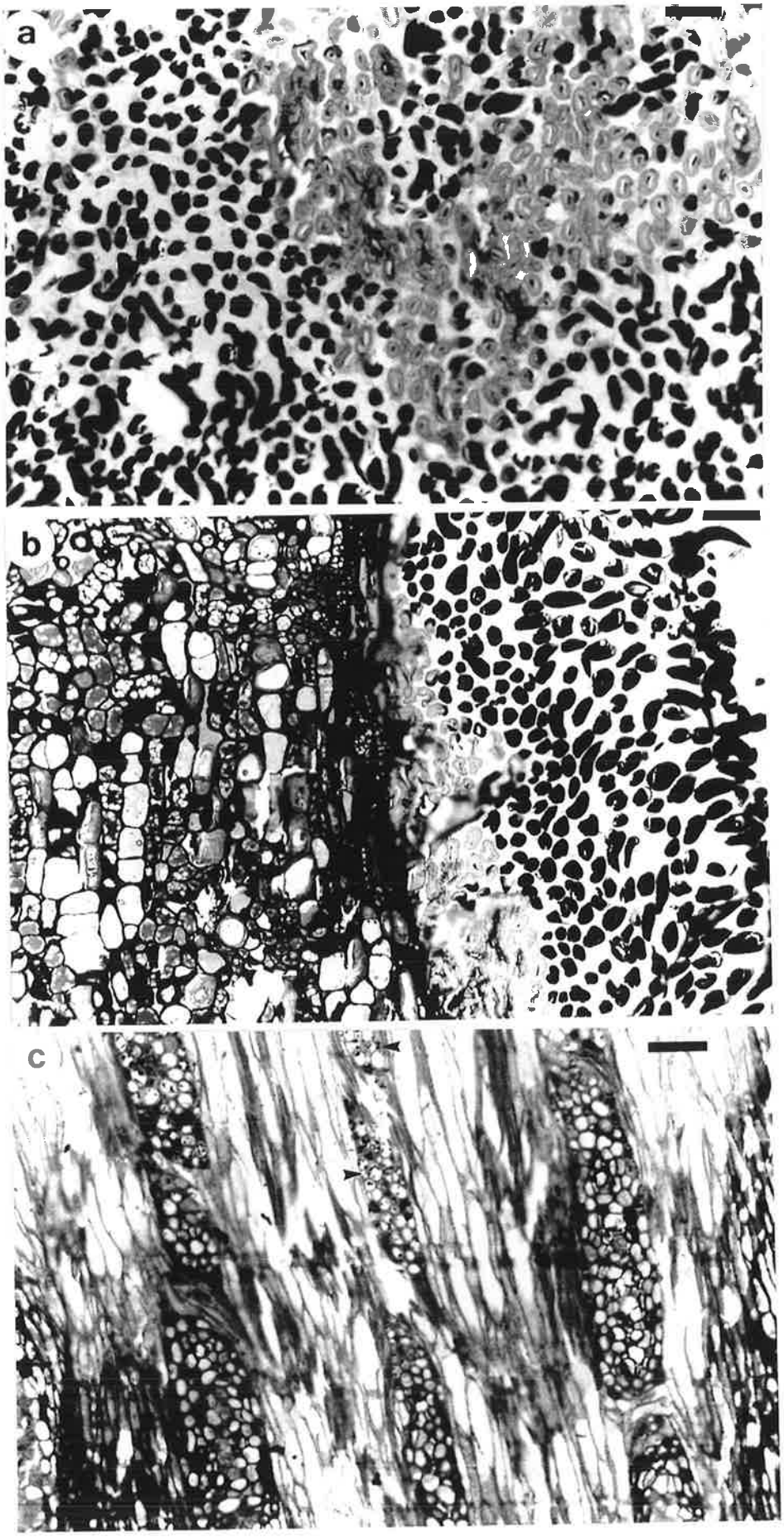
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Plate 3.13 *B. hookeriana* CSI and PSI collected in autumn 1994, TS

(a) One large occlusion in the cortex between the leaf trace and the vascular bundles of the CSI. Bar = 100 μm

(b) Overview of secondary wood development in the PSI. Bar = 500 μm

(c) Secondary growth of the PSI showing uniseriate vascular rays, phloem fibres and a leaf trace surrounded by non-xylary fibres in the cortex. Bar = 100 μm

(d) Occluded cortical cells and phloem fibres forming a continuous barrier of non-living tissue between the conductive tissue and the cortex in the PSI. Bar = 50 μm

(e) The leaf trace and phloem of the vascular bundles in (c) shown at higher magnification. Note the less active vascular cambium and the periphloeic band of phloem fibres and occluded cells. Bar = 100 μm

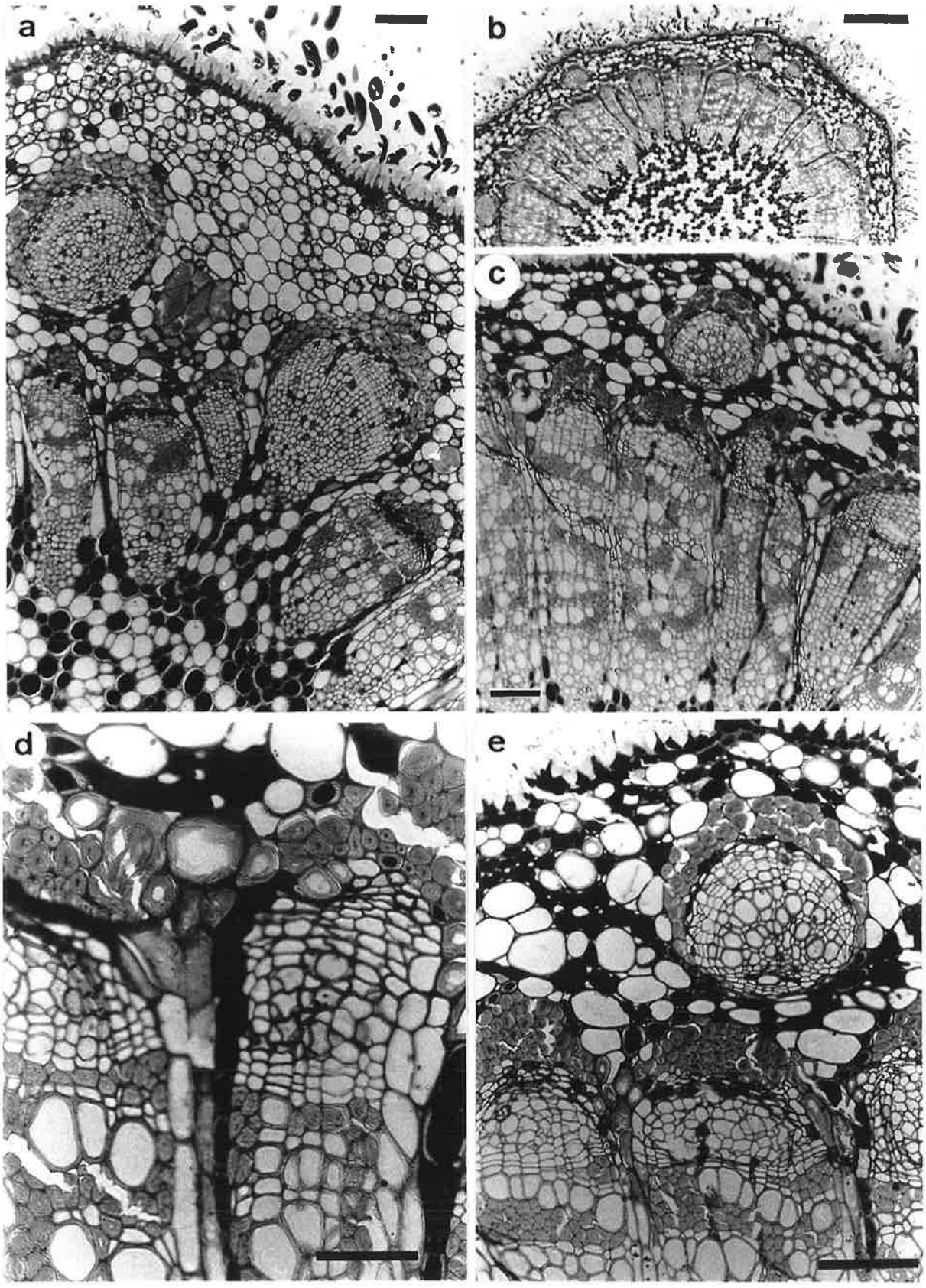
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Plate 3.14 *B. prionotes* CSI and PSI collected in autumn 1994, TS

(a) CSI stem section depicting the heavily staining phenolics in the pith and the cortex extended around the leaf traces. Bar = 500 µm

(b) PSI is rich in phenolics and a thick layer of superficial cork has developed. Large amounts of starch, visible in the interfascicular parenchyma, is transported to the pith and stored. Starch grains are also present in the cortex. Bar = 100 µm

(c) Leaf trace in the CSI completely surrounded by secondarily thickened fibres. The xylem is located centrally and is surrounded by the cambium and the phloem (amphicribral). Note the starch grains in the neighbouring cortical cells. Bar = 100 µm

(d) Superficial cork consisting of regular files of cells with suberised cell-walls and bounded by the cuticle at the surface and the phellogen immediately beneath. Bar = 50 µm

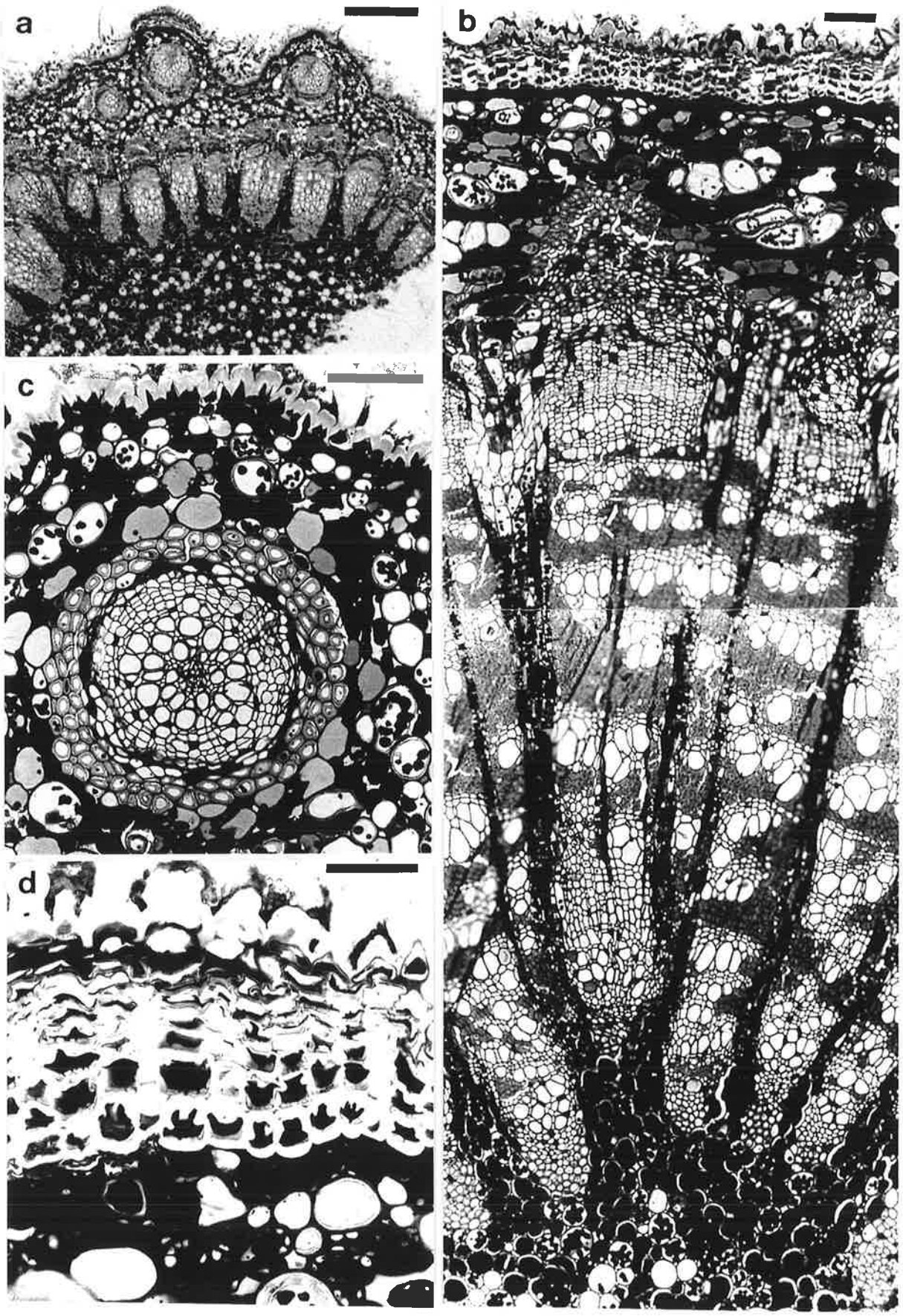
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Plate 3.15 Comparison of *B. coccinea* CSI and PSI collected in autumn 1995, TS

(a) General stem anatomy of the CSI comprised of collateral vascular bundles separated by interfascicular rays. Note the semi-regularly spaced leaf traces in the cortex, the thick cuticle and the surface trichomes. Bar = 500 μm

(b) General stem anatomy of the PSI. Bar = 500 μm

(c) High power detail of (a) showing the phloem fibres in contact with the primary phloem of the vascular bundles; fewer pith fibres are at the margin of the pith. Note the numerous intercellular spaces in the cortex and the absence of starch grains. Bar = 100 μm

(d) High power detail of (b) showing the secondary development of the xylem in the PSI. Large intercellular spaces between cortical cells are also present and starch grains can be seen in the cells of the interfascicular rays (ir) and pith. Bar = 100 μm

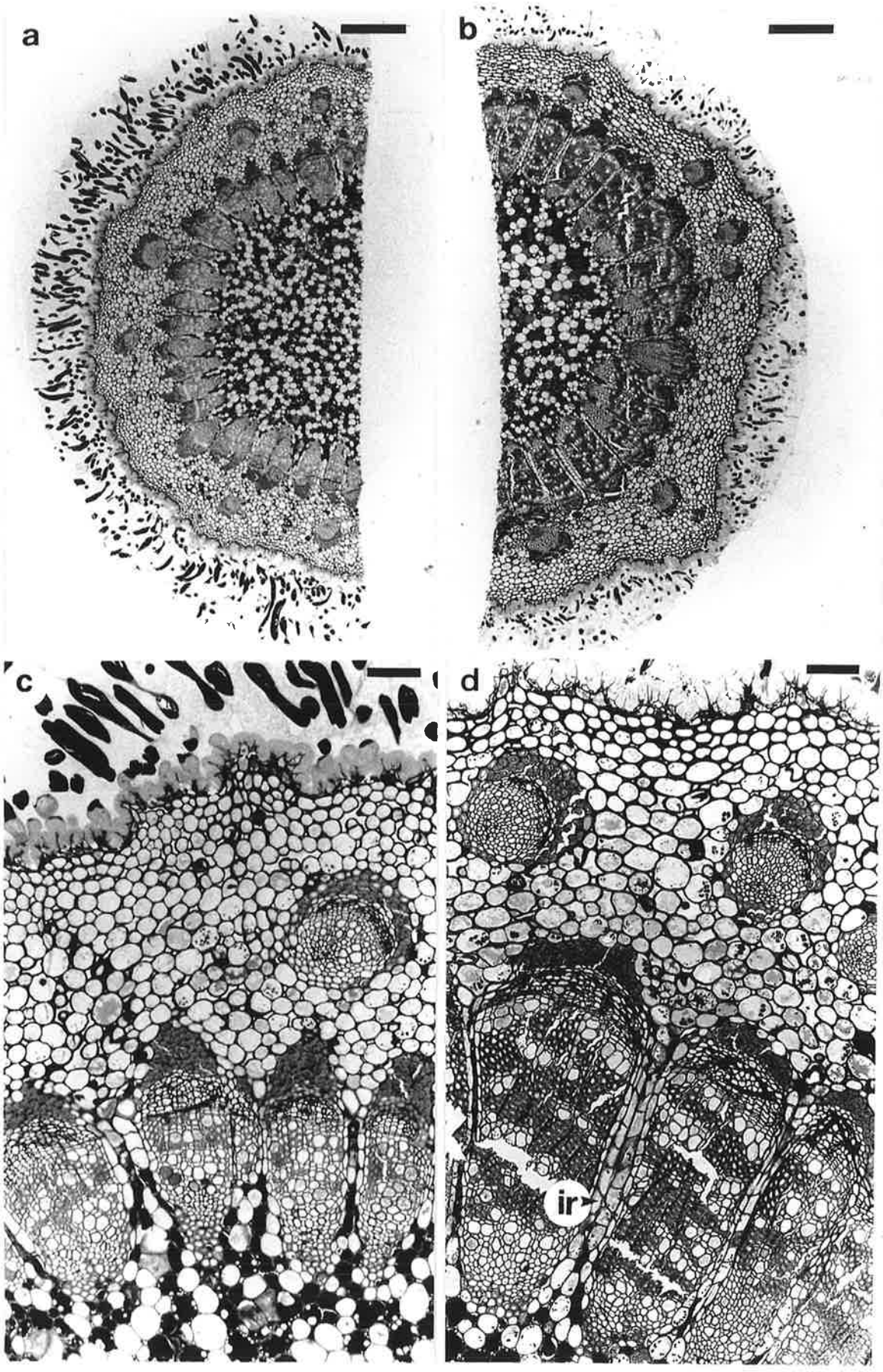
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Plate 3.16 *B. coccinea* CSI, TS

(a) Anatomy of the CSI collected in autumn 1995. Higher magnification colour detail of this internode is presented in Plate 3.25a. Bar = 500 µm

(b) Loosely arranged cortical cells in stem collected in autumn 1995 showing thick cuticle, intercellular spaces and cell occlusion (arrow). Colour staining of this section is presented in Plate 3.25b. Bar = 100 µm

(c) Interfascicular cambium (arrow) is visible in the interfascicular region in stem collected in autumn 1994. Neighbouring vascular bundles each have uniseriate vascular rays. Bar = 100 µm

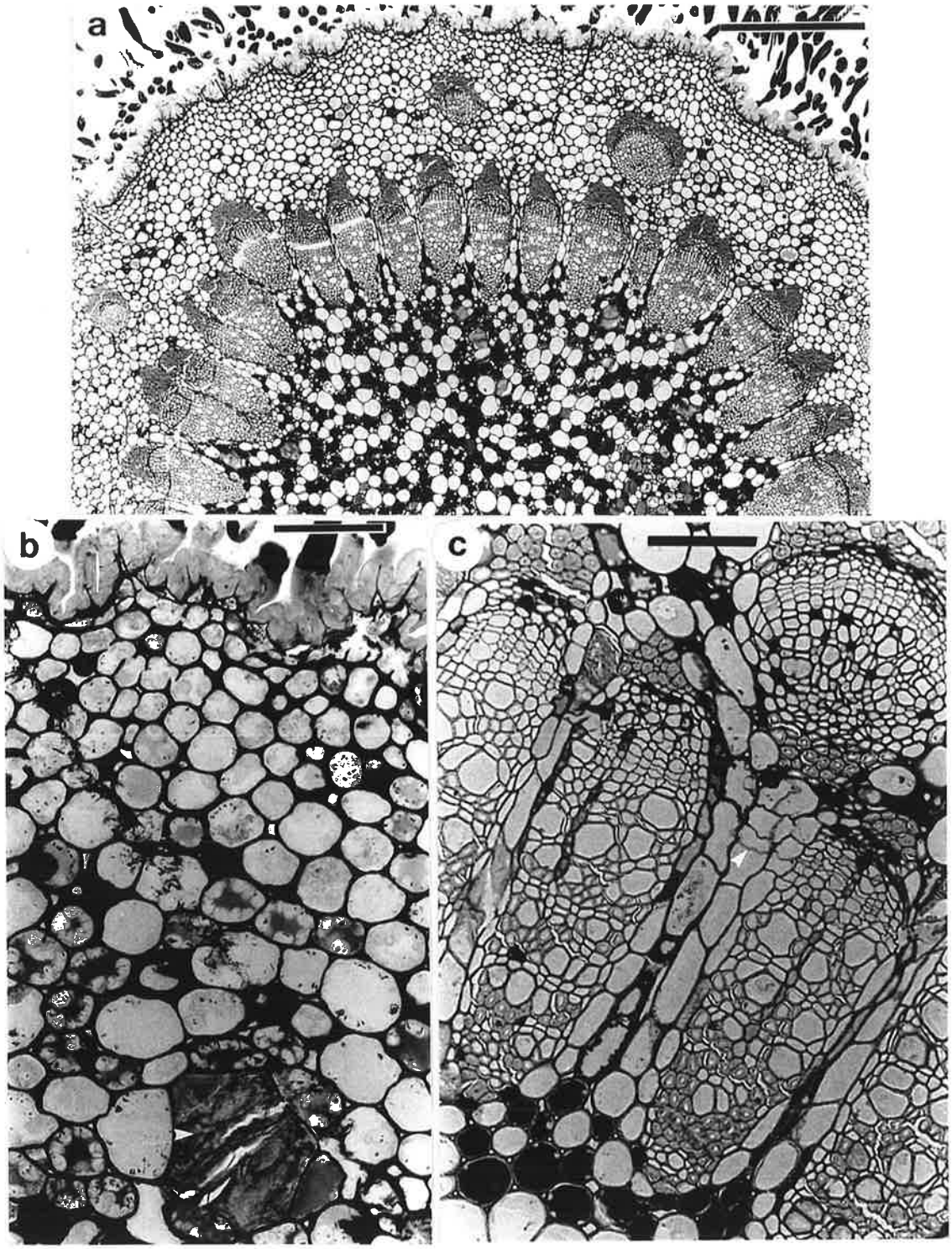
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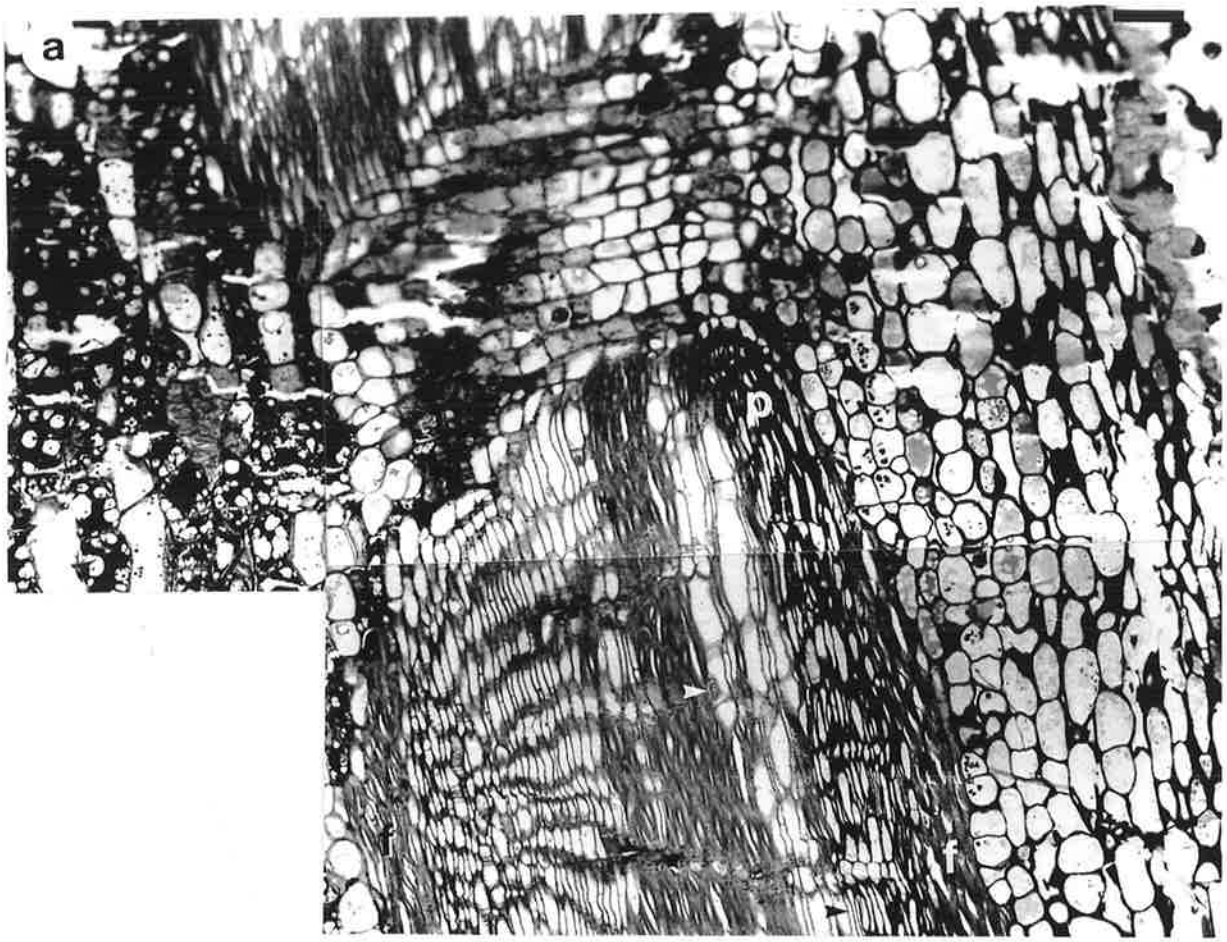
Plate 3.17 Stem anatomy in *B. coccinea* PSI collected in autumn 1995, LS

(a) Radial section through the cuticle (RHS), cortex, vasculature and pith. Phloem (RHS) and pith (LHS) fibres (f) are visible at the extremities of the vasculature. Phloem elements (p) appear as darker tissues adjacent to the large xylem vessels at the centre of the micrograph and a pair of cambial initials are visible at the lower edge (black arrow). Note the increase in size of the xylem vessels from the primary xylem growth at the left of the vasculature to those in the secondary xylem located centrally. An intervascular pit region is also visible (white arrow). Bar = 100 μm

(b) Radial section through the vasculature at a different location to (a) showing the organisation of the heterocellular interfascicular ray parenchyma relative to the vertical vasculature and several occluded cells at the interface to the cortex (arrow). Note the long intercellular spaces between the cortical cells. Bar = 100 μm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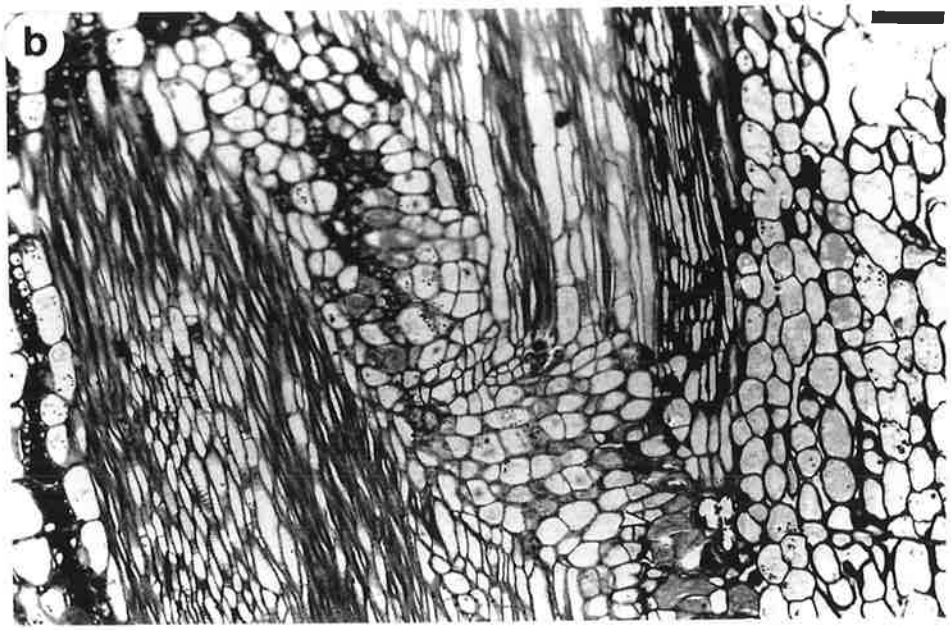
b

Plate 3.18 *B. coccinea* PSI collected in autumn 1995, LS

(a) Radial view of the PSI from the cuticle through the cortex, vasculature and pith of the PSI. Bar = 500 μm

(b) Higher magnification of the radial ray parenchyma of (a) showing the detail of the obliquely sectioned xylem crossing over between vascular bundles (centre of micrograph). Note the heterocellular nature of the surrounding ray. Bar = 100 μm

(c) Vessel-ray pits (arrows) at the juncture of marginal rows of square ray parenchyma and vertical xylem vessels from (a). These pits resemble intervascular pits observed in LS (Plate 3.26b). Note the starch grains in the ray cells. Bar = 50 μm

(d) Tangential section of the PSI through the cuticle (RHS), several vascular bundles and the intervening ray parenchyma. Note the elongate outer cortical cells compared to the isodiametric cells of the mid and inner cortex region. Bar = 500 μm

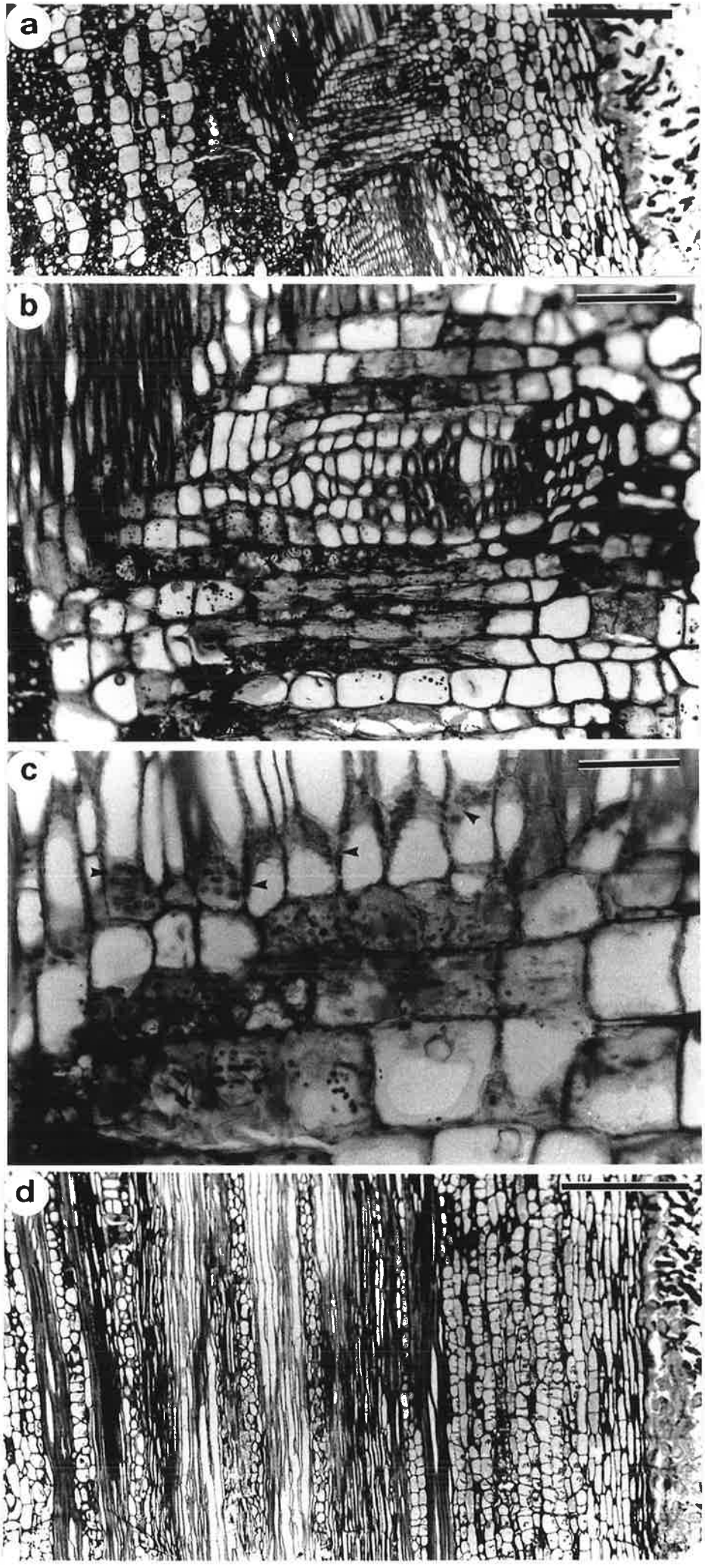
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Plate 3.19 Comparison of CSI and PSI of *B. spinulosa* var. *spinulosa* stem collected in spring 1994, TS

(a) & (b) Overview of the stem anatomy of each internode with the CSI on the left. Bar = 500 µm

(c) Detail of the superficial cork, narrow cortex, phloem fibres and vascular bundles of the CSI in (a). Bar = 100 µm

(d) Detail of the superficial cork, narrow cortex, phloem fibres and vascular bundles of the PSI in (b). Bar = 100 µm

(e) Occluded cortical cells at the distal end of the interfascicular ray between the two vascular bundles of the PSI. Note the starch grains in the interfascicular parenchyma. Bar = 100 µm

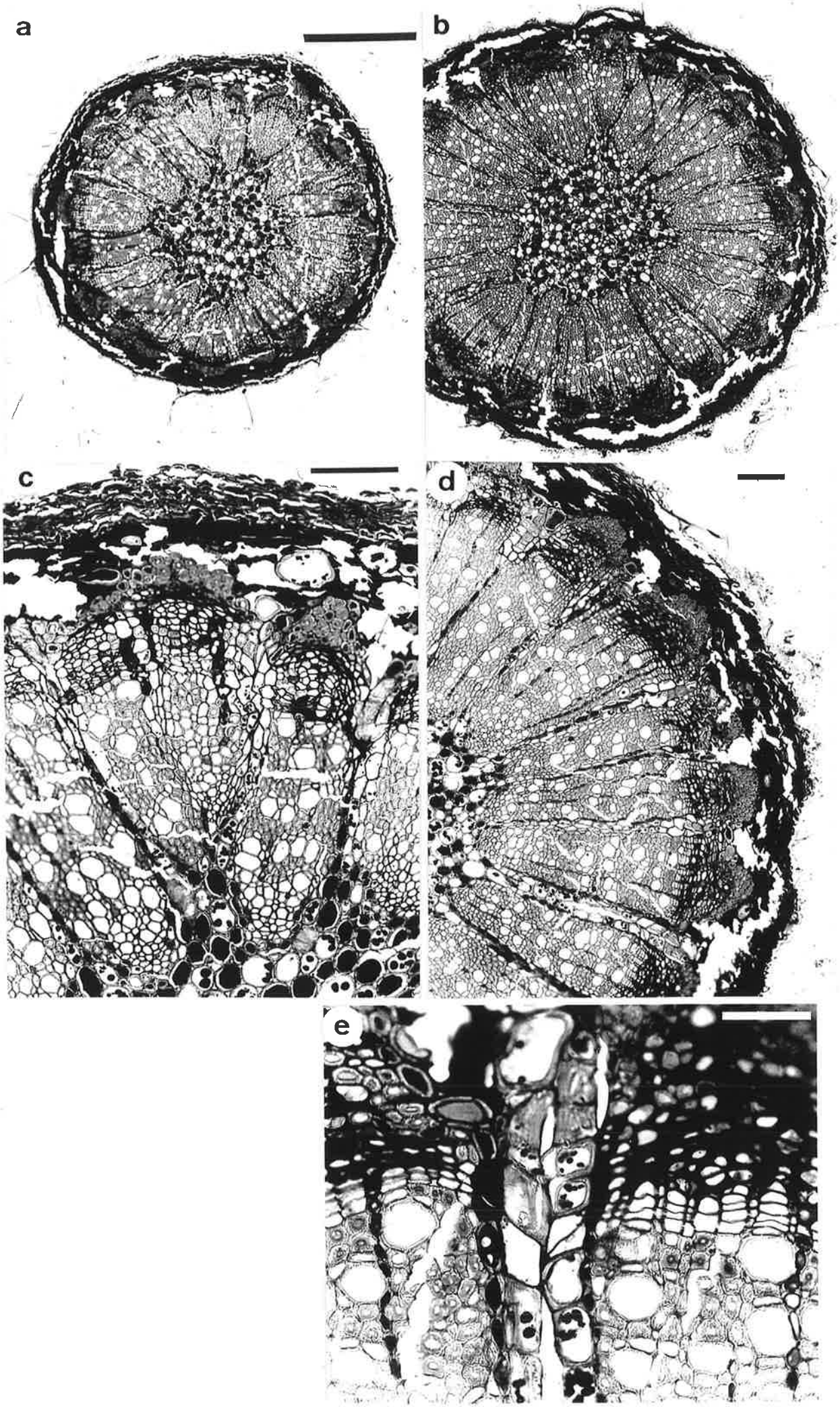
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Plate 3.20 Comparison of the CSI and PSI of *B. spinulosa* var. *cunninghamii* stem collected in spring 1994, TS

(a) & (b) Overview of the stem anatomy of the CSI (LHS) with developing leaf trace and petiole, and the PSI (RHS). Bar = 500 µm

(c) Detail of the developing cork beneath the cuticle, cortex, vascular bundles and active pith containing starch in the CSI in (a). Bar = 100 µm

(c) The thicker superficial cork layer of the PSI in (b) consists of a regular column of cork cells; the cortex is rich in phenolics. Note the occluded cells between the vascular bundles adjacent to the phloem. Bar = 100 µm

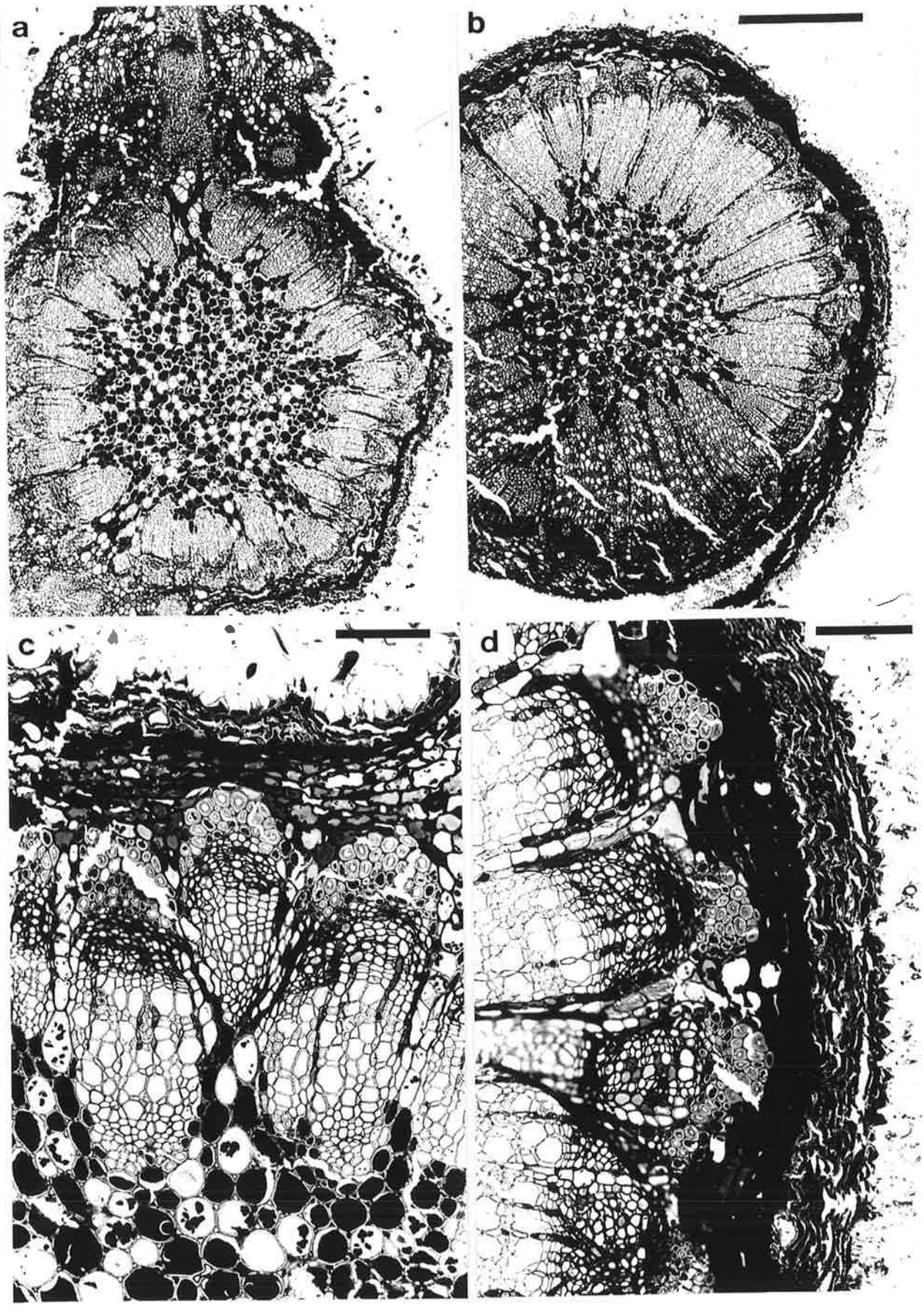
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Plate 3.21 *B. spinulosa* var. *collina* PSI collected in autumn 1994, TS

(a) General stem anatomy, showing the superficial cork, cortex, vasculature and pith Bar = 500 μm

(b) In addition to the cork, numerous non-living cells including occlusions and phloem fibres are present in the cortex. Uniseriate vascular rays extend into the secondary phloem and secondary xylem. Bar = 100 μm

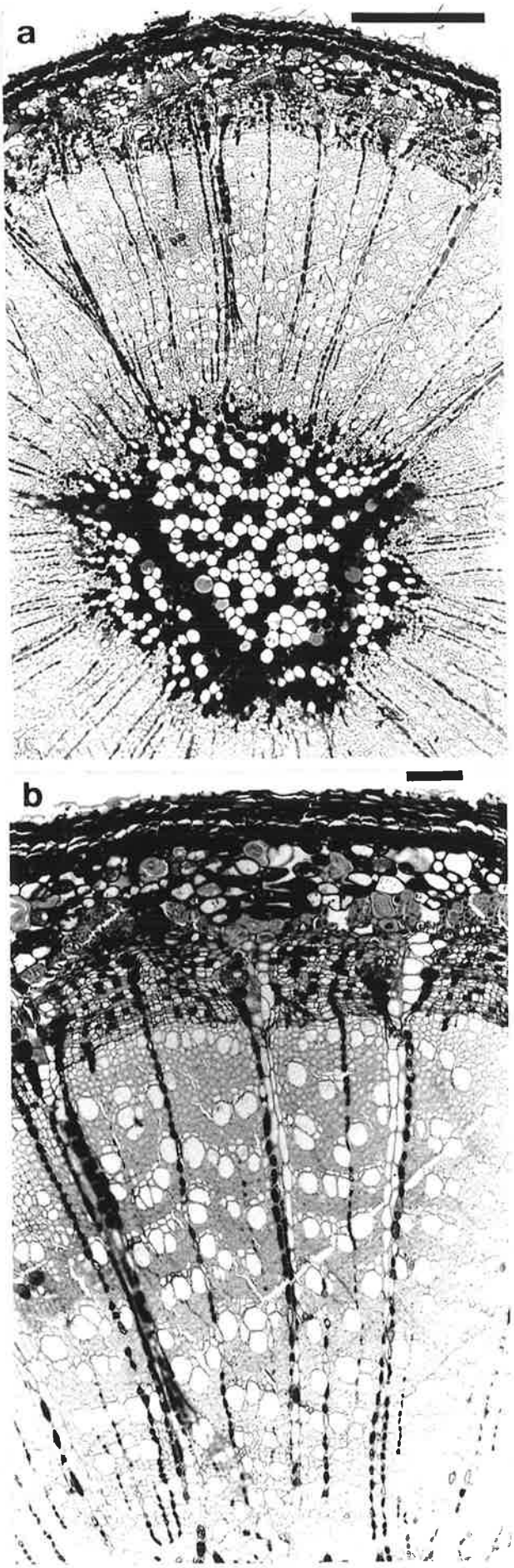
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Plate 3.22 Comparison of the CSI and PSI of *B. ericifolia* stem collected in spring 1994, TS

(a) CSI depicting the anatomy in the current season growth. Note the heavy stained cortex, the starch grains in the pith and the thick cuticle. Two petioles are present at either side of the stem and the vascular trace to the next leaf is visible (RHS). Bar = 100 μm

(b) PSI depicting the secondary growth of the stem. Note the heavily stained cortex, starch grains in the pith, uniseriate vascular rays and the developing petiole at the top centre. Bar = 100 μm

(c) Detail of (a) showing the actively dividing cambium with four or more initials, the large intercellular spaces in the cortex and the thick cuticle. Bar = 100 μm

(d) Detail from the section in (b) displaying the actively dividing cambium, two partially occluded cells blocking the distal end of the interfascicular ray (arrows) and superficial cork (upper edge). Bar = 50 μm

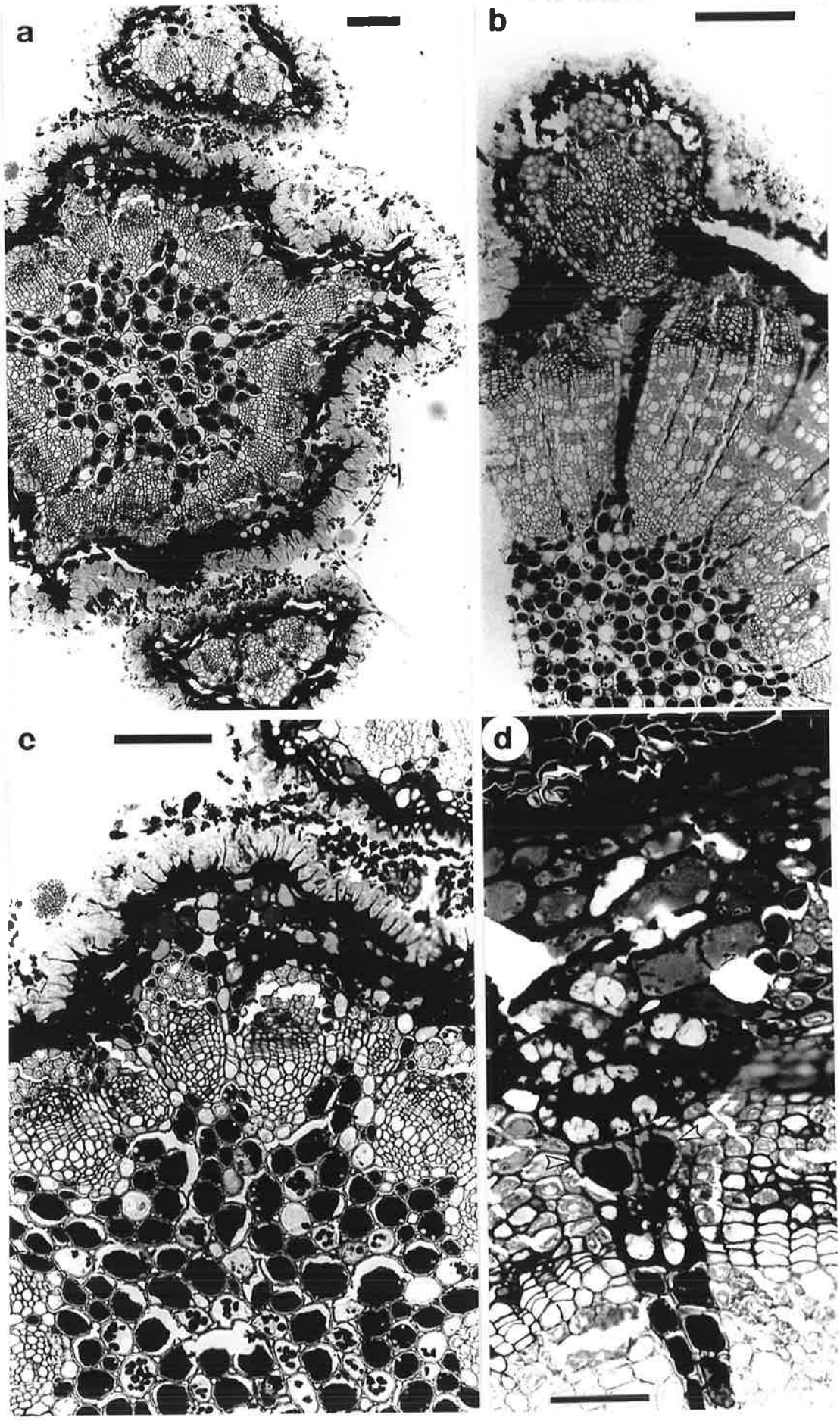
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Plate 3.23 *B. ericifolia* stem from PSI of bush-house grown potted plants collected in autumn 1994, TS

(a) Cortex, vasculature and leaf trace. Note the large intercellular spaces between cortical cells, the developing superficial cork periderm, several uniseriate vascular rays, particularly in the phloem, the wide interfascicular region and cell occlusions. Bar = 100 µm

(b) Vascular bundles showing vascular rays and large xylem vessels interspersed by fibre tracheids. Note the lenticels (arrows), which have developed in the cork periderm, the large intercellular spaces in the cortex and the pith. Bar = 100 µm

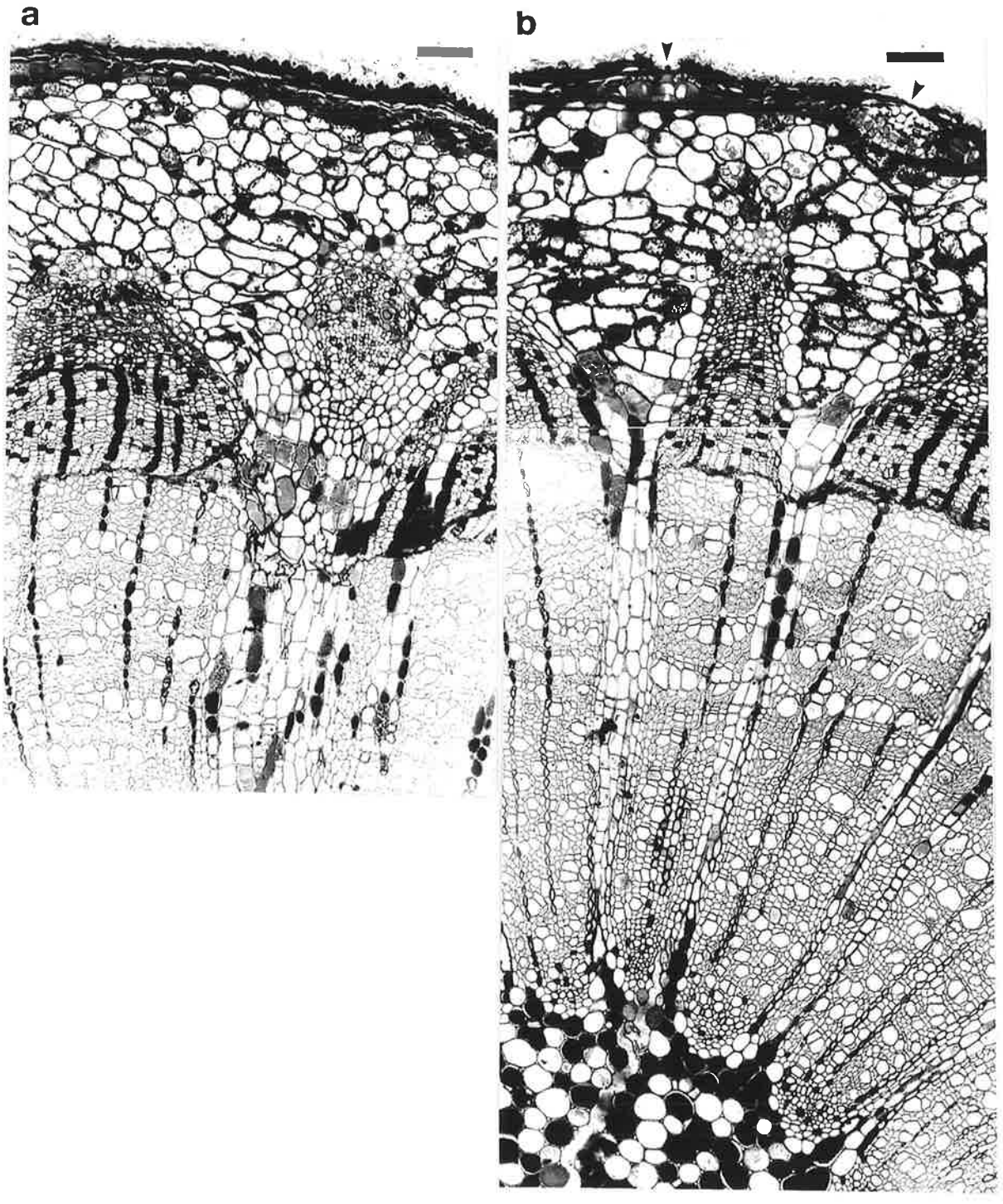
a
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Plate 3.24 Comparison of the CSI and PSI of *M. integrifolia* stem collected in autumn 1994, TS

(a) & (b) Overview of the internode stem anatomy of the CSI (LHS) and PSI (RHS). Note the starch grains in the interfascicular rays and the pith, and the thick layer of superficial cork at the margin of the narrow cortex. Bar = 100 µm

(c) Lower magnification of (b) showing cork layer breaking and sloughing-off due to the dilation of the stem width as secondary growth occurs, and the heavy staining of the ray parenchyma. Bar = 100 µm

(d) Occluded cells in the cortex between vascular bundles blocking the distal end of the interfascicular ray. Together with the phloem fibre cells, the occluded cells form a non-living barrier. Cells are occluded by successive depositions, until the living contents are obliterated. Bar = 50 µm

(e) Superficial cork (phellem) generated by the phellogen, the living layer of cells directly beneath consists of regular columns of four to five cells. Note the cuticle and cortical cells rich in phenolics. Bar = 50 µm

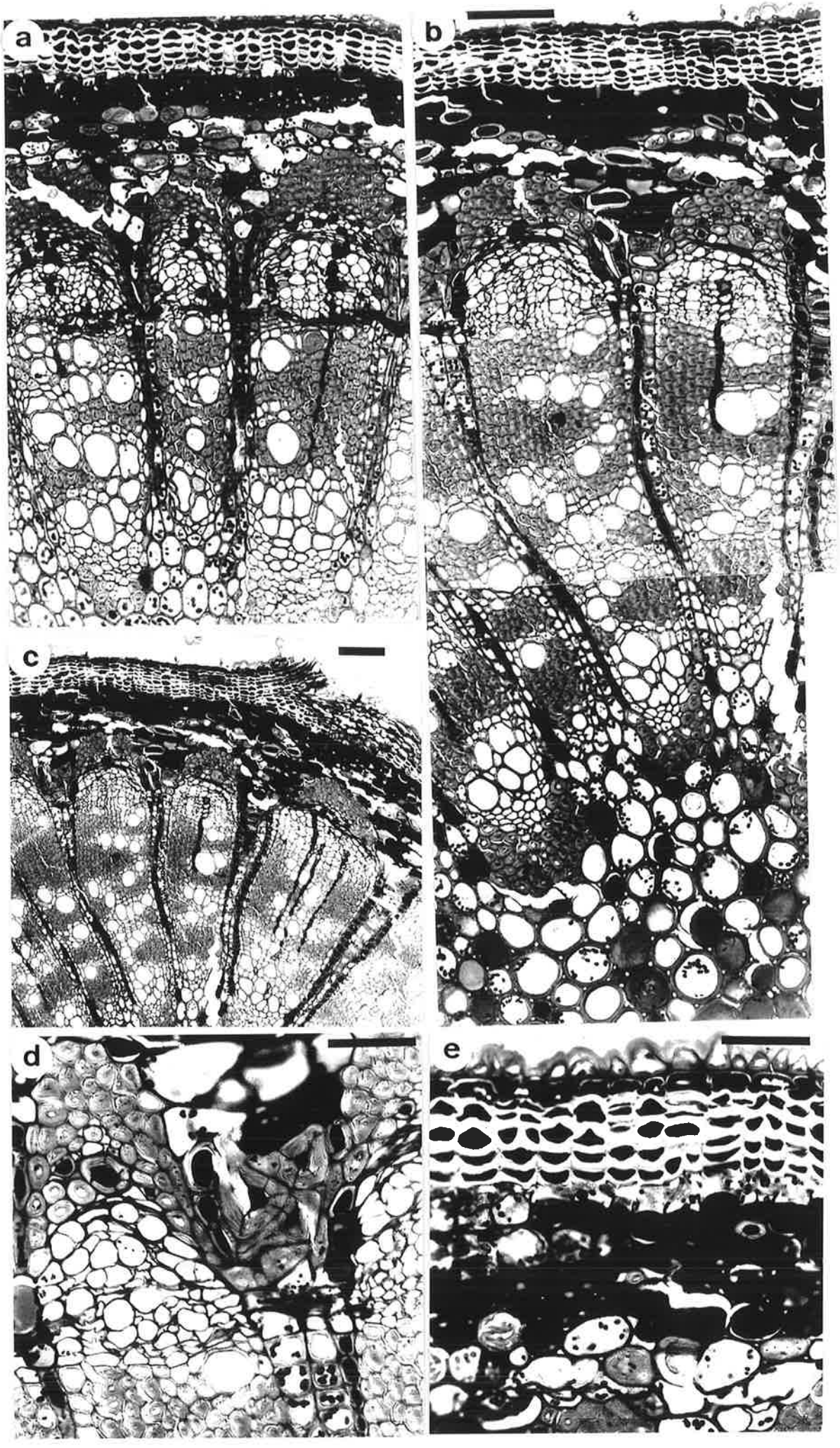
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Plate 3.25 Differential staining of *Banksia* stem stained with PAS - TBO

(a) TS of the CSI collected in autumn 1995 of *B. coccinea* showing the differential staining of tissues. Note occluded pith cells (arrow) stain aqua blue, similar to that of the cuticle; secondarily thickened fibres stained pink-purple. Bar = 100 μm

(b) TS of occluded cells (arrow) in the cortex of the CSI of *B. coccinea* collected in autumn 1995 stain similarly to the cuticle (top of photomicrograph). Bar = 100 μm

(c) Tangential LS of *B. coccinea* collected in autumn 1994. Note the occluded interfascicular parenchyma (aqua-blue, LHS), the long intercellular spaces and the leaf trace (RHS) in the cortex. Bar = 500 μm

(d) TS of *B. serrata* collected in spring 1994 showing aqua stained mass of occluded cortical cells (o) in close proximity to phloem fibres (pf), which stained pink-purple, and are surrounded by phenolic rich cortical cells. Bar = 50 μm

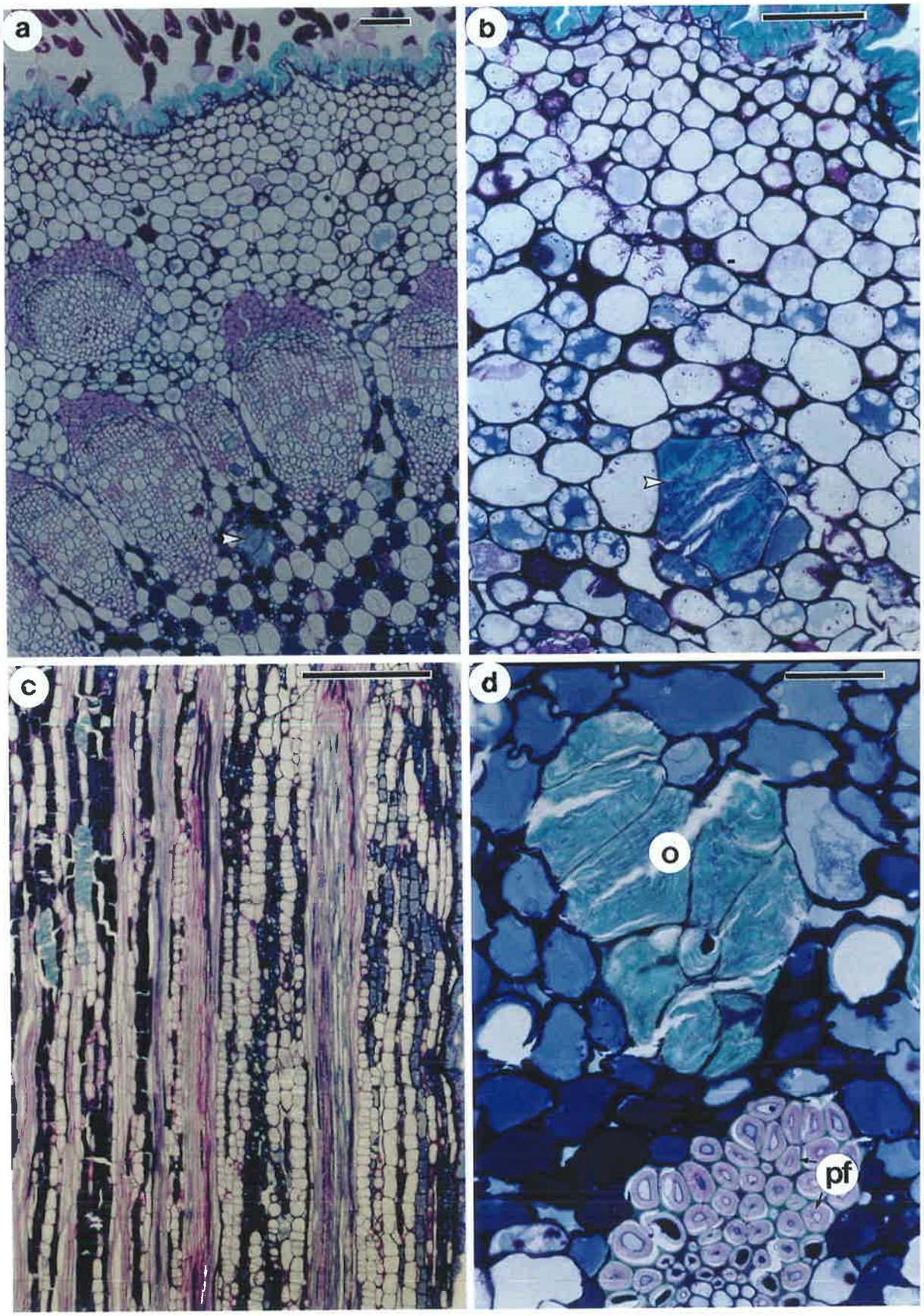
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Plate 3.26 *Banksia* stems

(a) Light micrograph of *B. ericifolia* CSI collected in spring 1994 showing differential staining of the tissue with PAS-TBO. Bar = 100 μm

(b) LS of CSI of *B. coccinea* collected in autumn 1994 showing primitive long vessel with annular secondary thickening (black arrows), circular inconspicuous intervascular bordered pits (white arrows) and dividing interfascicular parenchyma (RHS). Bar = 50 μm

(c) PSI of *B. menziesii* collected in autumn 1995 stained with PAS-SBB showing starch grains in the interfascicular ray parenchyma and the pith. SBB has stained the cuticle and cork black (top of photomicrograph). The contents of the occluded cortical cells (black arrows) have remained unstained by SBB and PAS, suggesting that the deposits in occluded cells are not lipid or polysaccharide based. Bar = 500 μm

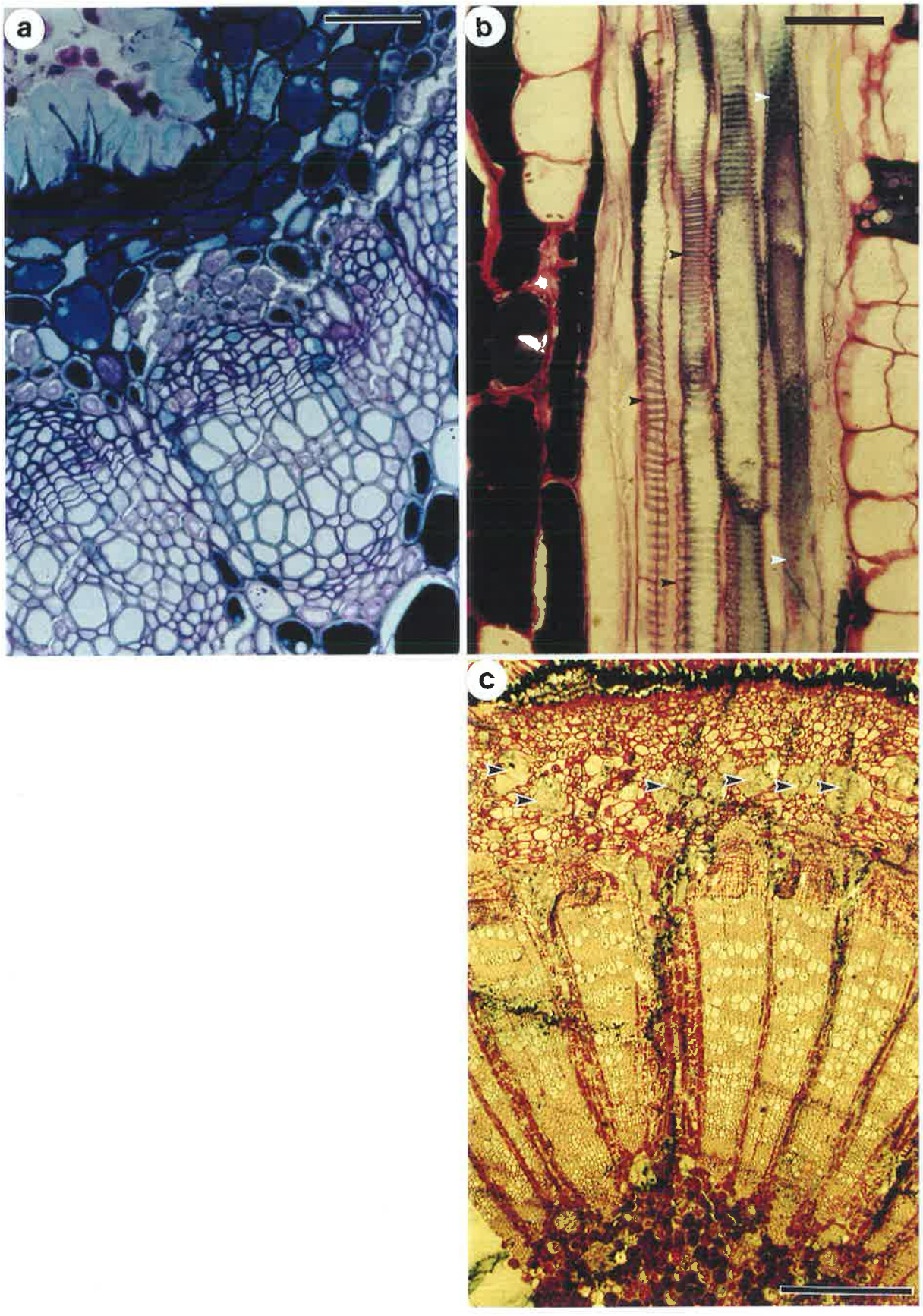
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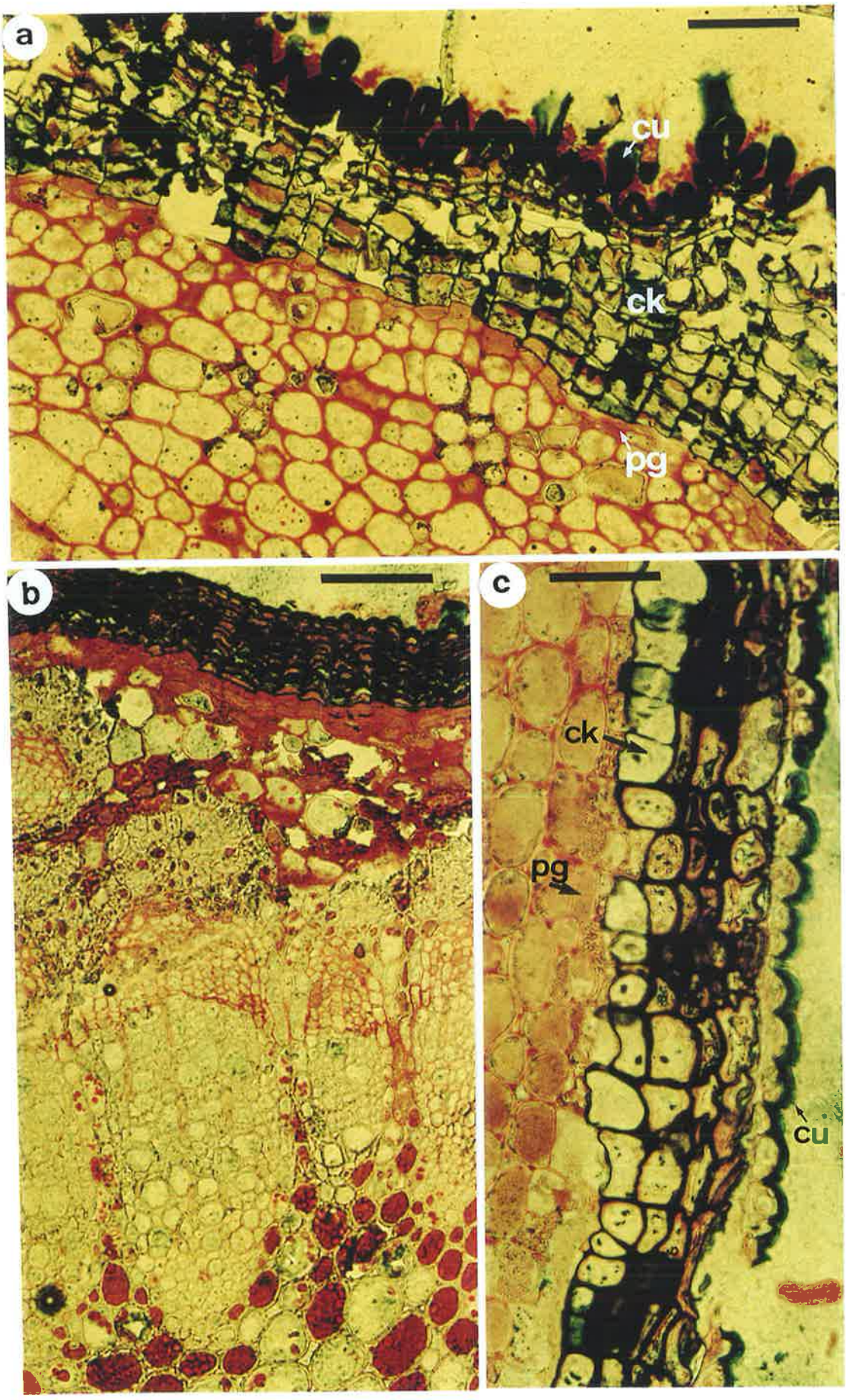
Plate 3.27 Sudan Black B staining of superficial cork (phellem) in *Banksia* stems collected in autumn 1995

(a) Suberised cell walls of cork (ck) stained black in PSI of *B. menziesii* stem. Note the phellogen (pg) and the cuticle (cu). Bar = 100 μm

(b) CSI of *B. spinulosa* var. *cunninghamii* stem. Note the phenolic rich cells (stained red) beneath the cork in the cortex and the starch grains in the interfascicular rays and pith. Bar = 100 μm

(c) PSI of *Macadamia integrifolia* stem showing regular columns, four to five cells thick of suberised cork cells (ck) generated by the phellogen (pg) beneath the cuticle (cu). Bar = 50 μm

Note: the regular columns of cork cells stained black from the inter-collation of the Sudan Black B stain in the lipid - suberin complex of the suberised cork cell walls. Tissues were first stained with Periodic acid-Schiff's reagent which stained other tissues pink.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Discussion

This study of the comparative stem anatomy of commercially important *Banksia* species supplements earlier anatomical studies on the characteristics of mature wood of the Proteaceae (Chattaway 1948b; Johnson & Briggs 1975), particularly the *Banksia* genus (Chattaway 1948a). The Proteaceae is an ancient family displaying primitive type wood structures as observed here in the members of the *Banksia* genus. Like many members of the Australian flora, *Banksia* has adaptations that aid survival in dry mediterranean, highly xeric habitats. Such adaptations include: dense trichomes, thick cuticle, cork, the presence of extra-xylary and xylary fibres, as well as specialised vasicentric fibres which ensure conduction throughout the growth ring during periods of drought (Carlquist 1985). Such structures, inherent in the tissues used for propagation, often serve to inhibit the processes of callus or root initiation and emergence required for successful vegetative propagation. This study has identified interspecific and seasonal differences in the location and relative proportions of tissues vital to (cortex, cambium, phloem, interfascicular rays, ray cell initials) or impairing (fibres, occlusions, cork, vascular rays) rooting or grafting processes. Thus, this research helps detect the most suitable season and type of material to use in the vegetative propagation of *Banksia*.

Banksia is a difficult to propagate species. This may be due to the presence of inert non-living impediments in the cortex and other living tissues responsible for callus production usually required for root initiation and successful graft formation. The occurrence of various events (rapid phloem senescence, lignification of the cell wall of the phloem) or structures (e.g., cork periderm, secretory canals) in tissues responsible for root initiation have been reported to block root initiation or root emergence (Beakbane 1969). Periphloic fibres found frequently in difficult to root species block the distal end of the rays that abut the cortex. This is the location of the initial cell divisions preceding callusing in *Banksia* grafting (refer to Chapter 4) and probably root initiation. Occluded cortical cells together with periphloic fibres form a continuous non-living barrier between the living tissues of the secondary phloem and the cortex, and may therefore preclude the development and emergence of root initials or callus in *Banksia*. Results from this study of the CSI and PSI of material collected in autumn found significant differences in the amounts of the main types of living tissues, such as the cortex (and hence the distance from the cambium to the surface), vascular bundles (in particular phloem), and vascular rays. Few significant differences were, however,

observed in spring material. This suggests propagules collected at six to eight weeks of age in late spring would be more desirable than older sub-terminal material from the PSI or material collected in autumn. McCredie *et al.* (1985a) concluded that six to eight week old non-woody material collected in late spring was more suitable for scion material used in *Banksia* grafting, than six to eight months old previous season material. Rapidly growing material of this age produced in early spring may also have reduced concentration of phenolics in cortical cells, vascular rays in the secondary phloem or in the interfascicular ray cells: the sites for root initiation (Beakbane 1969) and callus initial formation in successful grafts (Larson 1994).

Although the cortex constitutes the greatest proportion of living tissues in early stem growth in all *Banksia* species, there is interspecific variation and this may be important in matching scion and rootstock tissues in interspecific grafting, as discussed in Chapter 4. Even though the amount of cortex directly influences the distance of the cambium to the surface of the stem, in budding it is the cambial activity, which is more important. Bark-slip occurs when the cambium is most active and this is necessary for the insertion of the scion bud beneath the bark of the rootstock in T-budding, or for the easy removal of the bud and patch in patch-budding. Furthermore, in propagules taken at times of greater cambial activity a larger number of newly differentiated cells is available to re-differentiate and form root or callus initials. The CSI produced in spring has been observed to have the greatest cambial activity in *Banksia*. Using the PSI is undesirable as the phloem and cambium of the PSI form smaller percentages of the vascular bundles. This is because the PSI is undergoing slower extension growth than the rapidly growing CSI, which is recommended for use in vegetative propagation.

The percentage of vascular bundles increases from spring to the following autumn, in line with the peak phase of vegetative growth for *Banksia* over the summer (Taylor & Hopper 1988). In spring wood the percentage of vascular bundles increased by 5% between the CSI and PSI across all species and the percentage pith decreased. The vascular bundle analysis shows that the increase in vascular bundle size is attributed mainly to the deposition of secondary xylem in the bundles of the PSI and thus the greater volume of lignified, fibrous tissue. This has implication for the choice of propagules used in vegetative propagation where semi-hardwood is required. Less sclerified, woody material is most suitable and hence in *Banksia* the material of the CSI produced before the growth peak over summer, when sclerification occurs, is most suitable.

Although the percentage of leaf traces in the cortex decreases as a proportion of the cortex in PSI, their presence alone may influence vegetative propagation, particularly grafting, in an undesirable manner. The insertion of bud material on top of secondary thickened fibres, which surround the leaf trace or the periphloic fibres located between the phloem and the cortex, is likely to be unsuccessful. In Chinese chestnut (*Castanea* spp.), budding success was greatly impaired when scion buds were positioned directly on phloem fibre bundles (Huang *et al.* 1994), which are an anatomical feature of chestnut, similar to the phloem fibres found in *Banksia*. However, despite the presence of periphloic fibres, *Macadamia* is readily patch grafted (Henry 1976; Stephenson 1980) and the lack of internodal leaf traces, due to leaves occurring in whorls at the node, may be the reason for the successful insertion of buds.

The suberisation of cells in the outer cortex may be detrimental to vegetative propagation. The process of suberisation is followed by cork deposition, which has been shown to interfere with root emergence and reduce rooting potential (Beakbane 1969). It may be possible to reduce or delay the cork production by growing propagule source plants under controlled environmental conditions, such as shading or without water stress. Alternatively, young tip material that has not yet developed cork could be used as propagules. The presence of cork may not be a barrier in patch grafting, as it would be removed and replaced with the inserted bud patch. On the other hand the presence of cork in stem cuttings for rooting may be a physical barrier to root emergence in *Banksia*.

Vascular rays observed in *Banksia* occurred with greater frequency and were significantly longer in stems grown in autumn compared to stems sampled in spring, the CSI tending to have fewer and shorter vascular bundles than the PSI. The vascular rays contain largely phenolics, as observed from the PAS/TOB staining. Phenolics may be inhibitory to rooting or callus formation in propagation, so the choice cuttage or scion material would be late CSI, which has the shortest and lowest number of rays and hence the lowest amount of phenolics, particularly in the region of the secondary phloem. However, ray cell initials are sites for root initiation in deciduous fruit trees and may also play a role in root initiation in *Banksia*.

Tissues sclerification varies according to genotype (Beakbane 1969) and environmental factors, such as the absence of light, which retards the sclerification process (Maynard & Bassuk 1996) or xeric environment (Metcalfe & Chalk 1979). Sclerification in young *B. serrata* seedlings induced by water stress resulted in morphological and

physiological changes making seedlings drought resistant, although it has been suggested that these changes can be attributed in part to slower growth rates (Tibbits & Bachelard 1981). As growth slows in late summer, xylem tracheids and vessels become smaller creating greater resistance to water flow through xylem vessels (Carlquist 1985; Pate *et al.* 1995). The abundance of vasicentric tracheids in *Banksia* xylem (Carlquist 1985) aids the survival of *Banksia* and other proteaceous species in extremely xeric habitats. The presence of vasicentric tracheids may help explain the suggested physiological basis for the relationship between frost and drought tolerance of *B. marginata*, which occupies a wide range of extreme habitats in Tasmania, in which water is limited (Blake & Hill 1996).

Radial vasculature (Chattaway 1948a) was not observed in the interfascicular rays of the species studied. This may be due to the early stage of development of the interfascicular rays in the material examined. Chattaway (1948a) also observed that radial vasculature was rare in some species, but prevalent in *B. serrata.* As radial vasculature was not detected, it seems that these structures, observed in mature wood by other researchers, do not influence cambial activity and hence vegetative propagation using CSI and PSI. It may however play a role in how successful a scion bud grafts to mature wood in *Banksia* rootstocks.

Generally, interfascicular cells increase proportionally to the increase in xylem from one season to another. These tissues act as a starch storage area and those interfascicular cells abutting the cortex are sites of callus division in *Banksia* grafting (refer to Chapter 4) and root initial formation in other woody species. However, in *Banksia* cortical cells at the distal end of the interfascicular rays often become occluded with advancing age, forming a continuous barrier with the non-living adjacent phloem fibres, and separating the living cells of the phloem and interfascicular rays on the inside from the cortex on the outside. Such as barrier would impair processes of cell division and root emergence from initiation sites inside the barrier.

The presence of secondary metabolites and storage compounds increases with age, as observed in growth produced over two consecutive seasons. The accumulation of these compounds may have a positive or negative bearing on vegetative propagation in *Banksia*. Carbohydrates are stored as starch granules and possibly lipids in tissues close to the vascular tissue, interfascicular and pith cells, in current season growth, and additionally in cortical cells adjacent to the phloem in previous season's wood. Starch storage, commonly found in other woody tree species (Bamber 1964; Brewer & Scott

1983), is a temporary energy reserve, which can be resorbed during periods of high energy demand, such as in growth, flowering, regeneration after fire or after pruning. The large rays, characteristic of mature *Banksia* wood (Dadswell & Record 1936), may act as the location for starch deposition, as the heartwood forms and the pith cells die. Active pith in young stems in *Banksia* is a major site for starch storage. Saccharides and polysaccharides produced during starch degradation can easily move into neighbouring vascular tissue to be translocated to energy sinks throughout plant. Starch reserves are also influenced by seasonal variations and growing conditions, and different levels of starch accumulation in different species may affect success rates in the grafting and propagation by cutting. New growth for the *Banksia* species studied peaks during the summer in their natural habitats and a lower frequency of new shoots are produced at other times, mainly late autumn and early winter (Taylor & Hopper 1988). Shoot growth is stimulated in south eastern Australia at temperatures above 18.5°C (Groves 1978). Consequently, taking cutting material in the spring before starch reserves are completely mobilised and resorbed during the peak growth flush over the summer would be advisable. However, it may be advisable to ascertain precisely the times of peak starch accumulation in the species being used.

Other secondary metabolites such as endogenous flavonoids are present in soft tissues of *Chamaelaucium uncinatum* and were shown to have a promotive effect on rooting compared to other phenolics found exclusively in hardwood tissues in this species (Curir *et al.* 1993). The differences observed in the staining of phenolics in current season's soft wood and previous season's semi-hardwood in *Banksia* may likewise indicate that new growth had a lower concentrations of unfavourable phenolics and may have a higher rooting potential. Identification and quantification of the phenolics present in the living tissues of *Banksia* stems and their seasonal and species distribution is needed before drawing further conclusions.

Concentrations of other secondary metabolites, such as cyanogenic glycosides known to be present in the Proteaceae, particularly in the subfamily *Grevilleoideae* (Hale 1989; Swenson *et al.* 1989; Lamont 1993), and in *Macadamia* (Dahler *et al.* 1995) show seasonal and tissue specific variation. In vegetative tissues concentrations have been shown to be highest in young shoots. This however, may not be a problem for *Banksia* due to preliminary research showing sixteen members of the *Banksia* genus not to be cyanogenic (Swenson *et al.* 1989).

The field material examined in this study was taken from reproductively mature plants. The model for flower initiation in *Banksia* involves extension growth of a branch's terminal bud in each growth season until floral initiation. Conversion of the vegetative apical meristem to a floral meristem is stimulated largely by environmental factors, and upon the conclusion of extension growth new lateral shoots arise from axillary buds (Fuss *et al.* 1992). Floral initiation occurs several seasons before the flower is ready for harvest. To obtain suitable material in the vegetative phase with greater rooting potential for propagation, the source plants should be systematically pruned to remove florally initiated shoots and generate material of the desired age in the vegetative growth phase.

Many species of *Banksia* are native to acid sandy soils (Groves 1978; George 1987) and a base pre-treatment of cuttings, for example with NaOH, may break base labile ester bridges found in the cell walls of acidophilic plants. This would cause loosening of the plant cell walls, thus promoting water permeability, hormone absorption and possibly root emergence (Lee *et al.* 1976).

To conclude, the assessment of CSI and PSI in *Banksia* stems has identified CSI from late spring to be the most appropriate propagule to use in either grafting or cutting propagation. This type of stem tissue is in an active, vegetative phase of growth and hence has a greater proportion of newly differentiated cells available for re-differentiation to callus or root initials. Furthermore, it has accumulated fewer secondary metabolites, such as phenolics, and fewer occluded cells in tissues known to be the sites of callus and root initiation. If collected before the summer growth peak, it would have a higher level of storage carbohydrate needed to sustain the propagule during the initial phase of establishment. Also, cork is less likely to have developed in the first season, and the degree of periphloic sclerification is lower, allowing root emergence and callus proliferation.

Further research to clarify the location of root initiation sites in *Banksia* cuttings and the management practices for stock plants is needed to minimise factors detrimental to rooting and grafting success. The quantification of both beneficial, as well as inhibitory secondary metabolites is needed to firmly establish the role these play in the vegetative propagation of member of the *Banksia* genus.

Chapter 4

Banksia Grafting

Abstract

Self-, intra- and interspecific whip grafts between five species of *Banksia* from across the genus were conducted using *B. serrata* and *B. spinulosa*, var. *cunninghamii* as rootstocks, and *B. coccinea, B. ericifolia* and *B. menziesii* as scions. These species were chosen to represent a range of phylogenetic relationships. Seedling rootstock and scion material was used, with the exception of *B. coccinea,* which was clonal. The external appearance of the scions and rootstocks at the graft union was assessed visually using a scoring scale and the percentage survival calculated at nine and 21 weeks and at 12 months. At nine weeks up to 15% of some interspecific graft combinations were alive; intraspecific grafts of *B. serrata* were the only graft combination surviving to twelve months (7% success rate) and beyond. Histological sections of graft unions of two, four and 12 weeks post-graft were examined to assess the key events occurring in unions of *Banksia*. Early events of exudate production and cell necrosis were observed in all samples. Callus production had commenced at two weeks in the cortical cells near the phloem fibre caps and in the area of the interfascicular cambium; callus visible at four weeks was produced by cortex, cambium and phloem tissue. At 12 weeks callus had proliferated to fill the gap between the stock and scion interface. This work provides a basis on which further research into the improvement of grafting for commercial purposes can be based.

Introduction

The objective of the research presented in this chapter is to improve the commercial production of banksias for international and domestic cut-flower markets by increasing the understanding of grafting of this genus. Grafting is a method of vegetative propagation with inherent advantages over cutting propagation that can be used for the clonal propagation of elite plants. Understanding the key events in graft success and failure by interspecific, intraspecific and auto- (self-) grafting of species from across this genus will help improve the chances of successful grafting in *Banksia*. This may also aid conservation of threatened and endangered species (Ellyard 1987; George 1987; Taylor & Hopper 1988; Lamont *et al.* 1995) and the preservation of bio-diversity in this large, ancient genus.

The general low level of sexual self-compatibility and predominance of outcrossing results in a high degree of genetic variability in *Banksia* species (Maguire & Sedgley 1997). Many *Banksia* species display a large amount of phenotypic variation of morphological characteristics. This has positive and negative ramifications for the commercial development of species of this genus. Variation is a source of novel floral forms and colours, and possibly of other agronomically important traits, such as disease resistance. However, variation is undesirable in commercial production where uniformity and predictable performance in plantations is crucial to supplying uniform product. Thus an asexual means of propagation is necessary to produce commercial volumes of a selected genotype bearing desirable characteristics. A strategic approach using grafting could achieve this. Efficient production based on grafting requires clonally produced *Banksia* rootstocks and scions to achieve consistent, reliable performance.

In this study, a broad approach to the selection of species used was undertaken because the taxonomic relationship of the commercially important, but phylogenetically isolated species, *B. coccinea*, is not clear. A recent classification places it into the monotypic section *Coccinea*, a sister section to *Oncostylis* (Maguire *et al.* 1996).

This introduction briefly overviews information on grafting in woody plants and the previous work undertaken on grafting Proteaceae, native Australian plants, in particular *Banksia*, and how a strategic approach can be used to overcome many aspects hindering the further development of *Banksia* as a cutflower species. Other factors relating to the

plant, environment, technique and the nature of graft interface interactions are also highlighted, as these must be considered for successful grafting.

Grafting woody plants

The advantages of grafting have been observed and put into practice since antiquity (Garner 1988) and the chronological history of its use and development documented (Hartmann *et al.* 1990). In horticulture grafting is most widely used for the improved performance of deciduous temperate pome and stone fruits: apple (*Malus*), pear (*Pyrus)* and *Prunus* species (plum, peach, apricot, cherry); nut trees: almond (*Prunus dulcis),* walnut (*Juglans nigra*), chestnut (*Castania*) and pecan (*Carya*); and vines: wine and table grapes (*Vitis*), kiwifruit (*Actinidia chinensis*), passion fruit (*Passiflora edulis*) and black pepper (*Piper nigra*); also for olive (*Olea*), as well as perennial, subtropical and tropical species like citrus, cocoa (*Theobroma),* carob (*Ceratonia siliqua*), avocado (*Persea americana*), mango (*Mangifera indica*), custard apple (*Annona cherimoya*) and *Macadamia.* Grafting is also a common technique used in non-woody annual crops such as tomato (*Lycopersicon esculentum*), cucumber and melons (*Cucumis*) (Barth 1992).

In ornamental horticulture, rose propagation is founded on grafting and budding techniques, as is the production of many amenity trees. Intergeneric grafts of ornamental indoor foliage plants (Leonhardt 1996) are sold as novelty specimens; the production of dwarf forms (Lockard & Schneider 1981) also rely on the ability to graft desirable forms onto dwarfing rootstocks. In forestry grafting takes advantage of the precocity it affords for breeding and for the clonal propagation of plus-trees or for seed orchards (Copes 1970; Wojtusik & Felker 1993; Pianhanurak *et al.* 1996).

The main aim of the practice of grafting is to improve performance via the use of reliable and tested scions, and thus controlling vigour. Commercial routine grafting largely employs joining closely related genotypes, the rootstocks and scions usually being cultivars of the same species. Less frequently used are interspecific (Addison & Tavares 1952; Wojtusik & Felker 1993; Errea *et al.* 1994a), or intergeneric grafts (Leonhardt 1996). Generally, the greater the phylogenetic distance between the scion and rootstock genotypes, the less chance of vegetative compatibility in grafting.

Scion vigour may be improved through grafting by the removal of the effect of root disease causing bacteria (Boyhan *et al.* 1996), viruses (Biricolti & Chiari 1994; Heuss-LaRosa *et al.* 1995) or fungi (Garner & Beakbane 1968; Pianhanurak *et al.* 1996;

Pereira-Lorenzo & Fernandez-Lopez 1997). Conversely, some phytoplasmas or mycoplasmas can be beneficial in producing increased branching in the scions of grafted ornamental poinsettia cultivars (Ruiz-Sifre *et al.* 1997). Different rootstocks can influence scion vigour and the degree of branching, as seen in apple (Lockard & Schneider 1981; Ouellette *et al.* 1996).

Grafting can be employed to overcome other production limitations imposed by soil stress factors, such as salinity (Wiesman 1995), calcareous, alkaline or acidic soils (Ben-Ya'acov & Michelson 1995), poor aeration and waterlogged soils (Grauke & Barr 1996), the presence of soil borne pests such as nematodes (Schneider *et al.* 1995; Alcaniz *et al.* 1996) or *Phylloxera* in *Vitis* spp. (Wolpert *et al.* 1994). In this way a desirable scion can be propagated in an otherwise non-productive situation by using rootstocks which are not negatively influenced by soil limitations.

Summarising, grafting is essentially a horticultural practice applied for the following reasons (adapted from Garner (1988) and Hartmann *et al.* (1990)):

- uniformity and reliability of production
- perpetuating plant varieties not otherwise conveniently propagated by asexual means
- obtaining the benefits of certain rootstocks (e.g. disease resistance, adaptability to special climates or soil conditions, size control/vigour influence on the scion)
- changing the cultivar of established plants or combining more than one genotype on one plant
- shortening the time to reproductive maturity, termed precocity; this is used to avoid the juvenility delay in flowering or fruiting, or else to hasten reproductive maturity of seedling selections in hybridisation programs
- obtaining special forms of plant growth (weeping or dwarfed forms, conveyed due to the effects of stock dominance or which is inherent in the scion)
- repairing damaged parts of trees; overcoming stock/scion incompatibility, and invigorating weak plants (by inarching or bridge grafting)
- elucidation of viral diseases and diagnostic detection of viruses using indexing, or studying plant growth and structure

Several other benefits of grafting have been observed, including increased opportunity for sexual crossing via vegetative rapprochment (Biffen 1902; Crane & Marks 1952;

Evans 1955); rejuvenation of ontogenetically older plant material by serial grafting (Siniscalco & Pavolettoni 1988; Hartmann *et al.* 1990; Pianhanurak *et al.* 1996) and overcoming juvenility to achieve early flowering by autografting (Salomon & Reuveni 1994). However, grafting has not been widely used to take advantage of these phenomena in endemic Australian plant species.

Grafting in Proteaceae not endemic to Australia

Grafting of species from the southern hemisphere Proteaceae family, endemic to countries other than Australia, is not uncommon. Intergeneric grafting of South African proteaceous genera is possible (Ben-Jaacov & Ackerman 1989), however, interspecific grafting is most commonly used on members of the *Protea, Leucadendron* and *Leucospermum* genera. The main proponent of the research in this field is Israel, which produces the bulk of Europe's proteaceous cut-flowers (Malter 1994). Israel has been successful in circumventing soil type limitations like high pH and phosphorous levels, as well as soil borne diseases and nematodes by employing different grafting techniques on suitable rootstocks (Ben-Jaacov *et al.* 1992a). Cutting grafts have been used for the rapid propagation of *Leucadendron* and *Leucospermum* under mist to produce grafted plants on lime tolerant rootstocks (*Leucadendron coniferum* cv. "Orot"), which is ready for planting within four months. Graft-take was reported to be 40% to 100 % (Ackerman *et al. 1996*). Also, field-grafting of *L.* "Orot" rootstocks already under cultivation has been reported (Shemi 1996). Alternatively, clonally propagated rootstocks derived from cuttings were grafted using a wedge graft (Ben-Jaacov *et al.* 1992b) or top-cleft or side grafted; similar methods were used on *Protea* spp. (Ben-Jaacov *et al.* 1992a). Growing grafted plants on "ridges", a cultivation technique developed in Israel, has also been reported to improve yields of cutflowers bearing robust stems (Shemi 1996).

Intraspecific grafting was used to clonally propagate a brilliant maroon selection of *Protea longifolia* by T-budding scion material from this single plant onto seedling derived sibling plants already under cultivation in the same plantation (Harington 1988). *Protea* grafting and budding is also widely used in the Australian protea growing industry (Moffatt & Turnbull 1993).

Grafting of Australian native plants

The first records of grafts of Australian native plants date back to the nineteenth century when grafting was widely practised in Britain (Hockings 1976). Although the bulk of literature on grafting Australian native plant species is largely anecdotal and lacking

scientific rigour, it provides a basis on which to strategically develop grafting as a norm for the production of native plants for the cutflower and amenity horticulture industries.

The techniques and equipment used are similar to those used in grafting any plant. However, Elliott and Jones (1982) describe these in detail, using examples based on Australian natives. There are several other accounts by other practitioners on the methods that have brought them success (Burke 1983a; Abell 1988), including the "mummy" graft developed by Merv Hodge, Queensland. This method involves wrapping the entire scion in a single layer of stretchable plastic, *Nescofilm,* thus protecting the scion from desiccation (Hodge 1988, 1990). The leaves are removed from the scion, leaving only the leaf petioles to protect the subtended axillary bud. Buds burst through the plastic, which eventually breaks down under UV exposure.

Why graft Australian native plants? In addition to clonal propagation, a major possible benefit afforded through the rootstock is *Phytophthora* resistance, and the avoidance of other possible soil-stress factors (salinity, soil type). Advantages passed on through the scions are precocity, vigour, longevity and suitability to climatic factors, such as humidity (Hodge 1990), heat and frost.

Non-Proteaceae

Species with commercial application in amenity or ornamental horticulture have received the most attention with regard to grafting. Examples are the Sturt Desert Pea and intergeneric grafts of 31 species of the short-lived *Prostanthera* onto *Westringia fruticola* to extend its lifespan (Hartmann *et al.* 1990; Dawson 1996a). Table 4.1 presents an overview of literature on grafts across a wide range of non-proteaceous native plants. The grafting of quandong is the only instance amongst them on grafting of an indigenous food species.

Proteaceae

There has been a long-standing interest in propagation of Proteaceae in Australia (Costin 1985). Grafting of seven proteaceous genera is possible: *Grevillea* (Dupee & Clemens 1981; Burke 1983b; McKenzie 1984; Hodge 1988, 1990; Dawson 1996a) and *Hakea* (McKenzie 1984, 1994, 1996; Abell 1988; Hodge 1988, 1990; Pratt 1994; Dawson 1996a), have showed the most success, followed by *Macadamia* (Henry 1976; Stephenson 1980; Alexander 1986), *Telopea* (Dawson 1996a), *Isopogon* (Anthony 1994; LeBoeuf 1994), *Dryandra* (McKenzie 1984; Hodge 1988; Dawson 1996a) and *Banksia* (Table 4.2).

Table 4.1 Successful grafts of non-proteaceous native plants and source literature

GENUS	REFERENCE
Darwinia	Wrigley 1973; McKenzie 1984; Douglas 1994; Pratt 1994; Dawson 1996a
Eucalyptus	McKenzie 1984; Siniscalco & Pavolettoni, 1988; Hodge 1990; Hoopman 1996
Thryptomene	McKenzie 1984; Meyers *et al.* 1993
Verticordia	Wrigley 1973; Dawson 1996a
Kunzea	Wrigley 1973; McKenzie 1984; Dawson 1996a
Leptospermum	McKenzie, 1984
Callistemon	Wrigley, 1973
Eremophila	Hodge 1990; McDougall 1993; Dawson 1996a
Hibbertia	Hodge, 1990,
Allocasuarina	Meyers *et al.*, 1993
Prostanthera	Wrigley 1973; Hodge 1990; Dawson 1996a
Westringia	Wrigley 1973; Dawson 1996a
*Clianthus**	Wrigley 1973; McKenzie 1981b, 1984; Dawson 1996a
Correa	McKenzie 1984; Hodge 1990
Chamelaucium	McKenzie 1984; Hodge 1990
Boronia	Wrigley 1973; McKenzie 1984; Dawson 1996a
Quandong	Byrne 1996

* syn. *Swainsonia*

Intergeneric grafts of *Hakea* spp. on *Grevillea robusta* proved to be superior to grafts on *Kunzea flavescens* or *Baeckea virgata* (Hodge 1990); however, the recommended rootstock is *Hakea salicifolia*. Grafts of *Telopea mongaensis* rootstocks with scions of *Telopea speciosissima* and *T. speciosissima* hybrids and selections have survived ten, 17 and 15 years respectively (Dawson 1996a). Selected *Telopea* genotypes identified for frost tolerance (Dawson 1996b) could be used in an interspecific cross-breeding program to provide hybrids for grafting, thus extending the growth of this highly prized proteaceous cutflower species.

In the 1970s the fledgling *Macadamia* industry required methods of clonal propagation of superior nut trees, endemic to the Queensland tropics. Although grafting initially showed little success, a variation of the patch budding method - the punch bud graft - (Henry 1976; Stephenson 1980; Elliot & Jones 1982), was developed and is used for clonal propagation in the macadamia industry.

Limited grafting of *Dryandra* has been attempted (McKenzie 1984; Hodge 1988) and survival has been poor. Grafts of *D. fraserii* onto *Banksia integrifolia* rootstocks took slowly and buds eventually burst (Hodge 1988). Intergeneric grafts of *D. praemorsa* on *B. spinulosa*, var. *spinulosa* survived 60 months (McKenzie 1984), but did not survive on *B. integrifolia* or *B. ericifolia* rootstocks. However, compatibility of *Dryandra* with *B. serrata* appears to be satisfactory, although the percentage success is low (Wrigley 1973).

Banksia

Although there is tremendous horticultural potential within the *Banksia* genus, clonal propagation has proven extremely unreliable (Wrigley 1973; Hodge 1990) and is the key missing factor for further commercial development of selections and hybrids, both natural and from controlled crosses. Many of the natural hybrids are between eastern coast species (George, 1987), which are less horticulturally important as floral forms, but may prove horticulturally beneficial as rootstocks. Two scientific reports on grafting of *Banksia* exist (McCredie *et al.* 1985a; Barth & Bennell 1987) amidst a plethora of informal reports undertaken by talented enthusiasts, details of which are summarised in Table 4.2.

It is difficult to draw long-term conclusions from the grafting work of Barth and Bennell (1987) using *B. coccinea* and *B. menziesii* scions on *B. integrifolia* and *B. marginata* rootstocks, both of which are from series *Salicinae*, because the duration of observations was only 20 weeks post-grafting. It is also difficult to determine whether *B. integrifolia* performed better than *B. marginata* as a rootstock for either the autumn or the spring grafting. However, their work does present data from which general trends can be drawn. The scion, *B. menziesii*, is from the same sections as the rootstocks, although from different series (series *Banksia*). This species showed improved success rates during the autumn grafting using scion wood cinctured four weeks prior to collection. Despite slow growth, 73% of cinctured scions had survived at 20 weeks, 40% showing signs of growth. The comparatively low success rate using cinctured wood in spring suggests the seasonal accumulation of starch in the scion plant at this time was inadequate, or that other seasonal factors were influencing the results. Cincturing of *B. coccinea* scion wood, on the other hand, did not improve success rates in autumn or spring grafting. After an initial increase in success rates at eight and 12 weeks, the grafting success rate declined at 20 weeks. The success rate in uncinctured scion plants was 31% to 36% in the autumn and spring respectively. The scion wood used by Barth and Bennell (1987) was hardened current season's wood collected from mature, flowering, cultivated plants in autumn and spring.

In contrast, McCredie *et al.* (1985a) found that young, non-woody scion material, six to eight weeks old, produced in late spring was superior to the initially used six to eight month old wood from the previous season's growth. Although the study of McCredie *et al.* (1985a) was inconclusive on the benefits of the hot callus grafting method used, the study confirmed the suitability of *B. integrifolia* as a rootstock over six other species

trialed: *B. ericifolia* var. *ericifolia*, *B. oblongifolia*, *B paludosa*, *B. robur*, *B. serrata*, *B. spinulosa* var. *collina*. The species *B. integrifolia* was recommended due to (i) the ease of grafting afforded by this species' long internodes, (ii) the apparent compatibility with several horticulturally important species (*B. prionotes*, *B. victoriae*, *B. hookeriana* and *B. speciosa*, which are from a sister series in section *Banksia*), (iii) its tree habit, an important trait possibly conveyed through stock dominance, (iv) the lack of a lignotuber and prolific basal shoots, (v) its ease of propagation by cutting, important for clonal rootstock production, (vi) its cultivation in a wide range of soil types and (vii) its high resistance to soil borne diseases, especially, *Phytophthora cinnamomi* and *Pythiaceae* root rots. This report also mentions the suitability of *B. integrifolia* as a rootstock for *B. elegans*, as well as an unusual hybrid of *B. hookeriana* and *B. menziesii*.

Of the many combinations of scions and rootstocks presented in the informal reports (Table 4.2), *B. solandri* on *B. integrifolia* was one combination with the greatest longevity (12 years) (McKenzie 1984; Dawson 1996a). The grafted bush shown in Plate 4.1a illustrates the success of this combination. *B. integrifolia* seems to be a commonly used rootstock (McKenzie 1984; Hodge 1990) and is recommended as having the greatest potential as a rootstock due to its resistance to *Phytophthora* (McCredie *et al.* 1985a; Barth & Bennell 1987). McKenzie (1984) reports that although some combinations on *B. integrifolia* survived, growth was extremely slow, as in the graft of *B. speciosa* on *B. integrifolia* depicted in Plate 4.1b & c. He observed incompatibility symptoms appearing months or even years after grafting. These were: (i) a clean break at the union, (ii) slow or stunted growth, (iii) a gross disparity between the stock and the scion stem diameter (Plate 4.1c), (iv) a tendency of the stock to produce shoots continually and (v) abnormalities in the union, e.g. a furrow appearing around the stem bark at the graft union, indicating discontinuity of the growing tissue beneath the bark. McKenzie went on to report that grafting of *B. grandis*, *B. solandri*, *B occidentalis* and *B. verticiliata* on *B. integrifolia* rootstocks looked most promising.

Other graft combinations of *Banksia* surviving over ten years at the Australian National Botanic Gardens, Canberra, were *B. media* (24 years) and *B. speciosa* (11 years) on *B. serrata; B. canei* (15 years) and *B. grandis* (16 years) on *B. ericifolia*; *B. praemorsa* (14 years) on *B. spinulosa,* and *B. occidentalis* on *B. robur* (23 years) (Dawson 1996a), the latter three combinations of scion and rootstocks being from different taxonomic sections. Commercial trials of cotyledon grafts have been carried out using eastern species of *Banksia* as rootstocks (Pratt 1994). However, cotyledon grafting is not

recommended as a grafting method to avoid root disease because the graft union is at soil level and the risk of the aerial part of the grafted plant becoming infected by soil borne pathogens is high. All these examples stand as proof that grafting of species from across the genus *Banksia* is possible.

A strategic approach to vegetatively propagating desirable *Banksia* forms on a commercial scale must be possible by incorporating scions produced from selection and interspecific breeding programs with rootstocks developed specifically for grafting (e.g. resistant rootstocks which are closely related to the scion of the desired genotype) by refined grafting techniques. Selections of colour variants of *B. coccinea*, Waite Flame and Waite Crimson (Sedgley 1995a) and a natural hybrid of *B. prionotes* and *B. hookeriana*, registered as Waite Orange (Sedgley 1991) exist already. Hybridisation technique have been developed (Fuss & Sedgley 1991a) and used in controlled crossing experiments (Sedgley *et al.* 1994, 1996). The application of these techniques could be used in the generation of suitable rootstocks and scions for grafting. In this manner hybrids and elite selections could be clonally propagated for industry, thus aiding the uptake rate of new, improved cultivars by industry.

The majority of cultivated forms, as well as endangered species with commercial potential (e.g. *B. cuneata*), are highly susceptible to root diseases which are known to penetrate the aerial parts of the plant (McCredie *et al.* 1985b). Consequently, the development of diagnostic methods to identify diseased material is crucial and could be applied to prevent infected material entering developmental, pilot and bulking-up phases of commercial propagation schemes. Highly susceptible species could be used to detect cryptic disease agents (fungi, mycoplasma, viruses) in plus mother plants, using methods similar to those applied in plant virus indexing.

The identification of disease resistant genotypes, or genotypes bearing other desirable traits (Zhen-Xiang Lu *et al.* 1996) would be of tremendous value in breeding and selection programs. Progress in this direction has been made within the Proteaceae through the development of molecular techniques for DNA extraction (Maguire *et al.* 1994), the use of isozymes as genetic markers (Vithanage & Winks 1992) and RAPDs

Table 4.2 Overview of literature on grafting in *Banksia*

Rootstock species	Scion species	Numbers and success	Grafting details	Reference
B. integrifolia	*B. coccinea* *B. menziesii*		Stock: 12 –15 cm high seedlings. Scion: mature field material, girdled 4 wks before, 10 cm long piece of current seasons growth. Grafting: wedge; parafilm & wax covered with a plastic bag. Assessment: 8, 12, 20 wks for no. alive, scion development, speed of callusing/union	Barth & Bennell, 1987
B. marginata	*B. coccinea* *B. menziesii*			
B. integrifolia var. *integrifolia*	*B. brownii* *B. hookeriana* *B. prionotes* *B. sceptrum* *B. speciosa* *B. victoriae* *B. integrifolia,* var. *integrifolia*	1/10 successes at 120 d 10/18 successes at 120 d 1/7 successes at 120 d 1/12 successes at 120 d 2/5 successes at 120 d 2/9 successes at 120 d 5/6 successes at 120 d	Stock: 18 months old seedling PC resistant species: *B. ericifolia* var. *ericifolia, B. oblongifolia, B. paludosa, B. robur, B. serrata, B. spinulosa* var. *collina, B integrifolia* (*B. integrifolia* 12 months plant struck from cutting) Scion: 6 - 8 week old growth produced in late spring (most suitable); scion material was kept cool and misted before grafting which look place within 4 h from time of scion collection Methods: a scalpel was used to prepare stocks and scions; blades were rinsed in 1.5% sodium hypochlorite; grafts were wrapped in *Parafilm* "M" and placed in a hot callusing tube (ambient internal temperature of 27°C) for 25 d	McCredie et al., 1985a
B. integrifolia *B. ericifolia* *B. ericifolia* *B. serrata* *B. robur* *B. spinulosa* *B. serrata*	*B. brownii* *B. canei* *B. grandis* *B. media* *B. occidentalis* *B. praemorsa* *B. speciosa*	5 years 15 years 16 years 24 years 23 years 14 years 11 years	Australian National Botanic Garden, Canberra; "years" refers to the age of the oldest survivor	Dawson, 1996a Wrigley, 1973
B. integrifolia*	*B. solandri**	successful	*First record of grafting, Kew Gardens, in 1880s, this specimen reached 20' and >12" diameter stock & scion: seedlings at cotyledon stage Grafting: cotyledon grafting (as described for Desert pea, (McKenzie, 1981b); simple wedge) Assessment: compatibility, incompatibility symptoms, survival, age of oldest plants Average success rate: 75% - 80%	McKenzie, 1981a
B. integrifolia	*B. grandis*	most successful		
	B. solandri	most successful		
	B. verticillata	most successful		
	B. lemanniana	most successful		
	B. littoralis	most successful		
	B. benthaminana	successful		
	B. brownii	successful		
	B. laevigata	successful		
	B. lanata	successful		
	B. occidentalis	successful		
	B. pilostylis	successful		
	B. victoriae	successful		
	B. violacea	successful		

Table 2 continued

Rootstock species	Scion species	Numbers and success	Grafting details	Reference
B. serrata	*B. burdetii*	most successful	7/15 scion species lasted > 2 years	*Continued,* McKenzie, 1981a
	B. speciosa	most successful		
	B. menziesii	most successful		
	B. candolleana	successful		
	B. lemanniana	successful		
	B. prionotes	successful		
	B. victoriae	successful		
B. spinulosa var. *spinulosa*	*B. elderiana*	best scion, slow growth	22 scions tested, in most cases 1 to 3 plants grafted, in one case 8	
	B. praemorsa	best scion, slow growth		
	B. brownii	best scion, slow growth		
	B. laricina	slow growth		
	Dryandra praemorsa	best scion, slow growth	Intergeneric	
B. marginata	*B. grossa*	1/1 survived 18 months	7 scion species tested, none of which are major commercial species	
	B. lanata	2/3 survived 18 months		
	B. laricina	1/3 survived 18 months		
	B. nutans	1/1 survived 12 months		
	B. oreophila	0/2 survived 5 months		
	B. praemorsa	0/1 survived 4 months		
	B. sphaerocarpa	0/1 survived 6 months		
B. ericifolia	*B. coccinea*	0/1 survived 4 months	Few scions tried, only *B. nutans* survived > 2 yrs	
	B. nutans	best scion (4 yrs)		
	B. occidentalis	0/4 survived 6 months		
	B. sphaerocarpa	0/2 survived 18 months		
	Dryandra praemorsa	0/1 survived 6 months	Intergeneric	
	Dryandra polycephala	0/2 survived 6 months	Intergeneric	
B. verticillata	*B. coccinea*	1/2 survived 3 years		
	B. elderiana	0/1 survived 6 months		
	B. micrantha	0/1 survived 8 months		
	B. oreophila	0/1 survived 6 months		
	B. scabrella	0/1 survived 10 months		
B. lemanniana	*B. burdetti*	0/1 survived 4 months		
	B. oreophila	0/1 survived 4 months		
B. robur	*B. oreophila*	0/1 survived 5 months	Lignotuberous, hardy rootstock	
B. saxicola	*B. incana*	0/1 survived 12 months	Worth further testing; closely related to *B. integrifolia*	
	B. leptophylla	2/5 survived 2 years		
	B. scabrella	1/2 survived 2 years		

Rootstock species	Scion species	Numbers and success	Grafting details	Reference
B. ericifolia *B. integrifolia* *B. lemanniana* *B. marginata* *B. saxicola* *B. robur* *B. serrata* *B. spinulosa*, var. *spinulosa* *B. spinulosa*, var. *collina* *B. verticillata*	Many different scions used		Three methods were employed: cotyledon graft (McKenzie, 1981a), wedge graft and cutting grafts	McKenzie, 1984, 1988
B. spinulosa	*B. occidentalis* *B. brownii* *B. canei* *B. menziesii* *B. candolleana* *B.* giant candles *B. ericifolia* var. *ericifolia* *B. marginata* *B. spinulosa* var.*cunninghamii* *B. integrifolia* var. *integrifolia* *B. sphaerocarpa* *B. lindleyana* *B. media* *B. laevigata* *Dryandra quercifolia* *D. falcata*		Wedge; six month seedling stock; scion also from seedlings (grown in 3" pots); *Nescofilm* was applied as grafting tape Intergeneric Intergeneric	Peisley, 1989
B. ericifolia	*B. occidentalis* *B. brownii*			
B. marginata	*B. occidentalis* *B. brownii*			
B. integrifolia var. *integrifolia*	*B. occidentalis* *B. brownii*			

Table 2 continued

Rootstock species	Scion species	Numbers and success	Grafting details	Reference
B. ericifolia	*B. brownii**	1	Four methods were employed: approach (all combinations), wedge graft (*), cutting grafts (#) and buddling (@); grafts were done at several times throughout the year Stock plants were grafted 4 – 8 cm from the base on the firm, green stem, 2 – 5 mm in diameter. Grafted plants were placed in a cold frame without additional heating *B. ericifolia* was also wedge grafted with *B. occidentalis* scions *B. integrifolia* was also wedged grafted with scions of: *B. baueri* *B. brownii* *B. laevigata* *B. media* *B. meisneri* *B. pilostylis* *B. praemorsa* *B. sceptrum* *B. speciosa* *B. verticillata* A wedge graft of *B. marginata* with scions of *B. brownii* and *B. praemorsa* was also conducted	Wilson, 1975
	B. coccinea	1		
	*B. laricina**	2		
	B. meisneri	1		
	B. nutans#*	3		
	B. quercifolia	1		
	*B. sphaerocarpa**	2		
B. integrifolia	*B. ashbyi*	1		
	B. coccinea	1		
	B. menziesii	2		
	B. prionotes	1		
	B. quercifolia	1		
	*B. sceptrum**	1		
	*B. speciosa**	1		
B. robur	*B. ashbyi*	1		
	*B. brownii**	3		
	B. hookeriana	1		
	*B. lemanniana**	1		
	B. menziesii	1		
	B. quercifolia	1		
	B. speciosa	1		
B. serrata	*B. menziesii*	4		
	B. prionotes	4		
	B. sceptrum	1		
	B. speciosa	2		
B. spinulosa var. *cunninghamii*	*B. ashbyi**	3		
	B. brownii#@*	7		
	B. burdetttii	1		
	B. caleyi	1		
	B. coccinea	4		
	B. hookeriana	1		
	B. lamanniana#*	1		
	*B. menziesii**	1		
	B. nutans	1		
	B. occidentalis#	3		
	B. quercifolia	1		
	B. speciosa	1		
	B. sphaerocarpa	1		

Table 2 continued

Rootstock species	Scion species	Numbers and success	Grafting details	Reference
B. conferta	*B. laevigata*, var. *lavigata* *B. speciosa*		Approach graft; successful species Cotyledon graft	Henderson, 1987
B. ericifolia	*B. meisneri* *B. nutans*			
B. serrata	*B. praemorsa* *B. lemanniana* *B. speciosa* *B. menziesii* *B. blechnifolia* *B. petiolaris* *B. baxteri*			
B. ericifolia	*B. leptophylla*			
B. integrifolia	20 spp.tried		Irrigated approach graft	Clemsha, 1988
B. oblongifolia	*B. robur*			
B. ericifolia	*B. nutans*, var. *nutans* *B. nutans*, var. *cernuella*			
B. Giant Candles	*B. ericifolia* *B. spinulosa*, var. *cunninghamii*			
B. spinulosa var. *cunninghamii*	*B. spinulosa*, var. *spinulosa* *B. lemanniana*			
B. integrifolia	*B. canei* *B. conferta*, var. *conferta* *B. conferta* , var. *penicillata* *B. integrifolia*,var. *aquilonia* *B. saxicola* *B. seminuda*		Cotyledon wedge graft on seedlings	Clemesha, 1992
B. ericifolia	*B. nutans*, var. *cernuella*			
B. integrifolia	*B. menziesii* *B. media*		Rootstock - advanced seedling	McDowell, 1988
B. integrifolia	*B. menziesii*			
six *Banksia* species	no details listed		380 grafts undertaken, 50% of resulting plants are in the Royal Botanic Gardens, Sydney	Abells, 1988
B. integrifolia	"small handful"			Hodge, 1990
B. integrifolia	*B. menziesii*		Others species tried but not successful	Moffat, 1993

for analysis of interspecific and intergeneric relationships (Maguire & Sedgley 1997; Maguire *et al.* 1997), as well as other DNA profiling techniques used in plant identification (Morell *et al.* 1995). These molecular biological techniques form the basis on which modern identification and screening methods can be further developed for the improvement and registration of cultivars of *Banksia*.

The plant

Generally, the factors which affect graft success and which are inherent in the plant cannot be readily changed. Factors such as genotype (Autio *et al.* 1996; Leonhardt 1996; Pereira-Lorenzo & Fernandez-Lopez 1997), morphological features and anatomy of the wood tissues (Garner & Beakbane 1968; Huang *et al.* 1994) and phase status (Juvenile or mature) (Salomon & Reuveni 1994) are possible to control only insomuch as the grafter has a choice of which genotype to use and the age of the plant grafted. Knowledge of anatomical features and physiology of wood (vasculature, carbohydrate reserves, cambium activity), plant morphology (internode length, phyllotaxy and hairiness) and developmental changes in any of these underpins well informed decisions made during the grafting process and the final success.

The levels of tissue carbohydrates are important for scion survival and harvesting scion material at or after active carbohydrate assimilation is desirable. This can be increased by cincturing of the scion mother plant to maintain the level of carbohydrate in the branches to be harvested for scion material (Doggrell 1976; Barth & Bennell 1987; Santamour 1988a; Hartmann *et al.* 1990). Other physiological processes, such as the ability to produce callus, phenolics, flavanols (Santamour 1988a), lignin and suberin (Copes 1970; Hawkins & Boudet 1996) and or cyanogenic capacity (Gur & Blum 1973; Moore 1984d; Hale 1989; Vivian-Smith 1995) may also influence graft success.

Environment

Environmental factors play a critical role in graft success, affecting both the rootstock and scion before, at and after grafting. These factors include air and soil temperature (Meinke & Karnatz 1990), which affect root and shoot growth (Howard & Oakley 1997); and photoperiod (Erwin 1996) - lengthening days increasing callus production.

General health status is dependent on nutrition. A poorer nutritional status leads to increased incidence of disease and pest infestation - both undesirable in grafting. Active root growth, which is often followed by a phase of shoot growth, is largely stimulated by appropriate nutritional, water and temperature balance. Actively growing plants have a high rate of cell division and proportion of newly differentiated cells, predisposing

them to a greater chance of producing callus - a usual prerequisite for graft knitting. Also, an active vascular cambium results in proliferation of cambial initials with unthickened cell walls, a prerequisite for bark-slip in rootstocks (Hartmann *et al.* 1990). Bark-slip is a necessary requirement for bud grafting.

Hot callusing is another method of increasing ambient temperatures in the immediate vicinity of the graft union aiding callus formation (Hartmann *et al.* 1990; Barnett & Miller 1994). This method has been trialed on *Banksia* (McCredie *et al.* 1985a), but without definite conclusions on the benefits this method affords over other grafting methods.

Technique

There is a seemingly inexhaustible number of grafting techniques to choose from. Deciding on the appropriate grafting or budding technique to use on endemic Australian species is dependent on the nature and amount of scion material available, and the knowledge and expertise of the grafter. In deciduous plants dormant wood is the usual source of bud-wood and it is also possible to take buds from non-deciduous plants. This is done with more reliability and with better results when bark-slip occurs. However, material used in grafting is usually a semi-hardwood, sub-terminal section of the stem. The choice of grafting methods has been widely acknowledged to influence the results, and is shown by the numerous reports where several methods have been trailed to determine the most successful grafting method to use (Skene *et al.* 1983; Wojtusik & Felker 1993; Boyhan *et al.* 1996; Pereira-Lorenzo & Fernandez-Lopez 1997).

Various ways of finishing off the graft are possible after fixing the scion on the rootstock. Here, too, the choice will influence the final outcome and may involve: applying pruning paint or wax, wrapping the union with plastic tape (non-electric grafting tape or elastic tape - *Nescofilm* or *Parafilm*), applying a rubber, fungicide-coated patch with clip, or clipping the graft together with a metal or plastic device. It has also been reported that the orientation of the scion on the stem of the rootstock affects graft success (Larson 1994).

The relationship between sexual and vegetative compatibility

Two models have been developed to explain vegetative graft compatibility-incompatibility in higher plants. The first is based on the notion of self/non-self recognition events akin to that of animal systems (Clarke & Knox 1979); the other is the morphogen-toxin threshold model (Moore 1984b) and accompanying research (Moore & Walker 1981a, b, c; Moore 1982a, b, 1983, 1984a, c, d).

Although the recognition of self and non-self in the animal kingdom is well understood, it is less well understood in the plant kingdom. It has been reviewed in the context of knowledge of animal and plant systems (Yeoman *et al.* 1978; Clarke & Knox 1979; Noggle 1979). Solanaceous (*Lycopersicon, Nicotiana, Nicandra*) or *Prunus* species have been the model systems used in initial research on the molecular events in self and non-self interactions, and have dealt mainly with sexual recognition events of the style-pollen tube interaction (Bacic & Clarke 1988; Bacic *et al.* 1988). Understanding the molecular mechanisms and processes peculiar to compatibility-incompatibility in vegetative grafts of higher plants, as opposed to those involved in wound responses, invasion of infecting agents or in sexual (pistil-pollen) recognition, has only just begun. Little is known on whether the cellular recognition events that take place in sexual systems in plants (i.e. pollination, germination and fertilisation) are similar to those that take place during somatic cell contact in grafting. Yeoman *et al.* (1978) considered the wider significance of grafting, and although grafting occurs naturally in the wild (e.g. the common occurrence of root grafting in forest soil), a selective advantage conferred on species that can be grafted is difficult to appreciate, unlike sexual compatibility or defence or wounding responses. Why would plants develop systems for vegetative recognition that are of no selective advantage to them? Nonetheless, the phenomena of grafting higher plants has been exploited by mankind over the centuries and clues to its mysteries must be provided by the cellular and molecular interactions which occur during graft formation.

Moore (1984b) states that the major events of compatible graft formation - cohesion, callus proliferation and vascular differentiation - can be explained without the involvement of cellular recognition events. He postulates a model for graft compatibility-incompatibility where grafts are only incompatible if the effect of naturally present promoters of graft formation, morphogens, is overridden by toxins which elicit graft rejection.

Andrews and Marquez (1993) conclude that it is difficult to establish a relationship between sexual self-incompatibility (SSI) and graft incompatibility. SSI is the recognition of self-pollen in the style to prevent inbreeding. Grafting, on the other hand, is the recognition of non-self and involves the direction of completely different metabolic pathways, and the production of different substances. Furthermore, SSI is more frequent than self- (auto-) graft compatibility, and a similar physiological system would not support such a difference. Rather than similar recognition systems for

vegetative and sexual incompatibility, anatomical and physiological similarities may explain the high correlation existing between vegetative and sexual compatibility in species not taxonomically distant from one another.

The phenomenon of "memory" of prior contact of non-self in animal recognition systems has long been investigated and is the basis of today's vaccination systems. Some higher plant species display a similar memory phenomenon on the invasion of infecting bacteria, fungi or viruses. After prior contact with disarmed, non-virulent agents, plants show a greater resistance to disease. The research points to a certain parallelism, which seems to exist between the host-parasite interaction and the relationship between stock and scion in members of the Solanaceae. Whether this can be interpreted as a memory component of a recognition system, as in the plant pathogen system described above, rather than a simple switching on of metabolic pathways, is not possible to delineate with our current level of knowledge. A closer understanding of the pathways and molecular biology is required to elucidate this relationship.

Several papers report the high correlation between sexual compatibility and vegetative tissue (graft) compatibility in plant genera such as the tropical reafforestation genus, *Prosopis* (Wojtusik & Felker 1993), in cocoa (*Theobroma*) (Addison & Tavares 1952), *Trifolium* (Evans 1955) and in apple-pear hybrids (Crane & Marks 1952), to name a few. In each case, species used in successful interspecific hybridisation could be combined in successful compatible grafts, thus indicating a high correlation between sexual and vegetative compatibility. However, the mechanisms of this relationship are not clearly understood. The species in these genera are geographically and reproductively isolated, with little gene flow between species. Consequently, the level of natural interspecific hybridisation was low. In *Theobroma* (cocoa) (Addison & Tavares 1952) the completeness of reproductive isolation prevents gene exchange between species; natural hybrids are less probable than in many other genera and, despite wide searches, no natural hybrids have been found. The cross-pollination of nine species mostly gave abnormally maturing fruit; some crosses, however, resulted in hybrid seed with low viability and one partially fertile hybrid adult plant. The species which produced progeny, at least hybrid seed, could be grafted successfully. Vice versa, where interspecific grafts of *Theobroma* died off, hybridisation was usually impossible. Similarly, *Trifolium* is another genus with over 250 species showing little interspecific compatibility and a high correlation between graft success and the success of hybridisation, or at least fertilisation (Evans 1955).

Sexual interspecific compatibility across the *Banksia* genus is also low and natural hybrids are infrequently found in the wild (George 1987). Reproductive isolation, which maintains genetic identity and barriers to hybridisation in closely related, co-occurring species, has also been reported in *Banksia* (Scott 1980; Lewis & Bell 1981), and outcrossing is the predominant natural breeding system (Paton & Turner 1985; Whelan & Goldingay 1986; Carthew *et al.* 1988, 1996; Ramsey & Vaughton 1991; Carthew 1992; Maguire & Sedgley 1997). Interspecific incompatibility and intraspecific heterogeneity arising from species preference for outcrossing (Maguire & Sedgley 1997) are limitations to reliably generating improved forms of *Banksia* by sexual interspecific hybridisation for either scions or rootstocks. However, the great genetic diversity within and between species is a resource that can be screened and selected to provide new cultivars with improved characteristics or for specific uses (eg. scions or rootstocks). Clonal propagation of *Banksia* is, however, the linchpin to realising the benefits of this for industry.

Physiological aspects of the graft union

The physiological processes and cytological events occurring in graft formation in compatible or incompatible graft combinations have been documented (Yeoman & Brown 1976; Yeoman *et al.* 1978; Jeffree & Yeoman 1983). Most experimental systems used for research into graft physiology involve solanaceous species (Roberts & Brown 1961; Parkinson & Yeoman 1982; Jeffree & Yeoman 1983; Barnett & Weatherhead 1988) or *Sedum telephoides* from the Crassulaceae Family (Moore & Walker 1981a, b, c; Moore 1983, 1984a, c). Reports on grafting in woody species mostly aim at solving species specific problems of cultivation and are less often used as experimental systems for research. Yet such reports do offer information on graft physiology in woody perennial plants.

Plant growth regulators important in the stimulation, growth and lignification of callus also play crucial roles in grafting. Generally, auxins are produced in the scion bud, moving to the graft interface where they facilitate callus production from various cell types (Larson 1994). Auxins also move basipetally via the phloem to the root zone where they induce cytokinin production (Kamboj *et al.* 1997). Cytokinin levels in the xylem sap of the dioecious *Leucadendron rubrum*, a proteaceous species from South Africa, showed seasonal fluctuations which correlate with growth patterns, peaking with vegetative bud break and influencing differentiation (De Kock *et al.* 1994). Insight to the seasonal fluctuations of endogenous hormone levels in *Banksia* scions and

rootstocks may assist in timing the collection of scions or rootstock material and the final success of grafting.

Enzymes also influence grafting. A large number of peroxidases, widely present in different isozymic forms, are regulatory components of various pathways, particularly lignification (Deloire & Hebant 1982; Santamour 1988a). Peroxidase has been identified in chestnut cambium (Santamour 1988b) and other species (Santamour 1988a), where suggestions for their use in the prediction of compatible combinations of scions and rootstocks have been made (Hartmann *et al.* 1990), although these have not been completely successful (Huang *et al.* 1994). Isoperoxidases are thought to be involved in intraspecific graft incompatibility displayed by some deciduous trees of the Rosaceae (Andrews & Marquez 1993).

Metabolites may accumulate at the graft interface and affect the surrounding tissues, finally influencing grafting. Phenolics and polyphenolics are examples of such metabolites. The phenolics in sweet cherry (*Prunus avium*) are dominated by flavanones, several of which have been identified in callus and phloem, and their synthesis is influenced by carbohydrate levels (Treutter *et al.* 1985). Stress leads to the accumulation of flavanols, such as the flavanol glucoside - prunin - in apricot (Errea *et al.* 1994b). The high levels of flavanols may simply reflect overall changes in stress metabolism. Stress associated with grafting may modify tonoplast or plasmalemma permeability allowing leakage of flavanol glucosides. Cellular damage may affect the diffusion and movement of phenolics, altering the phloem and cambium in the region of the union in apricots. Flavanoids have also been reported to react with peroxidase, which is important in lignification, and hence callusing and development of the tensile strength of the graft union (Andrews & Marquez 1993).

The release of hydrolytic acid phosphatase into the cytosol of cells along the graft interface between *Sedum telephoides* and *Solanum penneli* has been positvely correlated with lethal cellular senescence (Moore & Walker 1981c; Moore 1983). The effect of other metabolites, some of which have been identified as toxic, is unclear and varies between species. Incompatibility in Moore's model is based on an unfavourable balance of toxin-morphogen in the grafted plant parts. This model is supported by incompatible grafts of pear on quince, and peach on almond, in which cyanogenic glycosides have been identified as the toxic component (Gur & Blum 1973; Moore 1984d). Five percent of higher plants are estimated to be cyanogenic (Lamont 1993) and over 75 cyanogenic glycosides have been documented (Poulton 1990). Many proteaceous taxa contain

poisonous substances, including cyanogenic glycosides (Everist 1974; Swenson *et al.* 1989; Larson 1995). Everist (1981) reports numerous proteaceous species that are cyanogenic: six *Grevillea* species, three *Hakea* species, five *Macadamia* species, two species of *Xylomelum*, as well as representatives from the *Lambertia* and *Lomatia* genera. Swenson *et al.* (1989) examined 155 species of the Proteaceae, and 44 were cyanogenic. Two cyanogenic glycosides extracted from eight species were identified as the tyrosine derived glycosides: dhurrin and proteacin. Of the 16 *Banksia* species tested by Swenson *et al.* (1989), none was cyanogenic, but a list of the species tested is not given.

In the Proteaceae, in general, the glycoside dhurrin is present predominantly in leaves, whereas flower and floral buds contain mostly dhurrin and the diglucoside, proteacin (Swenson *et al.* 1989). In *Macadamia* the amounts of cyanogenic glycosides vary with species, stage of development and tissue type. Concentrations increase in cotyledons during germination, dropping off during subsequent leaf maturation. The immature first leaf has the highest level (Dahler *et al.* 1995; Vivian-Smith 1995). *Grevillea* species have high cyanide levels in immature seeds, young leaves and germinating seedlings (Lamont 1993). Preliminary studies show that cyanogenic glycosides play a significant role in graft rejection in *Hakea* and *Grevillea* (Hale 1989), both genera having a high cyanogenic capacity (Swenson *et al.* 1989).

A phytoplasma infection has been identified as the cause of the beneficial factor used for the production of branching in commercial poinsettia cultivars. This is transferred from free branching poinsettia cultivars to varieties with restricted branching approximately ten days after approach grafting of the two varieties. Transmission occurs via the callus bridge connecting the two plants, even before vascular connections are established (Ruiz-Sifre *et al.* 1997). It is possible that numerous transmissible factors move across the graft union once the cells of the scion and rootstock come into contact. This movement may either be symplastic or apoplastic (Wang & Kollman 1996). Other agents transmitted across the graft union are floral induction factors (Lockard & Schneider 1981; De Kock *et al.* 1994; Weiss 1994; Kamboj *et al.* 1997), bacteria (Boyhan *et al.* 1996), viruses and viroids (Cohen *et al.* 1991; Heuss-LaRosa *et al.* 1995), nutrients and other constituents of the xylem and phloem sap. These may have a positive or negative bearing on graft formation.

Structural aspects of the graft union

There are many reviews on graft incompatibility-compatibility (Rogers & Beakbane 1957; Tubbs 1973a, b; Santamour 1988a). *In vitro* grafting experiments have been carried out under sterile conditions to examine structural (Parkinson & Yeoman 1982) and ultrastructural (cell wall deposition and vascularisation) events in graft formation (Moore 1984d; Gebhardt & Goldbach 1988; Wang & Kollman 1996). The majority of literature however deal with *in vivo* studies on graft formation in a wide cross-section of plants.

The sequence of structural events in successful grafts is not unique to grafting, occurring also in the wounding response. Furthermore, the initial phase of graft formation is very similar for compatible and incompatible grafts. The reported sequence of structural events occurring in successful grafts (Andrews & Marquez 1993) is:

(i) cell damage, production of exudate and generation of a necrotic layer

(ii) extension of living cells into the necrotic zone

(iii) callus bridge formation: interdigitating parenchyma cells invading the necrotic zone

(iv) increased tensile strength between the scion and the rootstock, with dictyosome-mediated secretion of cell wall precursors to aid cohesion

(v) differentiation of a new vascular cambium in callus

(vi) the production of secondary xylem and phloem, and establishment of vascular connections between the scion and rootstock.

The origin of callus is from various tissues in the outer bark region, including the cortex, phloem rays and newly formed cambial derivatives (Larson 1994). As the formation of the callus bridge develops, the tensile strength of the graft increases (Moore 1983, 1984a). The last two events listed above are peculiar to successful graft compatibility, whereas the others may occur in incompatible grafts.

The sequence of events which occur during graft formation at a structural and cellular level for the woody species of mango, *Prunus*, spruce and fir have also been documented.

Mango

Apical side-veneer grafts of six week old mango seedlings (Asante & Barnett 1997) produced exudate immediately they were grafted. The first sign of cell divisions were visible in the cortical parenchyma and phloem rays of the stock at day three and the next day a necrotic layer was also present. By day five callus proliferation from immature secondary xylem near the cut surface between the rays was penetrating the necrotic layer; pith cells enlarged and divided and a fan shaped proliferation of callus was apparent by day 25. Columns of callus perpendicular to the cut bridged the union by day 10 and extended into the pith zone. By day 30 interdigitating callus masses filled the gap between the scion and rootstock, and at day 60 the new cambium had formed, joining the scion and rootstock. A periderm formed over the outer surface of the callus and the necrotic layer was obliterated. The graft was completely healed by day 90.

Prunus

After seven days in compatible and incompatible chip-bud grafts of *Prunus* spp. (Errea *et al.* 1994a) callus cells were forming from the scion and rootstock. At 11 to 14 days a callus bridge formed, a new cambium developed centrally between the scion and rootstock producing some vascular connections, however, weaker vascular connections were established in incompatible grafts. Bud burst ensued. At day 21 differences could be identified in callus: in compatible grafts callus was regular, compact and had fewer extracellular spaces than callus produced in incompatible grafts, which was deposited irregularly, had thicker cell walls and stained heavily for extracellular substances. Also, the graft union did not develop uniformly throughout the graft interface.

Sikta spruce

In sikta spruce (Weatherhead & Barnett 1986; Barnett & Weatherhead 1988) resins were secreted onto the outer surfaces, initially aiding adhesion of the scion and stock, but later hardened to form an impenetrable barrier. Callus was produced by cambial derivatives and ray parenchyma of the scion and rootstock (seven days). Vascular cambium developed through mixed callus, linking the scion and rootstock cambia in a similar manner to *Prunus*. It is suggested that preceding general cell walls fusion, pectinaceous beads form on the surface of the callus cell walls that facilitate adhesion of the scion and rootstock.

Fir

Suberin zones and wound xylem areas are two indicators for delayed internal incompatibility in grafted fir (*Pseudotsuga menziesii*), which first appears after 12 months (Copes 1970). The suberin zones are initiated in the cortex and develop into the phloem and cambium. The wound xylem areas form only where suberin zones had previously developed into the phloem or cambium. This resulted in incompatibility in mature trees in fir seed orchards in North America.

Study rationale

The main reasons for grafting *Banksia* are: (i) to clonally propagate elite plants or hybrids not otherwise conveniently propagated; (ii) to obtain the benefits of certain rootstock characteristics (e.g. disease resistance, adaptability to special climates or soil conditions, size control/vigour influence on the scion) and (iii) to shorten the time to reproductive maturity, thus avoiding the juvenility delay in flowering. The cutflower industry needs a supply of clonal, elite plants and selections on which to base future plantings. This is presently lacking and the majority of *Banksia* plantations are derived from inherently variable seedlings. Grafting onto rootstocks tolerant to *Phytophthora* and heavy, alkaline soil types would enormously increase the potential production area for *Banksia*, which is currently limited to well drained, deep acid sands. Furthermore, *Banksia* seedlings have a long juvenile phase (refer to Table 5.1) which could be shorted by using grafted plants. Such outcomes provided by grafting would be advantageous to the commercial production of *Banksia.*

This study uses two eastern Australian seedling rootstocks, *B. serrata* and *B. spinulosa* var. *cunninghamii,* for self- (auto-), intraspecific and interspecific grafting to one eastern Australian scion species, *B. ericifolia,* and two western Australian species, *B. coccinea* and *B. menziesii.* Species from various taxonomic groupings were selected. Using this system, histological sections of ungrafted and grafted tissues were studied with the aim of increasing our presently limited knowledge on the key features of successful and unsuccessful grafts in *Banksia.*

Materials and Methodology

Plant material

Descriptions of the five species used in this grafting trial - *B. serrata, B. spinulosa* var. *cunninghamii, B. coccinea, B. ericifolia* and *B. menziesii* - are from George (1987), Taylor & Hopper (1988) and Maguire *et al.* (1996). All further references to *B. spinulosa* in this Chapter refer to variety *cunninghamii*. The two rootstock species are both from eastern Australia, but are from different sections of the subgenus *Banksia*: *B. serrata* is from section *Banksia* (series *Orthostylis*) and *B. spinulosa*, var. *cunninghamii* from section *Oncostylis* (series *Spicigerae*) (George 1987).

The two scion species from Western Australia, *B. menziesii* and *B. coccinea,* were previously classified in the same section, section *Banksia* and different series. *B. menziesii* remains in section *Banksia*, series *Orthostylis*. However, *B. coccinea* has been reclassified into a monotypic section, section *Coccinea*, which is more closely allied to section *Oncostylis* (Maguire *et al.* 1996). The third scion species, *B. ericifolia*, is an eastern species from the same section and series as *B. spinulosa*, section *Oncostylis*, series *Spicigerae* (George 1987).

The three species from the higher rainfall area of the eastern seaboard are tolerant to *Phytophthora*. Although the West Australian species, *B. coccinea* and *B, menziesii* are susceptible to *Phytophthora,* variation in susceptibility exists (Cho 1983; McCredie *et al.* 1985c).

Rootstock species

B. serrata Linnaeus f. (1782)

B. serrata is a tree to robust shrub endemic to the east coast of Australia where it grows on coastal dunes or sand over limestone (Blue Mountains), with an almost continuous distribution from Wilson's Promontory (Victoria) to Cooloola, Queensland (annual rainfall 800 - 1000 mm); a small population in north western Tasmania also exists. No lignotuber is present, but it has a thick bark, the plants usually re-shooting after fire from epicormic buds (Wrigley and Fagg 1989). New shoot growth occurs over the summer, and it flowers from January to June. This species is of economic importance as an ornamental, and also as a timber and honey source (Lazarides & Hince 1993).

***B. spinulosa* Smith, var. *cunninghamii* (Sieber ex Reichenbach) A.S. George (1981)**

B. spinulosa var. *cunninghamii* has a scattered distribution from near the Queensland – NSW border to the Dandenong Ranges, east of Melbourne, receiving a rainfall of 750 – 1000 mm. It is a shrub or small tree up to 4 m growing in understorey of forests, in soil ranging from sandy to heavier loams and clays at altitudes of 500 to 999 m (Taylor and Hopper, 1988). New shoot growth is largely over the summer months. Unlike the other *B. spinulosa* varieties it is non-lignotuberous, requiring seed for regrowth and it is tolerant to *Phytophthora* (McCredie *et al.* 1985b). Hybrids between *B. spinulosa* var. *cunninghamii* and *B. ericifolia* var. *ericifolia* have been recorded inland from Wollongong, NSW.

Scion species

***B. coccinea* R. Brown (1810)**

B. coccinea is from the south coast of Western Australia ranging from Albany to the Stirling Ranges and east to the Young River (rainfall 400 – 800 mm). It grows as a small tree usually 2 – 4 m in height and in deep white or grey sands in heath, tall scrubland or woodlands. New shoot growth peaks over summer and this species does not have a lignotuber and is killed by fire, regenerating from seed. It may take up to five years to reach flowering, which peaks in spring. *B. coccinea* is susceptible to the soil borne disease, *Phytophthora cinnamomi* (McCredie *et al.* 1985b).

***B. ericifolia* Linnaeus f. (1781)**

The distribution of this species is largely along the coast of NSW north of Sydney (rainfall 800-900 mm). *B. ericifolia* is a large shrub or small tree growing up to 4 m and is a common component of sandy coastal heaths. Although thought to be non-lignotuberous, resprouting after fire has been observed in some plants. It is also tolerant to *Phytophthora* (McCredie *et al.* 1985b). Flowering peaks from May to July and new shoot growth is predominantly from late spring to summer. Hybrids with *B. spinulosa* var. *cunninghamii* have been reported.

***B. menziesii* R. Brown (1830)**

B. menziesii is a west coast species from Western Australia, found commonly in woodlands and scrublands on deep sands from Kalbarri southwards to Waroona, eastwards to the Darling Escarpment, in 350 – 900 mm rainfall. It grows as a lignotuberous tree to 10 m or as a shrub to 3 m. Non-lignotuberous small trees have also been reported near Mt Lesueur. This species is moderately susceptible to *Phytophthora*

(McCredie *et al.* 1985b). New shoot growth occurs from late spring throughout the summer, peaking in December and January (Taylor and Hopper 1988). Flowering occurs from March to August. This species is widely cultivated throughout southern Australia as a commercial cutflower species. Natural stands are also important in honey production (Lazarides & Hince 1993).

Seed source and germination

Seed for three species, *Banksia serrata, B. menziesii* and *B. ericifolia* was obtained from the West Australian seed supplier, Nindethana Seed Services (RMB 939 Woogenilup, WA 6324). *B. spinulosa*, var. *cunninghamii*, seed was kindly provided by Mr Alf Sankin, Mt Waverley, Melbourne: collection 10 from Narrow Neck Peninsula, near Katoomba in the Blue Mountains, NSW (grid ref.: 325.828 (1:250,000); the voucher specimen is held at National Herbarium of Victoria).

Seed was sown directly into tamped, no. 3-4 vermiculite in deep seed flats (300 mm x 400 mm x 12 mm) (Plate 4.2a); larger seeds were covered with 1 cm layer of vermiculite, smaller seeds requiring less. These were watered in with a systemic fungicide, *Previcur* (Shering-Höchst, active constituent: 600 g/L propamocarb) at a rate of 1.5 mL/L of water and applying 2 L/m^2 , to control damping-off. Seed flats were placed in full sunlight in a well ventilated glasshouse at 18°C. Hand watering with a fine rose was applied daily; in hot weather, when the top layer of vermiculite dried out, the watering rate was increased to two or three times a day.

Emergence occurred between three to five weeks after sowing (*B. serrata* 4½ weeks (Plate 4.2a); *B. menziesii*, 3 – 4½ weeks; *B. spinulosa,* 3½ weeks). The germination rate[20] varied between 50% to 100% depending on the species (*B. serrata*, 65%, *B. menziesii*, 100%, *B. spinulosa*, 90%). *B. spinulosa* was susceptible to damping-off and black fly larva attack, if the medium was continually moist during the post-emergence phase.

B. coccinea scion material was sourced from a clonally propagated selection of a deep red, small flowering banksia (local code: KC76-165, propagated 9/1/92) held in the Department of Horticulture, Viticulture and Oenology, Faculty of Agricultural and Natural Resource Sciences, University of Adelaide. These plants were grown in 300

[20] Number of seedlings pricked out divided by the number of seeds sown

mm pots under shade-house conditions, pruned nine months prior to grafting, fertilised (*Osmacote N*17+P1.6+K8.7) and hand watered regularly.

Plant cultivation and preparation for grafting

Germinated seeds were pricked out at the cotyledon or first true leaf stage and planted into potting medium in 10 cm pots (Plate 4.2b) or 0.5 L nursery bags (from *PolyProduct*). The potting medium was a low phosphorus native plant mix (*Nuerth,* 80% composted pine bark (graded size proportions: 1: 2: 2 = 4mm: 6mm: 8 mm), 20% coarse sand, and trace elements; with pH 6 and an air filled porosity of 16 to 22%) to which *Osmacote* slow release fertiliser (N17+P1.6+K8.7, at 1g (¼ tsp.)/bag) was added. The seedlings were placed on high benches in a shade house and hand watered as required.

At 18 months, rootstocks were potted up into 150 mm (2 L) (Plate 4.2d) or 200 mm (4.5 L) pots using the same medium, whilst scion plants and the remainder of *B. spinulosa* rootstocks were potted into 1.2 L nursery bags (Plate 4.2c) and grown under the same conditions. Three liquid feeds of *Aquasol* (*Yates-Hortico*, 7% total nitrogen, 12% phosphorus, 5.5% potassium; 30:1 dilution; application rate: 500 mL/200 mm pot, otherwise liquid feeds were sprayed on) were applied at two, four weeks and at the time of grafting. One week prior to grafting plants were moved from the shade house into a glasshouse maintained at 18°C. Rootstock plants were top-dressed with *Osmacote* and sprayed with *Carbaryl* (active constituent: 500 g/L *Carbaryl*, application rate: 2.5 g/L) against leaf looper infestation.

Pest and disease control

During the growing of rootstock and scion plants, pesticide application was necessary to control damping-off (application of *Previcur*, refer above), thrip (application of *Orthene;* active constituent: 750 g/L Acephate applied at 1.3 g/L), mealy bug (application of *Lebaycid*, active constituent: 550 g/L Fenthion @ 0.1 – 1 mL/L), stem and collar blackening (application of *Benlate*, active constituent: 500g/kg Benomyl, applied at 50 g/L) and leaf looper (application of *Carbaryl*, refer above).

Grafting

Grafting was conducted in early spring (mid August), 1996 in a glasshouse with a mean daily average temperature of 16.8°C (Figure 4.1). Two rootstocks, *B. spinulosa* and *B. serrata* (Plate 4.2c & d) were grafted with scions to form eight possible combinations (Table 4.3). A

randomised design blocked into days was generated; the treatments (grafting combination) were randomised over each day block to remove any variation due to time of grafting or grafter fatigue. Twenty one (21) grafts per combination were carried out each day; the total number of grafts per combination is listed in Table 4.3.

Semi-hard wood scion material was cut from the mother plants. The bulk of leaves were removed leaving the petiole and the bottom portion of the leaf (ca. 1 cm) to protect the bud present in the leaf axil; 1 cm of foliage was left at the tip of the scion in the case of *B. ericifolia*. Where possible a "knuckle" – a region of stem with short internodes (Fuss *et al.* 1992) - was used. This material was surface sterilised by dipping for one minute in a 1% available chlorine solution (1:4 dilution of *White King*, 4% available chlorine) with a drop of surfactant (*Triton*) and placed in distilled water until required. The number of buds on the scion, together with its length and diameter were recorded (Table 4.5) before each scion piece was grafted.

The tops of rootstocks were sprayed with a 2% available chlorine solution plus surfactant, whilst secateurs and grafting knife[21] were surface sterilised between grafts with a dip in 70% ethanol. This solution was also used to clean working surfaces.

The diameter of the scion was matched as closely as possible to the rootstock, aligning the scion with one side of the rootstock, if the scion was smaller in diameter than the rootstock. These were grafted using a long oblique whip graft, fastened firmly in place with a 10 to 13 mm strip of *Nescofilm* (Plate 4.9d) and sprayed with *Previcur* (1.5 ml/L). Once dry, the scion was wrapped in a single layer of *Nescofilm* (Plate 4.9a & b). The equipment and solutions used are displayed in Plate 4.1d.

Post-graft plant maintenance

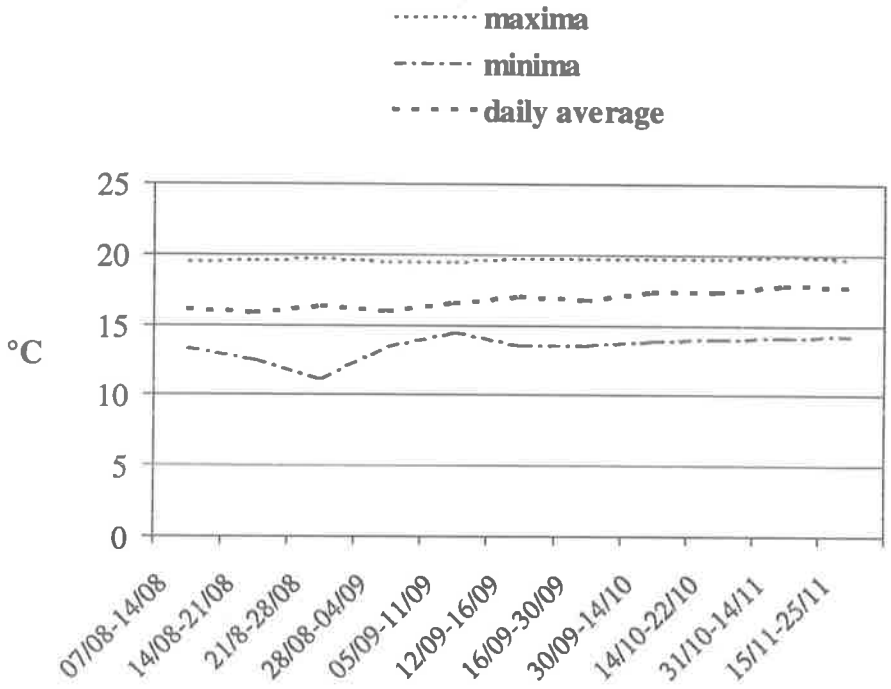
The grafted plants remained under glasshouse conditions with shade blinds semi-drawn for the entire sampling and observation period. The mean daily average temperature was 16.8°C with an average maximum and minimum of 20.6°C and 13.4°C, respectively (Figure 4.1). A brief spike in maximum temperature occurred on 25.09.96 due to a malfunction in the glasshouse cooling system and reached a temperature of 28°C on the south side and 31.5°C on the north side of the glasshouse. This did not affect the average data presented in Figure 4.1.

[21] A fixed blade grafting knife, overall length 15.5 cm, American patent: *Tina*, no. 683

Table 4.3 Graft combinations

COMBINATION DESIGNATION	ROOTSTOCK	SCION	TOTAL NO. GRAFTS
s-self	*B. serrata*	*B. serrata* (same plant – self)	105
s-intra	*B. serrata*	*B. serrata* (different plant – intraspecific)	105
s-cocc	*B. serrata*	*B. coccinea*	105
s-eric	*B. serrata*	*B. ericifolia*	105
s-menz	*B. serrata*	*B. menziesii*	105
s-sc	*B. serrata*	*B. spinulosa*	105
sc-self	*B. spinulosa*	*B. spinulosa* (same plant)	84
sc-cocc	*B. spinulosa*	*B. coccinea*	84

Figure 4.1 Glasshouse maxima, minima and average daily temperature for the duration of the grafting trial (time axis not to scale)



Spring 1996 (not to scale)

The stocks of grafted plants were regularly disbudded. This was particularly necessary for *B. serrata* which produces basal buds prolifically. Plants were hand watered taking care to avoid water stress, as well as avoiding contact of the graft union with free water. The tape was removed to prevent ring-barking of the plant after graft-take had occurred. *Nescofilm* did not breakdown under glass-house conditions.

Scion – rootstock scoring

Plants were scored at weeks 9 and 21, and 12 months post-grafting using a scoring scale (Table 4.4) developed for this trial.

Table 4.4 Scoring scale used for assessing graft union

	SCORE	ATTRIBUTES
Scion	1	Healthy, all green tissue (zero infection)
	2	Withered, wilted with no blackening or apparent infection
	3	≤ 20% fungal infection or blackening
	4	20% ≤ 50% "
	5	50% ≤ 80% "
	6	80% < 100% "
	9	100% infection/death
Rootstock	1	Healthy
	2	Some fungal infection at graft union
	3	Upper 10 cm of rootstock dead
	9	Whole rootstock plant dead

Microscopy

Random destructive samples of five graft unions of each graft combination were taken at two, four and twelve weeks post-graft. Ungrafted (control or day zero) stem wood was taken from two plants of each species at the beginning of grafting.

Secateurs were used to cut the rootstock a few centimetres below the graft union; the scions, with attached rootstock parts, were placed in water and taken into the laboratory for further dissection. The *Nescofilm* was carefully cut away and the graft union scored, cut to less than 10 mm and placed in 3% glutaraldehyde in 0.025 M phosphate buffer, pH 7 at 4°C. If the graft union was > 10 mm (the size of the embedding capsule), it was cut into smaller segments.

After 24 hours in glutaraldehyde, samples were subjected to dehydration through alcohols, fixation, infiltration and embedding in 2-hydroxyethyl-methacrylate (Feder & O'Brien 1968). Approximately twenty transverse sections (4 μ thickness) of the mid portion of the graft union were stained using Periodic acid-Schiff's reagent and Toluidine Blue O, mounted in *Micromount* on glass slides and cover slipped. (Refer to Chapter 3, Wood Anatomy for details on histological, microtoming and staining methods and techniques.) Sections were viewed and photographed using a transmitted light microscope (*Zeiss, Axiophot Pol Photomicroscope*); *Ilford Delta Professional* (ISO 400/27 or 100/21) black and white film was used to produce photomicrographs under standard darkroom procedures.

Statistical analysis

The mean and standard deviation for the scion length and diameter (Table 4.5) were generated using *Excel 97*. The pair-wise comparison of means was conducted using the z test, critical $z \geq 2.5$ (means statistically different if $z \geq 2.5$, confidence level of mean: $P < 0.05$). The z values were generated by dividing the difference of the means with the square root of the sum of the standard errors of each mean[22]. The percentage survival[4] of grafts was calculated and the mode scion and rootstock scores were generated using *Excel 97*.

[22] $z = (mean_x - mean_y)/\sqrt{(se_x^2 + se_y^2)}$

Results

Measurements of the length, diameter and number of buds for the scion material used for each species were made and the mean, standard deviation and range are presented in Table 4.5. Scion length ranged from 93.4 mm for *B. ericifolia* to 111.9 mm in *B. serrata*. The mean scion diameter varied from 3.3 mm in *B. ericifolia* to 5.2 mm in *B. serrata*. The mean number of buds per scion showed the greatest variability due to the different phyllotaxy of the scion species, ranging from 6.8 buds/scion in *B. menziesii* to 93.4 in *B. ericifolia*, the latter having small closely spaced leaves.

Scion – rootstock scoring and graft survival

Scions and rootstocks were scored at 9 and 21 weeks, and 12 months post-grafting using a scoring scale (Table 4.4). The mode scion and rootstock scores for each graft combination, together with the percentage survival[23] are presented in Table 4.6. At nine weeks post-grafting both self- and intraspecific grafts of *B. serrata* showed 22% survival of scion and rootstock, whilst *B. ericifolia* and *B. spinulosa* scions on *B. serrata* showed a grafting survival of 15% and 7% respectively, and intraspecific grafts of *B. spinulosa* 2%. All scions were dead at nine weeks in three graft combinations: *B. coccinea* and *B. menziesii* on *B. serrata* rootstocks and *B. coccinea* on *B. spinulosa* stocks. Scions of *B. coccinea* and *B. menziesii* had become wilted and withered, with no apparent infection (mode scion score = 2), whereas dead scions in other combinations were obviously infected (mode scion score = 6).

At 21 weeks post-grafting, intraspecific grafts of *B. serrata* had an 8% survival rate and auto-grafts of *B. serrata* one percent. At twelve months after grafting only intraspecific graft combinations of *B. serrata* were alive (7% success); both scions and rootstocks scored "healthy" (score: 1,1). These are shown at 18 months in Plate 4.10.

[23] % survival = no. scions alive (scored ≤ 5)/no. rootstocks alive (scored ≤ 2 (Table 4.4)); not including destructively sampled plants

Table 4.5 Scion mean length, diameter and bud number for each of the graft combinations on two rootstocks. Means followed by the same letter are not significantly different - each rootstock is analysed independently. P≤0.05, critical *z*-value = 2.5

SCION	LENGTH, mm		DIAMETER, mm		NO. BUDS/SCION	
	Range	Mean (±s.d.)	Range	Mean (±s.d.)	Range	Mean (±s.d.)
on *B. serrata* rootstocks:						
B. serrata – self	40 - 165	109.8 (22.4) b	3.3 – 7.8	5.2 (0.9) d	2 – 27	9.6 (4.8)
B. serrata – intraspecific	50 - 165	111.9 (24.0) c	2.8 – 7.4	4.9 (1.0) d	3 – 18	10.4 (3.5) b,c
B. ericifolia	10 - 130	93.4 (16.7) a	2.0 – 5.5	3.3 (0.8) a	10 – 145	93.4 (16.7) d
B. spinulosa	65 - 145	98.4 (15.8) a,b	2.0 – 6.4	4.1 (0.8) b	3 – 36	11.4 (5.2) c
B. menziesii	60 - 160	98.2 (21.4) a,b	2.8 – 6.9	4.4 (0.8) b,c	2 – 14	6.8 (3.0) a
B. coccinea	10 - 150	104.2 (18.7) b	2.9 – 6.6	4.5 (0.8) c	4 – 20	10.1 (4.3) b
on *B. spinulosa* rootstocks:						
B. spinulosa – self	65 – 150	107.3 (17.7) a	2.3 – 6.0	4.0 (0.8) a	4 – 27	12.8 (4.0) b
B. coccinea	60 – 160	105.1 (22.9) a	2.7 – 7.6	4.2 (1.1) a	3 – 25	10.7 (5.8) a

Table 4.6 Number and percentage of graft survivals at nine and 21 weeks, and 12 months post-grafting. The numbers in parenthesis [ ] are the mode of the rootstock and scion scores (refer to Table 4.4). This data does not include plants used as destructive samples.

		ROOTSTOCK – SCION COMBINATIONS													
		No. of grafts alive & mode scores [rootstock, scion]													
Time post-graft	Block (day)	s-self		s-intra		s-cocc		s-eric		s-menz		s-sc		sc-self		sc-cocc	
9 weeks	1	6	[1,6]	3	[1,6]	0	[2,2]	3	[1,6]	0	[2,2]	0	[1,6]	0	[2,6]	0	[2,2]
	2	4	[1,6]	13	[1,1]	0	[2,2]	9	[1,6]	0	[2,2]	3	[1,6]	1	[2,6]	0	[2,2]
	3	5	[1,6]	3	[1,6]	0	[2,2]	1	[2,6]	0	[2,6]	3	[2,6]	1	[9,9]	0	[2,9]
	4	5	[1,4]	2	[1,6]	0	[2,2]	1	[1,6]	0	[2,2]	1	[2,6]	0	[9,9]	0	[9,9]
	5	1	[2,6]	0	[2,6]	0	[2,2]	0	[2,6]	0	[2,2]	0	[2,6]	-		-	
	Total count	21	[1,6]	21	[1,6]	0	[2,2]	14	[1,6]	0	[2,2]	7	[2,6]	2	[9,9]	0	[9,9]
	% survival*	**22**		**22**		**0**		**15**		**0**		**7**		**2**		**0**	
21 week	1	0		1	[1,1]			0				0		0			
	2	0		5	[1,1]			0				0		0			
	3	0		0				0				0		0			
	4	1	[1,4]	1	[1,1]			0				0		0			
	5	0		0				0				0		0			
	Total count	1		7				0				0		0			
	% survival*	**1**		**8**				**0**				**0**		**0**			
12 months	1	0		1	[1,1]												
	2	0		5	[1,1]												
	3	0		0													
	4	0		0													
	5	0		0													
	Total count	0		6													
	% survival*	**0**		**7**													

* % survival = no. scions alive (scored ≤ 5)/no. rootstocks alive (scored ≤ 2 (Table 4.4)); not including destructively sampled plants;
self = same plant; intra = intraspecific; s = *B. serrata*; cocc = *B. coccinea*; eric = *B. ericifolia*; menz = *B. menziesii*; sc = *B. spinulosa* var. *cunninghamii*;

Microscopy

Ungrafted material

Photomicrographs of sections of ungrafted rootstock and scion stems (day 0 controls) show the general anatomy and main features of the tissues before grafting (Plate 4.3 and Plate 4.4). Cuticle was present in all species, however cuticle in association with cork was observed in two species, *B. ericifolia* (Plate 4.3b) and *B. spinulosa* (Plate 4.4a & b). The beginning of cork development was observed in *B. menziesii*, which had a dark blue layer, 1 - 2 cells thick beneath the cuticle (Plate 4.3c).

The heavily stained cortical cells were highly vacuolate. Newly divided cells were observed in the cortex of *B. ericifolia* (Plate 4.3b), *B. menziesii* and *B. spinulosa* (Plate 4.4b). Leaf traces were observed in the cortex of *B. coccinea* (Plate 4.3a), *B. menziesii* (Plate 4.3c) and *B. spinulosa* (Plate 4.4a).

Cambial initials varied in number from two to six, *B. ericifolia* and *B. menziesii* having the highest number of initials. Interfascicular ray parenchyma, which separate the vascular bundles (Plate 4.4c) and which were present in all species, consisted of regular, elongate seriate cells, in one to two rows; interfascicular cambial cells were also present adjacent to the cambium of the vascular bundles.

The area of xylem tissue, including the fibre cell caps at either end of the vascular bundles, varied with respect to the area of the whole section for each of the species examined. The xylem of *B. ericifolia* (Plate 4.3b) and *B. spinulosa* (Plate 4.4a) sections accounted for the largest proportion (ca. 80%) of the section area, followed by *B. menziesii* (ca. 50%) (Plate 4.3c), *B. serrata* (ca. 30%) (Plate 4.3d) and *B. coccinea* (20%) (Plate 4.3a).

The pith cells were round, thinner walled and larger than the cortical cells. Pith cells were uniform in appearance. Starch granules were visible mainly in the interfascicular and pith cells of *B. ericifolia, B. serrata* (Plate 4.3d & Plate 4.4d) and *B. spinulosa* (Plate 4.4b) and to a lesser extent in the cortex (*B. serrata*). However, *B. coccinea* (Plate 4.4c) and *B. menziesii* (Plate 4.3c) had few or no starch grains in the interfascicular parenchyma or pith.

Grafted material

Four of the five samples of each graft combination at each sampling time were sectioned for microscopy. Microscopic observations of sections, based on the frequency of occurrence of key features, are summarised in Table 4.7. Photomicrographs of sections of grafted plant tissue sampled two, four, and 12 weeks after grafting illustrate the initial

response to grafting and the progression of tissue growth or collapse (Plate 4.5 to Plate 4.8). The experimental unit taken at a point mid-way along the graft union is shown in Plate 4.5a, which illustrates good matching of tissue proportions, especially the cambium and cortex tissues that are fundamental to grafting success. In contrast, Plate 4.5b shows poor matching between *B. coccinea* scion and *B. serrata* rootstock two weeks after grafting: there is neither matching of the cambium nor the cortex – most of the scion cortex is aligned with the wood tissue of the rootstock. Higher magnification shows cell necrosis at the edge of the scion cortex, together with exudate production (Plate 4.5c). These features – cell necrosis and exudate production - are early responses to grafting. Exudate was observed in all graft combinations (Table 4.7) and was produced predominantly by the cortical, cambial and interfascicular cells. The frequency of cell necrosis (features 2, 3 and 4 in Table 4.7) increased over the 12 week sampling period and was more prevalent in the scion than in rootstock tissues.

Another reaction of tissue to grafting was the occurrence of irregular cell divisions. Irregular divisions in the cortex and the region between the phloem fibre caps (Plate 4.6a), as well as in the interfascicular cambium and phloem (Plate 4.6b), had begun as early as two weeks after grafting. However, appreciable callus was first observed at four weeks and was produced from the cortex, cambium and phloem tissues of *B. ericifolia* scion grafted onto *B. serrata* stocks (Plate 4.6c). Callus was observed in the xylem and pith regions of the graft union at 12 weeks for the same graft combination (Plate 4.7a & b). Here, the callus initiated in the region of the cambium and cortex continued to divide, filling the space between the scion and rootstock. In some instances there was close contact between the scion and rootstock tissues where the cambium and outer living tissues were not aligned and where no callus formed (Plate 4.8). These scions were still healthy at four weeks and received a score of 1. At nine weeks bud burst was observed in *B. ericifolia* scions on *B. serrata* (Plate 4.9a & c) and in intraspecific grafts of *B. serrata* (Plate 4.9b & c). At 18 months after grafting, only intraspecific grafts of *B. serrata* were alive and growing vigorously (Plate 4.10).

Table 4.7 Microscopy observations of ten features in rootstocks and scions at two, four and 12 weeks

Feature	Number and percentage of observations (of eight)											
	Two weeks				Four weeks				12 weeks			
	Rootstock		Scion		Rootstock		Scion		Rootstock		Scion	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Exudate from cut surface	8	100	8	100	8	100	8	100	8	100	8	100
Degradation of cortical cells at cut surface	3	38	3	38	4	50	4	50	3	38	2	25
Degradation of cortical cells overall	0	0	1	13	2	25	2	25	2	25	2	25
Degradation of cambial cells	0	0	1	13	0	0	1	13	2	25	1	13
Divisions in cortex & region between phloem fibre caps	6	75	3	38	6	75	5	63	5	63	5	63
Divisions in interfascicular cambium irregular	6	75	4	50	5	63	4	50	5	63	4	50
Callus knitting at cortex & between phloem fibre caps	0	0	0	0	2	25	2	25	2	25	3	38
Callus knitting at cambium/phloem	0	0	0	0	0	0	1	13	1	13	1	13
Callus knitting at xylem/pith region	0	0	0	0	0	0	0	0	1	13	1	13
Pith unchanged	8	100	/	/	8	100	/	/	7	88	4	50

/ = absence of pith in tissue sections

Plate 4.1 *Banksia* grafting

(a) Prolifically flowering *B. solandri* on *B. integrifolia* at Geoff McKenzie's property, Ocean Grove, Victoria

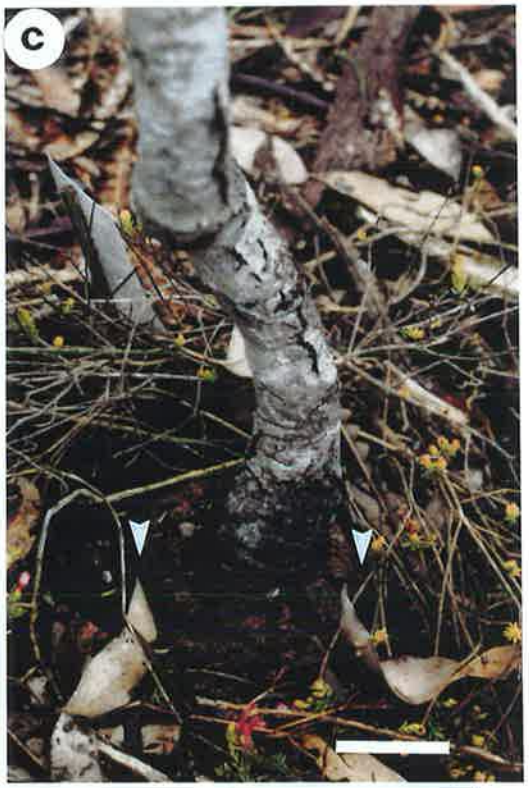
(b) Slow growing *B. speciosa* at Geoff McKenzie's property, Ocean Grove, Victoria grafted onto *B. integrifolia* rootstock; the size disparity in the graft union is shown in (c). Bar = 30 cm

(c) Close-up of (b) displaying the size disparity between the scion and rootstock (arrows indicate external diameter of the rootstock). This disparity may indicate a latent incompatibility between the two graft components. Bar = 10 cm

(d) Solutions and equipment used in grafting trials (back left to right): 1% bleach, 2% bleach spray, *Previcur* spray, 70% ethanol, distilled water, *Nescofilm*, *Tina* budding knife and secateurs.


a


b


c


d
BLEACH
DISTILLED WATER
NESCOFILM

Plate 4.2 Rootstock cultivation

(a) *B. serrata* seedling emergence 4½ weeks after sowing in seed flats (300 mm x 400 mm x 12 mm) containing vermiculite

(b) *B. serrata* seedlings pricked out into 10 cm pots at the first true leaf stage

(c) Eighteen month old seedling rootstocks of *B. spinulosa* var. *cunninghamii* potted into 1.2 L black plastic nursery bags

(d) Eighteen month old *B. serrata* seedling rootstocks potted into 150 mm pots

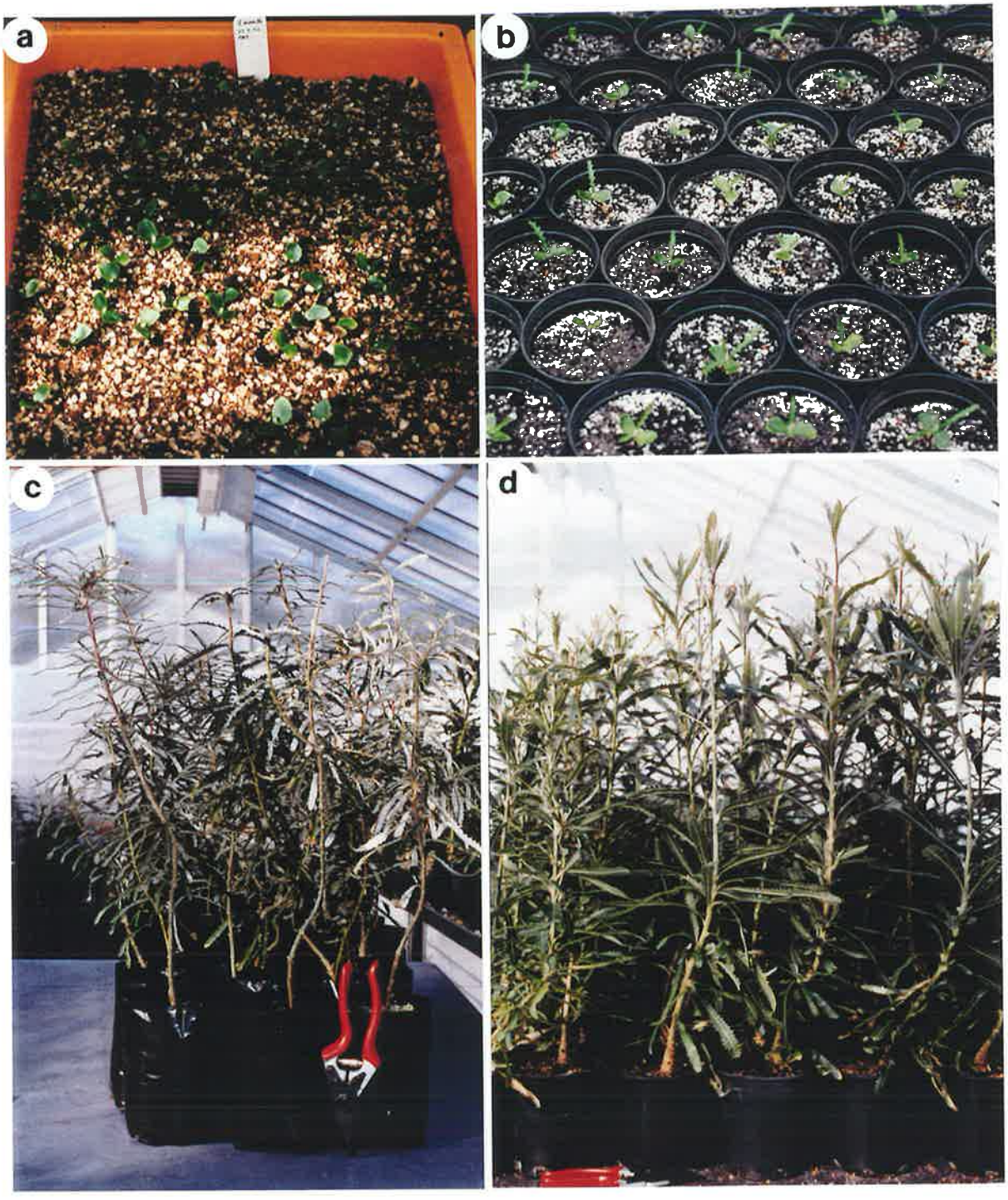

a
b
c
d

Plate 4.3 Light micrographs of ungrafted *Banksia* stems, TS

(a) *B. coccinea* showing leaf traces (l) in the cortex (c), collateral vascular bundles (v) and pith (p). Detail shown in Plate 4.4c. Bar = 500 μm

(b) *B. ericifolia* showing similar tissues as in (a), however, the vascular bundles account for a greater proportion of the cross sectional area and the cortex is comparatively small. Note the presence of newly divided cortical cells (arrows) and cork (ck). Bar = 500 μm

(c) *B. menziesii* showing tissue proportions similar to *B. coccinea* and leaf traces in the cortex. The build-up of phenolics in one or two rows of cortical cells immediately beneath the cuticle indicates the beginning of cork development. Note the low incidence of starch grains in the interfascicular rays and pith. Bar = 500 μm

(d) *B. serrata* stem section depicts a large central pith with cells storing starch grains, surrounded by a narrower band of vascular bundles and cortex, a detail of which is shown in Plate 4.4d. Bar = 500 μm

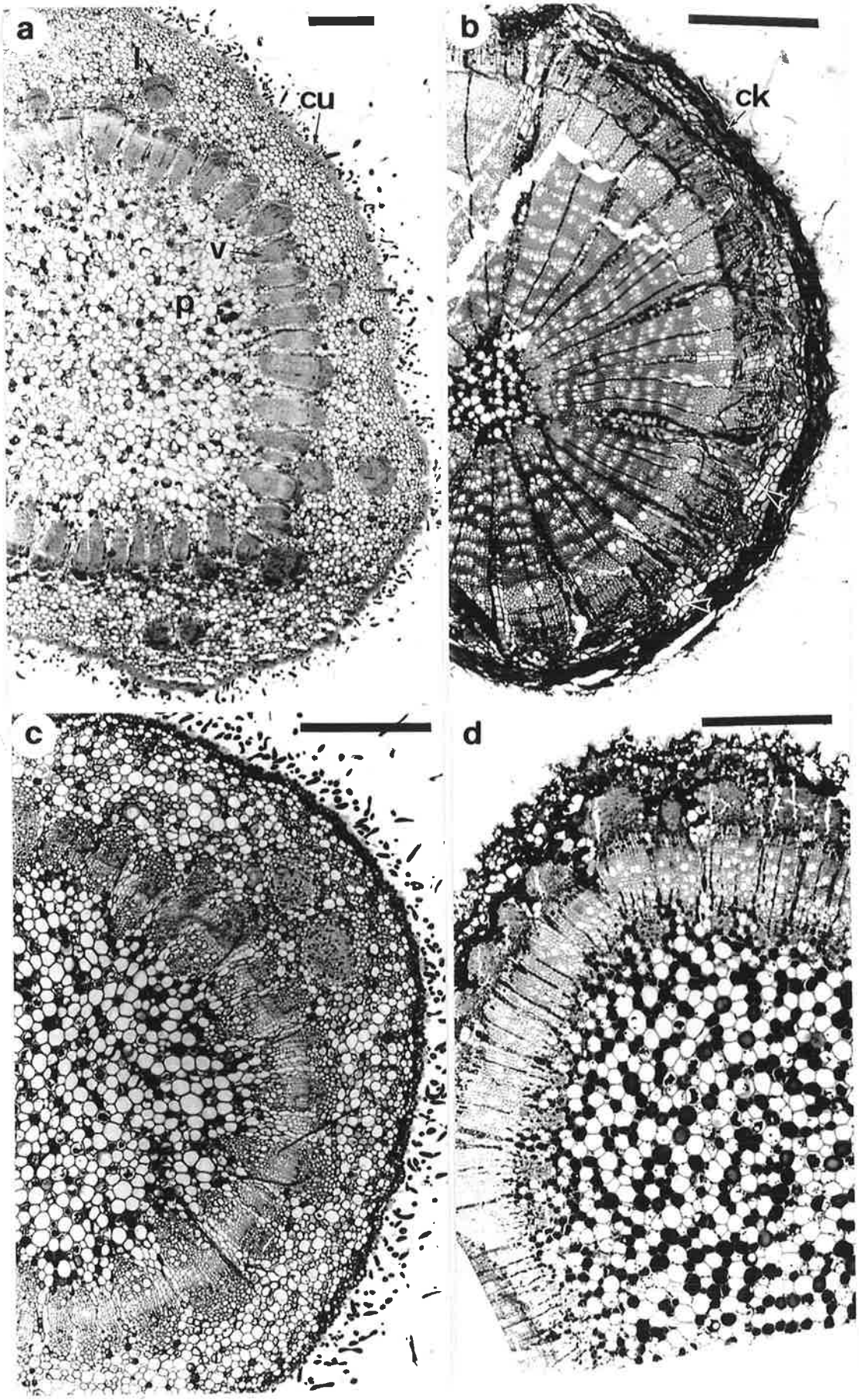

a
l
cu
v
p
c
b
ck
c
d

Plate 4.4 Light micrographs of ungrafted *Banksia* stem, TS

(a) *B. spinulosa* var. *cunninghamii* showing cork developing at the margin of the cortex which is shown in detail in (b), the presence of a leaf trace (bottom RHS) and the large proportion of vascular tissues. Bar = 500 μm

(b) *B. spinulosa* var. *cunninghamii* vascular bundles, cortex and developing cork (ck). Thin walled newly divided cells and large intercellular spaces are visible in the cortex. Bar = 100 μm

(c) *B. coccinea* detail of Plate 4.3d depicting the cuticle (cu) at the cortex margin and vascular bundles with phloem fibres (pf) at the margin to the cortex, cambium (ca), interfascicular ray parenchyma (ip), interfascicular cambium (ic), vascular rays (r). Note the general lack of starch grains. Bar = 100 μm

(d) *B. serrata* detail of Plate 4.3d showing the narrow, heavily stained cortex, abundant phloem fibres and the presence of starch grains in the pith and interfascicular cells. Bar = 100 μm

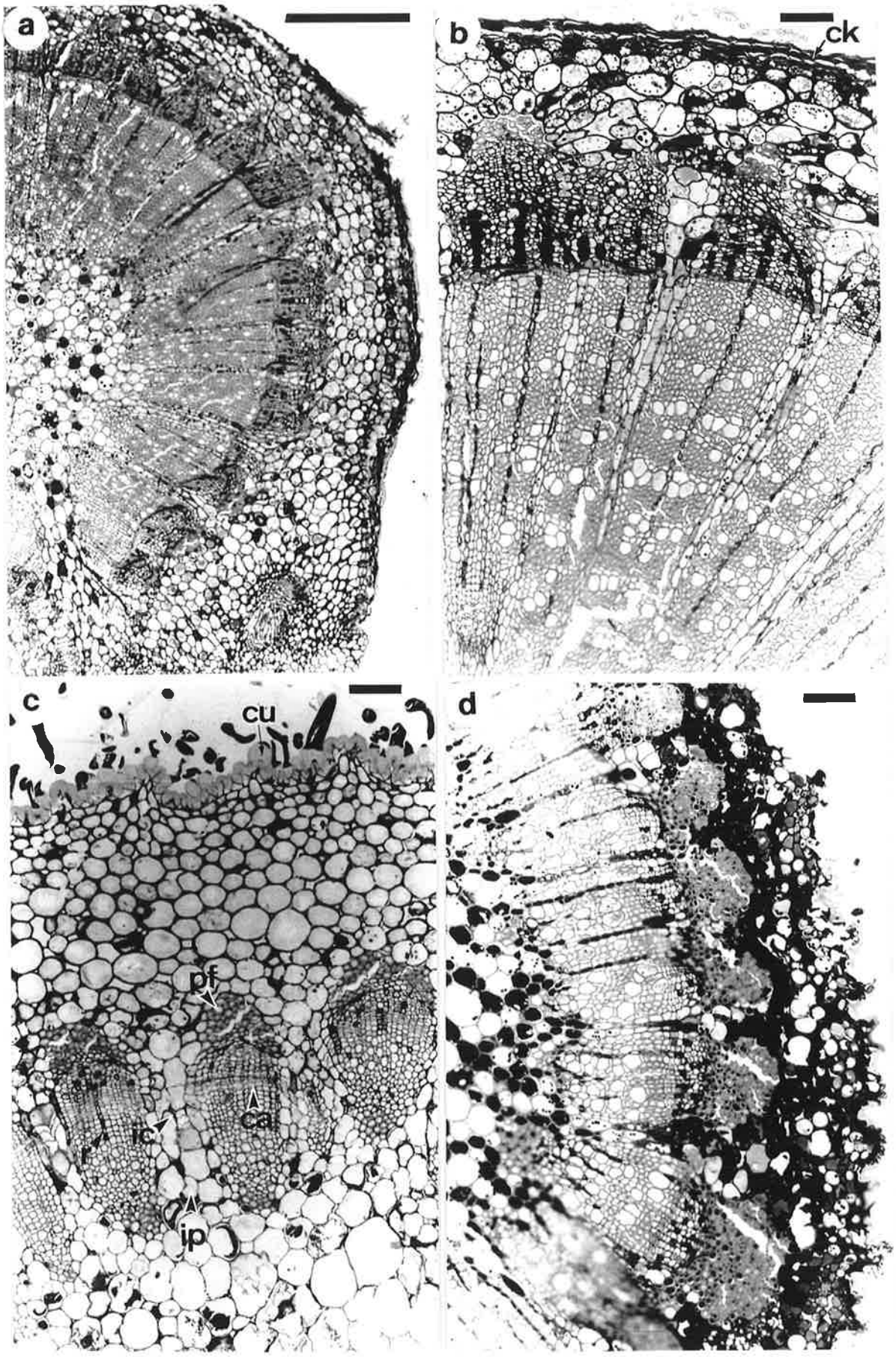

a
b
ck
c
cu
pf
ca
ic
ip
d

Plate 4.5 Light micrographs of transverse sections taken mid-way along the graft union

(a) *B. coccinea* scion (LHS) on *B. serrata* rootstocks (RHS) at four weeks, showing the experimental unit of well matched proportions of tissue types in the scion and rootstock. Bar = 500 μm

(b) Poorly matched graft between *B. coccinea* scion (LHS) and *B. serrata* rootstock (RHS) at two weeks after grafting. Note that no living cortical cells are in contact. Bar = 500 μm

(c) Exudate production (small arrows) and cell necrosis (large arrows) – both early responses to grafting. In this example of *B. coccinea* scion (top RHS) and *B. serrata* rootstock, two weeks post-graft, mismatching of outer living tissues of the cortex is visible and the top of the vascular bundles of the rootstock are in contact with the scion cortex. Bar = 100 μm

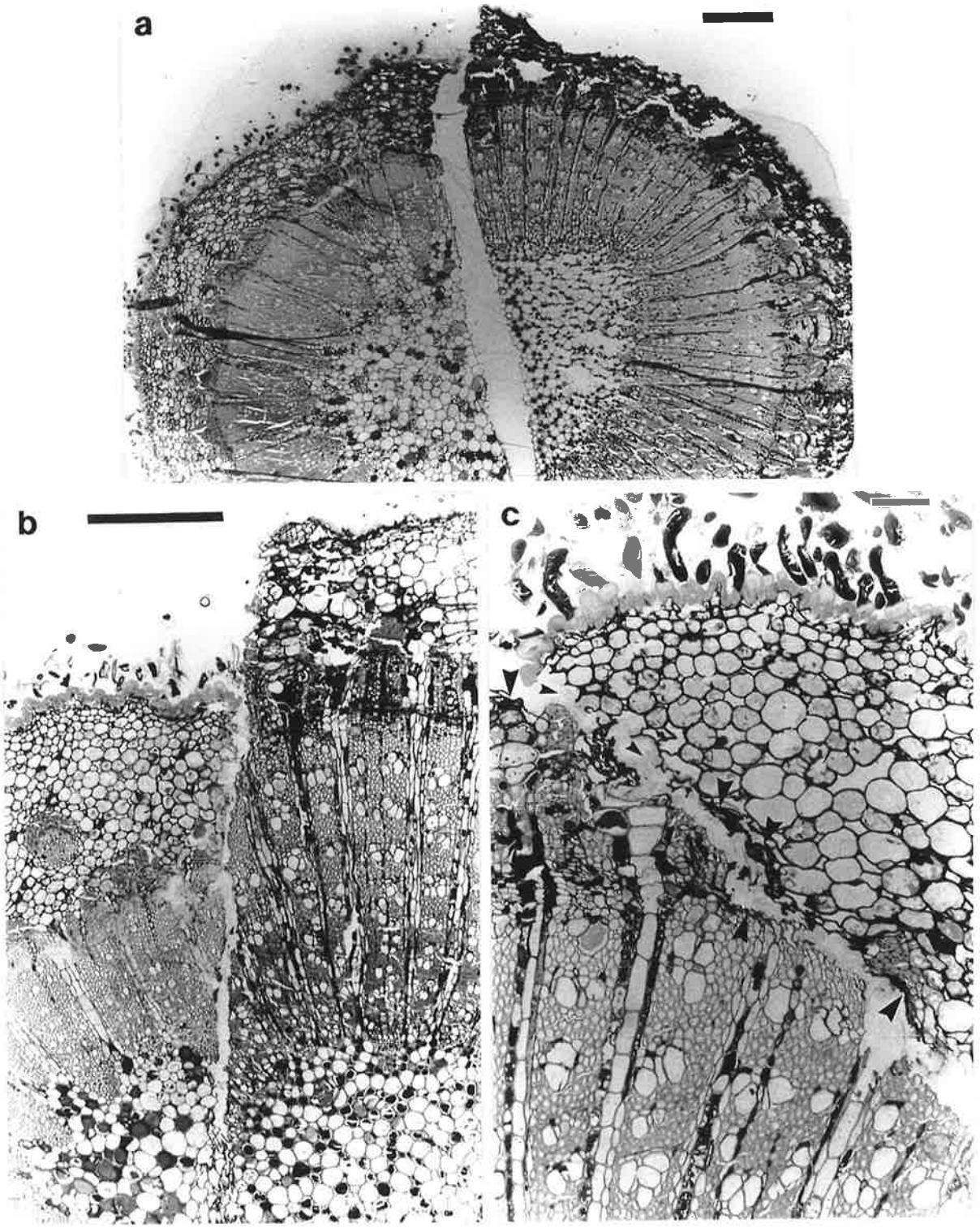

a
b
c

Plate 4.6 Light micrographs of transverse sections taken mid-way along the graft union

Cellular divisions and callus initiation

(a) *B. menziesii* scion (LHS) on *B. serrata* rootstock (RHS) two weeks after grafting. Note the irregular cell divisions (arrows) in the cortex and the region between the phloem fibre caps. Bar = 100 μm

(b) High magnification of (a) showing divisions in the interfascicular cambium and phloem cells (arrows). Bar = 100 μm

(c) Callus produced from the cortex (small arrow), cambium and phloem (large arrows) of *B. ericifolia* scion grafted onto a *B. serrata* (edge RHS) four weeks after grafting. Bar = 100 μm

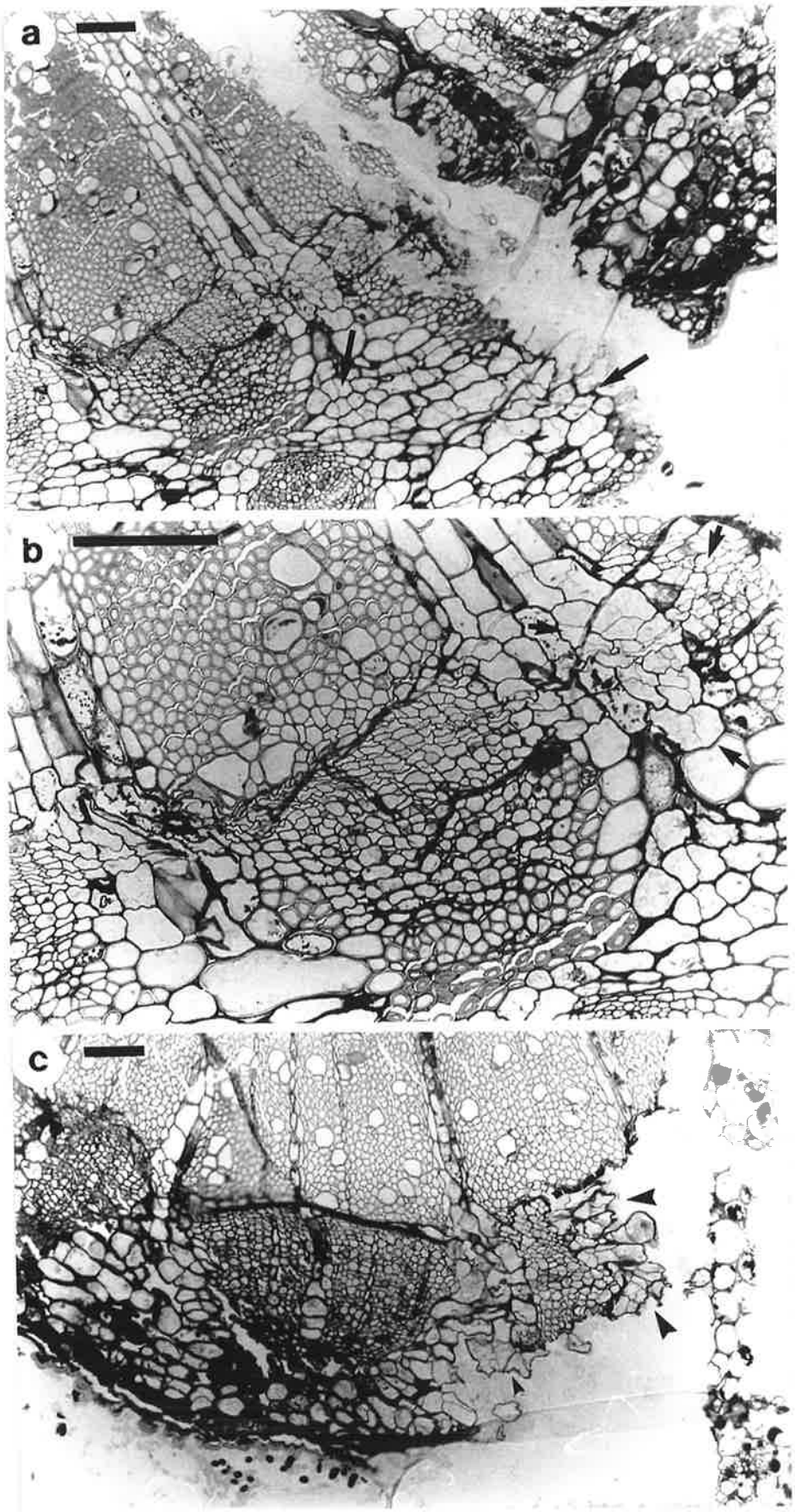

a
b
c

Plate 4.7 *B. ericifolia* grafted onto *B. serrata*, 12 weeks post-graft

Light micrographs of a graft of *B. ericifolia* scion (RHS of photomicrographs) grafted onto a *B. serrata* stock 12 weeks after grafting. Callus, initiated in the region of the scion cambium, phloem and cortex (large arrows) fill the space between the scion and rootstock (small arrow).

(a) Bar = 500 μm

(b) Bar = 100 μm

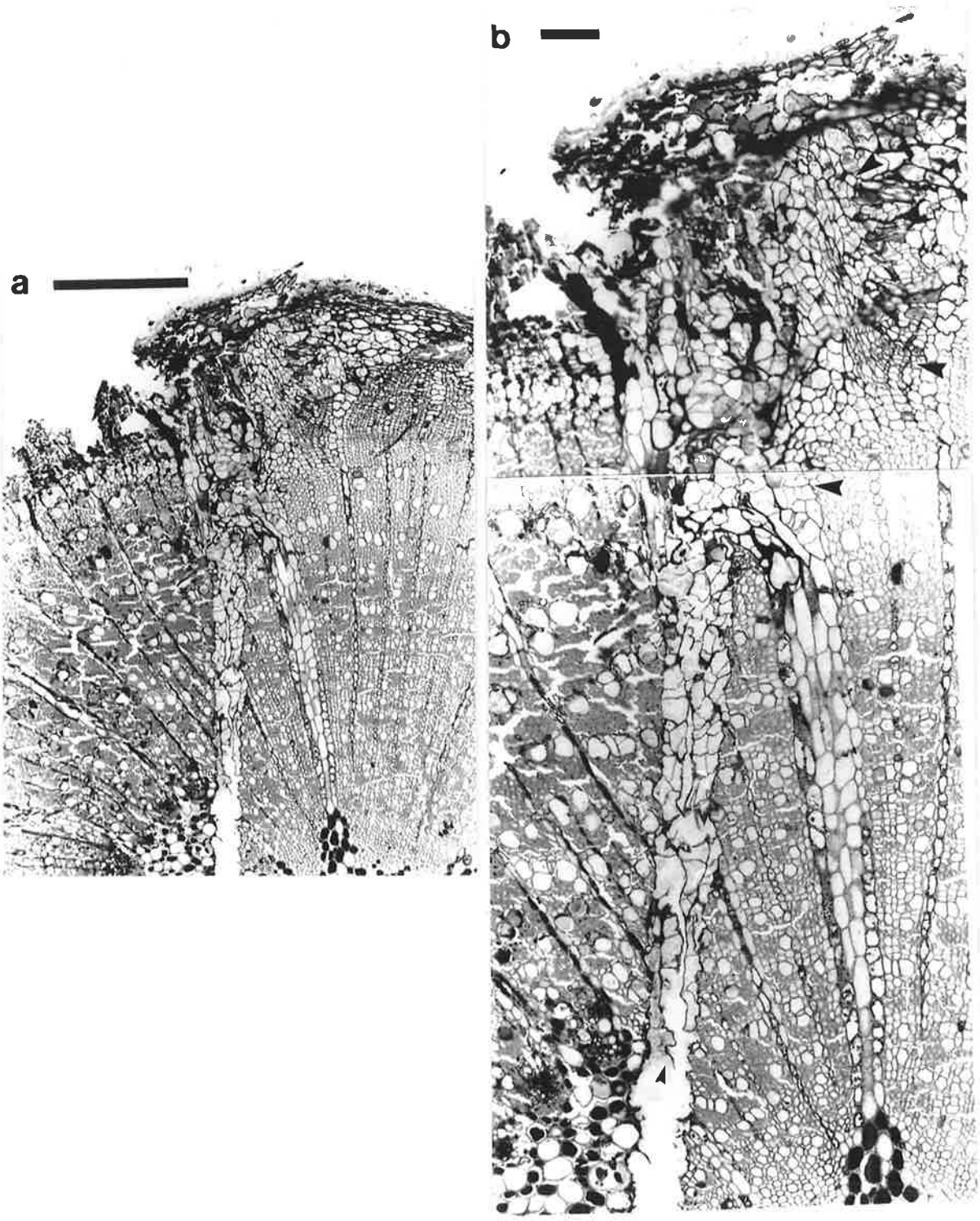

a
b

Plate 4.8 Light micrographs of transverse sections taken mid-way along the graft union

Collage of light micrographs of transverse sections of an intraspecific graft of *B. serrata* four weeks after grafting illustrating the close contact between the scion and rootstock tissues of the graft, however, without callus production. This graft received a (1,1) score (see Table 4.4) for scion and rootstock health. Arrows indicate the line of graft union. Note the cambia of the scion and rootstock are not aligned. Bar = 100μm

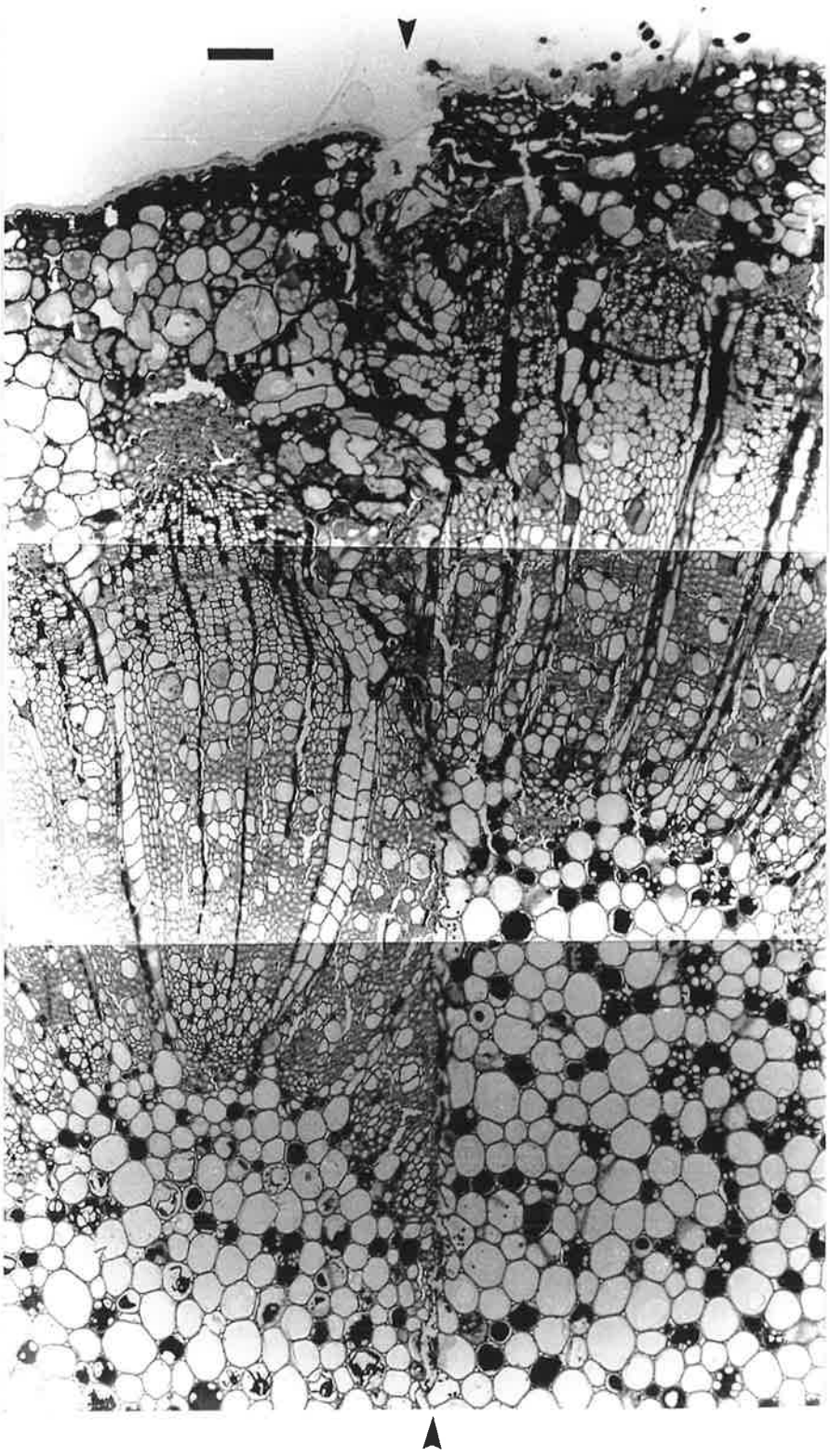


Plate 4.9 Grafting technique and bud burst

(a) "Mummy" graft of *B. ericifolia* on *B. serrata* nine weeks after grafting showing scion bud bursting (large arrow) through a single layer of *Nescofilm* used to wrap the entire scion. The small arrow indicates the distal end of the rootstock. Bar = 2 cm

(b) Buds bursting (large arrows) from the scion of an intraspecific graft of *B. serrata,* nine weeks after grafting. The small arrow indicates the distal end of the rootstock. Bar = 1 cm

(c) Grafted *B. serrata* rootstocks in 20 cm pots of (a) and (b) above. The LH pot is the *B. ericifolia* scion and RH pot is the intraspecific graft of *B. serrata.* Note the numerous lateral shoots arising from many of the leaf axils of the rootstock. Bar = 10 cm

(d) Method used to attach the scion to the rootstock (*B. spinulosa* var. *cunninghamii*). Firstly the two components were bound together using *Nescofilm* (small arrows). All leaves where removed from the scion. The scion was wrapped in a single layer of *Nescofilm*, as shown in (a) and (b) above, to reduce desiccation. Bar = 5 cm

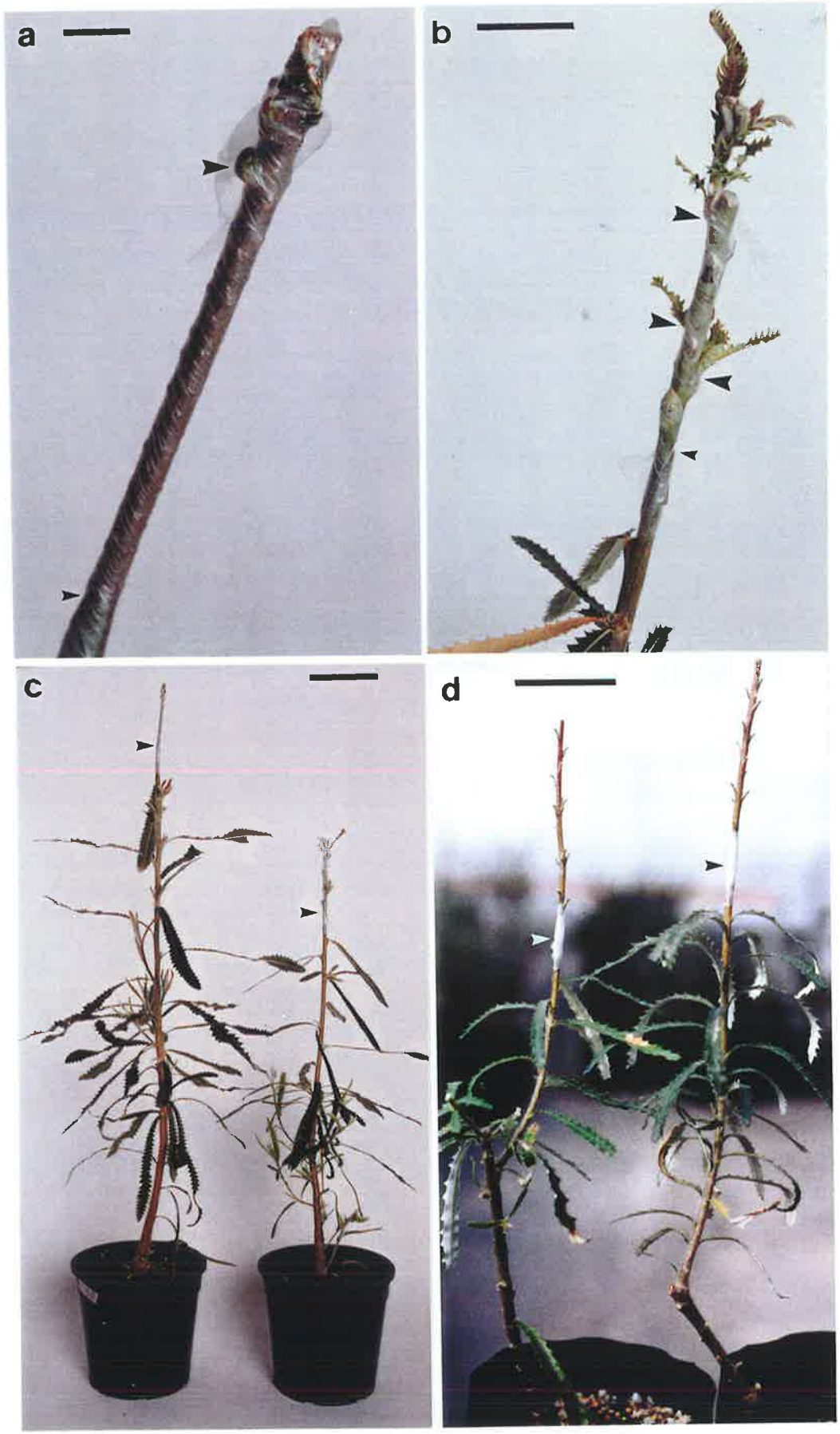

a
b
c
d

Plate 4.10 Intraspecific graft of *B. serrata* 18 months post-graft

(a) Successful intraspecific grafts of *B. serrata* greater than 2 m in height, 18 months post-graft. All basal shoots have been removed, allowing the scion buds to form the plant's canopy. The graft union is at the level of the yellow tie, about 80 cm above ground level. (Scale: 2 m measuring pole)

(b) Prolific basal shooting of a rootstock at ground level. Bar = 2 cm

(c) Prolific shooting of the rootstock at the "knuckle" (small arrows) - a region of short internodes, about 25 cm above ground level, which is established after the first season's growth. The basal shoots were removed shortly after grafting. The graft union is approximately 60 cm above ground level (outlined arrow). Scale in cm

(d) Close-up of the graft union (between the arrows) in (c). Bar = 2 cm


a
b
c
d

Discussion

The majority of scientific literature on grafting reports research on long-domesticated horticultural species, which have been highly selected and bred. These are mostly from the vegetable (Solanaceae, Crassulaceae), fruit (pome, stone and citrus), nut (almond, hazelnut, chestnut, walnut), amenity and forestry industries and use closely related genotypes as stocks and scions which results in high levels of grafting success. The research generally falls into two groups: research aiming to improve the empirical performance of the horticultural species used, or studies on the physiological and anatomical nature of graft formation and plant-plant recognition. This aim of this study aims at improving the empirical performance of interspecific grafts of *Banksia* species, which have undergone little selection or domestication, and which are taxonomically distant and geographically isolated. Hence, it is not unreasonable to expect low success rates in interspecific grafts of *Banksia*.

Intraspecific grafting of *B. serrata* was successful, but the low success rate for self- and intraspecific grafts may indicate the unsuitability of the whip method for grafting *Banksia*. Different methods have been shown to influence the rate of success (Skene *et al.* 1983; Wojtusik & Felker 1993; Boyhan *et al.* 1996; Pereira-Lorenzo & Fernandez-Lopez 1997). Alternative methods include a variation of the patch graft, known as the punch graft, which has been developed for *Macadamia* and is now routinely used in the Queensland *Macadamia* nut producing industry (Henry 1976; Stephenson 1980; Alexander 1986). A similar grafting technique modelled on this could be developed for *Banksia,* which may achieve greater success rates than currently possible. This method avoids mismatching of scion and stock tissues observed in this study.

Early events in graft development or failure were the same regardless of the scion-stock combination. However, the timing was different. Exudate and cellular debris, which remain on the cut surfaces during grafting, was observed in all graft combinations. This may serve some as yet unknown function in graft formation or failure. Cell necrosis was apparent along the cut edge of the cortex in both the scion and the rootstock by week two. This is due to the deleterious effects of normally compartmentalised cellular contents and the debris, which are produced on cutting of the wood tissues. Unlike mango (Asante and Barnett 1997) or *Prunus* (Errea *et al.*1994a), where the callus bridge was formed within 14 days, *Banksia* showed slower union development. The on-set of callus production had occurred in *Banksia* two weeks post-graft as irregular cell

division observed in both the scion and stock. These were most prevalent in the mid-cortex region and in the cortex between the phloem fibre caps close to the cut edges, but also observed in the phloem and cambium of the vascular bundles and interfascicular cambium. It is possible that these irregular cell divisions started earlier than two weeks after grafting. Altering the timing and conditions of grafting *Banksia*, such as different glasshouse temperature or season, may improve results. Slow callus development observed in *Banksia* may result in scion death before union establishment. Slow callusing had been suggested before as a cause of low root initiation success in cuttings (Davis *et al.* 1988).

In other species the initial divisions originate in living cells, which are actively dividing, or have recently divided or differentiated, e.g. cells in the cortex, phloem and interfascicular rays (Barnett & Weatherhead 1988; Errea *et al.* 1994a; Asante & Barnett 1997). Dividing cells did not form regular files of callus cells as observed in mango, where the first cell divisions were observed in the cortex and phloem ray cells three days after grafting (Asante & Barnett 1997). In *Banksia* grafts, irregular, amorphous callus was observed only at four weeks after grafting at the cut edge of the scion and stock. No cell divisions or callus production was observed in the xylem or pith regions, unlike mango grafts which formed callus from the pith (Asante & Barnett 1997). The amorphous callus, which filled the gap between the graft components in some combinations of *Banksia*, was derived from cells of the cortex, interfascicular cambium, phloem and cambium of the vascular bundles adjacent to the cut edge of both graft components, but more so in the scion of *B. ericifolia*. These cells produced continually dividing callus, proliferating past the xylem and pith and filling the gap between the grafted surfaces. Callus produced by *B. ericifolia* by week 12 was irregularly deposited, similar to that observed in incompatible grafts of *Prunus* (Errea *et al.* 1994a). However, due to the low overall success rate, it is possible that samples which had been sacrificed and observed by microscopy to produce callus may not have formed successful long-term grafts. Furthermore, the progression of graft development may vary over the graft interface. Observations made mid-way, as was undertaken, may not represent what is occurring at the distal or proximal ends of the graft union. More information could be gained by sampling at the distal and proximal end of the graft union where there may be an accumulation of hormones and nutrients, rather than midway along the graft.

The wounding response, and consequent callusing in *Banksia,* is much slower than in other commonly grafted plant species and varies greatly within the genus. *B. ericifolia*

is relatively quick to callus. This is reflected by the ease of cutting propagation of this species (Elliot & Jones 1982; Wrigley & Fagg 1989). Other species, such as *B. coccinea* and *B. menziesii*, require a greater length of time before callus is formed (Bennell & Barth 1987; Sedgley 1995b). Hence, in these species the vascular connections between the stock and the scion required in grafting would take longer to develop. A greater amount of callus is reported to be produced by the rootstock portion of the graft, a trend observed in this study. In some cases, the scion was supported by the rootstock through direct cellular contact and without the formation of a callus bridge. Apoplastic contact by the adherence of free cell walls via extracellular pectinacious beads, or symplastic cellular contact via plasmodesmata between the cell walls of cut surfaces of scions and rootstock, as seen in sitka spruce (*Picea sitchensis*) (Barnett & Weatherhead 1988), may have played a role.

From the material sampled in this study it was not possible to determine if a cambial bridge (Barnett & Weatherhead 1988) developed in the callus is present in successful unions. Cambial bridge formation involves linking the cambium of stock and scion, thus restoring the cambial continuum. Position of cells (relative to hormone and nutrient gradients) (Warren Wilson & Warren Wilson 1960) is one of the key determinants mooted for the *de novo* formation of cambia within newly formed callus in graft unions. Few *Banksia* graft unions produced callus, and those which did, developed very slowly. For members of this genus, sampling beyond the 12 week period would be necessary to study the *de novo* development of new cambia in the callus between the scion and rootstock, the establishment of vascular connections, and the later development of a periderm (Asante & Barnett 1997).

Prolific starch grains observed in some (*B. ericifolia, B. serrata, B. spinulosa*), but not in other (*B. coccinea, B. menziesii*) species used in this trial indicate differing metabolic status or seasonal accumulation of nutrients. The accumulation and storage of carbohydrate in the form of starch granules is desirable (Barth & Bennell 1987; Santamour 1988a), especially in the mother plants used as a scion source. Carbohydrate reserves are required for the maintenance of the scion until cambial bridging and vascular connections are established and buds have burst, providing a means for photosynthesis. This may account partly for the very low success rate of some species, such as *B. coccinea* and *B. menziesii*. Both these West Australian species may have different seasonal patterns of carbohydrate assimilation, and it may therefore be

necessary to determine those patterns in scion mother plants before deciding on the most suitable time to harvest scion material.

Although the cortex constitutes the greatest proportion of living tissues in early stem growth in all *Banksia* species as observed in Chapter 3, interspecific variation in the amount of cortex exists. The relative proportion of cortex is important in matching scion and rootstock tissues in grafting as seen here. The cells in the mid-cortex and cortex between the secondary phloem are sites of cell division and callus initiation. To achieve knitting of the living tissues of stock and scion in whip or similar grafts, optimising the contact between the cortex of the stock and scion should be maximised. This would be the case in interspecific grafts of *B. coccinea, B. menziesii* and *B. serrata,* which have comparable amounts of cortex. Mismatching and sub-optimal contact would occur if these species were grafted to species with narrow cortex regions, such as *B. spinulosa* varieties.

One of the distinguishing features of *Banksia* wood anatomy is the lack of continuous vascular cambium (Chattaway 1948b; Ilic 1991), where the phloem and xylem are arranged in discrete vascular bundles, which are separated by one or several rows of interfascicular cells. These discrete vascular bundles are clearly visible in young *Banksia* wood, becoming less well defined after the third growth season when progressive production of xylem compacts the interfascicular cells between the vascular bundles. The presence of discrete vascular units and the lack of continuous vascular cambium, as found in commonly grafted species, may be one reason for the difficulty encountered in grafting *Banksia*. Furthermore, it is possible that the radial vasculature reported elsewhere in some *Banksia* species, e.g. *B. serrata, B. aemula*, (syn. *B. serratifolia*), *B. grandis* and *B. ilicifolia* (Chattaway 1948a) may compound these difficulties. Radial vasculature was reported to penetrate the cambium in mature trees. However, this radial vasculature was not observed in the transverse sections of two year old wood in this study. Chattaway (1948a) also observed that the occurrence and amount of radial vasculature varied greatly in different species, and it may be rare in some of the species used in this study.

As the stem matures in woody angiosperms, lignification and the production of sclerenchyma and occluded cells in the cortex increases leaving fewer living cells as potential root initiation sites or callus initiation sites as required for grafting (Maynard & Bassuk 1996). Etiolation and partial shading of scion stock plants (Hartmann *et al.* 1990) is known to retard the sclerification process and may hence be beneficial in the

management of scion mother plants for use in the grafting of *Banksia*, the cortex of which has many sclerotic fibres and occluded cells (refer to Chapter 3). Sourcing young scion material at the end of the first season's rapid growth phase would also minimise the risk of using plant material with a high degree of cortical cell occlusion, especially in the region between the phloem fibre caps of neighbouring vascular bundles and the adjacent interfascicular region.

The confluence of cell contents from the cut cells at the surface of the scion and rootstock in grafted *Banksia* will most likely impair the functioning of intact cells immediate to the graft interface. Cyanogenic glycosides are known to occur widely in the Proteaceae (Everist 1974), especially the subfamily *Grevilleoideae* (Swenson *et al.* 1989), and have been shown to influence grafting in *Hakea* and *Grevillea* (Hale 1989). It is possible that these compounds, or other phenolics, accumulate at the graft interface in *Banksia* where they are exposed to the degradative enzymes (β-glycosidases and α-hydroxynitrile lyase) at a slightly acidic pH. Under such conditions cyanogens would be catabolised to generate the respiratory toxin, hydrogen cyanide, which would have a detrimental effect on the living cells involved in graft formation. Toxic cyanogens have been identified to be the causes of graft incompatibility in Rosaceae species (Gur & Blum 1973; Moore 1984d). Two such glycosides, dhurrin and proteacin, have been identified in the family Proteaceae (Swenson *et al.* 1989; Lamont 1993; Vivian-Smith 1995). Dhurrin has been localised predominantly in the vegetative organs (Swenson *et al.* 1989), and cyanogenicity is greatest in young leaves (Lamont 1993; Dahler *et al.* 1995). Although examination of sixteen species of *Banksia* showed none to be cyanogenic (Swenson *et al.* 1989), further research is needed to determine whether other *Banksia* species are cyanogenic, and if so, the age and type of tissue in which the compounds are concentrated and how they are metabolised. Then it may be possible to assess whether these compounds play a role in graft physiology in *Banksia*.

To summarise, the stages of successful graft formation in *Banksia* are the production of cellular debris, exudate and cell necrosis by two weeks, the occurrence of irregular cell divisions by week two and the on-set of callus production by week four, depending on the species, followed by callus proliferation by week twelve. In unsuccessful *Banksia* grafts the events observed in the early stages of graft decline are exudate production and cell necrosis, followed by irregular cell divisions and callus development by two weeks, which was not sustained over the long term. Collapse of the cambium and cortical cells was apparent by week two.

Considering the taxonomic relationship of species used in interspecific grafts, it is possible to speculate on the likelihood of the success or failure of a particular combination. Species from the same series, such as *B. menziesii* and *B. serrata* from series *Banksia*, might be expected to have a chance of successful union, but at nine weeks post-grafting all scions had withered. The unclear taxonomic position of *B. coccinea* in the *Banksia* genus makes it difficult to select closely related species as recipient rootstocks for this commercially valuable species. A recent reclassification places *B. coccinea* into a monotypic section, *Coccineae* - a sister section to section *Oncostylis* (Maguire *et al.* 1996) - based primarily on lower stylar compatibility. Thus, *B. coccinea* grafted with members of section *Oncostylis*, such as the rootstock species *B. spinulosa* var. *cunninghamii* (series *spicigerae*), should be further trialed.

Recommendations and future research direction

This study provides fundamental information on graft development in *Banksia* and may provide a basis for future grafting work and commercial development of *Banksia* species. Further grafting systems must, however, take a multi-faceted approach looking at (i) plant chemistry, (ii) grafting technique and conditions, and (iii) nature of both scion and rootstock plant material.

Firstly, a greater understanding is required of the seasonal and developmental fluctuations in internal levels of compounds influencing graft success (carbohydrates and starch, hormones (auxins, cytokinins), cyanogenic glycosides, cyanolipids or phenolics) and whether practices like etiolation, banding, shading and cincturing are beneficial. This would facilitate improved selection and management of source plants and the collection of plant material at the most suitable time and age for grafting. A histological study of grafts in the closely allied *Macadamia* would be beneficial to understanding the anatomy and physiology of successful grafts in the *Banksia* genus.

Secondly, optimal grafting methods must be developed for *Banksia* species, such as (punch) budding or grafting. Understanding seasonal growth patterns is crucial for determining the correct timing for grafting, as well as for bark-slip which is necessary for budding or patch grafts. Immediate post-graft conditions and management of plants (such as misting, temperature, day length and disbudding practices) must also be optimised. A successful technique of punch budding has been established for grafting *Macadamia;* it may be possible to apply this system to members of the *Banksia* genus.

Thirdly, research on and development of appropriate plant material must occur. Rootstocks need to be systematically developed together with scion material. Selection and hybrid breeding programs must focus on generating novel scions (e.g. flower colour and form, flowering time), together with improved rootstocks. Clonal hybrid rootstocks may be necessary to increase the chances of successfully grafting closely related scion genotypes on a commercial scale, and thus avoiding the variability associated with seedling stocks. Selection and breeding programs must focus on genotypes that are high-yielding (vigorous growth and flowering), disease resistant and easy to clonally propagate (high rooting potential), but also which are graft compatible. Whether the ontogenetic age of the source material plays a role, i.e. juvenile compared to mature plant material, also requires further research. Selection and development for both scion and rootstock genotypes will be imperative to the successful development of this industry into the next millennium.

Chapter 5

Lignotubers in *Banksia*

Abstract

The first report on the anatomical structure and early development of cotyledonary nodes and lignotubers in *Banksia* is presented. Serial sectioning through cotyledonary nodes of post-emergent seedlings of non-lignotuberous *B. serrata* and lignotuberous *B. menziesii* was undertaken. In *B. serrata* sampled at 15 weeks exogenous axillary buds are present in the cotyledon and leaf axils, the buds comprising of a bud apex and prophylls; the base of the cotyledons were not fused, and accessory and adventitious buds were not observed.

In *B. menziesii* sampled at eight and 26 weeks the fused base of the cotyledons forms a thick sheath of parenchymatous tissue around the stem, creating a narrow encircling lumen between the protective sheath and the stem in which three types of buds arise. Exogenous axillary buds arise in the axils of the each cotyledon and first true leaves. Endogenous accessory buds arise in the cortical tissue at either side of the axillary buds. Exogenous adventitious buds on the adaxial surface of the protective sheath are generated serially from meristematic tissue along the fusion line of the bases of the cotyledons on each side of the stem axis. In addition, adventitious buds occur singly on the adaxial wall of the sheath facing the lumen in close proximity to vascular bundles. After 26 weeks phenolics and tannins accumulate in the outer sheath in *B. menziesii,* which later becomes a woody encasement protecting the hidden axillary, accessory and adventitious buds.

Seedling habit and growth of the lignotuberous species *B. menziesii*, which has hidden buds and *B. spinulosa* var. *spinulosa*, which has visible superficial buds, differed from one another and from *B. serrata* and *B. spinulosa* var. *cunninghamii* which are not lignotuberous. Although the length of the hypocotyl remains more or less constant, the dimensions at the cotyledonary node increase in both lignotuberous and non-lignotuberous varieties, particularly in lignotuberous species.

Removal of apical dominance in *B. spinulosa* var. *spinulosa* by pruning accelerates extension growth of pre-existing lignotuber shoots; lignotuber bud growth is influenced by seasonal changes, increasing rapidly during autumn, at a slower rate during spring and over summer it was almost negligible.

This study confirms that the *Banksia* cotyledonary node is an organ bearing buds ready for rapid regeneration after destruction of aerial plant parts; it later constitutes the lignotuber.

Introduction

Literature on *Banksia* lignotubers has addressed their presence or absence within the genus (George 1987; Taylor & Hopper 1988; Thiele 1993), or their ecological role (Lamont *et al.* 1994; Richardson *et al.* 1995), particularly in regard to regeneration after fire, as either sprouters or seeders (Zammit 1988; Pate *et al.* 1990; Bowen 1991; Bowen & Pate 1993). The bulk of literature on lignotuber anatomy, development and biogeography is based on eucalypt species (Kerr 1925; Chattaway 1958; Ladiges 1974; Ladiges & Ashton 1974; Bamber & Mullette 1978a, b; Mullette 1978; Carr *et al.* 1984a, b; Myers 1995). This report presents anatomical descriptions of early lignotuber structure in *Banksia menziesii*, comparing it with *B. serrata*, a non-lignotuberous species, which sprouts from epicormic buds along the stem. In addition, exploratory data on early seedling growth and habit of four *Banksia* species, together with the response of *B. spinulosa* var. *spinulosa* to pruning are presented. The emphasis on vegetative propagation in preceding Chapters will be continued here, as lignotuber resprouting in *Banksia* is potentially a means of vegetative propagation of species within the genus. There is also potential to take advantage of the lignotuberous trait in other horticultural applications, as discussed later.

This introduction presents a definition of lignotubers as they are currently understood and overviews the state of knowledge on their occurrence in the Australian flora, their morphology in *Banksia* and their anatomical structure in *Eucalyptus*. Finally, aspects relevant to the use of lignotubers in horticultural applications, such as vegetative propagation, selection and breeding programs are presented.

Definition of the term 'lignotuber'

The term 'lignotuber' was coined by Kerr (1925) to describe the swellings at the base of the stem in many eucalypts and angophoras, which, in earlier works reviewed by her, were widely thought to be a pathological phenomenon. However, she notes the observations made by another author (Jönsson, 1901, reference not sighted) where lignotubers were reported to develop under conditions of unfavourable nutrition, and removal of leaves, buds and branches accelerated their growth. The usage of the term 'lignotuber' is generally associated with eucalypts. This is reflected in the literature on lignotubers, which is primarily on eucalypts in the Australian flora. The following

definition from the botanical dictionary of the Society for Growing Australian Plants also reflects this:

> "*Lignotuber*: of eucalypts and other myrtaceous plants, a conspicuous swelling at the base of the stem, at or below soil-level, bearing dormant buds. An adaptive feature of survival value, the development of the buds to suckers is stimulated by destruction or loss of top growth." (Debenham 1970)

Pate and Beard (1984) consider the lignotuber as a specialised root structure for the purposes of their survey of West Australian sand plain (Kwongan) flora. Although lignotubers arises from the lower stem or hypocotyl and is therefore not strictly a root, they define them as woody, roughly spherical swellings of noticeably greater diameter than the roots and shoots which emanate from them, located just beneath the soil surface. George (1987) similarly defines the lignotuber as a woody swelling just below ground level which can produce new shoots and roots after fire, giving the example of a mallee root. James (1984) suggested that the term 'lignotuber', rather than 'burls' - a vague term describing any woody structure with a swirled grain - be used to describe the ontogenetically produced, swollen stem base or root crown occurring in these plant families. Her review further highlights how little is known on Australian proteaceous lignotubers, because only five South African species, four *Leucadendron* and one *Protea,* are listed in her table of families of lignotuberous woody plants. The only Australian examples cited are eucalypts; *Banksia* is not mentioned. This report is timely since it addresses the vast gap in our knowledge on the anatomy and function of lignotubers in *Banksia*.

Occurrence, function and size of lignotubers

Lignotubers occur in families in mediterranean ecosystems in various parts of the world (Californian chaparral, South African fynbos, Chilean matorral, European macchia and Australian Kwongan) (James 1984). In Australia, lignotubers are one of several highly specialised modifications widely occurring in endemic flora. They are found in genera of the families Proteaceae, Myrtaceae, Casaurinaceae and Epacridaceae, as indicated in a survey of 429 plants species of the Kwongan, the indigenous term for the mediterranean, sand plain communities of sclerophyll shrubland in south west Australia (Pate & Beard 1984). Lignotubers were found in 24 out of the 74 proteaceous species from ten genera: *Grevillea, Hakea, Isopogon,*

Dryandra, Petrophile, Lambertia, Adenanthos, Persoonia, Conospermum and *Banksia*. In family Myrtaceae eleven *Eucalyptus* species, two species each of *Melaleuca* and *Calothamnus,* and one species each of *Darwinia, Verticordia* and *Eremaea* were lignotuberous, as well as one species from each of the families *Casuarinaceae* and *Epacridaceae*, i.e. *Allocasuarina* and *Conostephium*. The 44 lignotuberous species accounted for one quarter of the 163 species of woody shrubs and tree species in the survey.

Root morphology is highly consistent at a species level, but a genus or family can display a range of rooting patterns. In a taxonomic sense, Western Australian species known to bear some form of specialised subterranean storage organ are not common, representing ca. 1.4% of dicotyledons, 9.8% of monocotyledons and 5.8% of ferns and gymnosperms (Pate & Dixon 1982). In the Dicotyledoneae, schlerophyllous woody shrubs and trees of the families Proteaceae, Myrtaceae, Fabaceae, Malvaceae and Sterculaceae, are more likely to have specialised underground storage organs that are lignotuberous.

In the genus *Banksia*, at least half of the species are lignotuberous (Thiele 1993). George (1987) reports 36 species have fire tolerant trunks or lignotubers, with bark at least 1 cm thick. Table 5.1 shows *Banksia* taxa known to be lignotuberous. The distribution of lignotuberous taxa throughout the different series in the taxonomic groups indicates there is no direct phylogenetic relationship between lignotuberous species. Generally, lignotubery is thought to have evolved at several times in different taxa (James 1984). Furthermore, there is no association with east or west distribution of species. Lignotuberous *Banksia* species are not limited to a specific habitat, as species range in distribution from tropics (*B. dentata*), to swamps (*B. robur*), to dense temperate forests (*B. spinulosa*), the arid zone (*B. audax*), exposed coastal aspects (*B. marginata*) and high altitudes (*B. spinulosa* var. *neoanglica*). Within the range of distribution of a species there can be marked differences in the lignotuberous phenotype. This is exemplified by *B. menziesii,* which occurs as a (lignotuberous) mallee shrub at the xeric end of its distribution, north of Perth in Western Australia. In the xeric environs, temperatures are higher, fires are more prevalent and the height of this species declines. Where fires are rare or absent, it retains its dominant trunk, developing into a tree (Lamont *et al.* 1994). Similar observations on lignotuber variability within *Eucalyptus viminalis* showed that low rainfall populations display

greater lignotuber development than those from high rainfall areas (Ladiges 1974; Ladiges & Ashton 1974).

Interestingly, Series *Prostratae* is the only series where all species are lignotuberous, which may indicate a relationship between the prostrate and lignotuberous habit. These species also have the largest cotyledons within the genus (Thiele & Ladiges 1996). Since lignotubers develop at the cotyledonary node at the point of attachment of the cotyledons, the large size may be advantageous in lignotuber development. Moreover, the large thick cotyledons would afford greater protection to hidden buds than thinner cotyledons with a narrow point of attachment. Of the lignotuberous species listed in Table 5.1, seventeen have large, flabellate cotyledons, whereas the other 14 have narrower, spathulate cotyledons. Although the cotyledons are generally larger than average, no one type of cotyledon appears to be associated with the lignotuber trait.

The presence or absence of the lignotuberous habit is consistent in all species of *Banksia*, with the exceptions of *B. marginata* and *B. violacea*, some populations of which are lignotuberous and some are not (Thiele 1993) (Plate 5.2).

Most of the species which are horticulturally important cut-flower species are non-lignotuberous and are from three series in Section *Banksia*, i.e. *Crocinae, Banksia* and *Cyrtostylis*, the latter two series having a mixture of lignotuberous and non-lignotuberous species. All four species in Series *Crocinae: B. prionotes, B. victoriae, B. hookeriana* and *B. burdettii* are cut-flower species (Karingal Consultants 1994) and non-lignotuberous. The majority of plantations of these four species are based on seedlings, as clonal propagation is difficult. The large colourful floral displays of these species, which have lead to their development, are important to the sexual reproductive success of these essentially reseeder species. Of the horticulturally important species, those reproducing from seed generally produce flowers earlier (<5 years) than species regenerating from lignotuber resprouts (9 – 10 years) (Table 5.1).

Table 5.1 Lignotuberous species of *Banksia*

Taxonomy[1]	Lignotuberous[2] habit		Lignotuberous species & years to flowering[3] in brackets		R a n g e	Species important to amenity and ornamental horticulture[4]
Subgenus *Isostylis*	-	0/3[5]				
Subgenus *Banksia*						
Section *Banksia*						
Series *Salicinae*	+/-	4/10	*B. marginata**		E	
			B. paludosa (10)		E	
			B. oblongifolia (5 – 7)		E	
			B. robur (3 – 5)		E	
Series *Grandes*	-	0/2				
Series *Quercinae*	-	0/2				
Series *Bauerinae*	+	1/1	*B. baueri* (5 – 7)		W	
Series *Banksia*	+/-	2/8	*B. menziesii* (6 – 10) †		W	*B. speciosa* (3 – 4)
			B. candolleana (10)		W	*B. baxteri* (3 – 4)
						B. sceptrum (3 – 4)
Series *Crocinae*	-	0/4				*B. prionotes* (3 – 5)
						B. hookeriana (3 – 4)
						B. burdettii (3 – 4)
						B. victoriae (3 – 4)
Series *Cyrtostylis*	+/-	6 or 7/13	*B. attenuata* (10) †		W	*B. praemorsa* (4 – 5)
			B. lindleyana (6 – 8)		W	*B. ashbyi* (3 – 5)
			B. benthamiana (4 – 5)		W	
			B. audax (10)		W	
			B. lullfitzii (10)		W	
			B. elderiana		W	
			B. elegans (10 – 15)			
Series *Prostratae*	+	6/6	*B. goodii* (6 – 8)		W	
			B. gardneri	var. *gardneri* (4 - 6)	W	
				var. *heimalis*	W	
				var. *brevidentata*	W	
			B. chamaephyton (8)		W	
			B. repens (6 – 8)		W	
			B. blechnifolia (4 – 5)		W	
			B. petiolaris (3 – 5)		W	
Series *Tetragonae*	-	0/3				
Section *Coccineae*	-	0/1				*B. coccinea*
Section *Oncostylis*	+/-					
Series *Spicigerae*	+/-	1/8	*B. spinulosa*	var. *spinulosa* (8)	E	*B. ericifolia* (4 – 6)
				var. *collina*	E	*B. spinulosa* var. *cunninghamii* (8)
				var. *neoanglica*	E	
Series *Dryandroideae*	-	0/1			E	
Series *Abietinae*	+/-	5/13	*B. sphaerocarpa*	var. *sphaerocarpa* (8 – 10)	W	
				var. *caesia*	W	
				var. *dolichostyla*	W	
			B. micrantha (> 5)		W	
			B. grossa (> 5)		W	
			B. incana (> 5)		W	
			B. violacea (> 4 - 5) *		W	

[1] After George (1981, 1988), Maguire (1996)
[2] Source: Thiele & Ladiges (1996)
[3] Years from seed to flowering, after George (1987)
[4] RIRDC report by Karingal Consultants (1994)
[5] Number of lignotuberous species of the total number in the series
E, endemic to east Australia
W, endemic to west Australia
* polymorphic populations with or without lignotubers
† ornamental cut-flower species

Intervarietal differences in the lignotuberous predisposition exist in *Banskia*. Varieties from three species: *B. gardneri, B. sphaerocarpa* and *B. spinulosa* all exhibit the lignotuberous habit (Table 5.1), except for *B. spinulosa* in which one of the four varieties, *B. spinulosa* var. *cunninghamii,* is not lignotuberous. This variety regenerates from seed (Taylor and Hopper, 1988; George 1987).

Lignotubers have two main interrelated functions as regenerative and storage organs. Usually underground and referred to in the literature as the root crown, the specialised lignotuberous structure has the functions of vegetative regeneration after the aerial parts of the plant have been defoliated, or destroyed, usually by fire. Defoliation or damage to the aerial parts of the plant stimulates series of shoot-buds on the upper surface of the lignotuber to sprout, thus giving it the capacity to rapidly regenerate a new shoot system (Chattaway 1958; James 1984; Pate & Beard 1984). Plants that resprout in this manner are long-lived slowly reproducing individuals, typically with a very high success in self-replacement after fire and utilising limiting environmental resources with high efficiency in high density situations (Pate & Beard 1984). The rapid regeneration after defoliation, usually by fire, is supported by the reserves of stored nutrients conserved by the plant in the woody thickened root crown or the lignotuber. In *Eucalyptus obliqua* it has been concluded that the lignotuberous tissue is not more efficient than roots or stem as storage tissue and that the prime function is related to the large number of buds in a protected osition (Carrodus & Blake 1970). The fire resistant trunks, as well as woody lignotubers of resprouter species may serve as nutrient reserves. There are few data on the composition of lignotubers, however, in mature proteaceous plants the accumulation of phosphorus as polyphosphates in lignotubers has been recorded (Jeffrey 1968) and it is likely that there is an increase in storage products with age. In *Eucalyptus gummifera* starch was identified in the axial and radial parenchyma of the sapwood and the phloem of the lignotuber, twice as much as in the stem, giving a greater starch storage capacity. These reserves decline during rapid growth and can become completely depleted (Bamber & Mullette 1978a).

Lignotuberous species all exhibit similar root morphology, consisting of a woody taproot, with horizontal side branches of similar diameter (Pate & Beard 1984). Root depth is correlated to sprouting ability in chaparral plants; species with root systems deeper than 3.5 m are sprouters (James 1984). Lignotuber size, and hence sprouting

ability, depends on age and species. Sizes range from 10 cm to 1 m, weighing several kilograms in eucalypts; the size of the subterranean lignotuber of *Eucalyptus gummifera* was estimated as covering 75m^2 (Mullette 1978). *Banksia oblongifolia* lignotubers in the field have been reported to be covering an area greater than10 dm^2 (Zammit 1988).

Morphology and anatomy of lignotubers

Lignotubers in the *Banksia* genus display two widely differing structures distinguished by the type of buds and their position on the stem. In the first type, the buds are on the surface visible to the naked eye, and are positioned at the cotyledonary node or there and also at the lower leaf nodes. Species with superficial epicormic buds visible to the naked eye are exemplified by the lignotuberous *B. spinulosa* varieties: *spinulosa*, *collina* and *neoanglica,* and also by *B. robur* (Plate 5.1). In *B. robur*, buds and occasionally inflorescences are located along the lower stem (Plate 5.1b) as well as at the cotyledonary node. These structures are clearly visible and located above ground level.

The second type of bud structure is concealed beneath the surface at the cotyledonary node only, its presence noticeable by the swelling and uneven surface of the top of the hypocotyl at the cotyledonary node in seedlings, as exemplified by *B. menziesii*. The latent buds are hidden in a woody swelling, which develops at the cotyledonary node and forms the lignotuber (Plate 5.5 c and d). Generally, these bud structures are woody and subterranean in the adult plant. Both types of latent bud-containing structures have the prime function of resprouting after disturbance of the aerial parts of the stem. The different forms appear to be survival adaptations, having developed under different environmental conditions. The woody, subterranean-type gives maximum protection to species from xeric climates with high temperatures and low soil moisture, which are prone to fire. Superficial dormant buds appear in species from mesic climates receiving higher rainfall, such as in forest or other moist habitats. It remains to be determined whether the component buds of either form are ontogenetically, anatomically or physiologically similar.

As the ability of lignotuberous structures to sprout is influenced by plant age, lignotuber size and root depth (James 1984), the potential maximum number of buds formed may also be influenced by these factors. Chattaway (1958) concluded from her anatomical study of 47 eucalypts that only those species with concealed buds below as well as above the axillary buds on the main stem had the capability to form lignotubers at the

cotyledonary node and the first true leaf nodes. In *Banksia* little is known of the bud composition at leaf nodes. The phyllotaxy of leaves along the stem is "scattered", sometimes crowded (George, 1987), and rarely in whorls (as in *B. occidentalis, B. verticillata, B. seminuda, B. littoralis*). In *B. serrata* the leaf node morphology is trilacunar, that is having three leaf gaps (Metcalfe & Chalk 1979). In this species a maximum of three shoots develops at leaf scars along the main stem (personal observations from grafting experiment reported in Chapter 3). This species also has a thick bark, and leaf scars are not visible on the surface. However, as in the development of epicormic buds in eucalypts (Chattaway 1958), leaf scars no longer visible on the stem surface are the sites of dormant epicormic buds, remaining beneath the surface and supplied by vascular traces, which can be observed in longitudinal sections. The exact anatomy of buds in *Banksia* is unknown and requires further anatomical observations, and so it is not possible to draw a conclusion similar to that made by Chattaway (1958) on the relationship between concealed buds present either side of the axillary buds and the ability to form lignotubers. The fact that most *Banksia* species form lignotubers only at the cotyledonary node may indicate that this relationship is not critical in the *Banksia* genus.

As the only anatomical detail on lignotubers is for eucalypts (Kerr 1925; Chattaway 1958; Carrodus & Blake 1970; Bamber & Mullette 1978a; Mullette 1978; Carr *et al.* 1984a, b), the main aspects of their anatomy will be briefly overviewed focusing on those parts relevant to this study.

The buds described in eucalypts can be grouped broadly into naked and concealed buds (Chattaway, 1958). Naked buds occur at the stem apex and in the leaf axils (axillary buds) and contribute to rapid growth in height in young plants. The concealed buds, on the other hand, serve as replacement buds enabling rapid regeneration after fire or defoliation by other means. The origin of these buds is a patch of meristematic tissue located in the axil of naked axillary buds and their subtending leaves; they often occur on the other side of the axillary shoot or bud as well, between it and the main stem. However, the concealed buds of the cotyledonary node and the first few true leaf nodes constitute the developing lignotuber. Chattaway concludes that the predisposition for lignotuber formation in eucalypts is the presence of a double set of concealed buds (i.e. on either side of the naked axillary buds found in leaf axils, the leaves occurring in pairs, opposite one another at the leaf node). Most concealed buds develop exogenously,

arising from the cortex in very young lignotubers, finally located on the surface. Otherwise they develop endogenously, arising from the phloem in older lignotubers, but remaining beneath the surface surrounded by tissue.

In eucalypts the lignotuber appears as a swelling of the stem above the axillary bud group. During further growth the cambium extends into this swelling, the meristems of concealed buds are induced and further buds develop around the periphery at the base of existing buds (exogenous) or deep with in the cortical or phloem tissue of the lignotuber (endogenous). Other buds develop in the axils of the existing ones, forming bud clusters, which continue to proliferate, constituting the permanent bud tissue of the lignotuber.

A protective flap of regular rows of tannin filled cells surrounds young concealed buds. It is often difficult to distinguish the difference between endogenous and exogenous buds as they are set deeply in the leaf axils protected by this darkly staining flap, where the base of the leaf petiole and the axillary shoot are pressed closely together.

The time taken for lignotuber development varies with the season, species and location. They can develop within a few weeks in summer, rapidly becoming lignified, whereas in winter the time required for lignotuber swelling to occur is delayed.

Vascular strands, which develop to supply the meristematic patches that form the concealed buds at leaf nodes persist in mature trees, whereas the traces to the axillary bud and petiole are eliminated. The meristematic patch, the eventual site of an epicormic bud, is immediately outside the strand, and is almost perpendicular to the main stele in older stems. The leaf and shoot scars are eliminated as the periderm forms. The remaining meristematic patches rapidly form buds when apical dominance is removed by defoliation of the main stem and the rudimentary strand subtending it connects to the vasculature of the newly developing leaf bud. In this manner, epicormic sprouts can form within a very short period of time.

The difference observed in eucalypt stem and lignotuber anatomy is quantitative rather than qualitative (Chattaway 1958; Bamber & Mullette 1978a).

Horticultural application and genetics

In eucalypts, lignotubers are used in commercial plantations for the production of juvenile foliage for the cut-flower industry (Plate 5.3). The regenerative potential of the

lignotuber at the cotyledon and within the first 1 m of the base is the basis of continued production, and buds can be induced continually over numerous seasons to produce uniform foliage products. However, basal sprouting may be undesirable in other horticultural production systems, and can be circumvented by using cutting propagation, the lignotuber not forming in the cutting derived plant. In vegetative propagation tissues arising from the base of the main stem are considered juvenile and have a greater propensity to form root initials. As lignotuberous tissues are derived from the cotyledonary node, or the first few leaf nodes in eucalypts, they are ontogenetically young. Shoots arising from lignotubers would also be ontogenetically juvenile and also have a greater rooting potential compared to ontogenetically older material taken from higher on the source plant. This has not been tested in *Banksia*, but if it is the case, lignotuberous mother plants managed appropriately by pruning and grown under optimal conditions would be a source of uniform propagules for a specific genotype. Such a system is ideal for clonal propagation and could be applied to the production of clonal rootstocks or desirable genotypes for pot plants or cut-flower production. Generally, the lignotuberous trait has not been observed in the plants arising from rooted cuttings taken from lignotuber shoots. This is of importance in clonal rootstock propagation where basal sprouting is not favoured because it sequesters nutrients required for graft formation and scion establishment.

Selection and breeding of *Banksia* could possibly be used to introduce the lignotuberous trait into non-lignotuberous species (Brits *et al.* 1986). This would be useful in the clonal propagation of the majority of cut-flower species that are non-lignotuberous (Table 5.1) and difficult to propagate.

When present in a species, it appears that the expression of the lignotuberous trait is highly regulated and tissue specific, being expressed in ontogenetically young nodal tissue. It is only observed at the cotyledonary node and first few leaf nodes, where the trait is maintained and continues to function in the intact plant. Rooted cuttings taken from lignotuberous shoots do not, however, develop lignotubers. Hence there is a high tissue specificity and stringent regulation in ontogenetically older plant material. *B. spinulosa* var. *spinulosa* and *B. spinulosa* var. *cunninghamii* provide the basis of a system where the genes involved in lignotuber formation can be studied, isolated and identified using molecular biology techniques. Overlying these genetic controls are environmental factors (temperature, fire, etc.) which exert considerable effects on the

Plate 5.1 Superficial lignotuber buds in *B. robur* and *B. spinulosa*

a) *B. robur* lignotuber buds on a two year old potted seedling. Bar = 1 cm, scale in cm

b) Superficial buds long the stem of an established tree of *B. robur*; note the inflorescences (arrows). Bar = 5 cm

c) Cotyledonary node of a pruned *B. spinulosa* var. *spinulosa* plant (2½ year old pot plant from pruning trial). Bar = 1 cm

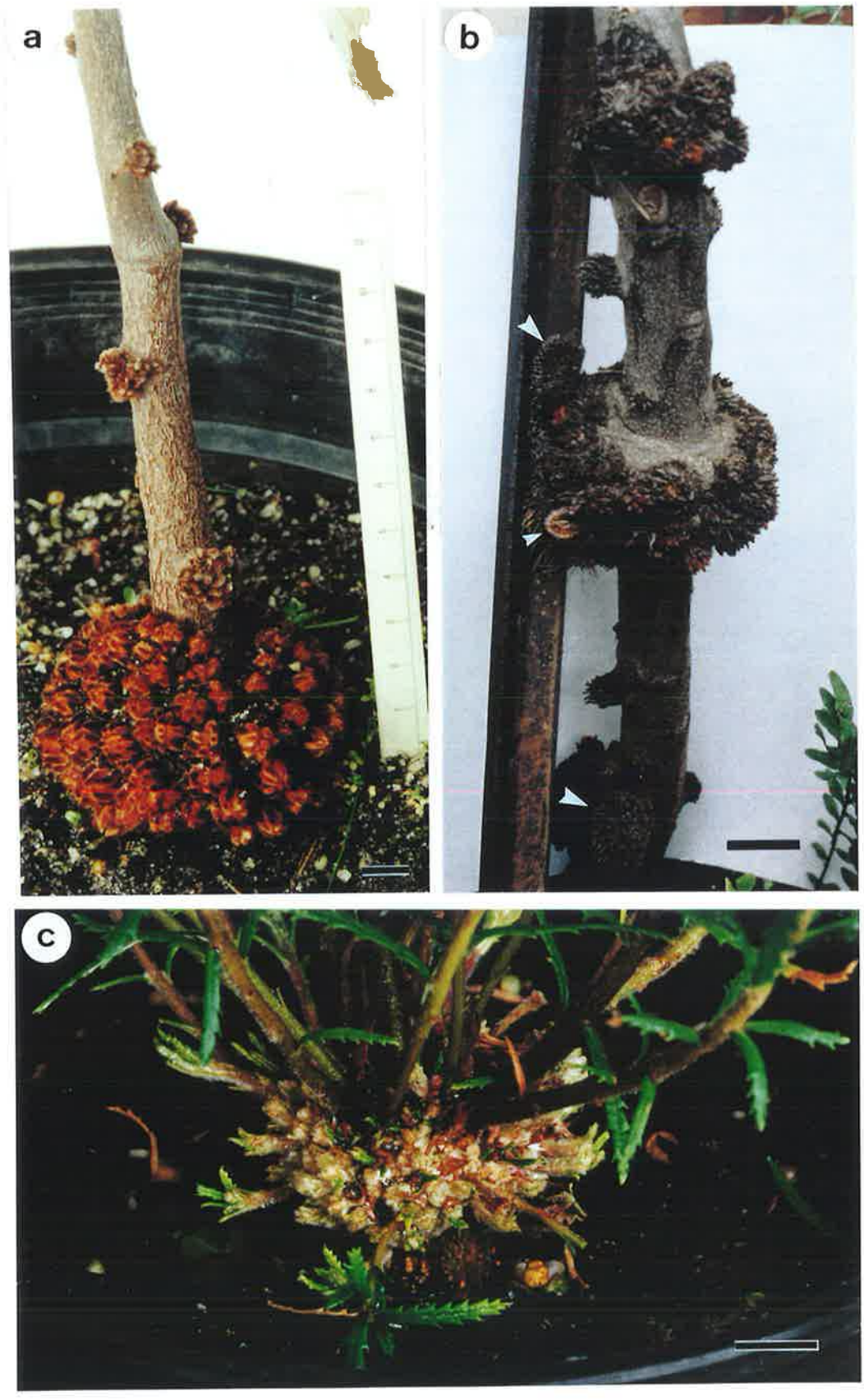

a
b
c

Plate 5.2 *B. serrata* and *B. marginata*

a) *B. serrata* pot plant with thickened base, 1995. *B. serrata* is reported to be non-lignotuberous. Bar = 5 cm

b) The same plant of *B. serrata* as in (a), one year later (1996). Bar = 1 cm

c) *B. marginata* tubestock seedling with thickened stem base (arrow) from which multiple shoots have arisen; bar = 1 cm

d) *B. marginata* tubestock seedling with a single main stem, but no thickened stem base; bar = 2 cm

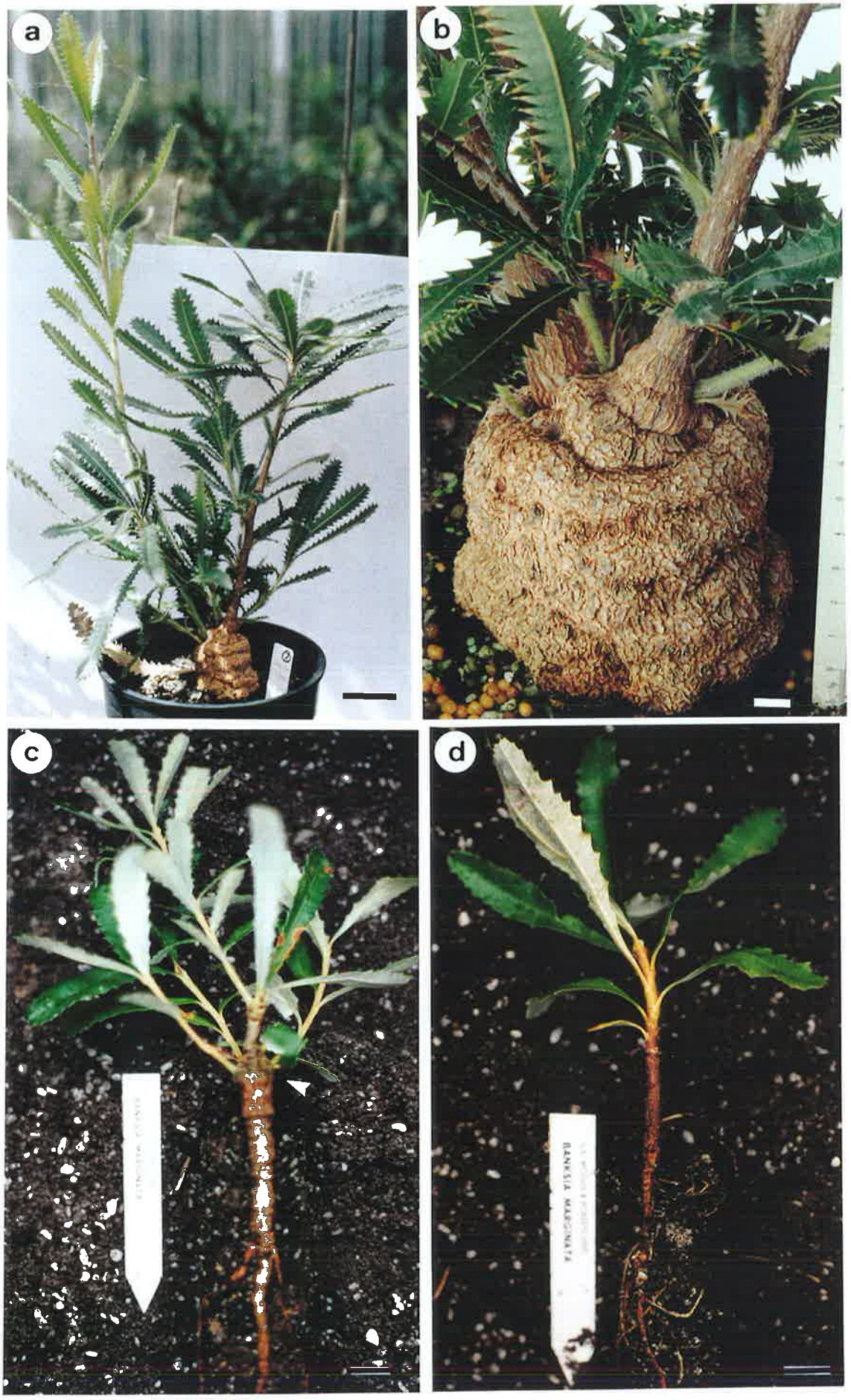
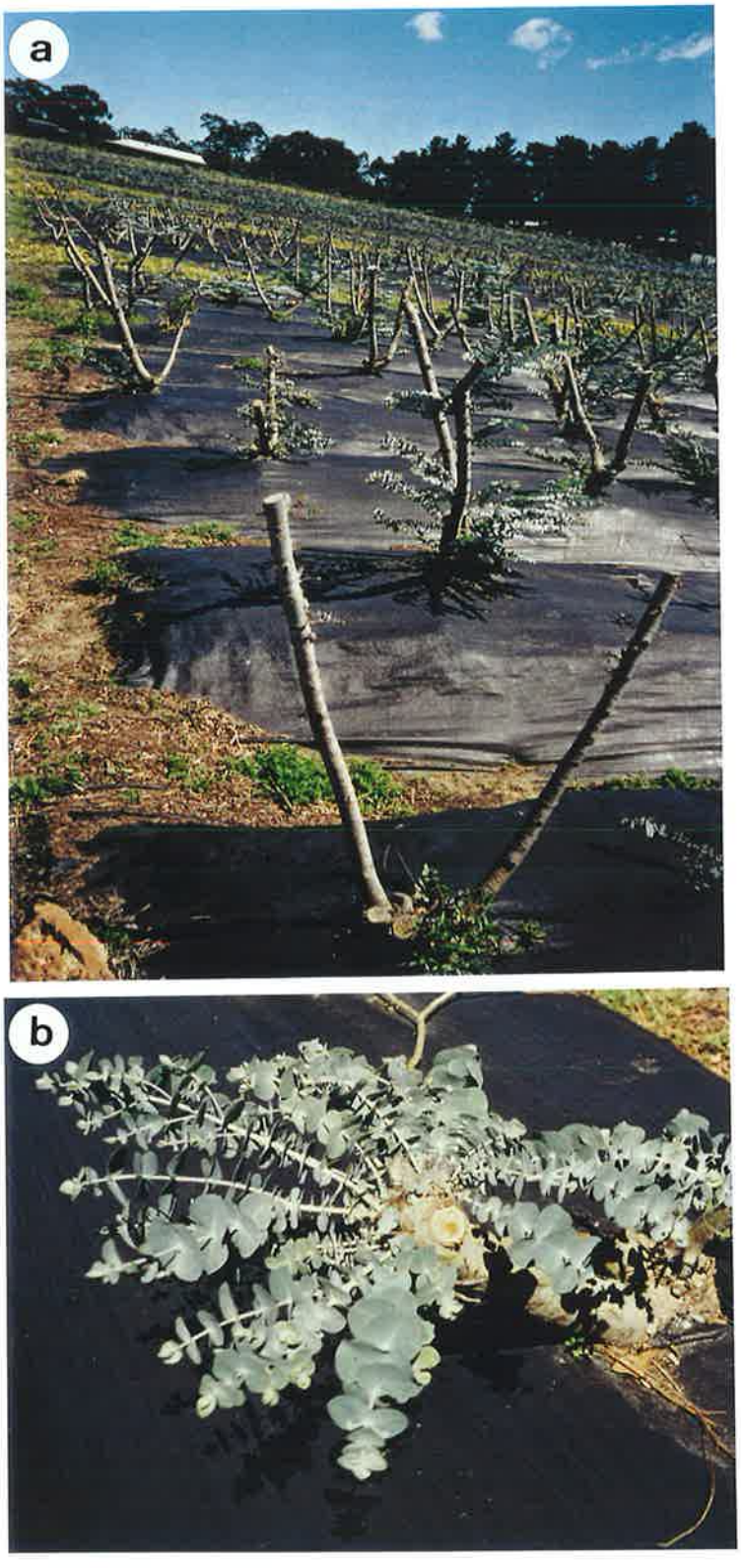

a
b
c
d
BANKSIA MARGINATA

Plate 5.3 Horticultural application of lignotubers

Commercial plantation for the production of eucalypt cut-foliage (*Eucalyptus pulverulenta)* in northern NSW.

a) Large scale plantation of *Eucalyptus* trees pruned to about 1 metre height above ground level.

b) Eucalypt resprouting from lignotuber and lower stem after pruning ca. 30 cm from the ground.


a
b

phenotype observed in field plants throughout their geographic range. Therefore, lignotubers in *Banksia* provide a resource yet to be used in the horticultural production of members of this genus.

Study rationale

Lignotubers occur in more than 50% of *Banksia* species, yet little information on them in this genus exists. With the increased interest in commercial growing of *Banksia* in plantations as a highly valued cut-flower species, lignotubers provide a source of propagules for vegetative propagation with high rooting potential, as these structures are considered ontogenetically juvenile. In this respect, several questions arise based on our preliminary knowledge of lignotubers in *Eucalyptus*: do *Banksia* lignotubers have the same morphology and anatomy? Do these bud bearing regenerative structures have a finite number of buds or can bud development be induced? Do lignotuber shoots have a greater rooting potential than other explant sources?

This study examines the morphology and anatomy of lignotubers in *Banksia* and how lignotuber buds respond to pruning. Fundamental information of this nature will enable informed decision making on how lignotubers can be used to benefit the amenity and cut-flower trade, the conservation of threatened species, as well as expanding our limited knowledge of regenerative structures in this genus.

Materials and Methodology

Plant material

Four *Banksia* species were used in this study of lignotubers in *Banksia*, two from Section *Banksia*, Series *Banksia*: *B. menziesii* and *B. serrata*, and two varieties of *B. spinulosa* from Section *Oncostylis*, Series *Spicigerae*, namely, *B. spinulosa* var. *cunninghamii* and *B. spinulosa* var. *spinulosa*, which are non-lignotuberous and lignotuberous, respectively. Details on habitat and growth characteristics of all but the latter of these are presented in Chapter 4. *B. spinulosa* var. *spinulosa* will be described here.

***Banksia spinulosa* var. *spinulosa* Sm.**

Of the four varieties of *B. spinulosa*, variety *spinulosa* is lignotuberous, as are varieties *collina* and *neoanglica*; variety *cunninghamii* is non-lignotuberous. This shrub of normally less than 2 m in height is scattered in four main regions along the eastern coast of Australia in Queensland and New South Wales, where it is more common, almost to the northern tip of the Australian Capital Territory. It is a variable, slow growing species, widely distributed on many different soil types in open forests on hills at altitudes of mainly 500 – 1000 m (Taylor and Hopper 1988). Southern clones are frost tolerant. This was one of the first varieties introduced into cultivation in the UK in 1788 (Wrigley and Fagg 1989) and is still of economic importance in Australia in amenity horticulture and as an ornamental, particularly as a miniature potted plant. It has also been used as a rootstock in intergeneric grafts with *Dryandra praemorsa*, and also as an interspecific graft rootstock (refer to McKenzie (1981), Peisley (1989) in Table 4.2).

Seed source and cultivation

Seeds of *B. serrata* and *B. menziesii* were purchased from Nindethana Seed Services (RMB 939 Woogenilup, WA 6324); *B. spinulosa* var. *spinulosa* was obtained from D. Orriell Seed Exporters (45 Frape Avenue. Mount Yokine, WA 6060) and *B. spinulosa* var. *cunninghamii* was acquired from the same source as the seed used in Chapter 3. Seeds were sown in autumn (02.04.94) as described in Chapter 3; germination rates of 67%, 100%, 56% and 90% were obtained from *B. serrata*, *B. menziesii*, *B. spinulosa* var. *spinulosa* and *B. spinulosa* var. *cunninghamii*, and emergence was 29, 30, 36 and 31 days after sowing, respectively. Seedlings were pricked out (04.05.94) and planted into 10 cm (0.5 L) pots in medium made of equal parts of peat (*Eurotorf*), perlite and

fine sand (Mt. Compass grey sand) with no other adjustments or additives. These were placed on raised benches in an evaporatively cooled glasshouse at 18°C, which had whitewashed glass throughout summer (September to April). Plants were hand-watered three times per week, which was later reduced to twice weekly due to compaction of the potting medium and the reduction in drainage. Increasing acidity of the potting medium over time (ca. pH 4, measured according to Handreck & Black 1989) was reduced by flooding the pots with lime water (1 g/L hydrated lime, and let settle) until pH 6.5 was attained. Slow release fertiliser (*Osmacote 17+1.6+8.7*, 5 kg/m^3) and trace element mix (*Micromax*, 0.75 kg/m^3) were applied.

Seedling growth measurements

To study seedling habit, growth measurements of five seedlings of four species: *B. serrata, B. menziesii, B. spinulosa* var. *spinulosa* and *B. spinulosa* var. *cunninghamii,* were taken at bimonthly intervals (30.05.94, 16.07.94, 28.09.94, 25.11.94, 16.02. 95, 31.03.95, 31.05.95, 24.07.95). The measurements taken were seedling height, leaf number, longest leaf length and cotyledonary node dimensions of width and breadth, as well as hypocotyl length. Plant height was measured from ground level to the tips of the youngest unfolding leaf; hypocotyl width was a measure of the diameter of the top of the hypocotyl parallel to the line of attachment of the cotyledon, and breadth was measured at right angles to the width. *Microsoft Excel* was used to analyse the results.

Destructive sampling and embedding for microscopy

For anatomical studies, five seedlings of *B. serrata* and *B. menziesii* were destructively sampled at eight, 15 and 26 weeks after sowing. Soil was washed off the roots and the cotyledonary node was dissected leaving intact the lower portion of the stem and the entire hypocotyl to the root junction. The sides of large, swollen cotyledonary nodes were scored with a scalpel to allow penetration of fixative. The cotyledonary node and adjoining stem and hypocotyl tissues were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer, pH 7 at 4°C, then dehydrated, infiltrated, and embedded using methods detailed in Chapters 2 and 3. Transverse serial sections were made through the node samples embedded in plastic. Every tenth section, 4μ in thickness, was placed on a glass slide, stained with Periodic acid-Schiff's reagent and Toluidine Blue O, and viewed using a transmitted light microscope and photographed as outlined previously.

Pruning challenge of *B. spinulosa* design and analysis

The pruning challenge of *B. spinulosa* var. *spinulosa* involved three treatments: (i) low pruning - cut at the location of the second or third true leaf on the main stem, (ii) high pruning - cut at the end of the first season's growth on the main stem and (iii) unpruned control treatment. At eighteen months after sowing, *B. spinulosa* seedlings were potted into 15 cm pots with *Nuerth* native potting mix and grown under shade-house conditions on raised benches for the duration of the pruning challenge trial. Plants were pruned in spring (29 September 1995) and fertilised with *Osmacote* (N17 + P1.6 + K8.7, 5 kg/m^3) and a trace element mix (*Micromax*, 0.75 kg/m^3), and hand-watered as required. Fertiliser was reapplied the following spring, 1996.

These plants were arranged in a randomised block design on benches in the shade-house. Measurements on the number of buds and the length of shoots were made at Day 0 and at four subsequent times at three to four month intervals (29.09.95, 18.12.95, 28.03.96, 09.07.96 and 21.10.96) until the plants were 2½ years old. Results were analysed using *Microsoft Excel*. Shoot lengths were estimated by counting the number of shoots falling into five different length categories: < 5 cm (which included buds < 2mm), 5 to 10 cm, 10 to 15 cm, 15 to 20 cm and > 20 cm. From this information, the average shoot length was derived using the following formulae.

Average shoot length:

$$\frac{(2.5 \times n_1) + (7.5 \times n_2) + (12.5 \times n_3) + (17.5 \times n_4) + (22.5 \times n_5)}{N}$$

Where n_1 = number of shoots < 5 cm, including buds < 2mm

n_2 = number of shoots 5 to 10 cm in length

n_3 = number of shoots 10 to 15 cm in length

n_4 = number of shoots 15 to 20 cm in length

n_5 = number of shoots > 20 cm in length

N = total number of shoots

Results

Lignotuber morphology and seedling habit

The morphology of initial lignotuber development differs between the *Banksia* species studied. In post emergent seedlings of *B. serrata* and *B. menziesii* the cotyledons are thick and rubbery without prominent veins, and the first pair of true leaves arises from the cotyledonary node perpendicular to the cotyledons. In *B. menziesii* the end of the hypocotyl proximal to the cotyledons is green, non-woody and swollen with a series of bumps on the surface (Plate 5.5c). *B. serrata* does not show the pronounced thickening of the cotyledonary node as observed in *B. menziesii* during seedling development, although shoots do arise from the stem immediately above the cotyledonary node (Plates 5.4b & 5.6a), and woody thickening at the stem base often occurs in older plants of *B. serrata* (Plate 5.2a & b). *B. menziesii* had the largest cotyledonary node dimensions, followed by *B. serrata*, *B. spinulosa* var. *spinulosa* and *B. spinulosa* var. *cunninghamii* (Figures 5.4 & 5.5). In *B. spinulosa* var. *spinulosa* the surface of the cotyledonary node is covered by a mass of superficial buds, forming a dense collar (Plates 5.5a, 5.6b & c), which later produce whorls of shoots two to three deep around the cotyledonary node (Plate 5.1c). *B. spinulosa* var. *cunninghamii* did not produce shoots from the cotyledonary node, however lateral shoots grew from leaf axils along the main stem (Plates 5.4d & 5.5a). These interspecific and intervarietal differences in lignotuber morphology are supported by data on the habit and plant growth characteristics of the species: plant height (Figure 5.1), maximum leaf length (Figure 5.2), leaf number (Figure 5.3), hypocotyl dimensions (width Figure 5.4 and breadth Figure 5.5) and hypocotyl length (Figure 5.6). *B. spinulosa* varieties have the smallest leaf length (Figure 5.2), but the greatest plant height (Figure 5.1) and leaf number (Figure 5.3). The size and number of leaves were lowest in *B. menziesii* and *B. serrata,* which also showed slower growth rates and hence lower plant habit for the 12 month growth period, *B. serrata* having a very compact habit with short internodes (Figure 5.1 & Plate 5.4b). The hypocotyl changes little in length for each species, remaining more or less constant (Figure 5.6). On the other hand, the cotyledonary node width and breadth showed increases in the 12 month growing period (Figures 5.4 & 5.5). Shoots were never observed below the cotyledonary node.

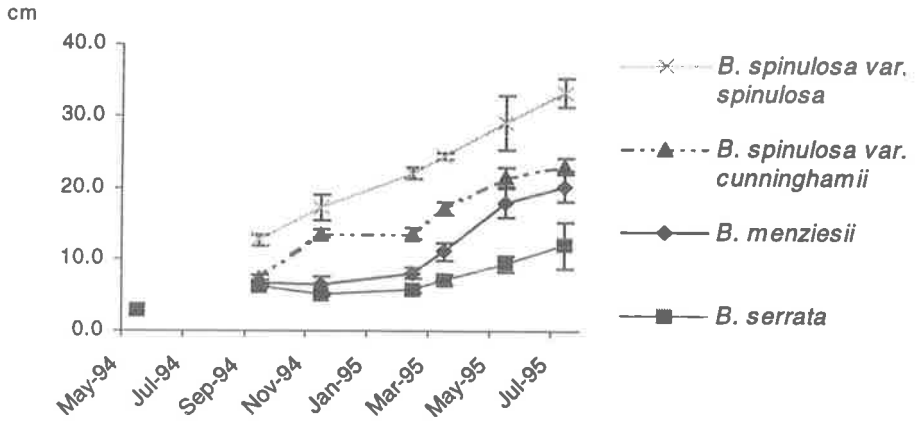


Figure 5.1 Seedling height (mean ± s. e.; $P \leq 0.05$)

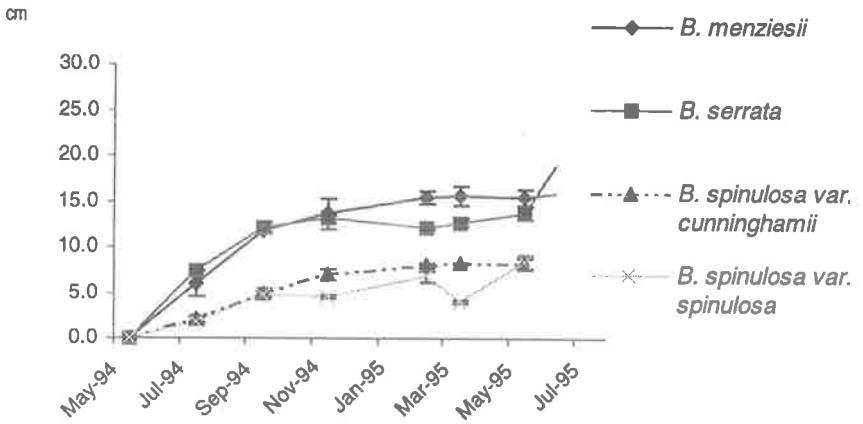


Figure 5.2 Maximum leaf length (mean ± s. e.; $P \leq 0.05$)

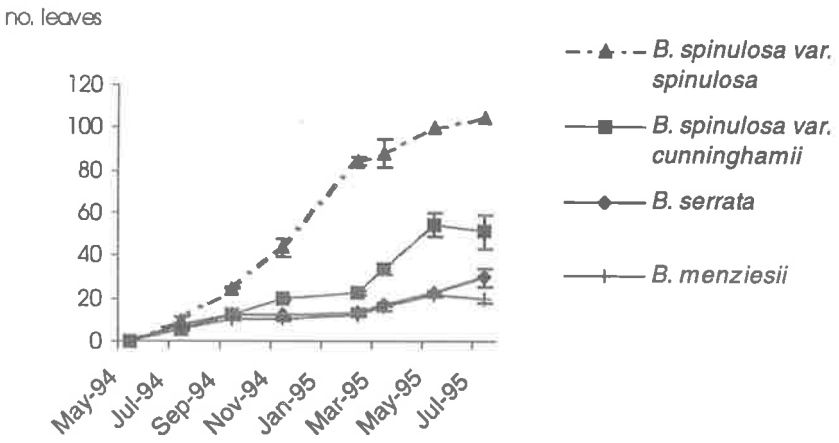


Figure 5.3 Leaf number (mean ± s. e.; $P \leq 0.05$)

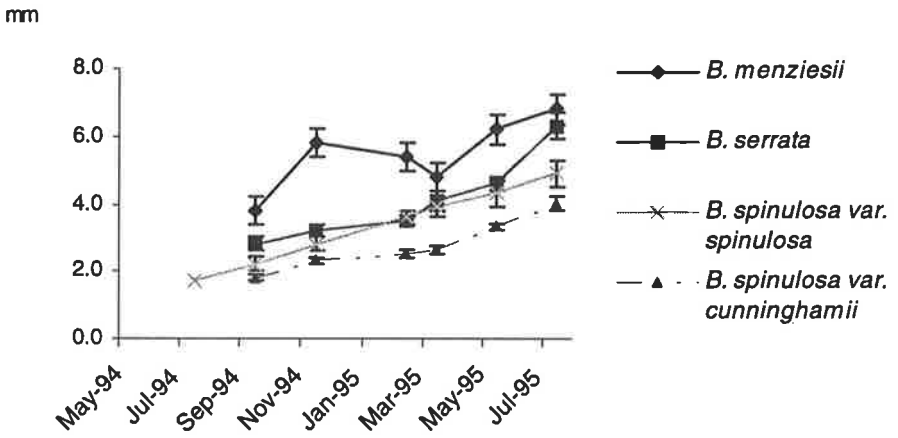


Figure 5.4 Cotyledonary node width, (mean ± s. e.; $P \leq 0.05$)

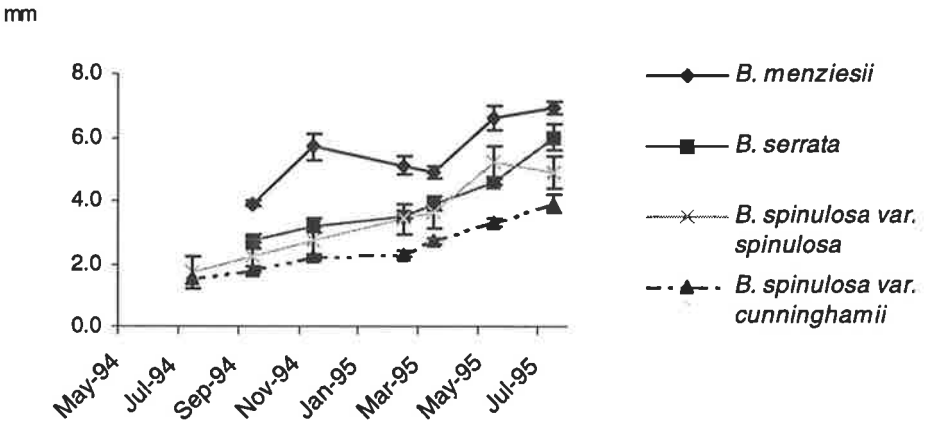


Figure 5.5 Cotyledonary node breadth (mean ± s. e.; $P \leq 0.05$)

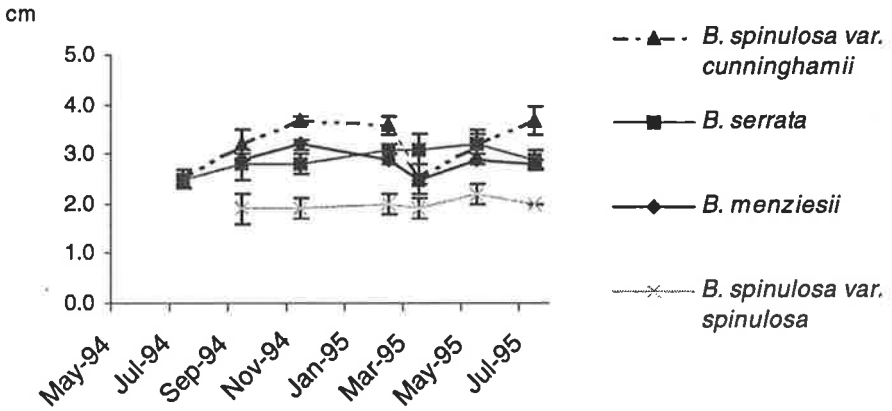


Figure 5.6 Hypocotyl length (mean ± s. e.; $P \leq 0.05$)

Plate 5.4 Seedling habit of four *Banksia* species

Habit of four *Banksia* species at 15 months after sowing at the time when final growth measurements were taken (July, 1995). Seedlings were removed from pots and roots partially washed.

(a) *B. menziesii* showing senesced cotyledons (large arrow) and testa (small arrow) still wrapped around the hypocotyl.

(b) *B. serrata* with cotyledonary node (arrow) and thicker hypocotyl compared to the main stem. Note the compact internodes and short plant height.

(c) *B. spinulosa* var. *spinulosa* showing prolific cotyledonary node sprouting and senesced cotyledon (arrow).

(d) *B. spinulosa* var. *cunninghamii* showing prolific lateral shoot production from leaf axils; no shoots are produced from the cotyledonary node determined by the position of the senesced cotyledons (arrows).

Scale in cm

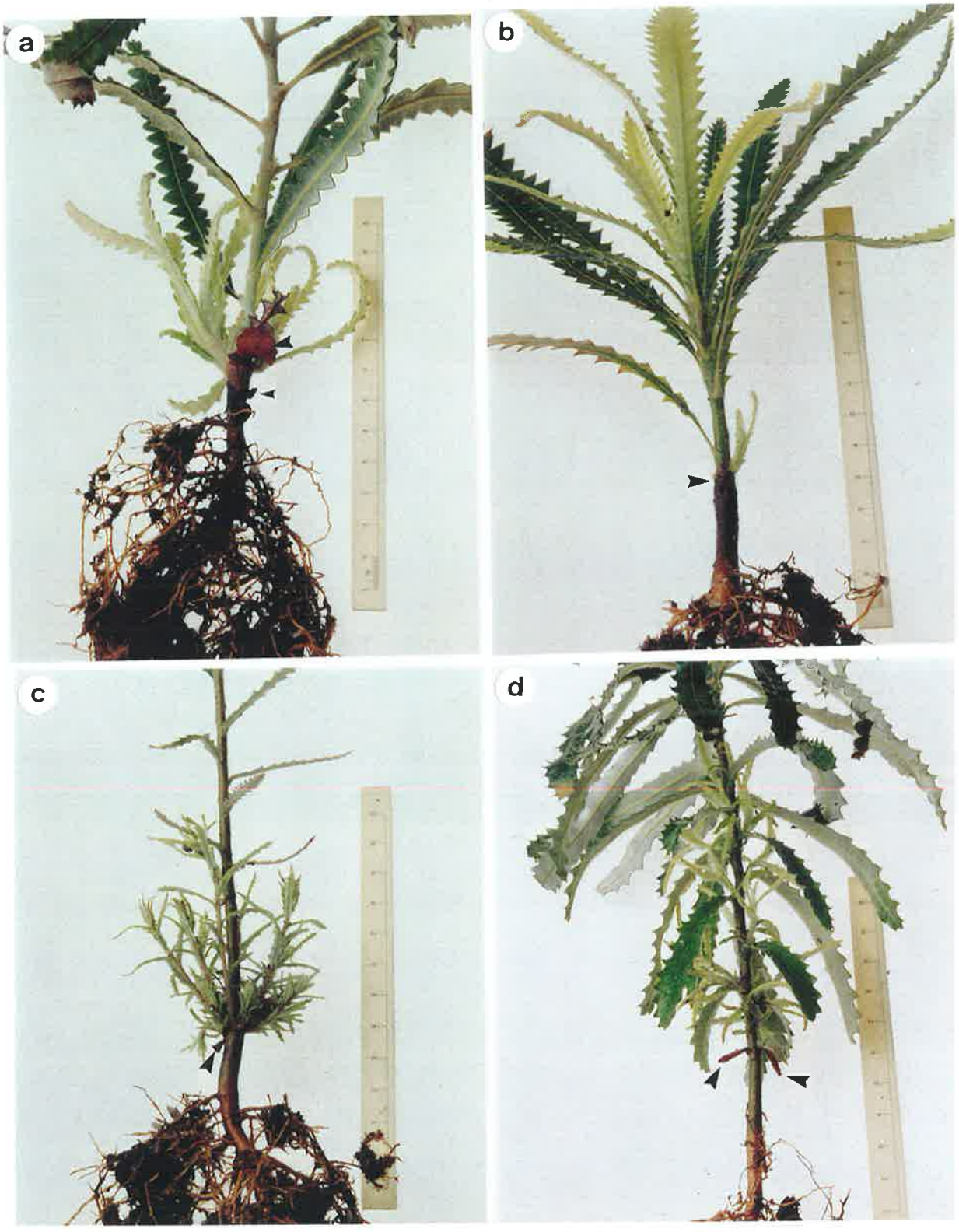

a
b
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Plate 5.5 *B. spinulosa* and *B. menziesii*

(a) Seedling habit of *B. spinulosa* var. *cunninghamii* showing apical and lateral shoot (arrows) growth; bar = 5 cm

(b) Seedling habit of *B. spinulosa* var. *spinulosa* showing variation in the length of shoots produced from the cotyledonary node (arrows) in seedlings of the same age; bar = 5 cm

(c) Lignotuber morphology in the early stages of seedling development in *B. menziesii* (five months from sowing) showing pronounced swelling of the upper hypocotyl beneath the cotyledons (black arrows) with rows of surface bumps (white arrows) which may indicate the presence of buds or vascular bundles beneath the surface. Note this individual has larger than average hypocotyl dimensions for a seedling of this age which average 3.9 mm in diameter (Figures 5.4 & 5.5); bar = 1 cm

(d) *B. menziesii* woody hypocotyl structure (at 14 months) with withered main stem (black arrow), three shoots resprouting from cotyledonary node and a senescent cotyledon (white arrow); bar = 0.5 cm

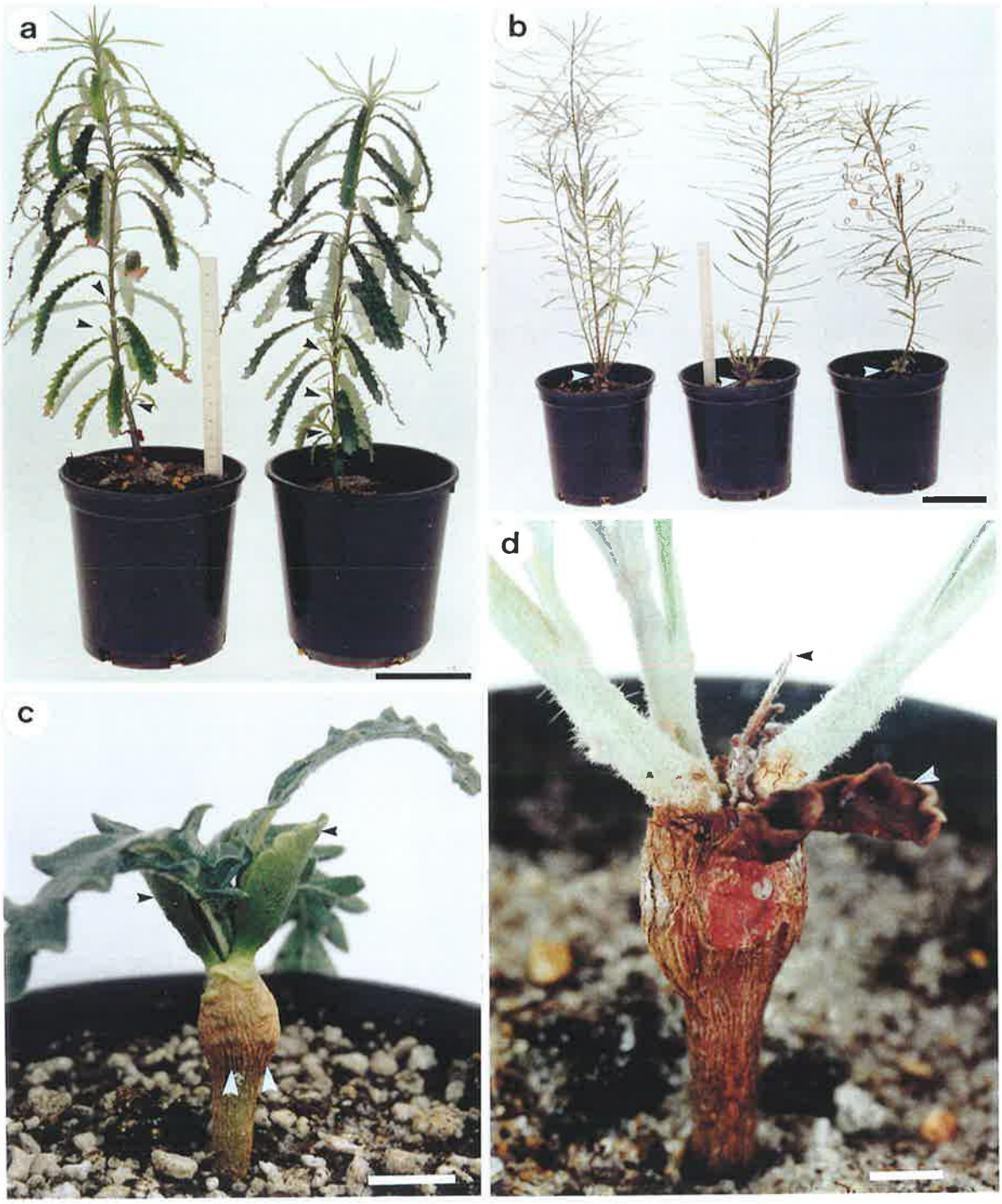
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Plate 5.6 Basal sprouting in *Banksia*

(a) Basal stem shoots (large arrows) from lower leaf scars and recurved senesced cotyledons (small arrows) in potted seedling of *B. serrata* (14 months). Average hypocotyl diameter of seedling of this age was 6 mm (Figures 5.4 & 5.5); bar = 0.5 cm

(b) Resprouting from cotyledonary node (arrow) in *B. spinulosa* var. *spinulosa* (14 months); hypocotyl = double arrow; stem = black arrow; bar = 1 cm

(c) Variation in sprouting from cotyledonary node (arrows) in *B. spinulosa* var. *spinulosa* seedlings (14 months); bar = 5 cm


a
b
c

Cotyledonary node anatomy of non-lignotuberous *B. serrata* at 15 weeks

The anatomy of the cotyledonary node of *B. serrata* is presented in Plates 5.7 to 5.9. The hypocotyl of *B. serrata* displays bilaterally symmetrical vasculature, and ligules are present at the base of the cotyledons (Plate 5.7a). The base of the cotyledon is continuous with the cortex at either side of the upper hypocotyl (Plate 5.7b & c), and vascular traces, which supply the median and lateral veins of the cotyledons (Plate 5.7d), can be observed (Plate 5.7b). The laminae of the cotyledons remain separate from one another and have a comparatively narrow area of attachment to the main stem. The stem cortex forms narrow protrusions at the base of the stem between the cotyledons (Plates 5.7c & d, 5.8e & f). An axillary bud is present in the axil of each cotyledon (Plate 5.8a & c) and consists of a bud apex and adjacent prophylls (Plate 5.8b & d). The first pair of true leaves develops at right angles to the cotyledons, each leaf subtending a large axillary bud (Plate 5.9a, b & c). The third and fourth leaves, each subtending an axillary bud (Plate 5.9d & e), develop perpendicular to the first pair of true leaves, however, their development is slightly staggered (Plate 5.9d), with the third leaf and bud at a more advanced stage. The location and number of axillary buds in the cotyledonary node are summarised in Table 5.2.

Cotyledonary node anatomy of lignotuberous *B. menziesii*

Seedling at eight weeks after sowing

The structure of the cotyledonary node in a post-emergent seedling (eight weeks) comprising two cotyledons and true leaves at an early stage of development is depicted in the photomicrograph series presented in Plates 5.10 and 5.11. The distinguishing feature is the fused bases of the cotyledons, which form a sheath around the region of the upper hypocotyl and base of the stem. Progressing from the hypocotyl through the cotyledonary node towards the stem, the vasculature of the stem is bilaterally symmetrical (Plate 5.10). Pairs of vascular bundles supplying the cotyledons can be observed in the cortex at either side of the upper hypocotyl. The lowest point of the air-filled lumen, which develops between the ensheathing base of the cotyledons and the stem, is evident between a cotyledonary vascular trace and the main stele (Plate 5.10b). Within a short distance (< 2 mm) the fused cotyledonary bases become separate from the base of the stem, which is then encircled by the narrow lumen (Plate 5.10c-f). The outer sheath of fused cotyledon bases consists of thick spongy cortical parenchyma, which is approximately as thick as the diameter of the inner stem in the eight weeks old

seedling. Vascular bundles identified in the sheath supply the median and lateral veins of the cotyledons. An axillary bud develops in the axil of each cotyledon (Plate 5.10d & f) from cortical tissue at the interface of the main stem and the upper hypocotyl. Adventitious buds develop from superficial tissues on the adaxial wall of the sheath in close proximity to the cotyledonary vascular bundles (Plates 5.10c & 5.11).

Seedling at 26 weeks after sowing

At twenty six weeks after sowing, *B. menziesii* shows a similar structure as described above at eight weeks including cotyledonary axillary buds and irregular occurrence of adventitious buds on the adaxial wall of the sheath facing the lumen (Plates 5.12 to 5.17). A schematic overview of the structure of the cotyledonary node and location of buds observed in the serial sections is shown in Figure 5.7. In addition to the axillary and adventitious buds observed at eight weeks, serial sections showed numerous adventitious buds generated along the line of fusion of the bases of the cotyledons on each side of the main stem axis (Plates 5.14 & 5.15) and the presence of accessory buds on either side of the cotyledonary axillary buds. An axillary bud was present in the axil of the cotyledon (Plates 5.12b & 5.13b), as well as in the axils of the first true leaves (Plates 5.16a, b & 5.17). The cotyledonary axillary buds have endogenous accessory buds set deeply in tissues at either side (Plates 5.12b - d, 5.13d & e). Tannins and other phenolics (Plate 5.18a & c) are concentrated in the enveloping sheath, which eventually becomes lignified (Plate 5.5d). At this stage the sheath is approximately half the thickness of the radius of the inner stem it surrounds. A summary of the location and estimated number of buds in *B. menziesii* is presented in Table 5.2.

Table 5.2 Bud location and numbers in *B. menziesii* at eight and 26 weeks, and *B. serrata* at 15 weeks

Bud type	Bud location	Bud number *B. serrata* 15 weeks	*B. menziesii* 8 weeks	*B. menziesii* 26 weeks
Axillary (exogenous)	Cotyledon axil	2	2	2
Accessory (endogenous)	Cotyledon axil	0	–	†6
Axillary (exogenous)	True leaf axil	#2	–	#2
Adventitious (exogenous)	Adjacent to ligule, LHS	0	–	11
	Adjacent to ligule, RHS*	0	–	11
	Other locations	0	2	4
Total		**4**	**4**	**36**

† 2 cotyledonary axils per node with a minimum of 3 accessory buds each
# 2 true leaf bases per cotyledonary node
* assumed bilateral symmetry
– indicates no observations made of this location

Plate 5.7 Cotyledonary node anatomy in non-lignotuberous *B. serrata* at 15 weeks

(a) Upper hypocotyl beneath the juncture of the cotyledon ligule (l) which can be seen at the top and bottom of the photomicrograph; deep scoring (*) of the hypocotyl sides was a necessary aid for fixation.

(b) Cotyledonary node ca. 120 µm above (a) at the juncture of the cotyledon ligules (arrows). Note symmetrical vasculature and the pairs of darkly stained vascular bundles in the cortex outside the central vascular stele at the top and bottom. The thick layer of cortex, which has been scored on both sides, forms the base of the cotyledons.

(c) Cotyledonary node ca. 120 µm above (b) showing the base of the cotyledons (c), which are separate from one another but joined to the central axis. Note the protrusion of cortex (arrows) which is the lowest part of the main stem.

(d) Cotyledonary node ca. 80 µm above (c) showing the cotyledons almost detached from the base of the main stem (gaps between the cotyledons and stem are indicated by narrow arrows). The median and lateral vascular bundles are visible in each of the cotyledons, as indicated in the RHS cotyledon only (outlined arrows).

Scale is the same for (a) to (d); bar = 500µm

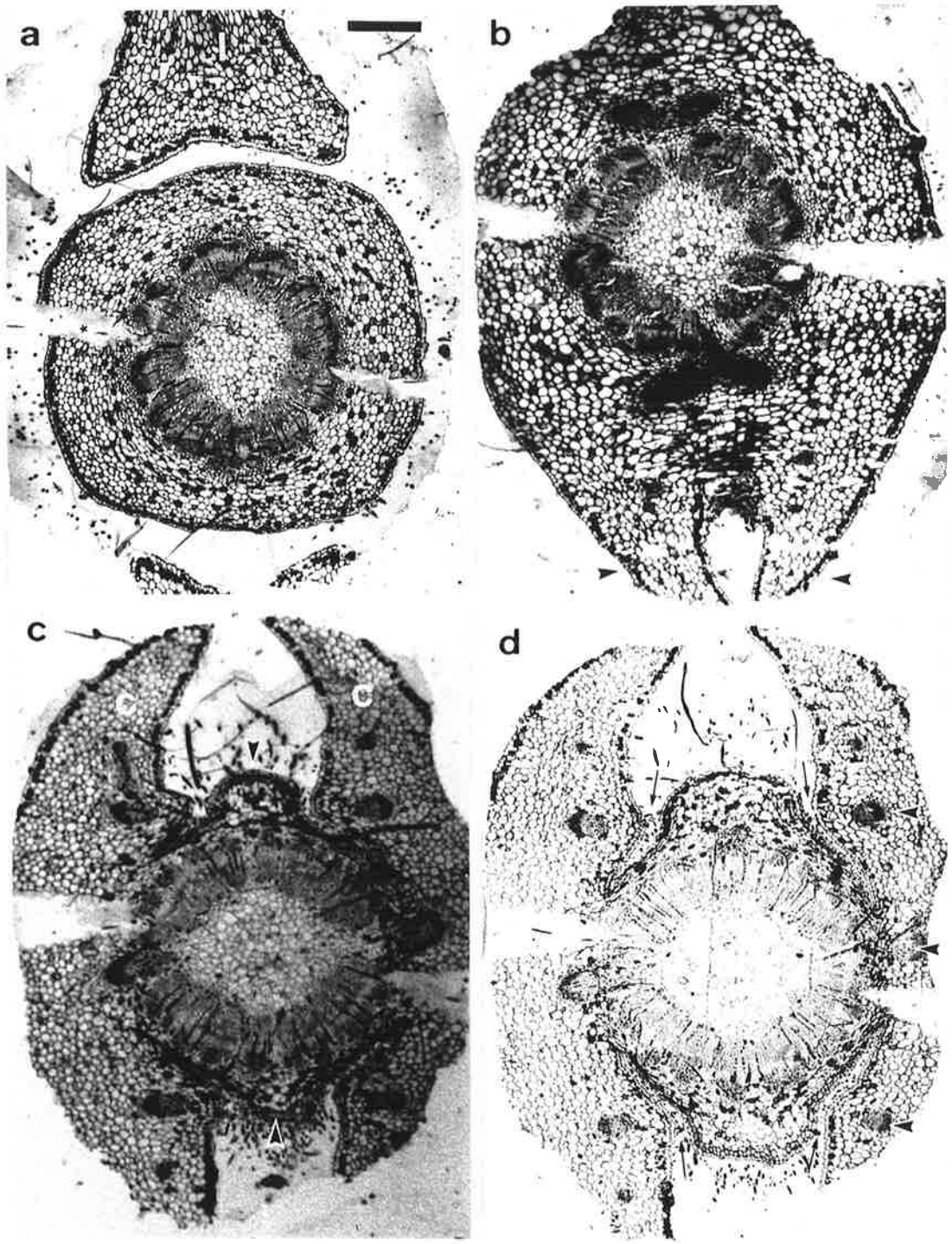

a
b
c
c
c
d

Plate 5.8 Cotyledonary node anatomy in non-lignotuberous *B. serrata* at 15 weeks

(a) Cotyledonary node ca. 160 µm above Plate 5.7d depicting the cotyledons unattached at either side of the stem base and the first cotyledonary axillary bud in the axil of the RHS cotyledon (arrow) shown in higher magnification in (b)

(b) First cotyledonary axillary bud in the axil of the RHS cotyledon from (a) showing the early arrangement of bud apex and prophylls

(c) Cotyledonary node ca. 120 µm above (a) showing the second cotyledonary axillary bud in the axil of the LHS cotyledon (arrow); note the vascular bundles (arrows) in the upper and lower cortex which are the vascular traces leading to the mid vein of the first true leaf pair.

(d) Second cotyledonary axillary bud in the axil of the LHS cotyledon in (c) showing the central meristematic bud apex with prophylls either side

Scale is the same for: (a) & (c), bar = 500µm; (b) & (d), bar = 100 µm

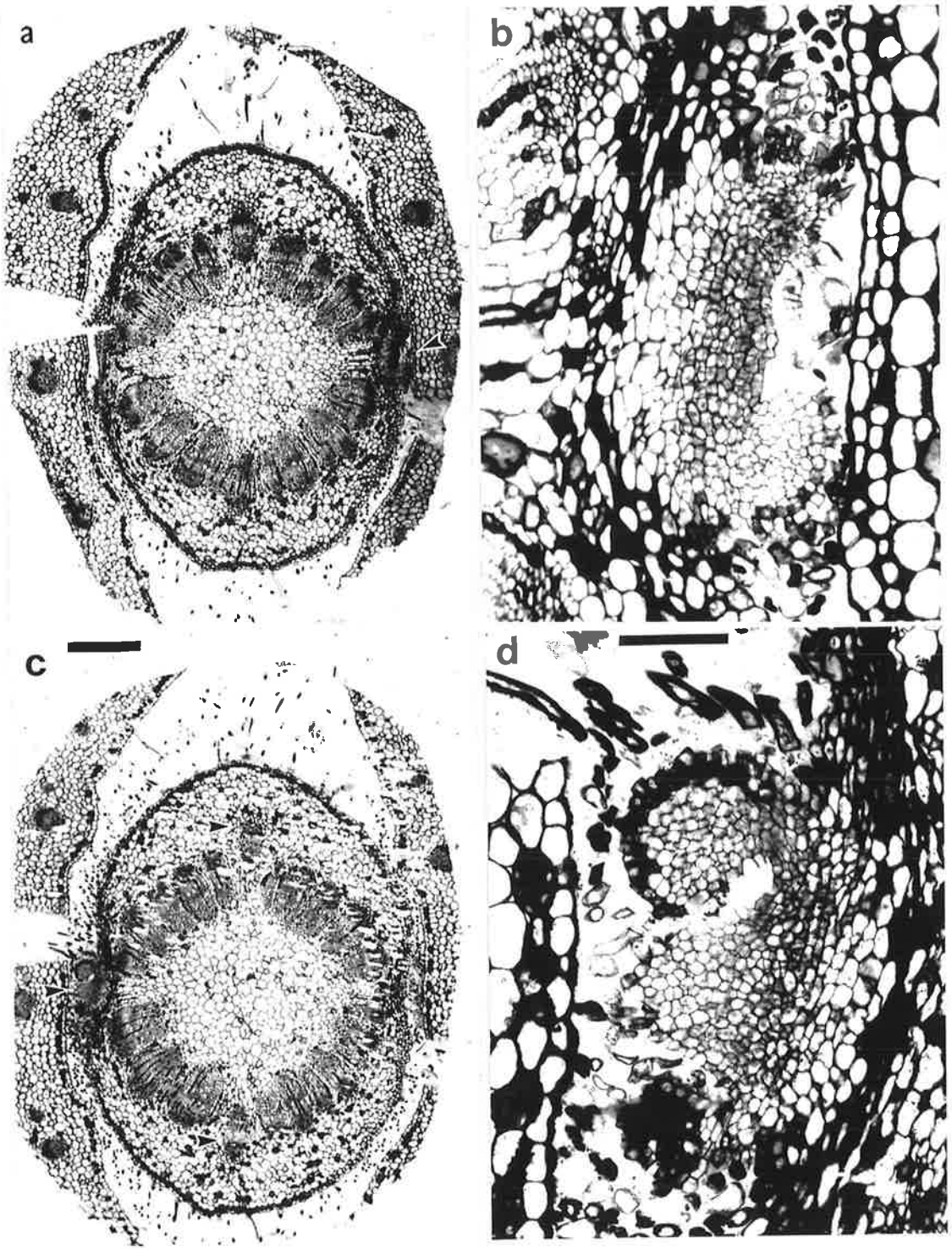

a
b
c
d

Plate 5.9 Cotyledonary node anatomy in non-lignotuberous *B. serrata* at 15 weeks

(a) Cotyledonary node ca. 720 µm above Plate 5.8c showing the leaf bases (long arrows) of the first two true leaves either side of the stem, each subtending an axillary bud (outlined arrows). Each bud is shown at higher magnification in (b) and (c). Note the remains of the adaxial surface of the cotyledon (tissue at top right) is only partially visible due to filing back of the block for sectioning.

(b) Axillary bud in leaf axil indicated in (a) (upper bud) showing bud apex and prophylls; the adaxial surface of the leaf base is at the top of the micrograph. Bar = 100 µm

(c) Axillary bud primordia in leaf axil indicated in (a) (lower bud) is less developed than the bud in (b). Note the leaf base is still intact with the cortex of stem on the lower RHS; the adaxial surface of the leaf base is at the bottom of micrograph.

(d) Cotyledonary node ca. 880 µm above (a) showing the first true leaf separate from the stem (top of micrograph; the second leaf is out of the area photographed). The leaf base of the third leaf (long arrow) and axillary bud (outlined arrow) are visible at the LHS of the stem; the axillary bud is shown at higher magnification in (e). The base of the fourth leaf is discernible on the opposite side of the stem by the thickened cortex and numerous vascular bundles. Bar = 500 µm

(e) Axillary bud is the axil of the third true leaf (adaxial side at top of micrograph) as shown in (d) depicting the meristematic bud apex between two prophylls (arrows).

Scale is the same for: (a) & (d), bar = 500µm; (c) & (e), bar = 100 µm

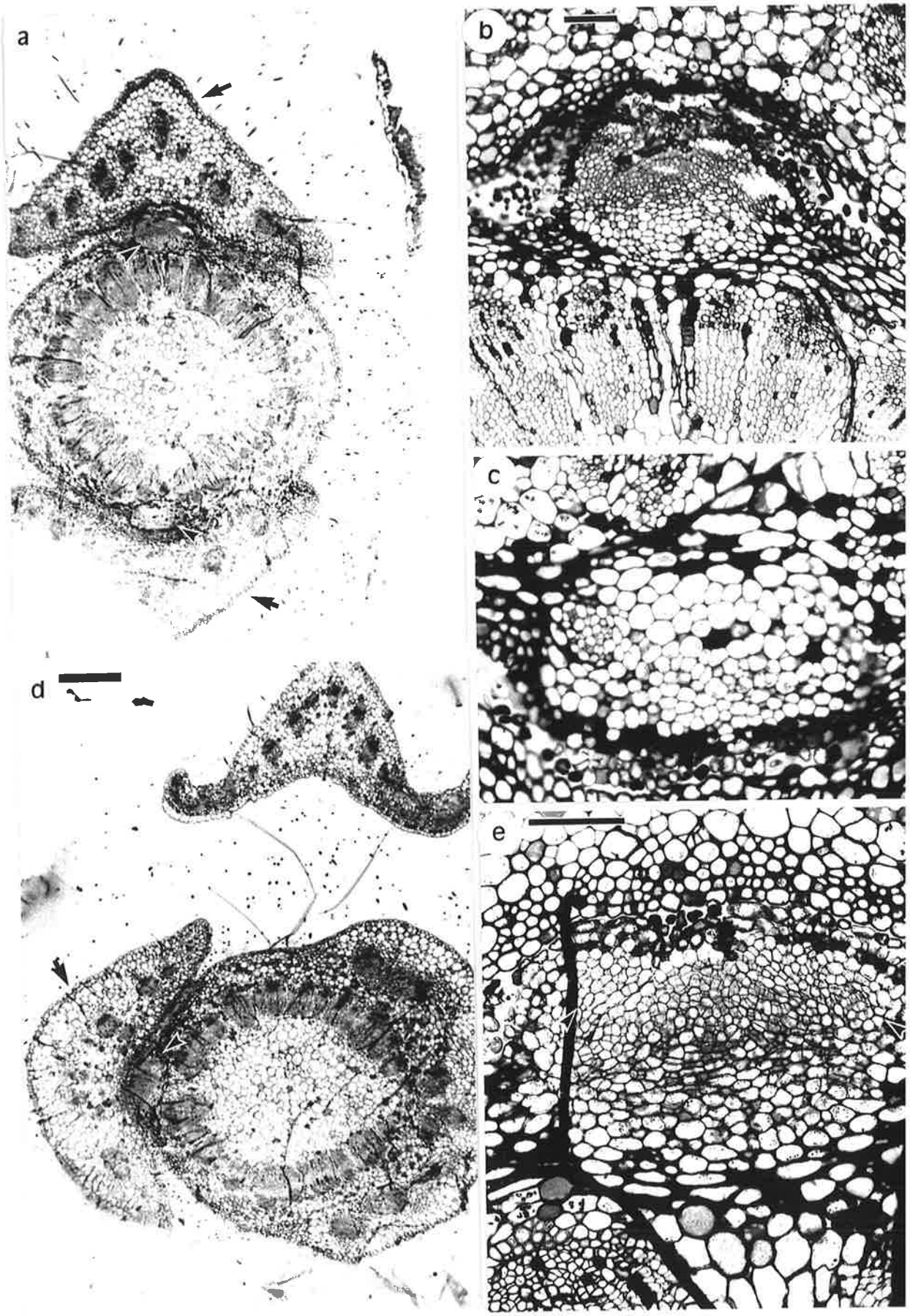

a
b
c
d
e

Plate 5.10 Cotyledonary node anatomy in lignotuberous *B. menziesii* at eight weeks

Cotyledonary node anatomy in a post-emergent seedling of *B. menziesii* (eight weeks after sowing): six photomicrographs of serial transverse sections through the cotyledonary node are presented, starting at the upper hypocotyl and progressing towards the base of the stem coving a distance of ca. 840 µm.

(a) Upper hypocotyl showing symmetrical vasculature and pairs of vascular traces supplying the median veins of the cotyledons (arrows).

(b) Base of cotyledonary node ca. 320 µm above (a). The lowest point of the lumen (large arrow) evident between the lower left cotyledonary vascular bundle and the main stele. Of the vasculature at the LHS, the outermost vascular bundle pair (outlined arrow) supplies the median vein the cotyledon, whereas the inner vascular bundle pair (small arrow) supplies the large axillary bud in the axil of the LHS cotyledon.

(c) Base of cotyledonary node ca. 200 µm above (b). The base of the cotyledonary axillary bud (large arrow), visible on the LHS, forms from cortical tissue at the base of the stem, where it interfaces with the top of the hypocotyl. A single adventitious bud meristem is visible, forming from superficial tissues on the adaxial surface of the ensheathing layer, in close proximity to the RHS vascular bundle of the bottom pair (small arrow).

(d) Cotyledonary node ca. 40 µm above (c). The cotyledonary axillary bud is visible on the surface of the stem, protected by the outer thick sheath. The adventitious bud in (c) is no longer visible.

(e) Cotyledonary node ca. 160 µm above (d). The outer of the RH pair of vascular bundles (outer arrow) is the median vein of the RH cotyledon and the inner (inner arrow) supplies the axillary bud in the axil of the RH cotyledon. Vascular bundles adjacent to the central stele of the stem, three each at the top and bottom, are leaf traces supplying the first pair of true leaves.

(f) Cotyledonary node ca. 120 µm above (d) showing the RHS cotyledonary axillary bud (arrow) and the base of the stem ensheathed by a thick sheath – the fused base of the cotyledons.

Bar = 1 cm

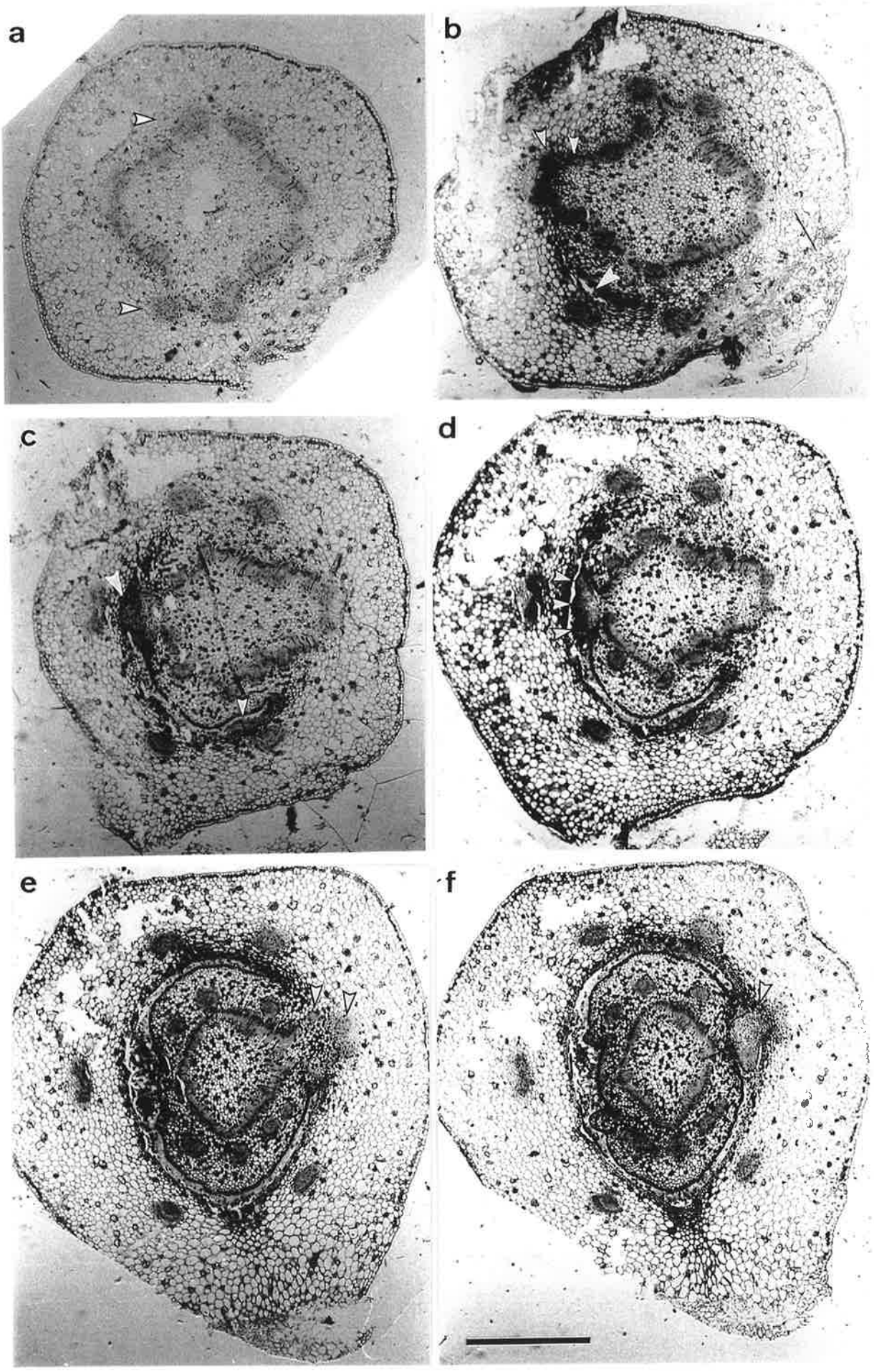

a
b
c
d
e
f

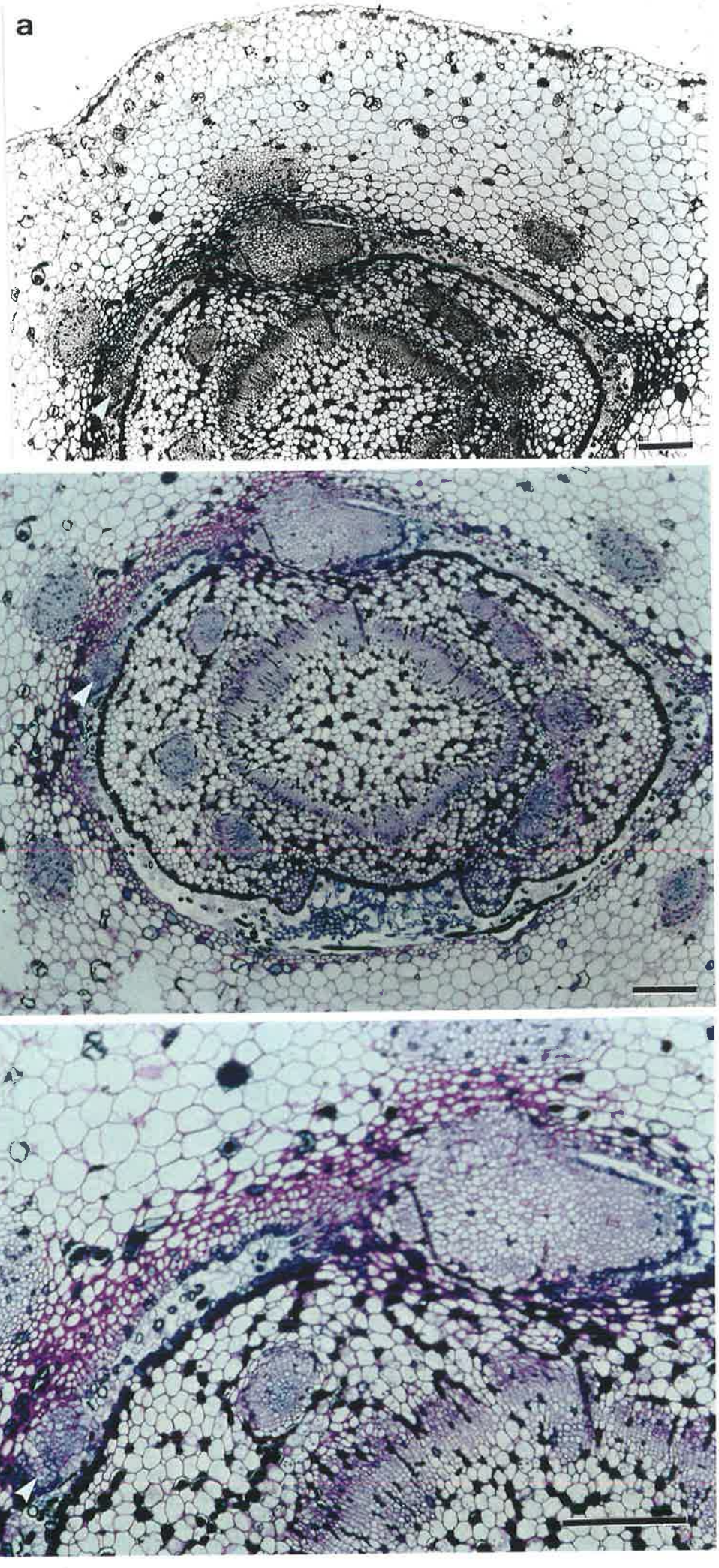
Plate 5.11 Cotyledonary node anatomy in lignotuberous *B. menziesii* at eight weeks

(a) Enlargement of the large axillary bud (centre) in Plate 5.10f showing the tissues of the thick ensheathing cotyledon base with an adventitious bud (arrow) forming on the inner surface at bottom left.

(b) Colour close-up of the same buds and the surrounding tissues as in (a)

(c) Higher magnification of the large axillary bud and the adventitious bud in (a). The adventitious bud (arrow) has formed from superficial tissues close to a vascular bundle on the adaxial surface of the sheath; note the close proximity of the vascular bundle to the inner surface.

Bars = 100µm


a
b
c

B. menziesii cotyledonary node, 26 weeks
sections presented on the LHS of Plates 5.12 to 5.17

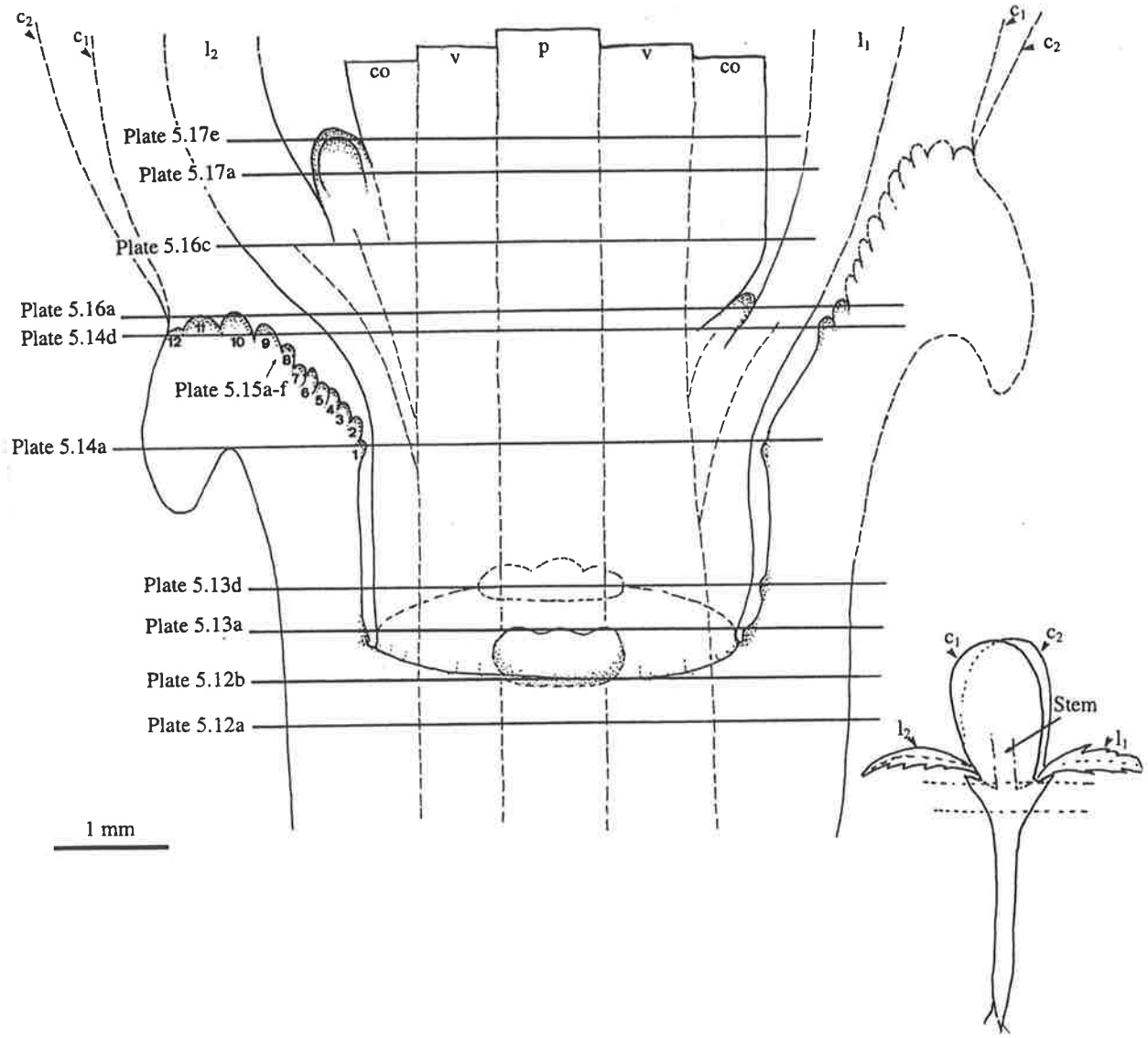


Figure 5.7 Median longitudinal aspect of *B. menziesii* at 26 weeks showing the location of the low power photomicrographs of transverse sections presented on the LHS of Plates 5.12 to 5.17

co = cortex	v = vascular stele	p = pith
l_1 = first true leaf base	l_2 = second true leaf base,	
c_1 = first (front) cotyledon	c_2 = second (rear) cotyledon	

Note: numbers at 'Plate 5.15a-f' above represent the buds shown in this plate; solid lines represent actual location of tissues, broken lines represent expected location

Plate 5.12 Cotyledonary node anatomy in *B. menziesii* at 26 weeks after sowing. TS

Transverse section of in *B. menziesii* seedling (refer to Figure 5.7)

(a) Upper hypocotyl beneath the cotyledonary node showing symmetrical vasculature. The dense cortical tissue (arrows, bottom of photomicrograph) marks the area immediately beneath the lowest point of the lumen. Bar = 500µm

(b) Base of cotyledonary node located ca. 360 µm above (a) showing the cotyledonary axillary bud (arrows) in the axil of the first cotyledon. Note the bases of the cotyledons are fused, ensheathing the node. Bar = 500µm

(c) and (d) Higher magnifications of axillary bud region in (b) showing the main axillary bud comprising of apex and accompanying prophylls (large black arrows) and the proliferation of accessory buds (outlined arrows) on either side. The accessory buds are deeply seated (endogenous) compared to the axillary bud, which originates from superficial tissues (exogenous). Bars = 100µm

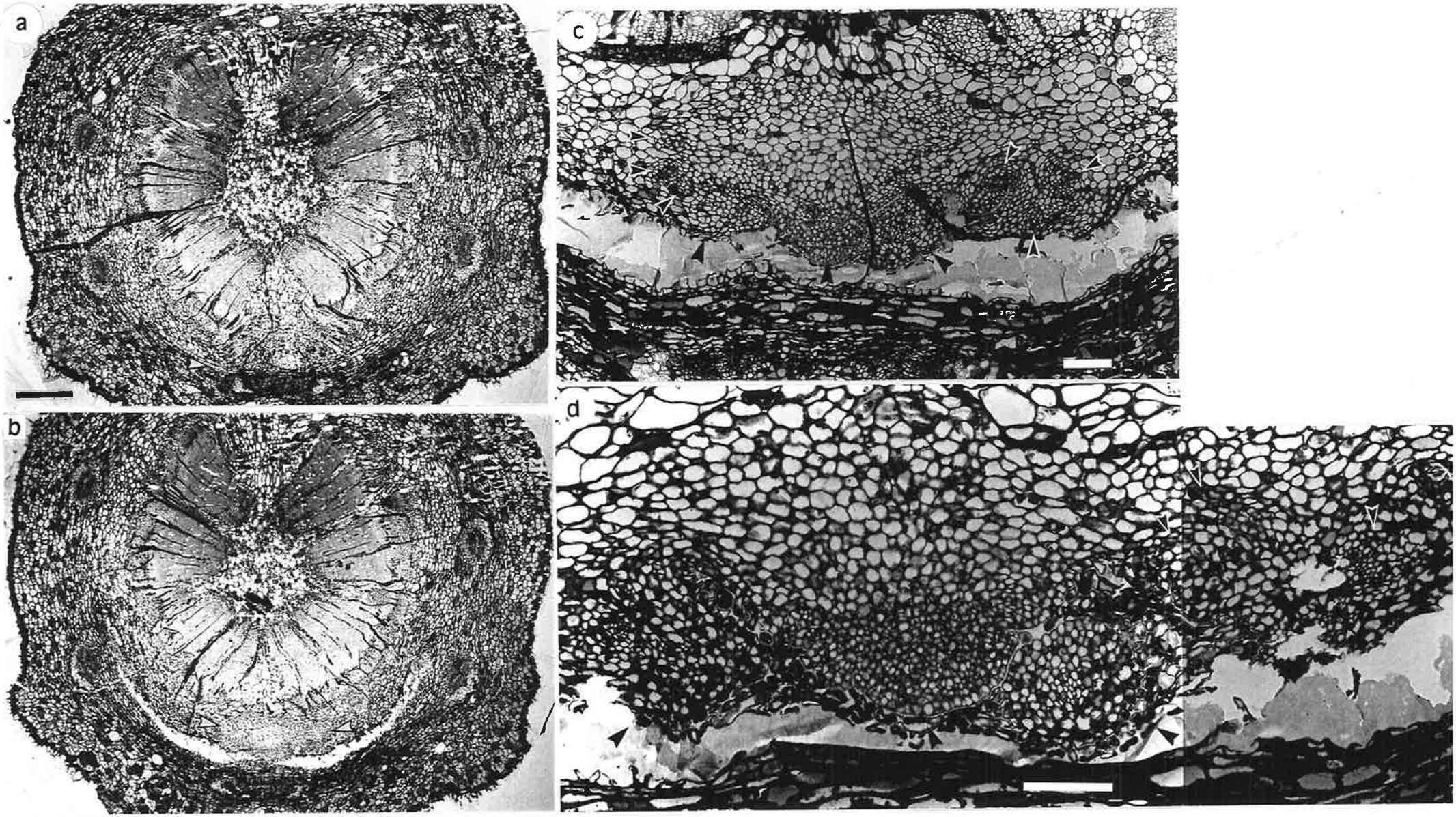

a
b
c
d

Plate 5.13 Cotyledonary node anatomy in *B. menziesii* at 26 weeks

(a) Cotyledonary node ca. 360 µm above Plate 5.12b showing the top of the prophylls of the first cotyledon axillary buds visible in the lumen (bottom of photomicrograph). Two adventitious bud meristems (arrows), visible on the LH and RH adaxial walls of the sheath, are shown in higher magnification in (b) and (c).

(b) Adventitious bud from LHS of (a) at an early stage of development

(c) Adventitious bud from RHS of (a) showing dome of meristematic tissue

(d) Cotyledonary node ca. 400 µm above (a) showing second cotyledonary axillary bud (top arrows), forming from the cortex of the stem (top of photomicrograph); a higher magnification of this region is shown in (e). An adventitious bud (outlined arrow, bottom RHS) is present on the adaxial wall of the sheath protruding into the lumen. The cortex of the central stem has three leaf traces (small black arrows) on either side, which supply the median and lateral veins of the first true leaves, the bases of which are visible in Plate 5.14d.

(e) Axillary bud region in the axil of the second cotyledon, a higher magnification of (d) showing the numerous endogenous accessory buds (arrows); the axillary bud is, however, not present in this section.

Scale is the same for: (a) & (d), bar = 500µm; (b), (c) & (e), bar = 100 µm

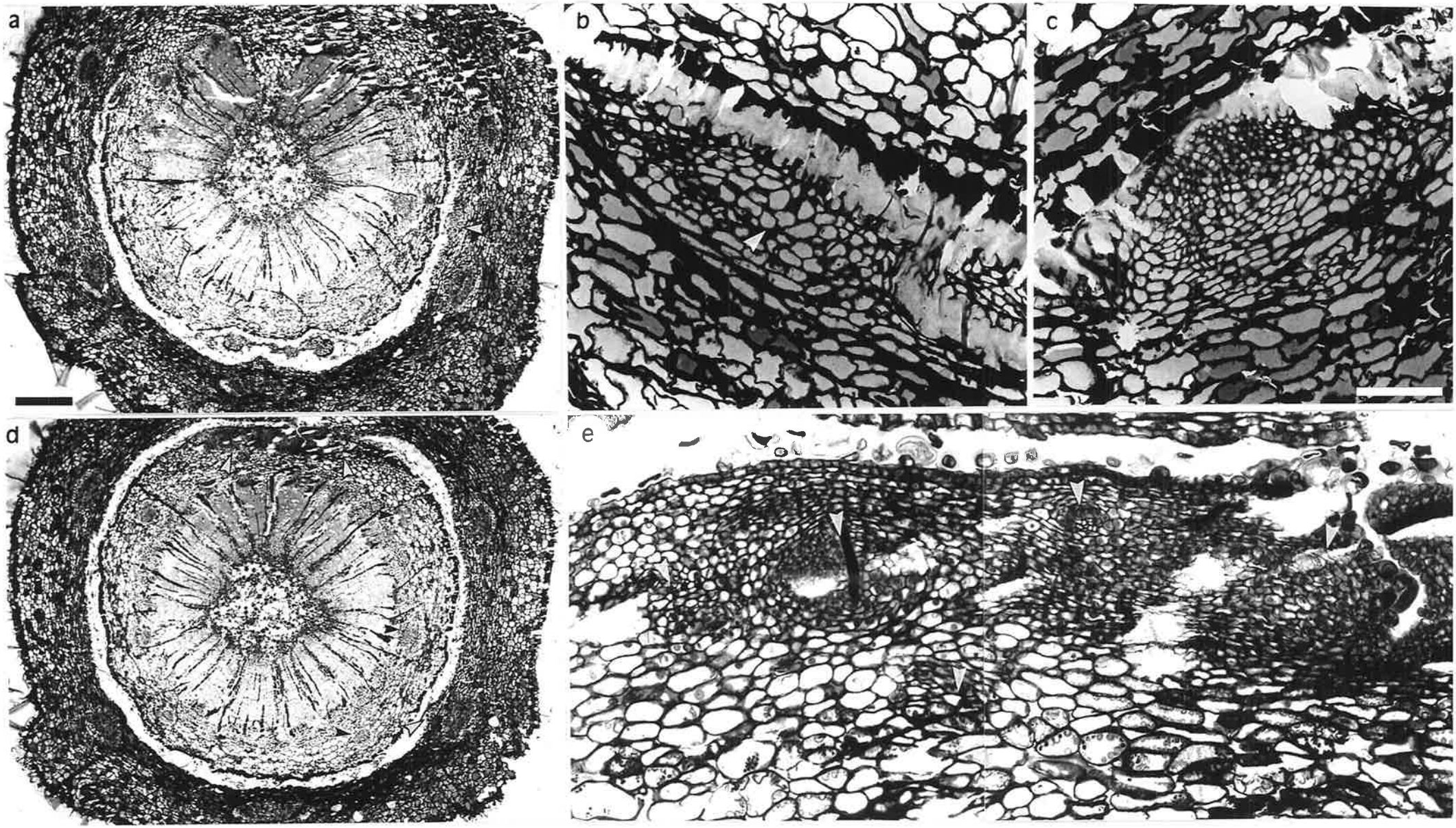

a
b
c
d
e

Plate 5.14 Cotyledonary node anatomy in *B. menziesii* at 26 weeks

(a) Cotyledonary node ca. 1130 µm above Plate 5.13d depicting the cotyledonary ligule (visible on the LHS) attached to the sheath of fused cotyledon bases. Two adventitious buds (arrows) have formed on the adaxial surface of the sheath. The LH bud comprising a single meristematic dome is shown at higher magnification in (b). The RH adventitious bud, comprising a meristematic region of superficial cells, is shown at higher magnification in (c). Note the base of the first true leaf on the RHS of the inner stem.

(b) Cotyledonary ligule (LHS) joined to main axis and LHS adventitious bud from (a)

(c) Meristematic region (arrow) from (a) showing an early bud primordia derived from superficial cells of the adaxial wall of the sheath (RHS of photomicrograph)

(d) Cotyledonary node ca. 960 µm above (a) showing the adventitious buds at the point where the fused bases of the cotyledons separate giving individual cotyledonary laminae on the LHS of the main axis; three buds (arrows) can be seen, with a possible forth. Another adventitious bud (arrow) is present on the inner adaxial surface of the sheath on the RHS immediately beneath the juncture between the opposite ligule (not shown) and the main axis. The axillary bud (outlined arrow) in the axil of the first true leaf is visible on the RHS of the central stem.

(e) Three adventitious buds (buds 9, 10 and 11, refer to Plate 5.15) at higher magnification from LHS of (d)

(f) Adventitious bud at higher magnification from (d) originating from the sheath of cotyledonary laminae still fused at the right side. The bud apex has prophylls on either side.

Scale is the same for: (a) & (d), bar = 500µm; (b) & (c), bar = 500µm; (e) & (f), bar = 100 µm

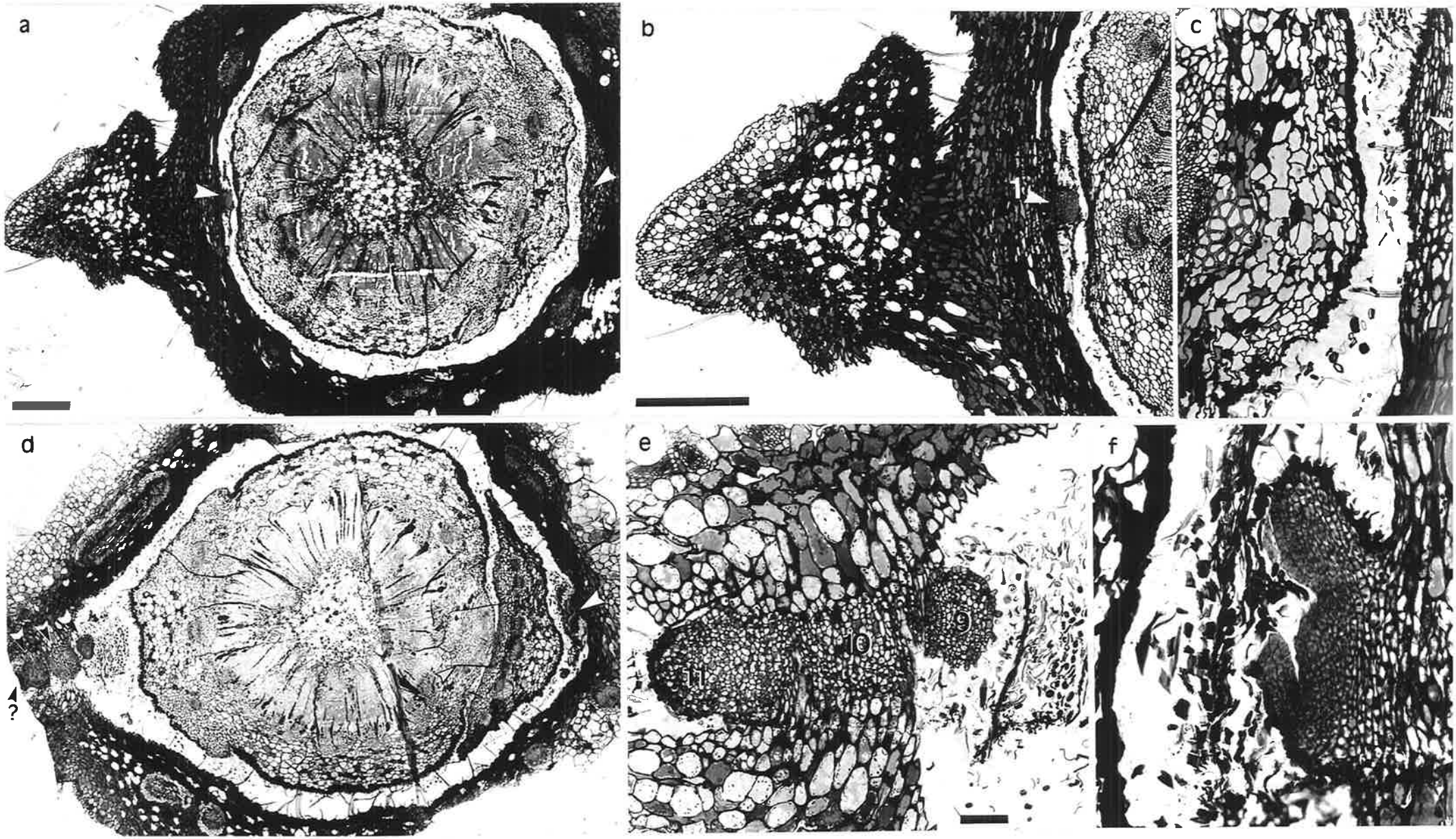

a
b
c
d
e
f
1
9
10
11
?

Plate 5.15 Adventitious bud series in the lumen adjacent to the cotyledonary ligule

Series of adventitious buds along the fusion line between the bases of the cotyledons on the LHS. Buds nos 2 to 8 are found between bud 1 (adjacent to the ligule in Plate 5.14a & b) and buds 9, 10 and 11 (Plate 5.14d & e).

(a) bud no. 2, 240 µm (ca. 60 sections) further on from bud no.1 in Plate 5.14a & b

(b) buds nos. 3 and 4, 120 µm (ca. 30 sections) on from (a)

(c) buds nos. 5 and 6, 120 µm (ca. 30 sections) on from (b), upper surface of bud 4 still visible

(d) 40 µm (ca. 10 sections) on from (c) showing the beginning of development of bud no. 7 in the axil above bud no. 6

(e) 80 µm (ca. 30 sections) on from (d) showing bud no. 7 and the upper surface of bud no. 6

(f) 40 µm (ca. 10 sections) on from (e) showing the top of bud no. 8 and the bud no. 9 on the adaxial wall of the sheath, seen also in Plate 5.14(e) together with the last two buds nos. 10 and 11

Scale is the same in (a) to (e), bar = 100µm

Scale in (f), bar = 500 µm

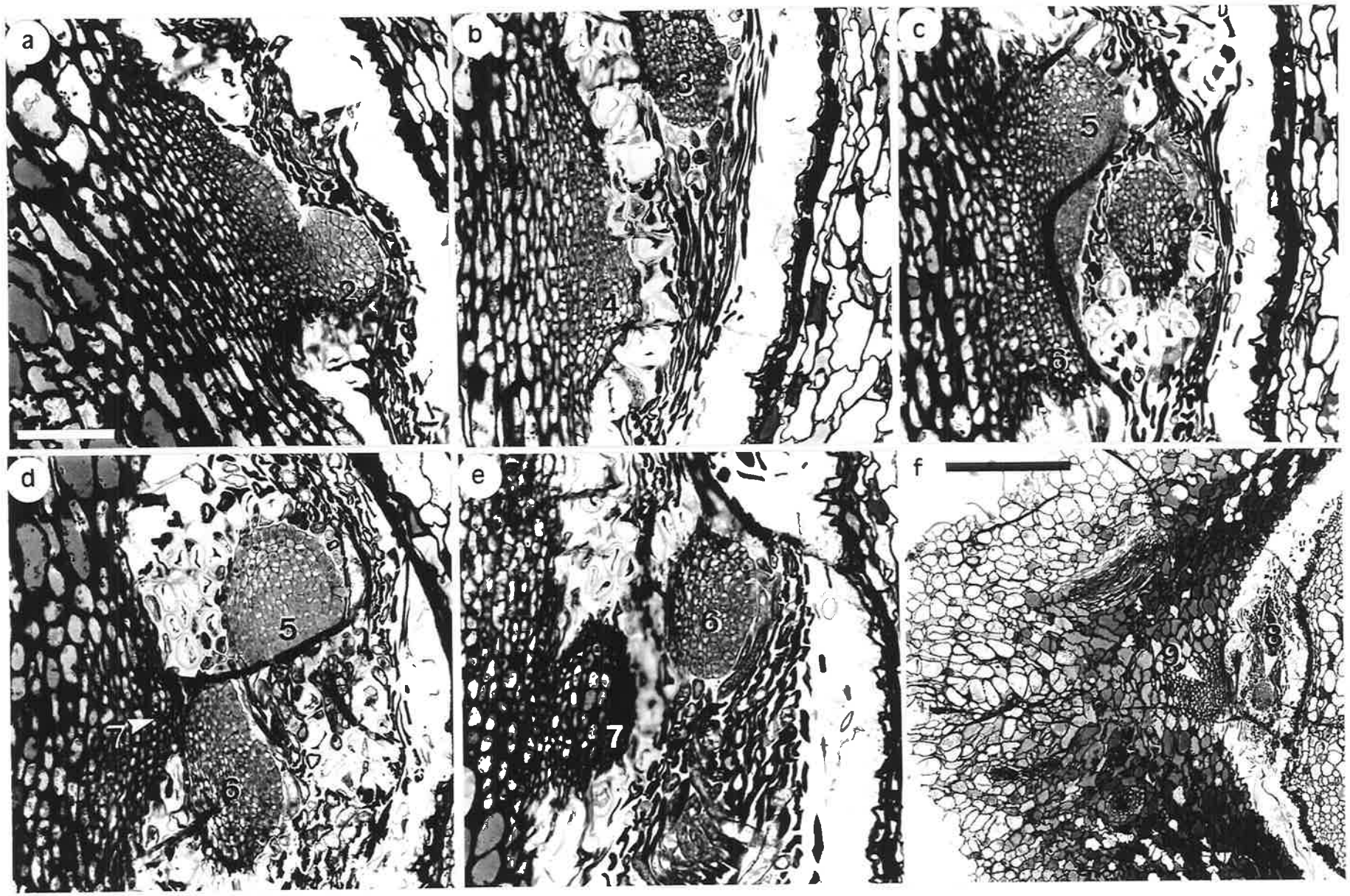

a
2
b
3
4
c
5
6
d
5
7
6
e
6
7
f
9
8

Plate 5.16 Cotyledonary node anatomy in *B. menziesii* at 26 weeks

(a) Cotyledonary node ca. 120 µm above Plate 5.14d consisting of the central stem on which the bases of the first true leaves are visible, the one subtending an axillary bud (arrows) shown at higher magnification in (b). The sheath formed by the base of the cotyledons has separated on the LHS, and the upper surface of adventitious bud no. 11 (bottom LHS arrow) is visible in the gap. Another adventitious bud (upper corner RH arrow) has formed on but still fused to the adaxial surface of the cotyledon bases on the RHS of the main axis.

(b) Enlargement of axillary bud in (a) showing the bud apex and adjacent prophylls (outlined arrows) arising from the cortex of the central stem. An adventitious bud on the adaxial wall of the sheath originates from superficial tissues. Bar = 100µm

(c) Cotyledonary node ca. 520 µm above (a) depicting an axillary bud (small arrows) in the leaf base (large arrows) axil on the LHS of the stem. Note the cotyledons, still in close proximity to the stem, have been filed back or are outside the area photographed.

Scale is the same in (a) & (c), bar = 500µm

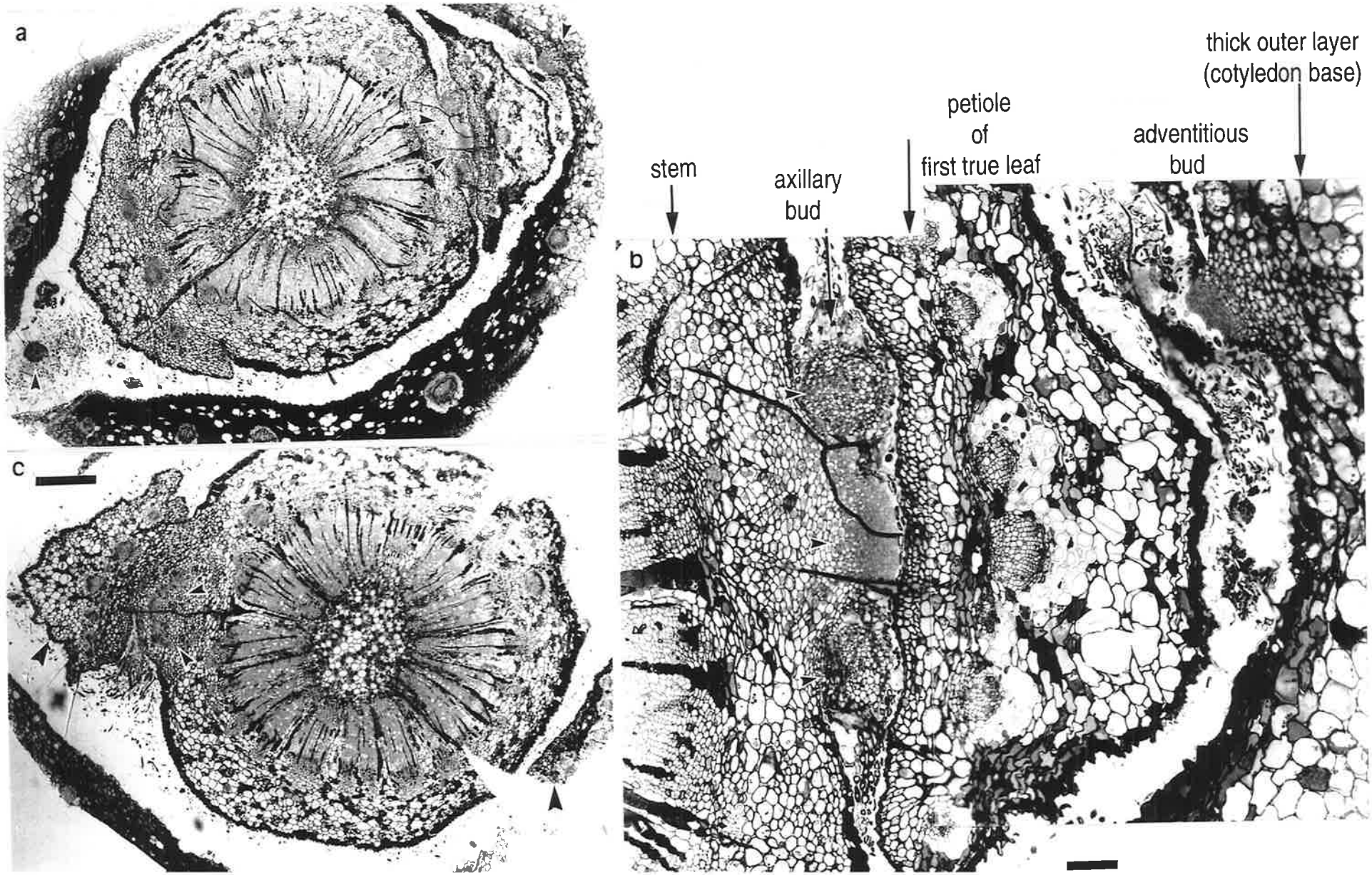

a
b
c
stem
axillary bud
petiole of first true leaf
adventitious bud
thick outer layer (cotyledon base)

Plate 5.17 Cotyledonary node anatomy in *B. menziesii* at 26 weeks

(a) Cotyledonary node ca. 280 μm above Plate 5.17c showing stem and axillary bud (arrow) in the axil at the second true leaf base (p) on the LHS, shown at higher magnification in (b)

(b) Axillary bud from (a) consisting of shoot apex and darkly stained prophylls either side

(c) Same axillary bud several sections above (b) shown at higher magnification in (d)

(d) Axillary bud from (c) at higher magnification showing the lobed structure of the shoot apex

(e) Base of stem ca. 280 μm above (a) is no longer ensheathed by the cotyledons. The apex and one prophyll of the second axillary bud are visible (arrows) and both leaf bases (p) are well apart from the stem (only one is visible).

Scale is the same in: (a) & (e) (bar = 500μm); (b) & (c) (bar = 500 μm)

Scale in (d): bar = 100μm

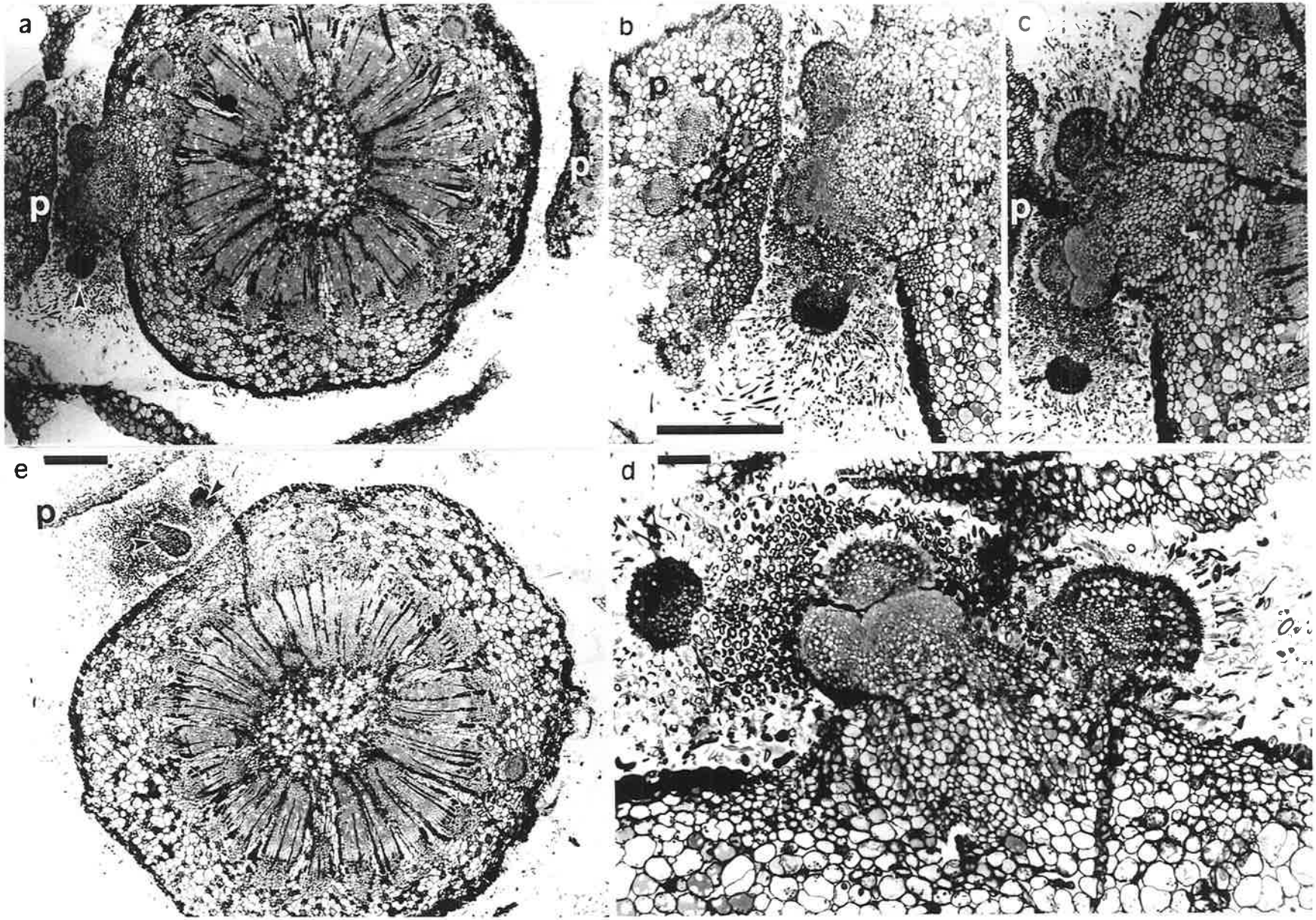

a
p
p
b
p
c
p
e
p
d

Plate 5.18 Cotyledonary node anatomy in *B. menziesii* at 26 weeks

Colour photomicrographs showing the Periodic acid - Schiff's and Toluidine Blue O staining of cotyledonary node tissues

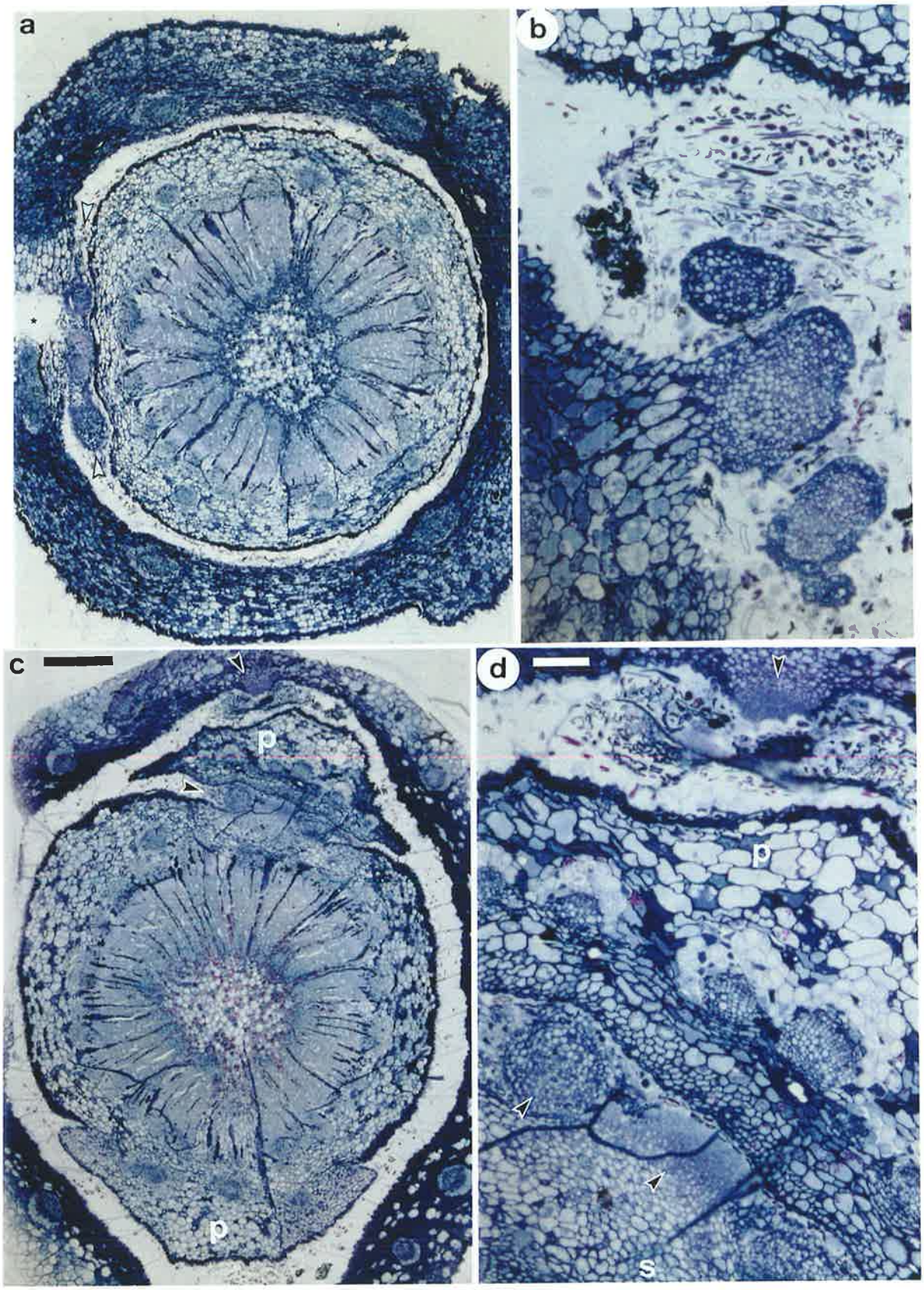
(a) TS of cotyledonary node between Plates 5.13d and 5.14a showing cotyledonary axillary buds (arrows) in the LHS lumen ensheathed by the fused bases of the cotyledons which are phenolic rich. Scoring (*) of the outer tissue layers was necessary to allow fixative penetration.

(b) Three adventitious buds located where the fused cotyledon bases separate to form each cotyledon lamina. The uppermost surface of the middle bud is visible in (c).

(c) TS of cotyledonary node (Plate 5.16a in black and white) showing axillary bud (arrows) between the leaf base (p) and main stem and adventitious buds (top and bottom arrows) arising from the sheath. The cotyledonary laminae have separated (long arrow, bottom centre) at one side of the stem axis. Note the starch in the interfascicular rays of the stem vasculature and the pith (pink staining).

(d) Axillary and adventitious buds (arrow top edge) from (c) at higher magnification. p = leaf base, s = stem

Scale is the same in (a) & (c), bar = 500µm; and in (b) & (d), bar = 100 µm


a
b
c
d
p
p
p
s

Pruning challenge of *B. spinulosa* var. *spinulosa*

Twelve months after pruning 18 month old *B. spinulosa* var. *spinulosa* potted plants the total number of lignotuberous shoots and buds was greatest in unpruned plants (Figure 5.8). However, shoots were significantly longer in pruned treatments (Figure 5.9), with the high pruning producing marginally longer shoots than the lower pruning regime at the end of the first season's growth. Generally, pruning increased the length of lignotuberous shoots already present. Figure 5.10 shows the percentage of different lengths of lignotuberous shoots produced in response to the three pruning treatments. Although the initial complement of buds in all treatments was similar (ca. 85% of < 5 cm buds and ca. 15% of 5 – 10 cm shoots), attrition of shoots occurred over the summer. This was followed by a period of rapid growth during the autumn, slowing over winter and increasing slowly in spring. In autumn and spring the trends is for pruned plants to form a higher percentage of long shoots (< 5mm). The pruning level also influenced plant survival because the harsher low pruning regime had a 25% survival rate after 12 months, whereas 55% and 70% survival was obtained for the high prune and control plants, respectively. Air and soil temperature maxima and minima recorded for the duration of the trial are presented in Figure 5.11. The mean maximum air temperature over summer (1995 and 1996) was ca. 35°C. The winter maximum mean air temperature was 14.2°C (July 1996).

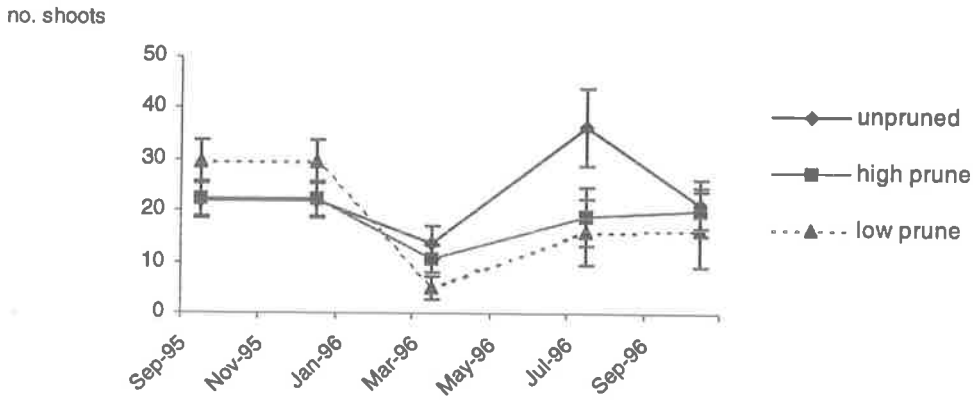


Figure 5.8 Total number of shoots and buds from *B. spinulosa* var. *spinulosa* pruned in September 1995 ($P \leq 0.05$)

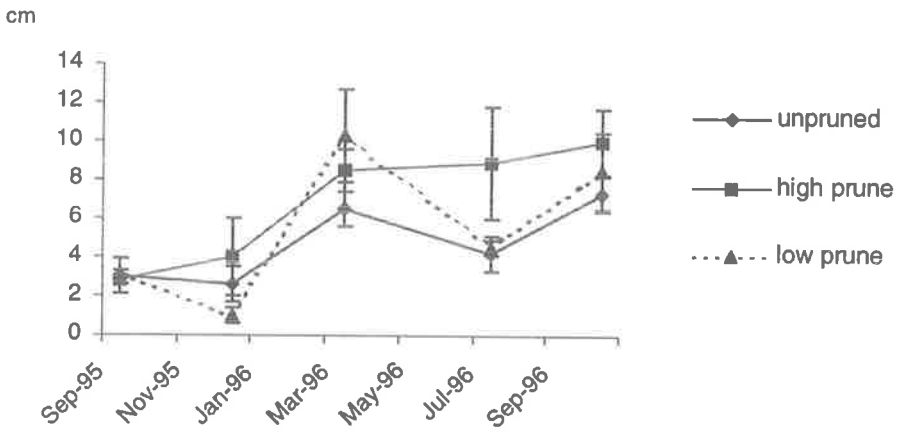


Figure 5.9 Average length of shoots from *B. spinulosa* var. *spinulosa* pruned in September 1995 ($P \leq 0.05$)

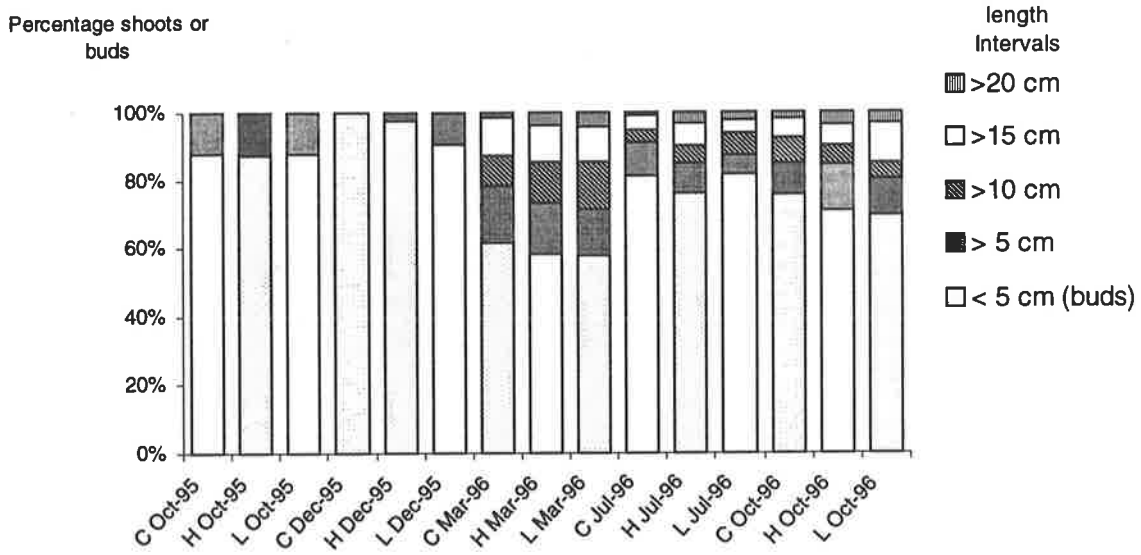


Figure 5.10 Percentage of bud (< 5 mm) and shoot lengths from the lignotuber of *B. spinulosa* var. *spinulosa* pruned in September 1995. C, control; H, high prune, L, low prune

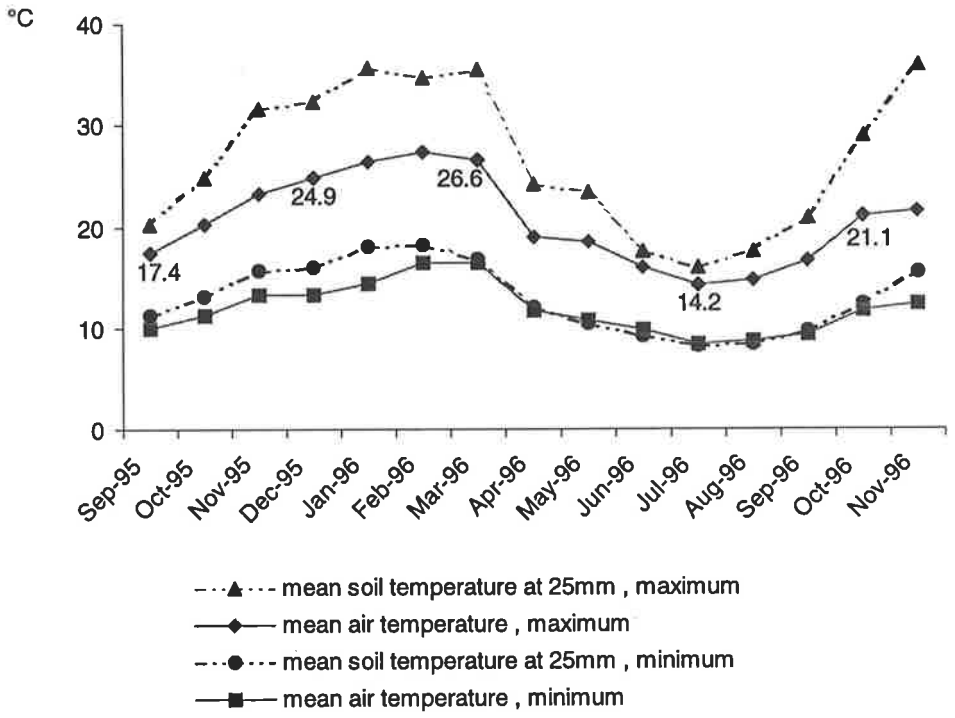


Figure 5.11 Air and soil temperatures for the duration of pruning trial; actual mean maximum air temperature given for sampling times; source: Waite Institute meteorological data

Discussion

In older *Banksia* plants the cotyledonary node is at or beneath ground level and develops into what is loosely termed the *lignotuber* in those species bearing the lignotuberous trait. The gross morphology of *Banksia* lignotubers shows buds are either hidden (Plate 5.5) or superficial (Plate 5.1), occurring usually at the cotyledon. As in other lignotuberous genera, lignotubers in *Banksia* fulfil a regenerative function. They have a reserve of buds, with the capacity to be induced in the event of defoliation, and nutrients to support rapid regrowth once the aerial part of the plant and its photosynthetic capacity have been destroyed. So, what are the anatomical differences in the cotyledonary node that affords lignotuberous plants regenerative capacity not found in non-lignotuberous plants? In particular, what types of buds are present, how are they protected and are they of finite number? This study answers some of these questions, providing information on anatomical features of cotyledonary nodes in post-emergent seedlings of *B. menziesii*, a lignotuberous species with hidden buds, and *B. serrata*, a non-lignotuberous species, as well as information on the response of *B. spinulosa* var. *spinulosa*, a lignotuberous species with superficial buds, to pruning. Furthermore, how this information has horticultural application for the clonal propagation of *Banksia* is also discussed.

The early stages of cotyledonary node development in *B. menziesii* seedlings produce the foundation of the resprouting lignotuberous structure observed in established plants. Anatomical observations confirm the widely held view that lignotubers are regenerative organs bearing buds that enable plants to rapidly regenerate new aerial parts after defoliation or destruction, usually by fire. The thick sheath formed from the fused bases of the cotyledons provides protection to cotyledonary axillary and adjacent accessory buds, the adventitious buds arising from the adaxial wall of the sheath, as well as the axillary buds found in the axils of the first pairs of true leaves. The axillary buds of the first pair of true leaves are considered to be integral to the cotyledonary node, which later constitutes the lignotuber, due to the short internode between them and the cotyledonary axil, and the fact that they are also protected by the cotyledonary sheath. Adventitious buds were the most numerous type of bud found in *B. menziesii*, accounting for 26 out of a total of 36 buds per cotyledonary node, whereas in *B. serrata* none were observed. The types of buds located in the cotyledonary node of *B. serrata* consist entirely of axillary buds located in the axils of the cotyledons and the first pair of

true leaves, totalling four buds per cotyledonary node (Table 5.2). In this species the base of the cotyledons are not fused. Although *B. serrata* is not lignotuberous (George, 1987), it often has a thick, swollen stem base (Plate 5.2a & b) and compact habit due to the small internodes produced in the first growth season (Figures 5.1 and 5.2). This results in many basal buds, which may develop into epicormic buds, explaining the prolific basal shooting observed in older plants of *B. serrata* (refer to Plate 4.10b & c). As the plant matures, epicormic buds (Chattaway 1958; Fink 1983) develop at the scars of leaf and axillary buds, becoming buried by the developing thick periderm characteristic of *B. serrata*. Each scar bears a lateral bud. If a young *B. serrata* seedling has, for example, 12 leaves at the end of its first summer (Figure 5.3), with little or no internode development, there will be 12 epicormic buds at the base of the stem which become overgrown by the periderm in the maturing plant. Whether this finite number of buds is static, or whether the formation of adventitious or epicormic buds can be induced in non-lignotuberous species is unknown and requires further research; such research is also needed to understand the physiology of axillary buds and possible accessory buds in this genus.

In *B. menziesii* and *B. serrata* the cotyledonary node anatomy is symmetrical, with three of the six vascular bundles in the outer sheath of the lignotuberous cotyledonary node of *B. menziesii* supplying each cotyledons. In *B. serrata* vascular traces of the cortex of the upper hypocotyl supply the cotyledons. The first pair of true leaves can be seen at the edge of the stem perpendicular to the cotyledons, each having three main vascular traces; and the second pair is set perpendicular to the first pair of true leaves. The vascular anatomy of the leaf nodes observed in *B. menziesii* and *B. serrata* agrees with other research (Metcalfe & Chalk 1979) showing the trilaclunar structure of leaf traces in the stele with a median and two lateral leaf traces for each leaf base.

The differences between the early ontogeny of the cotyledonary node in lignotuberous and non-lignotuberous *Banksia* species is visible from observations made in this study. The outer sheath in *B. menziesii* is derived from the basal tissue of the cotyledons, which are fused at the sides. This observation is also supported by the fact that many lignotuberous species of *Banksia* have thick large cotyledons, as in Series *Prostratea*, which have the largest cotyledons (Thiele 1996). As the cotyledons of *B. serrata* do not fuse, and although this species has a cotyledon of similar size to *B. menziesii* (Thiele 1996), the generative and protective structures of the sheath that are vital to lignotuber

function are not present. It would be interesting to examine individual lignotuberous and non-lignotuberous populations of *B. marginata* to explore if the fused cotyledon base is a predisposition for adventitious bud formation as seen in *B. menziesii* and whether this is the case in other species with hidden buds.

The outer protective sheath in post emergent seedlings at eight weeks is green living tissue that is thicker than the sheath in *B. menziesii* at 26 weeks. In later stages phenolics and tannins accumulate in the tissues of the sheath, which eventually becomes lignified, developing into a woody encasement protecting the internal axillary, accessory and adventitious buds. In some species, such as *B. oblongifolia*, a very large woody, subterranean lignotuber is formed attaining volumes in the range of square metres (Zammit, 1988). The ontogeny of such a structure in relation to the structures observed here require clarification, but are likely to originate from tissues originally derived from the cotyledonary node.

Unlike eucalypts, which regularly produce lignotubers at the first few leaf nodes as well as the cotyledonary node, *Banksia* lignotubers are usually produced at the cotyledonary node only. One exception, *B. robur,* produces masses of superficial buds at the base, as well as irregularly along the stem, probably at the sites of former leaf scars (Plate 5.1a & b). This species, and several others such as the lignotuberous varieties of *B. spinulosa*, display a distinctly different form of lignotubery, having superficial (exogenous) buds covering the surface of the cotyledonary node which are visible to the naked eye and without protection. In the other form *Banksia* lignotuber buds are hidden beneath the surface. This appears similar to the well described situation in eucalypts where either naked or concealed buds are hidden deeply in the tissues of the lignotuber. The early lignotuber or cotyledonary node of *B. menziesii* exhibits hidden buds similar to the types existing in eucalypts - axillary, accessory and adventitious buds - however, their location and the structure of the protective sheath around them is distinctly different to the protective tissues described in eucalypts. This raises the question: how does the anatomy of the cotyledonary node of *Banksia* species with superficial buds differ from that of species with hidden buds? The answer this question requires further research and microscopic examination. A comparison of species such as *B. spinulosa* var. *spinulosa* or *B. robur,* which have superficial buds, with species with hidden buds, such as *B. menziesii* or *B. oblongifolia*, may help clarify the nature of these structures.

The first signs of lignotuber development in *B. menziesii* are unlike that of eucalypts, in which swellings are initially produced on the stem above the concealed accessory buds, developing to a larger size above the axillary shoot and eventually spreading to the lower side, encircling the shoot (Chattaway 1958). In *B. menziesii* the swelling occurs more or less evenly around the circumference of the cotyledonary node only, below the point of attachment of the cotyledons. The buds in eucalypts are concealed and slowly enveloped as the node grows. In *B. menziesii* on the other hand, arrangement of exogenous adventitious buds, originating from superficial tissues on the adaxial wall of the sheath, is different to the endogenous adventitious buds buried deeply in the tissues of the cotyledonary and first leaf nodes in eucalypt (Graham *et al.* 1998). In *B. menziesii* the thick sheath affords protection to the buds in the lumen and axils of cotyledons and the first pair of leaves. The sheath therefore functions in a similar manner to the thickened swelling of the stem above the buds or via the protective phenolic-containing flap that covers the concealed buds in eucalypts (Chattaway 1958); however, no air-filled lumen is present.

It is possible to stimulate the buds through the removal of apical dominance by pruning, as observed in the pruning challenge of *B. spinulosa* var. *spinulosa*. A seasonal effect was observed in the activity of the exogenous lignotuber buds in pruned *B. spinulosa* var. *spinulosa* plants. Higher temperatures appear to inhibit new buds from shooting and promote extension growth in lignotuber shoots already present. Apical dominance is also accentuated by high summer temperatures (Blake & Carrodus 1970), and this may explain the high proportion of buds (< 5 cm length) relative to longer shoots observed in December in *B. spinulosa* var. *spinulosa* (Figure 5.10). In other lignotuberous species from the chapparal, California, a cessation of lignotuberous shoot growth is observed when air temperature is high and soil moisture low (James 1984). This may also explain the low number of shoots, as opposed to buds in *B. spinulosa* var. *spinulosa* over the summer. Stress is more likely to occur in potted plants with confined root systems, growing under conditions of high soil and air temperature. This seasonal effect is intensified by pruning, which causes latent buds on the lignotuber surface to commence extension growth earlier and form shoots relative to unpruned plants.

Furthermore, growth measurements of unchallenged seedlings showed prolific basal sprouting from the cotyledonary node, although the plants were not pruned. Plant stress due to the restrictive growth conditions in 10 cm pots and the lack of fertiliser may have

induced a resprouting response. The anatomical observations of the number and type of buds present in *B. menziesii* were also made on unchallenged seedlings. *B. menziesii* showed a greater diversity in the type and number of buds due to the presence of accessory and adventitious buds in addition to axillary buds, in comparison to non-lignotuberous *B. serrata,* which had only axillary buds. Further controlled challenge experiments conducted on *B. menziesii* by removing apical dominance, followed by microscopic observations would provide information on how the different types of buds respond and whether the induction of subsequent buds is possible after the original suite of buds observed in unchallenged cotyledonary nodes has been exhausted. Additional longitudinal sectioning is required to determine whether accessory buds form adaxially and abaxially to the cotyledonary axillary buds, similar to the arrangement to eucalypts. Freeze fracturing and scanning electron microscopy at the fusion line on the inner surface of the sheath in *B. menziesii* would provide precise information on the distribution and number of adventitious buds which occur there and elsewhere on the inner adaxial surface of the sheath.

The biomass production from lateral shoots in non-lignotuberous *B. spinulosa* var. *cunninghamii* is probably similar to that produced from the cotyledonary node in *B. spinulosa* var. *spinulosa*. However, the lignotuberous variety would have a greater potential to undergo continuous re-shooting if subsequent bud initiation in the lignotuber is stimulated. The extent to which this could occur depends on the season and the age of the plant; both influence the starch reserves present and whether they can be replenished between periods of intensive carbohydrate usage.

The horticultural application of the use of lignotuberous *Banksia* species may be of increasing importance in cut-foliage production. *Banksia* blooms are well known for their long vase life; foliage produced from coppicing or pruning of resprouting species may be beneficial in producing filler material of complementary vase life, capitalising on some of the novel leaf forms found throughout the genus.

Understanding the genetics of the lignotuber trait would aid crossbreeding programs targeted at introducing this trait into non-lignotuberous species. Such a phenomenon has been observed in the Proteaceae, where hybrids of lignotuberous *Leucadendron cuneiforme* and non-lignotuberous *L. cordifolium* developed lignotubers (Britts *et al.* 1986). This would be desirable in interspecific crosses of *Banksia*, especially in difficult to propagate cut-flower species, if lignotuber shoots were found to have improved

rooting potential. The inherent difficulty in producing interspecific *Banksia* hybrids (refer to Chapter 3) may render this difficult, although genotype selection for these characters may alleviate this. Lignotuberous *B. spinulosa* var. *spinulosa* and the closely related, but non-lignotuberous sister species, *B. spinulosa* var. *cunninghamii,* provide an ideal starting point for studying lignotuber genetics. Using molecular biology techniques, the identification of the gene or genes responsible for the presence or absence of lignotubers in these varieties may be possible, thus providing fundamental information, which may lead to the production of transgenic, lignotuberous *Banksia* cultivars which can be clonally propagated with ease.

Although exogenous (axillary and adventitious) and endogenous (accessory) buds may serve the same regenerative function in *Banksia* as in other genera, their anatomy and physiology may be different enough to influence their use as a source of cutting material for clonal propagation or as tissue culture explants. Several questions arise with respect to the use of these buds and the ensuing shoots for vegetative propagation: do lignotuber shoots exhibit juvenile wood characteristics as found in epicormic and basal shoots from other woody angiosperms (Hartmann 1990; Barrett *et al* 1997)? If so, do they have a greater rooting potential compared to the lateral shoots of mature branches from the same plant? Are they free of sclerotic cells in the primary phloem and other inert fibrous tissues (Beakbane 1961), rendering them better candidates for successful clonal propagation? Would explants from cotyledonary nodes or the cotyledons have a greater totipotency and propensity to regenerate whole individuals through organogenesis or somatic embryogenesis? Cotyledon and hypocotyl explants (Gutmann *et al.* 1996) are widely used in the vegetative propagation of recalcitrant woody species because of their greater regenerative capacity. However, these types of explant have not yet been tried in *in vitro* culture of *Banksia.* Further research is required to find solutions to these questions.

As mentioned earlier a comparative study is required to obtain a clearer picture of the anatomical structures in lignotuberous species with superficial and hidden buds, but work also needs to be carried out on their horticultural application, particularly in clonal propagation. The ontological development of the bud structures, how they respond to defoliation (pruning) and whether they are exhaustible would also provide information for use in horticultural applications of *Banksias* for cut foliage. A comparative study on the structure of the leaf and cotyledonary nodes in *Banksia* would be valuable in

determining whether the presence of concealed accessory buds of leaf nodes is a predisposition to lignotuber formation as observed in eucalypts (Chattaway 1958).

In conclusion, this first report on the anatomy of the cotyledonary node in *Banksia* provides a basis for further applied and fundamental research to address the currently under-used potential of the *Banksia* lignotuberous trait in floricultural applications. This report yet again highlights the diversity present in the genus, particularly in the morphological and anatomical forms of lignotubers. Yet there is little understanding of them or how they can be manipulated and used in selection and breeding programs to develop improved commercial *Banksia* cultivars for the cut flower and foliage industries. The buds observed in the cotyledonary node have the potential to be highly regenerative and could therefore be exploited in clonal propagation via tissue culture or conventional methods. Species, such as *B. menziesii* or other species currently less commercially significant but which may be used to introduce the lignotuberous trait into other commercially desirable forms, could then be developed further. These are the first steps needed to supply growers new improved cultivars, which in turn will help satisfy the seemingly insatiable demand of international markets for floral forms that are captivating and suitable to their end-uses.

Chapter 6

Concluding Discussion

The successful development of new floricultural crop species is founded on three interdependent elements: superior plant material, production technology and effective marketing strategies (Roh & Lawson 1996). Superior plant material, such as plus-tree selections or new hybrids, can only reach its potential if it can be clonally propagated and if it has an ornamental value in the market place, where it satisfies a specific market need. This is only possible if commercial scale production technology and propagation protocols exist for those species. Presently the domestication of endemic species, such as *Banksia*, relies mainly on seed, which has the disadvantages of its inherent variability and often poor or variable germination rates. Few examples of successful varietal improvement exist for species of the Australian flora, including *Banksia*, which have been taken through to the market place. The failure of endemic species to reach their commercial potential is due in many instances to the poor rooting of vegetative propagules. Consequently, the lack of high-yielding, quality clonal selections from existing species is limiting further development of the wildflower industry. The research outcomes presented in this thesis provide fundamental information for further successful development of relatively recently domesticated members of the *Banksia* genus by providing market information, which can be used as criteria in clonal selections and product preparation, as well as an insight into the vegetative biology of the genus, which is needed to improve clonal propagation.

The outcomes of market research presented in Chapter 2 focused on the main export destination of *Banksia* – Germany, providing details on market structure (points of access, parallel channels for dried and fresh product), end-uses of *Banksia* (main segment: grave floristry) and the need for improved quality (flower head to stem ratio, colour, stem length, grading uniformity) and appropriate services (information, consistency of performance and supply). Understanding the end-uses of *Banksia* in the German market allows tailoring of production and post-harvest management to increase customer satisfaction. For instance, the provision of fresh flowers with stem lengths of 30 cm is preferred to longer stems due to the current fashion for round table bouquets. Further opportunities for product development for other niches in the German market exist, such as smaller round blooms with 10 cm stems used as 'picks' in wreaths during the peak seasons of Advent, Christmas and the days of commemoration of the dead

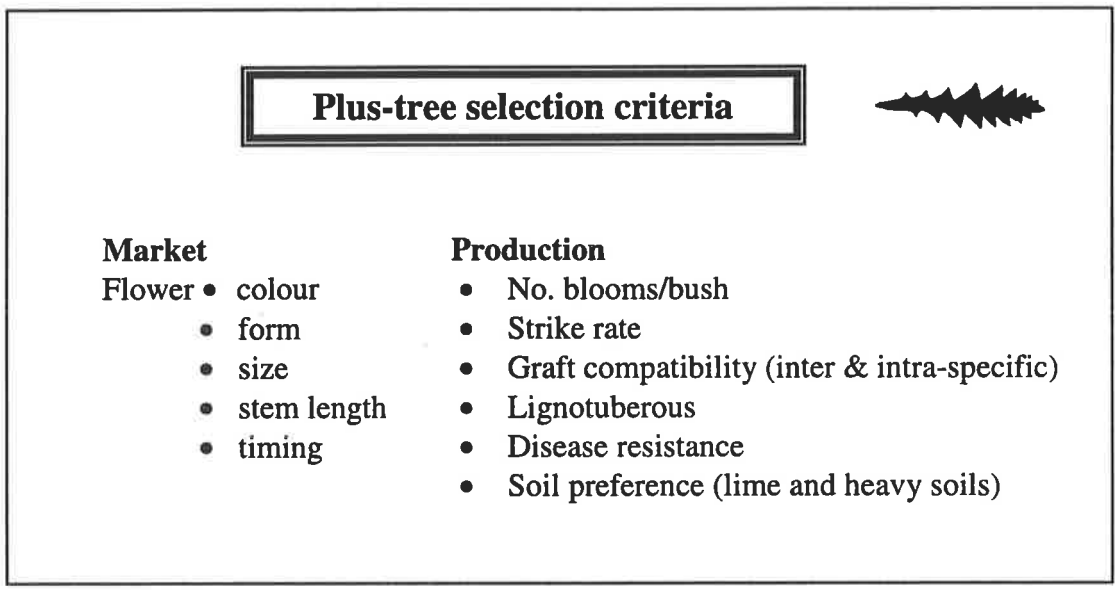
(early November). Furthermore, such market information must be applied in the selection of new improved varieties, together with the relevant production criteria (Figure 6.1). The availability of clonal selections developed to suit market needs gives growers greater control over production, leading to better returns, not to mention improved customer satisfaction. Production from high quality selections could be targeted at times of peak market demand by manipulation of the flowering period (achieved through production location, clonal selections, genetic manipulation or the use of different varieties), for example the extension of *B. coccinea* flowering to coincide with Christmas.

The market research questionnaire presented in Chapter 2 identified *Banksia* quality criteria requiring improvement. All the criteria identified are controllable production variables that are within growers' and exporters' power to improve; they could thereby achieve higher levels of customer satisfaction and maintain buyer interest in Australian grown product, as well as overall international competitiveness. Furthermore, due to the handling of *Banksia* by several members of the market channel (customers), quality should be viewed as 'anything that is a requirement of the end customers' (e.g. box size, number or blooms per box), thereby satisfying all 'customers' in the market channel.

Understanding the fundamental vegetative biology of the *Banksia* genus, shown to be largely unknown in the literature reviewed in Chapters 3, 4 and 5, is critical to overcoming and avoiding the impediments to propagation which have been encountered in this genus. The success to date of *Banksia* as a primary display flower in fresh and dried arrangements for niche markets here and abroad, without varietal improvement, has been due to this crop species' good internal value (long vase-life, high ornamental value, transportability) and high value per unit. However, to ensure future growth and international competitiveness, the flow of new, improved *Banksia* varieties suited to market end uses and with better production features (broader soil-type range, higher yielding, resistance to soil borne diseases) must be provided to industry.

The large number of *Banksia* taxa and widespread interspecific variability is a vast genetic resource, used only marginally and on a relatively *ad hoc* basis, from which superior plant material can be made based on production and market criteria (Figure 6.1). The identification of plus-tree selections is the first step in the varietal improvement program as portrayed in the Flow diagram in Figure 6.2. Outcomes from the research presented in Chapters 3 and 4 impact directly on the development of improved propagation for such plus-selections by conventional methods using cuttings

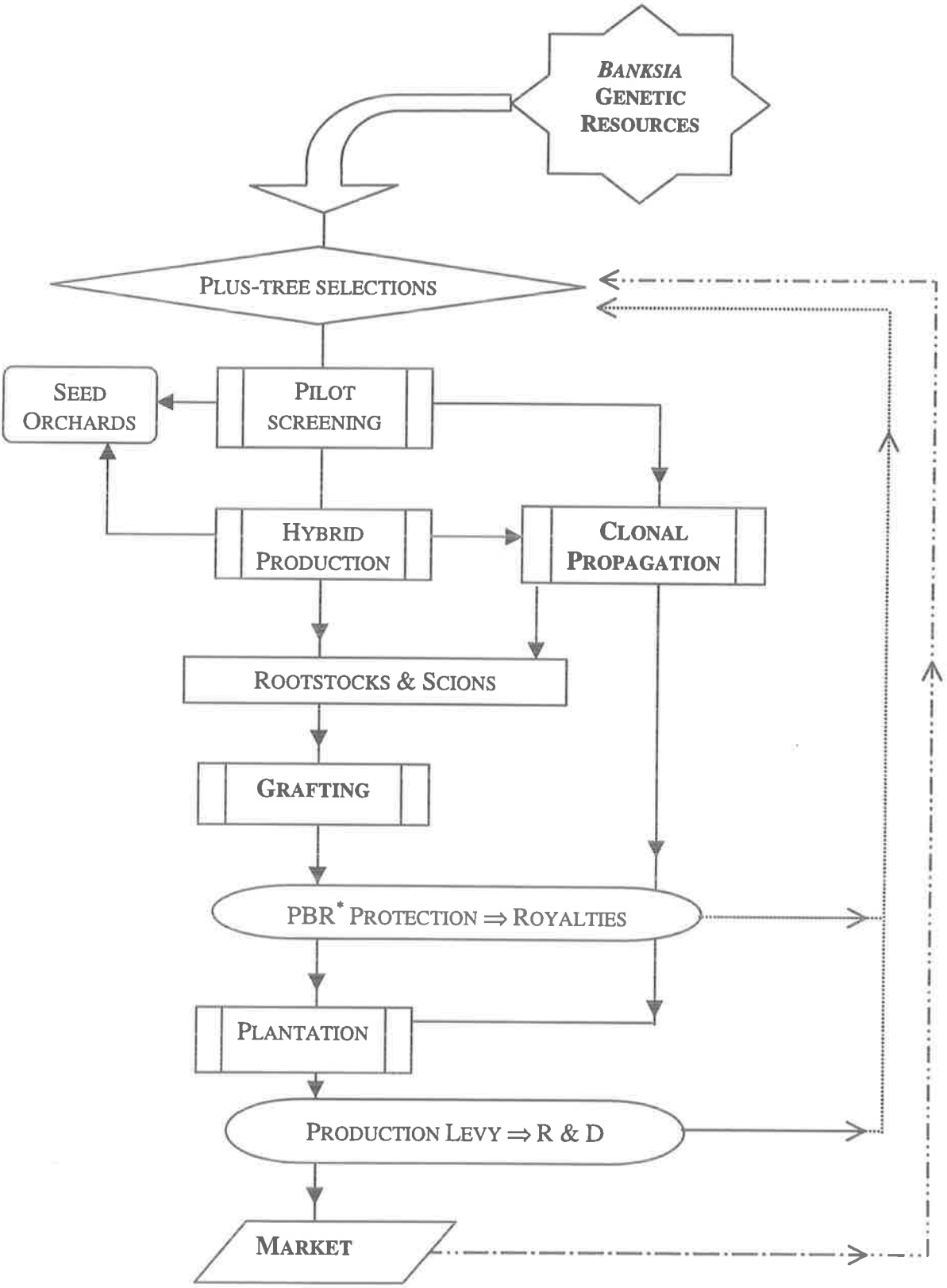
Figure 6.1 Market and production selection criteria of the varietal improvement of *Banksia*



or grafting. Although not studied in this work, the possibility exists of using the various bud types identified in Chapter 5 in *Banksia* lignotubers as explants for micro-propagation and tissue culture. At this stage, the development of the majority of Australian flora involves collection of germ plasm from plants in highly variable populations in their native habitats, largely xeric, harsh environments in the case of *Banksia*. The survival adaptations observed in Chapter 3 in stem material collected from the field (cork, fibres, vasicentric tracheids), together with the primitive nature of *Banksia* wood and our incomplete knowledge of lignotubers, as seen in Chapter 5, have lead to sub-optimal results in initial steps of domestication using asexual methods. Anatomical features in stem tissues, such as fibres, occluded cells, cork, and increased concentrations of secondary metabolites, are likely impediments of root initialisation and callusing in rhizogenesis and grafting, respectively.

Although management systems for propagation stock plants were not studied in this thesis, some inferences can be drawn. A pilot screening phase is the first step of bringing plus-selections of wild plants into domestication, requiring selection and testing of suitable genotypes with desirable characters (high-yielding, disease resistance, high cutting strike rate, graft compatibility). Cultivation under monitored conditions, as discussed in Chapter 3, should be aimed at optimising growth that minimised factors

Figure 6.2 Strategic varietal improvement of *Banksia* based on production and market linked selection criteria



* PBR: Plant Breeders' Rights

identified in reducing rooting and grafting potential. The optimisation of propagation protocols for specific species is required to identify plants with the best propagation characteristics. Managed hedge orchards pruned low to maintain juvenility and hence higher rooting success could be used as a source of propagules for the rapid multiplication of planting stock. Lignotuber resprouting or species with epicormic basal shoots, may be useful in this respect as a source of vegetative propagules. Once through the initial screening and testing phase, plants would be ready for bulking-up by either cutting propagation or grafting.

An understanding of the process of successful graft union, as shown in Chapter 4, together with a knowledge of the current difficulties encountered in grafting seedling-derived *Banksia* (slow time to callus, cell necrosis and collapse of living tissues) highlights the long term requirement for selection, testing and stock plant management to develop suitable, uniform rootstocks that can be clonally produced. This is particularly so in light of the low success of interspecific grafting attained here. Hybrids of closely related species or varieties may provide progeny with suitable rootstock characteristics and increased genetic compatibility. The early flowering of the grafted scion selections is desirable in seed orchard production, the production of hybrids and in testing progeny (earlier maturity gives earlier test results). Intraspecific grafting, shown in Chapter 4 to have the best chances of success in *B. serrata* can be immediately applied in the clonal propagation of selected elite scion genotypes (earlier or later flowering, terminal flowers or attractive colour variants) by grafting onto seedling or clonally produced sibling rootstocks of the same species. However, optimising the grafting protocols for *Banksia* species is mandatory, and should be based on successful models developed for macadamia and cashew (Coombs 1995).

In Chapter 5 the preliminary exploration of the biology of the cotyledonary node, which later develops into the lignotuber in 50% of *Banksia* species, showed this bud bearing organ to be an untapped resource for horticultural application. The cotyledonary node of *B. menziesii* has been identified as a source of three types of buds (axillary, accessory and adventitious) and therefore a possible source of explants for tissues and micro-propagation. Through genotype selection and defined management regimes, high-yielding plants or novel floral types could be rapidly made available via propagules collected from lignotuber shoots. Alternatively, lignotuber re-sprouting after pruning or coppicing could be used in the production of cut foliage with an extended vase life. Lignotuberous species, particularly eastern coast species or natural hybrids may be

useful in the development of clonal rootstocks that have greater tolerance to root diseases and grow in a range of soil types.

Although many questions arise from the research conducted in Chapter 5 on *Banksia* lignotubers, clear directions can be drawn from the outcomes, such as the strategic selection of lignotuberous species and their use in hybrid breeding. This would supply the wildflower industry with material to be used in different strategic paths (clonal rootstock production, stock plant management for the supply of clonal plant material, introduction into non-lignotuberous commercially important species through crossbreeding, cut-foliage production) for the improvement of *Banksia* production systems.

Such strategic varietal improvement programs bring additional benefits, such as maintaining control over unique Australian germ plasm and its identity as an endemic Australian species. PBR protected cultivars return royalties to industry for the further development of product quality, branding and market driven generation of successive floral products, as well as product diversification (value added dried, preserved and dyed flowers and foliage). The exposure of export markets to higher quality, as well as greater production diversity and capacity, generated through varietal improvement, would help maintain long-term buyer interest and reduce dependence on bush harvesting and the associated low export prices received for this product. This in turn would lead to improved conservation and management of natural stands, thus aiding the preservation of endangered species.

The work in this thesis supports the further development of *Banksia*. The varietal improvement of this genus, as outlined in Figure 6.2, must apply market information in the establishment of criteria used to select superior plant material, and in the preparation and value adding of product to be used in strategic marketing planning (appropriate combination of product, placement, price and promotion). Production technology must be founded on sound propagation protocols designed specifically for the species used. To achieve this requires an understanding of the unique biology and specialised characteristics evolved by Australian species to survive in extreme habitat and major climatic shifts. The research compiled in this thesis contributes to both these areas, thus providing knowledge needed for successful varietal improvement. A strategic approach to the development of new varieties will ensure that *Banksia* production has a continued and productive future in Australia.

References

Abell, P. (1988). Grafting Australian plants. *Australian Horticulture* **86**(11), 38-44.

Ackerman, A., Gilad, S., Mechnik, B., Shehori, Y., Ben-Jaacov, J. (1996). Cutting grafts for *Leucospermum* and *Leucadendron* a method for quick propagation by simultaneous rooting and grafting. Abstract in Proceedings of the International Protea Association, 8th Biennial conference, 4th International Protea Working Group Symposium, March 17-21, Israel, p2.

Addison, G., Tavares, R. (1952). Hybridization and grafting in species of *Theobroma* which occur in Amazonia. *Evolution* **6**, 380-386.

Alcaniz, E., Pinochet, J., Fernandez, C., Esmenjaud, D., Felipe, A. (1996). Evaluation of *Prunus* rootstocks for root-lesion nematode resistance. *HortScience* **31**, 1013-1016.

Alexander, D. McE. (1986). Practical hints for budding and grafting fruit and nut trees. CSIRO Australia

Alford, G. (1994). The development of conjoint analysis and value-pricing techniques to assess the market for value-added products from Australia's infant rural industries - a pilot study based on Australian native flowers and Japanese nationals resident in Australia. RIRDC Final Report, December 1994.

Andrews, P. K., Marquez, C. S. (1993). Graft incompatibility. *Horticultural Reviews* **15**, 183-232.

Anthony, J. (1994). An investigation of grafting of the genus *Isopogon* R.Br. Ex Knight (Proteaceae). Horticulture Project III, Bachelor of Applied Science (Horticulture) Thesis, Victorian College of Agriculture and Horticulture, Burnley Campus, Melbourne, Australia. 87 p.

Asante, A. K., Barnett, J. R. (1997). Graft union in mango (*Mangifera indica* L.). *Journal of Horticultural Science* **72**(5), 781-790.

Asper, H. (1984). Propagation of proteas. *International Plant Propagators' Society Combined Proceedings* **34**, 168-173.

Autio, W. R., Greene, D. W., Lord, W. J. (1996). Performance of 'McIntosh' apple trees on seven rootstocks and a comparison of methods of productivity assessment. *HortScience* **31**, 1160-1163.

Bacic, A., Clarke, A. E. (1988). Molecular approaches to understanding cellular recognition in plants. *Recent Advances in Phytochemistry* **22**, 61-81.

Bacic, A., Gell, A. C., Clarke, A. E. (1988). Arabinogalactan proteins from *Nicotiana alata*. *Phytochemistry* **27**(3), 679-684. (N)

Bamber, R. K. (1964). Sapwood and Heartwood. Technical Publication No.2, Forestry Commission of New South Wales.

Bamber, R. K., Mullette, R. K. (1978a). Studies of the lignotubers of *Eucalyptus gummifera* (Gaertn. & Hochr). II Anatomy. *Australian Journal of Botany* **26**, 15-22.

Bamber, R. K., Mullette, R. K. (1978b). Studies of the lignotubers of *Eucalyptus gummifera* (Gaertn. & Hochr). III Inheritance and chemical composition. *Australian Journal of Botany* **26**, 23-28.

Barnett, J. R., Miller, H. (1994). The effects of applied heat on graft union formation in dormant *Picea sitchensis* (Bong.) Carr. *Journal of Experimental Botany* **45**, 135-143.

Barnett, J. R., Weatherhead, I. (1988). Graft formation in sitka spruce: a scanning electron microscope study. *Annals of Botany* **61**, 581-587.

Barrett, C., Lefort, F., Douglas, G. C. (1997). Genetic characterisation of oak seedlings, epicormic, crown and micropropagated shoots from mature trees by RAPD and microsatellite PCR. *Scientia Horticulturae* **70**, 319-330.

Barth, G. (1988). Banksias for cut-flower production, Fact Sheet 21/87 South Australian Department of Agriculture.

Barth, G. (1992). Overseas study tour report 1991 - Holland, Germany & France. Floriculture production and marketing, Greenhouse production systems, floriculture research. Department of Primary Industries, South Australia, November 1992.

Barth, G., Bennell, M. (1987). Selection and grafting studies of *Banksia coccinea* and *Banksia menziesii*. *International Plant Propagators' Society Combined Proceedings* **36**, 220-224.

Beakbane, A. B. (1961). Structure of the Plant stem in relation to adventitious rooting. *Nature* **192**, 954-955.

Beakbane, A. B. (1969). Relationship between structure and adventitious rooting. *International Plant Propagators' Society Combined Proceedings* **19**, 192-201.

Beal, P., Joyce, D., Shorter, T. (1996). Tropical Grevilleas. *Australian Horticulture* **94**(9), 43.

Behnke, H. D. (1995). Sieve-element characters of the Proteaceae and Elaengnaceae: nuclear crystals, phloem proteins and sieve element plastids. *Botanica Acta* **108**, 514-524.

Ben-Jaacov, J., Ackerman, A. (1989). New approaches to the development of proteaceous plants as floricultural commodities. *Acta Horticulturae* **252**, 193-199.

Ben-Jaacov, J., Dax, E. (1981). *In vitro* propagation of *Grevillea rosmarinifolia*. *HortScience* **16(3)**, 309-310.

Ben-Jaacov, J., Ackerman, A., Gilad, S., Carmeli, R., Barzilay, A., Shchori, Y. (1992a). Grafting techniques and the use of rootstocks in *Leucadendron*, and other proteaceous plants. *Acta Horticulturae* **316**, 69-71.

Ben-Jaacov, J., Gilad, S., Ackerman, A., Carmeli, R. (1992b). Grafting and the use of rootstock in *Leucodendron* and other proteaceous plants. *Protea News* **12**, 17.

Bennell, M., Barth, G. (1987). Propagation of *Banksia coccinea* by cuttings and seed. *International Plant Propagators' Society Combined Proceedings* **36**, 148-152.

Ben-Ya'acov, A., Michelson, E. (1995). Avocado rootstocks. *Horticultural Reviews* **17**, 381-429. (N)

Besford, R. T., Hand, P., Peppitt, S. D., Richardson, C. M., Thomas, B. (1996). Phase change in *Prunus avium*: differences between juvenile and mature shoots identified by 2-dimensional protein separation and *in vitro* translocation of mRNA. *Journal of Plant Physiology* **147**, 534-538.

Bickford, S., Sedgley, M. (1994). Colour variation of *Banksia menziesii* blooms for the cut flower production. *Journal of Horticultural Science* **69**(6), 993-997.

Biffen, R. H. (1902). Notes on some grafting experiments. *Annals of Botany* , 174-176.

Biricolti, S., Chiari, A. (1994). Meristem culture and micrografting of *Passiflora edulis* f. *edulis*. *Advances in Horticultural Science* **8**, 171-175.

Blake, J., Hill, R. S. (1996). An examination of the drought and frost tolerance of *Banksia marginata* (Proteaceae) as an explanation of its current widespread occurrence in Tasmania. *Australian Journal of Botany* **44**, 265-281.

Blake, T. J., Carrodus, B. B. (1970). Studies on the lignotubers of *Eucalyptus obliqua* II Endogenous inhibitor levels correlated with apical dominance. *New Phytologist* **69**, 1073-1079.

Bowen, J. B. (1991). Fire response within the family Proteaceae: a comparison of plants displaying the seeder and resprouter mode of recovery. Ph.D. Dissertation, University of Western Australia, Department of Botany, Perth, WA.

Bowen, J. B., Pate, J. S. (1993). The significance of root starch in post-fire shoot recovery of the resprouter *Stirlingia latifolia* R. Br. (Proteaceae). *Annals of Botany* **72**, 7-16.

Boyhan, G. E., Abrahams, B. R., Norton, J. D. (1996). Budding method affects transmission of *Xylella fastidiosa* in plum. *HortScience* **3**, 89-90.

Bretton-Clark (1992). Conjoint Analysis. Software manual, 89 Headquarters Plaza; North Tower, 14th Floor, Morristown, NJ 07960 Fax: 201-993-1757.

Brewer, M., Scott, T. (1983). Concise Encyclopedia of Biochemistry. English ed. de Gruyter Lublishers, Berlin. 518 pages. (Translated from German to English, revised and extended by M. Brewer, T. Scott; original German edition: Brockhaus Verlag, Leipzig, ABC Biochemie, by HD. Jalcubke and H. Jeschkeit, 1976)

Brits, G. J. (1986). The influence of genotype, terminality and auxin formation on the rooting of *Leucospermum* cuttings. *Acta Horticulturae* **185**, 23-30.

Brits, G. J., Jacobs, G., Steenkamp, J. C. (1986). The pruning of proteas for cut flower production. In 'Flowers and Ornamental Shrubs, Farming in South Africa'. 1st ed. Vol. 15. RSA Department of Agriculture and Water Supply, Pretoria, pp. 27-31.

Bronner, R. (1975). Simultaneous demonstration of lipid and starch in plant tissues. *Stain Technology* **50**, 1-4.

Bunker, E. J. (1981). Growing certain Australian native shrubs and trees from softwood cuttings. *International Plant Propagators' Society Combined Proceedings* **31**, 130-133.

Bunn, E. (1992). Towards *in vitro* propagation of Western Australian Proteaceae. *Protea News* **12**, 17.

Burke, D. (1983a). Grafting native plants. *Australian Horticulture* **81**(3), 7-11.

Burke, D. (1983b). Growing Grevilleas in Australia and New Zealand. Kangaroo Press, Sydney, Australia. 80 pages.

Burton, N., Reid, A., Growns, D. (1994). Seasonal reminders. *The Floriculture Industry Newsletter* , 19-21.

Byrne, B. (1996). Quandong project up-dates - grafting quandong. In: The Australian Quandong Industry Association Newsletter, Autumn, AQIA Inc. PO Box 236 Upper Sturt, SA 5156.

Carlquist, S. (1985). Vasicentric tracheids as a drought survival mechanism in the woody flora of southern California and similar regions; Review of vasicentric tracheids. *ALISO* **11(1)**, 37-68.

Carr, D. J., Jahnke, R., Carr, S. G. M. (1984a). Initiation, development and anatomy of lignotubers in some species of *Eucalyptus*. *Australian Journal of Botany* **32**, 415-437.

Carr, D. J., Jahnke, R., Carr, S. G. M. (1984b). Surgical experiments on eucalypt lignotubers. *Australian Journal of Botany* **32**, 439-447.

Carrodus, B. B., Blake, T. J. (1970). Studies on the lignotubers of *Eucalyptus obliqua*. I The nature of the lignotuber. *New Phytologist* **69**, 1069-1072.

Carthew, S. M. (1992). Population genetic structure of *Banksia spinulosa*. *Heredity* **70**, 566-573.

Carthew, S. M., Ayre, D., Whelan, R. J. (1988). High levels of outcrossing in populations of *Banksia spinulosa* R.Br. and *Banksia paludosa* Smith. *Australian Journal of Botany* **36**, 217-223.

Carthew, S. M., Whelan, R., Ayre, D. J. (1996). Experimental confirmation of preferential outcrossing in *Banksia*. *International Journal of Plant Science* **157**, 615-620.

Cavanagh, A. K. (1997). The Biology, ecology and horticultural potential of *Banksia* L. f: A bibliography of recent literature. *The Victorian Naturalist* **114**(2), 77-82.

Chattaway, M. M. (1948a). Note on the vascular tissue in the rays of *Banksia*. *Journal of the Council of Scientific Industrial Research of Australia* **21**, 275-278.

Chattaway, M. M. (1948b). The wood anatomy of the Proteaceae. *Australian Journal of Scientific Research*, B **1**, 279-303.

Chattaway, M., M (1958). Bud development and lignotuber formation in eucalypts. *Australian Journal of Botany* **6**, 103-115.

Cho, J. J. (1983). Variability in susceptibility of some *Banksia* species to *Phytophthora cinnamomi* and their distribution in Australia. *Plant Disease* **67**, 869-871.

Clarke, A. E., Knox, R. B. (1979). Plants and immunity. *Developmental and Comparative Immunology* **3**, 571-589.

Clemesha, S. (1988). Grafted banksia by irrigated approach grafting. *Banksia Study Report* **8**, 12-15.

Clemesha, S. (1992). A further note on grafting. *Banksia Study Report* **9**, 50-51.

Cohen, Y., Gur, A., Barkai, Z., Blumenfield, A. (1991). Decline of persimmon (*Diospyros kaki* L.) trees on *Diospyros virginiana* rootstocks. *Scientia Horticulturae* **48**, 61-70.

Coombs, B. (1995). Horticulture Australia. The complete reference of the Australian horticultural industry, Moorescope Publishers Pty. Ltd., Melbourne

Copes, D. (1970). Initiation and development of graft incompatibility symptoms in Douglas fir. *Silvae genetica* **19**, 101-106.

Costin, R. (1985). Domestication of the Australian tropical Proteaceae. *International Plant Propagators' Society Combined Proceedings* **23**, 154-159.

Crane, M. B., Marks, E. (1952). Pear-apple hybrids. *Nature* **170**, 1017.

Criley, R. A., Parvin, P. E. (1993). New cut foliage from Australia, New Zealand and South Africa. *Acta Horticulturae* **337**, 95-98.

Curir, P., Sulis, S., Mariani, F., Sumere, C. F. van, Marchesini, A., Dolci, M. (1993). Influence of endogenous phenols on rootability of *Chamaelaucium uncinatum* Schauer stem cutting. *Scientia Horticulturae* **55**, 303-314.

Curtis, C. E. (1986). Nutrient deficiency symptoms of plants. Department of Agriculture and Rural Affairs, Agnote, order no.: 3641/86; Agdex 632.

Dadswell, H. E., Record, S. J. (1936). Identification of woods with conspicuous rays. *Tropical Woods* **48**, 1-51.

Dahler, J. M., McConchie, C. A., Turnbull, C. G. N. (1995). Quantification of cyanogenic glycosides in seedlings of three *Macadamia* (Proteaceae) species. *American Journal of Botany* **43**, 619-628.

Davis, T., D, Haissig, B., E, Sankhla, N. (1988). Adventitious root formation in cuttings. 1st ed. (Advances in Plant sciences Series, vol. 2.) Dioscorides Press, Portland, Oregon. 315 pages.

Dawson, I. (1996a). Grafting Australian Native Plants. Proceedings IV National Workshop for Australian Native Flowers, 28 - 30 September; p.97-104.

Dawson, I. (1996b). A hardy cutflower - waratah. *Australian Horticulture* **94**(11), 82.

Debenham, C. (1970). The Language of Botany. The society for Growing Australian Plants, Sydney, NSW. 208 pages.

De Kock, M., Theron, K., Swart, P., Weiler.E. W., Bellstedt, D. U. (1994). Cytokinins in the xylem sap of the dioecious fynbos shrub, *Leucadendron rubrum* Burm. f.: seasonal fluctuations and their possible interaction with morphological characteristics as expressed in the two sexes. *New Phytology* **127**, 749-759.

Deloire, A., Hebant, C. (1982). Peroxidase activity and lignification at the interface between stock and scion of compatible and incompatible graft of *Capsicum* on *Lycopersicum*. *Australian Journal of Botany* **49**, 887-891.

Dickson, P. R. (1997). Marketing Management. 2nd ed. The Dryden Press, New York. 813 pages.

Dixon, W. K, Thinlay, N., Sivasithamparam, K. (1984). Technique for rapid assessment of tolerance of *Banksia* spp. to root rot caused by *Phytophthora cinnamomi*. *Plant Disease* **68**, 1077-1080.

Doggrell, L. M. (1976). Commercial propagation of macadamias. *International Plant Propagators' Society Combined Proceedings* **26**, 391-393.

Donnelly, P. N. (1995). The use of mycorrhizal fungi during propagation. Regional conference, Hahndorf, South Australia; International Plant Propagators' Society Combined Proceedings; p.6.

Douglas, A. W. (1995). Proteaceae affinities. In 'Proteaceae'. 1st ed. Vol. 17. (Ed: McCarthy, P.) (Flora of Australia.) CSIRO, Melbourne, pp. 1-13.

Douglas, F. (1994). Grafted darwinias. *Australian Horticulture* **92**(7), 114.

Dubois, P., Joyce, D. (1989). Dyeing cut flowers and foliage. WA Department of Agriculture, Farmnote no. 31/89.

Dubois, P., Joyce, D. (1990). Preserving plant foliage with glycerine. WA Department of Agriculture, Farmnote no. 87/90.

Dupee, S. A., Clemens, J. (1981). Propagation of ornamental *Grevillea*. *International Plant Propagators' Society Combined Proceedings* **31**, 198-208.

Elliot, R., W, Jones, D. L (1982). Encyclopaedia of Australian Plants. Vol. 1 & 2. Lothian Publishing Company Pty. Ltd., Melbourne, Sydney, Auckland. (pp.281-305)

Ellyard, R. K. (1976). Effect of supplementary light and auxin application on rooting leafy cuttings of certain Australian species. *International Plant Propagators' Society Combined Proceedings* **26**, 395-401.

Ellyard, R. K. (1987). Conservation of Australia's endangered plants - a role for the Australian National Botanic Gardens. Australian National Botanic Gardens Occasional Publication, Ed. Armstrong, J.A., Canberra, Australia.

Errea, P., Felipe, A., Herrero, M. (1994a). Graft establishment between compatible and incompatible *Prunus* spp. *Journal of Experimental Botany* **45**, 339-401.

Errea, P., Treutuer, D., Feucht, W. (1994b). Characterization of flavanol type-polyphenols on apricot cultivar and rootstocks. *Advances in Horticultural Science* **8**, 165-169.

Erwin, J. E. (1996). Temperature and photoperiod affect grafted cactus scion necrosis. *HortTechnology* **6(4)**, 393-395.

Evans, A. M. (1955). Grafting and hybridization experiments in the Genus *Trifolium*. *Nature* **175**, 687-688.

Everist, S. L. (1974). Poisonous plants of Australia. 1st ed. Angus and Robertson, Australian. 650 pages.

Feder, N., O'Brien, T. P. (1968). Plant microtechnique: some principles and new methods. *Australian Journal of Botany* **55**, 123-142.

Fink, S. (1983) The occurrence of adventitious and preventitious buds within the bark of some temperate and tropical species. *American Journal of Botany* **70**(4), 532-542.

Fisk, E. L., Millington, W. F. (1959). Atlas of Plant Morphology, Portfolio 1 - Photomicrographs of root, stem and leaf. 1st ed. Burgen Publishing Company, Minneapolis, Minesota. 60 pages.

Foster, A. S., Gifford, E. M. (1959). Comparative Morphology of Vascular Plants. 1st ed. Freeman and Co., San Francisco. 555 pages.

Fuss, A. M., Sedgley, M. (1991a). The development of hybridisation techniques for *B. menziesii* for cut flower production. *Journal of Horticultural Science* **66**, 357-365.

Fuss, A. M., Sedgley, M. (1991b). Variability in cut flower production of *Banksia coccinea* R.Br. at six locations in southern Australia. *Australian Journal of Experimental Agriculture* **31**, 853-858.

Fuss, A. M., Pattison, S. J., Aspinall, D., Sedgely, M. (1992). Shoot growth in relation to cut flower production of *Banksia coccinea* and *Banksia menziesii*. *Scientia Horticulturae* **49**, 323-334.

Garner, R. J. (1988). The Grafter's Handbook. 5th ed. Cassell Publishers in association with The Royal Horticultural Society, London.

Garner, R. J., Beakbane, A. B. (1968). A note on the grafting and anatomy of black pepper. *Experimental Agriculture* **4**, 187-192.

Gebhardt, K., Goldbach, H. (1988). Establishment, graft union characteristics and growth of *Prunus* micrografts. *Physiologia Plantarum* **72**, 153-159.

George, A. (1981). The genus *Banksia* L.f. (Proteaceae). *Nuytsia* **3**, 239-474.

George, A. (1987). The Banksia Book. 2nd ed. Kangaroo Press, Sydney. 240 pages.

George, A. (1988). New taxa and notes on *Banksia* L.f. (Proteaceae). *Nuytsia* **6(3)**, 309-317.

George, A. (1996). Proteus in Australia - change here for? Abstract from International symposium on the Biology of the Proteaceae, University of Melbourne & Royal Botanical Gardens, Sept 29 2 Oct. p.23.

Gibson, O. E. (1974). An unorthodox method of rooting ripe cuttings of proteaceous plants in the open. *International Plant Propagators' Society Combined Proceedings* **24**, 296-297.

Gill, N. T., Vear, K. C. (1980). Agricultural Botany. Monocotyledonous crops. Vol. 2. Oxford University Press, Oxford. 259 pages.

Goody, J. (1994). The Culture of Flowers. Cambridge University Press, Cambridge. 462 pages.

Graham, A. W., Wallwork, M. A. and Sedgley, M. (1998). Lignotuber bud development in *Eucalyptus cinerea* (F. Muell., ex Benth), *International Journal of Plant Science*, in press

Grauke, L. J., Barr, R. (1996). Initial survival of pecan grafts on seedling rootstocks of pecan, water hickory, and their interspecific hybrid. *HortTechnology* **6(1)**, 45-46.

Groves, R. H. (1978). Growth of heath vegetation. IV. Effects of temperature on growth of *Banksia ornata, B. serrata, B. serratifolia*. *Australian Journal of Botany* **26**, 45-51.

Gur, A., Blum, A. (1973). The role of cyanogenic glycoside in incompatibility between peach scions and almond rootstocks. *Horticultural Research* **13**, 1-10.

Gutmann, M., Charpentier, J. P., Doumas, P., Jay-Allemand, C. (1996). Historical investigation of walnut cotyledon fragments for a better understanding of *in vitro* adventitious root initiation. *Plant Cell Reports* **15**, 345-349.

Hackett, W., P (1985). Juvenility, maturation and rejuvenation in woody plants. *Horticultural Reviews* **7**, 109-155.

Hackett, W., P (1988). Donor plant maturation and adventitious root formation. Ch. 1. In 'Adventitious Root Formation in Cuttings'. 1st ed. (Eds: Davis, D., D, Haissig, B., E, Sankhla, N.) (Advances in Plant Science Series, vol. 2.) Dioscorides Press, Portland, Oregon, pp. 11-25.

Hair, J. F., Jr., Anderson, R. E., Tatham, R. L., Black, W. C. (1995). Multivariate Data Analysis. 4th ed. Prentice Hall, Engkewood Cliffs, New Jersey. 745 pages. (Chapter 10, Conjoint Analysis, p. 556-599)

Hale, C. P. (1989). The role of cyanogenic glycosides in graft rejection in Australian species of hakea and grevillea. B. Agric. Sc. Honours thesis, Murdoch University, Perth, Western Australia. 45 p.

Hall, M. J. (1981). Propagation using fogging technique. *International Plant Propagators' Society Combined Proceedings* **31**, 376-380.

Handreck, K. A. (1991). Interaction between Iron and phosphorus in the nutrition of *Banksia ericifolia* L.f. var. *ericifolia* (Proteaceae) in soil-less potting mix. *Australian Journal of Botany* **39**, 373-384.

Handreck, K., Black, N. (1989). Growing Media for Ornamental Plants and Turf. 3rd ed. NSW University Press, Kensington, Australia. 401 pages.

Harington, B. (1988). Budding or top working of *Protea longifolia*. *International Protea Association Journal* **16**, 15.

Hartmann, H. T., Kester, D. E., Davies, F. J. (1990). Plant Propagation, Principles and Practice. 5th ed. Prentice-Hall, Englewood Cliffs, New Jersey. 647 pages.

Hawkins, S., Boudet, A. (1996). Wound-induced lignin and suberin deposition in a woody angiosperm *Eucalyptus gunnii* Hook.: histochemistry of early changes in young plants. *Protoplasma* **191**, 96-104.

Henderson, T. (1987). Successful *Banksia* grafting trials in Sydney. *Banksia Study Report* **7**, 36.

Henry, S. T. (1976). Punch budding of macadamias. *International Plant Propagators' Society Combined Proceedings* **26**, 393-395.

Heuss-LaRosa, K., Hammond, R., Crosslin, J. M., Hazel, C., Hammerschlag, F. A. (1995). Monitoring *Prunus* necrotic ringspot virus infection by hybridization with a cRNA probe after in vitro micrografting. *Journal American Society of Horticultural Science* **120(6)**, 928-931.

Hockings, F. D. (1976). Early records of propagation techniques for Australian native plants. *International Plant Propagators' Society Combined Proceedings* **26**, 362-369.

Hodge, M. (1988). A new method for grafting native plants. Dryandra Study Group, Newsletter No. 14, p8-14 July, 1988. Society for Growing Australian Plants. ISSN: 0723-151X.

Hodge, M. (1990). Grafting native plants. *Australian Plants* **15(124)**, 369-377.

Hoopman, T. (1996). Grafting - agronomy notes. in Gumnut Gossip, No. 16, May/June.

Howard, B. H., Oakley, W. (1997). Bud-grafting success in *Acer plantoides* 'Crimson King' related to root growth. *Journal of Horticultural Science* **72**(5), 697-704.

Huang, H., Norton, J. D., Boyhan, G. E., Abrahams, B. R. (1994). Graft compatibility among Chestnut (*Castanea*) species. *Journal American Society of Horticultural Science* **119 (6)**, 1127-1132.

Ilic, J. (1991). CSIRO Atlas of Hardwoods. 1st ed. Crawford House Press in association with CSIRO Publications, Bathurst. 525 pages.

James, C. (1997). Kornwestheim: Hier blueht's. *Deutscher Gartenbau* **33**, 1782.

James, S. (1984). Lignotubers and Burls - their structure function and ecological significance in mediterranean ecosystems. *Botanical Review* **50**, 225-266.

Jeffree, C. E., Yeoman, M. M. (1983). Development of intercellular connections between opposing cells in a graft union. *New Phytologist* **93**, 491-509.

Jeffrey, D. W. (1968). Phosphate nutrition of Australian heath plants. *Australian Journal of Botany* **16**, 603-613.

Johnson, L. A. S., Briggs, B. G. (1963). Evolution in the Proteaceae. *Australian Journal of Botany* **11**, 21-61.

Johnson, L. A. S., Briggs, B. G. (1975). On the Proteaceae - the evolution and classification of a southern family. *Biological Journal of the Linnean Society* **70**, 83-182.

Jones, R., Faragher, J. (1991). Cold storage of selected members of the Proteaceae and Australian native cut flowers. *HortScience* **26(11)**, 1395-1397.

Jones, R., Moody, H. (1993). Caring for cut flowers. revised ed. Department of Agriculture, Agmedia, Melbourne. 176 pages.

Joyce, D., Jones, R., Faragher, J. (1993). Postharvest characteristics of native Australian flowers. *Postharvest News and Information* **4(2)**, 61N-67N.

Joyce, D. C., Beal, P., Shorter, A. J. (1996). Vase life characteristic of selected *Grevillea*. *Australian Journal of Experimental Agriculture* **36**, 379-382.

Kamboj, J. S., Browning, J. D., Quinlan, P. S., Blake, P. S., Baker, D. A. (1997). Polar transport of [3H]-IAA in apical shoot segments of different apple rootstocks. *Journal of Horticultural Science* **72**(5), 773-780.

Karingal Consultants (1994). Australian Wildflower Industry - A Review. A report for Rural Industries Development Corporation. RIRDC Research Paper No 94/9.

Kerr, L. R. (1925). The lignotubers of eucalypt seedlings. *Proceedings of the Royal Society of Victoria* **37**, 79-97.

Kester, D. E. (1976). The relationship of juvenility to plant propagation. *International Plant Propagators' Society Combined Proceedings* **26**, 1976.

Kloosterboer, W., Maddock, E., Green, W., Craig, S. (1992). The New South Wales Cut Flower Industry, An Industry Survey and Situation Statement. NSW Agriculture, Sydney. 81 pages.

Ladiges, P. Y. (1974). Differentiation in some populations *Eucalyptus viminalis* Labill. in relation to factors affecting seeding establishment. *Australian Journal of Botany* **22**, 471-487.

Ladiges, P. Y., Ashton, D. H. (1974). Variation in some central Victorian populations *Eucalyptus viminalis* Labill. *Australian Journal of Botany* **22**, 81-102.

Lamont, B. B. (1993). Injury-induced cyanogenesis in vegetative and reproductive parts of two *Grevillea* species and their F1 hybrids. *Annals of Botany* **71**, 537-542.

Lamont, B., Whittens, V. A., Witkowski, E. T. F., Rees, R. G., Enright, N. J. (1994). Regional and local (road verge) effects on size and fecundity in *Banksia menziesii*. *Australian Journal of Ecology* **19**, 197-205.

Lamont, B. B., Wills, R. T., Witkoski, E. T. F. (1995). Threats to the conservation of south-western Australian Proteaceae. *Acta Horticulturae* **387**, 9-18.

Larson, P. L. (1994). The Vascular Cambium. Springer, Berlin. 725 pages.

Larson, R. L. (1995). Flora of Australia, Elaegnaceae, Proteaceae I. Vol. 16. CSIRO Australia, Melbourne. 521 pages.

Lazarides, M., Hince, B. (Eds.) (1993). CSIRO handbook of economic plants of Australia. 1st ed. CSIRO Australia, Melbourne. 330 pages.

LeBoeuf, G. (1994). Landscape potential for NSW Isopogon. *Australian Horticulture* **92**(2), 135-136.

Lee, C. I., Paul, W. P., Hackett, W. P. (1976). Root promotion on stem cuttings of several ornamental plant species by acid or base pre-treatment. *International Plant Propagators' Society Combined Proceedings* **26**, 95-99.

Leonhardt, K. W. (1996). Intergeneric graft compatibility within the family Araliaceae. *HortTechnology* **6**, 254-256.

Levey, V. (1973). Some aspects of grevillea propagation. *International Plant Propagators' Society Combined Proceedings* **23**, 420-422.

Lewis, J., Bell, D. T. (1981). Reproductive isolation of co-occurring *Banksia* species at the Yule Brook Botany Reserve, Western Australia. *Australian Journal of Botany* **29**, 665-74.

Loach, K. (1988a). Controlling environmental conditions to improve adventitious rooting. Ch. 18. In 'Adventitious Root Formation in Cuttings'. 1st ed. (Eds: Davis, D. D., Haissig, B. E., Sankhla, N.) (Advances in Plant Science Series, vol. 2.) Dioscorides Press, Portland, Oregon, pp. 248-273.

Loach, K. (1988b). Water relations and adventitious rooting. Ch. 8. In 'Adventitious Root Formation in Cuttings'. 1st ed. (Eds: Davis, D., D, Haissig, B., E, Sankhla, N.) (Adevances In plant Science Series, vol. 2.) Dioscorides Press, Portland, Oregon, pp. 102-116.

Lockard, R. G., Schneider, G. W. (1981). Stock and scion growth relationships and the dwarfing mechanism in apple. *Horticultural Reviews* **3**, 315-375.

Mabberley, P. J. (1987). The Plant Book. A portable dictionary of the higher plants. 1st ed. (ISBN 0 521 34060 8) Cambridge University Press, Cambridge. 706 pages.

Maguire, T. L., Collins, G. G., Sedgley, M. (1994). A modified CTAB DNA extraction procedure for plants belonging to the family Proteaceae. *Plant Molecular Biology Reporter* **12**, 106-109.

Maguire, T. L., Sedgley, M. (1997). Genetic diversity in *Banksia* and *Dryandra* (Proteaceae) with emphasis on *Banksia cuneata*, a rare and endangered species. *Heredity* **79**, 394-401.

Maguire, T. L., Sedgley, M., Conran, J. G. (1996). *Banksia* Sect. *Coccinea* (Proteaceae) a new section. *Australian Systematic Botany* **9**, 887-891.

Maguire, T. L., Conran, J. G., Collins, C. G., Sedgley, M. (1997). Molecular analysis of interspecific and intergeneric relationships of *Banksia* using RAPDs and non-coding chloroplast DNA sequences. *Theoretical and Applied Genetics* **95**, 253-260.

Malter, A. (1994). The European Market for protea: recent trends and lessons for the further product and market development. *International Protea Association Journal* **27**, 8-12.

Maughan, J., Merriman, P. (1986). Major diseases of proteaceous plants. Department of Agriculture and Rural Affairs, Agnote, order no.: 3554/86; Agdex 280/633.

Mauseth, J., D (1988). Plant Anatomy. 1st ed. The Benjamin/Cummings Publishing Company, Menlo Park, California. 560 pages.

Maynard, B. R., Bassuk, N. L. (1996). Effects of stock plant etiolation, shading banding and shoot development on histology and cutting propagation of *Carpinus betulus* L. *fastigiata*. *Journal American Society of Horticultural Science* **121**, 853-860.

McCarthy, E. J., Perreault, W. D., Jr., Quester, P., Wilkinson, J. W., Lee, K. Y., (1994) Basic Marketing. A Managerial Approach, 1st Australian edition, published by Richard D. Irwin. Inc., Sydney. 714 pages

McCormack, C., O'Neill, G. (1993). Learning to propagate an endangered grevillea. *Australian Horticulture* **91**(5), 52-54.

McCredie, T., Dixon, K., Sivasithamparam, K. (1985a). Grafting banksias to avoid root rot. *Australian Horticulture* **83**(4), 75-79.

McCredie, T., Dixon, K., Sivasithamparam, K., Watkins, P. (1985b). Seeking *Phytophthora* resistant banksias. *Australian Horticulture* **83**(4), 48-51.

McCredie, T., Dixon, K. W., Sivasithamparam, K. (1985c). Variability in the resistance of *Banksia* L. f. species to *Phytophthora cinnamomi* Rands. *Australian Journal of Botany* **33**, 629-637.

McCully, M. E., O'Brien, T. P. (1981). The study of plant structure. Principles and selected methods. 1st ed. Termarcarphi Pty. Ltd., Melbourne. 250 pages.

McDonald, M. H. B. (1989). Marketing Plans, how to prepare them: how to use them. 2nd ed. Butterworth-Heinemann, Oxford.

McDougall, J. (1993). Grafting for a better emu bush. *Australian Horticulture* **91**(6), 19.

McDowell, M. (1988). Grafted *Banksia*. *Banksia Study Report* **8**, 51.

McKenzie, B. L. (1973). Propagation of Proteaceae by cuttings. *International Plant Propagators' Society Combined Proceedings* **23**, 380.

McKenzie, D. (1981a). Grafted *Banksias*. *Banksia Study Report* **7**, 2-9.

McKenzie, D. (1981b). Grafted desert peas. *Australian Plants* **11(89)**, 228-230.

McKenzie, D. (1984). Grafting Australian native plants. *International Plant Propagators' Society Combined Proceedings* **34**, 135-141.

McKenzie, D. (1988). The grafting of Australian native plants. *Banksia Study Report* **8**, 54-62.

McKenzie, D. (1994). Grafting hakeas to improve performance. *Australian Horticulture* **92**(4), 19-23.

McKenzie, D. (1996). Grafting hakeas. *Society for Growing Australian Plants Journal* , p. 206. (November)

McLean, C. (1995). Mycorrhiza of the Epacridaceae and its use in propagation. *International Plant Propagators' Society Combined Proceedings*, p.4. (Regional Conference, Hahndorf, South Australia)

Meinke, H., Karnatz, A. (1990). Influence of air and soil temperatures on grafted and self-rooted *Passiflora* hybrids. *Scientia Horticulturae* **43**, 237-246.

Metcalfe, C. R. (1987a). Anatomy of the Dicotyledons. Magnoliates, Illiciates and Laurales. 2nd ed. Vol. III. Oxford University Press, Oxford. 224 pages.

Metcalfe, C. R. (1987b). Anatomy of the Dicotyledons. Wood structure and conclusions to the general introduction. 2nd ed. Vol. II. Oxford Univeristy Press, Oxford. 224 pages.

Metcalfe, C. R., Chalk, L. (1979). Anatomy of the Dicotyledons. Systematic anatomy of leaf and stem with brief history of the subject. 2nd ed. Vol. I. Oxford University Press, Oxford. 275 pages.

Metcalfe, M. (1994). Profit Forecasting. Kluwer Academic Publishers, Boston. 339 pages.

Meyers, D. (1993). Thryptomene - The grafting of Grampians thryptomene. *Australian Plants* **17**, 78.

Meyers, D., Beardsell, D., Kerr, R. (1993). Grafting the forest she-oak. *Australian Plants* **17**, 175.

Mibus, R. (1997). Germany – a major export market for Australian wild flower – banksia. RIRDC project Final Report

Middleton, R. (1978). Effects of water quality in relation to propagation. *International Plant Propagators' Society Combined Proceedings* **28**, 472-475.

Moffatt, J. (1993). Letter from Nanju Proteas, Ravensbourne. personal communication.

Moffatt, J., Turnbull, L. (1993). Grafting Proteas. Special ed. QDPI, Horticulture Centre, Redlands Research Station, Queensland, Cleveland, 47 pages.

Moore, R. (1982a). Further evidence for cell wall deposition during graft formation. *Annals of Botany* **50**, 599-604.

Moore, R. (1982b). Studies on vegetative compatibility in higher plants. V. A morphometric analysis of the development of a compatible and an incompatible graft. *Canadian Journal of Botany* **60**, 2780-2786.

Moore, R. (1983). Studies of vegetative compatibility-incompatibility in higher plants. IV. The development of tensile strength in a compatible and an incompatible graft. *American Journal of Botany* **70(2)**, 226-231.

Moore, R. (1984a). Development of tensile strength in conventional and approach grafts in *Sedum telephoides*. *Canadian Journal of Botany* **62**, 1580-1582.

Moore, R. (1984b). A model for graft compatibility-incompatibility in higher plants. *American Journal of Botany* **71(5)**, 752-758.

Moore, R. (1984c). The role of direct cellular contact in the formation if compatible autografts in *Sedum telephoides*. *Annals of Botany* **54**, 127-133.

Moore, R. (1984d). Ultrastructural aspects of graft incompatibility between pear and quince *in vitro*. *Annals of Botany* **53**, 447-451.

Moore, R., Walker, D. B. (1981a). Studies of vegetative compatibility - incompatibility in higher plants. I A structural study of compatible autograft in *Sedum telephoides* (Crassulaceae). *American Journal of Botany* **68**, 820-831.

Moore, R., Walker, D. B. (1981b). Studies of vegetative compatibility - incompatibility in higher plants. II A structural study of an incompatible heterograft between *Sedum telephoides* (Crassulaceae) and *Solanum pennelli* (Solanaceae). *American Journal of Botany* **68**, 831-842.

Moore, R., Walker, D. B. (1981c). Studies of vegetative compatibility-incompatibility in higher plants III The involvement of acid phosphatase in the lethal cellular senescence associated with an incompatible heterograft. *Protoplasma* **109**, 317-334.

Morell, M. K., Peakall, R., Appels, R., Preston, L. R., Lloyd, H. (1995). DNA profiling techniques for plant variety identification. *Australian Journal of Experimental Agriculture* **35**, 807-819.

Morgan, A., Fuss, A. (1994). Merging conservation with production in remnant bush. *West Australian Journal of Agriculture* **35**, 111-114.

Morley, B. D., Toelken, H. R. (1983). Flowering Plants in Australia. Rigby Publishers, Adelaide. 416 pages.

Mullette, K. J. (1978). Studies of the lignotubers of *Eucalyptus gummifera* (Gaertn.&Hochr). I The nature of the lignotuber. *Australian Journal of Botany* **26**, 9-13.

Myers, B. A. (1995). The influence of the lignotuber on hydraulic conductance and leaf conductance in *Eucalyptus behriana* seedlings. *Australian Journal of Plant Physiology* **22**, 857-863.

Nagao, M. A., Ho-a, E. B., Yoshimoto, J. M., Yoshimura, E. R., Notley, E., Fuchigami, L. H. (1994). Relationship between vegetative flushing and flowering of *Macadamia integrifolia* in Hawaii. *Scientia Horticulturae* **60**, 9-16.

Niccol, R. J., Regan, P. A., De Filipps, L. F. (1994). Simplified protocol for the micro-propagation of selected *Eucalyptus*. *Australian Forestry* **57**(4), 143-147.

Nixon, P. (1987). The Warratah. Kangaroo Press, Sydney. 80 pages.

Noggle, G. R. (1979). Recognition systems in Plants. *What's New in Plant Physiology* **10(2)**, 5-6.

Offord, C., Campbell, C. (1994). The Waratah. *Australian Horticulture* **92**(8), 52-57.

Ouellette, D. R., Unrath, C. R., Young, E. (1996). Manual and Chemical branch inducement on fall- and spring-planted 'Empire' apple on two rootstocks. *HortScience* **31**, 82-88.

Parkinson, M., Yeoman, M. M. (1982). Graft formation in cultured, explanted internodes. *New Phytologist* **91**, 711-719.

Pate, J. S., Beard, J. S. (1984). Kwongan, Plant Life of the Sandplain. 1st ed. University of WA, Perth. 284 pages.

Pate, J. S., Dixon, K. (1982). Tuberous, cormous and bulbous plants. 1st ed. Vol. 2. University of WA, Perth. 268 pages.

Pate, J. S., Froend, R. H., Bowen, J. B., Hansen, A., Kuo, J. (1990). Seedling growth and storage characteristics of seeder and resprouter species of mediterranean-type ecosystems of S. W. Australia. *Annals of Botany* **65**, 585-601.

Pate, S. J., Jeschke, W. D. (1993). Mineral uptake and transport in xylem and phloem of the proteaceous tree, *Banksia prionotes*. In 'Plant Nutrition - from Genetic Engineering to Field Practice'. (Ed: Barrow, N. J.) Kluwer Academic Publications,, pp. 313-316. (N)

Pate, J. S., Jeschke, W. D., Aylward, M. J. (1995). Hydraulic architecture and xylem structure of the dimorphic root system of south-west Australian species of Proteaceae. *Journal of Experimental Botany* **46**, 907-915.

Paton, D. C., Turner, V. (1985). Pollination of *Banksia ericifolia* Smith: birds, mammals and insects as pollen vectors. *Australian Journal of Botany* **33**, 271-286.

Pegrum, J. (1989). An overview of the Western Australian Native Plant and Protea Industries. WA Dept. of Agriculture, Perth.

Peisley (1989). *Banksia spinulosa* var *cunninghamii* performance as a grafting stock. *Banksia Study Report* **9**, 6-13.

Pereira-Lorenzo, S., Fernandez-Lopez, J. (1997). Propagation of chestnut cultivars by grafting: methods, rootstocks and plant quality. *Journal of Horticultural Science* **72**(5), 731-739.

Perry, D. K. (1987). Successfully growing Proteaceae. *International Plant Propagators' Society Combined Proceedings* **37**, 112-115.

Pianhanurak, P., Piyapan, P., Pianhanurak, C. (1996). A preliminary study of rejuvenation of teak by the budding technique. Information Note, ASEAN Forest Tree Seed Centre Project, Muak-Lek, Saraburi, Thailand. (ISSN: 0858-3099)

Poulton, J. E. (1990). Cyanogenesis in plants. *Plant Physiology* **94**, 401-405.

Pratt, L. (1994). Australian Native plantation cut flowers. The Sapphire Coast Producers Association Inc., project paper no. 1, 14 August.

Pullar, D., Cadman, R., Lewis, I., Morey, P. (1993). Winning the race - international competitiveness in Australian horticulture, case study no. 3, cut flowers. Horticultural Policy Council.

Ramsey, M., Vaughton, G. (1991). Self-incompatibility, protandry, pollen production and pollen longevity in Banksia menziesii. *Australian Journal of Botany* **39**, 497-504.

Record, S. J. (1936). Classification of various features of dicotyledonous woods. *Tropical Woods* **47**, 12-48.

Richardson, D. M., Cowling, R. M., Lamont, B. B., van Hensbergen, H. L. (1995). Coexistance of *Banksia* species in south-western Australia: the role of regional and local processes. *Journal of Vegetation Science* **6**, 329-342.

Roberts, J. R., Brown, R. (1961). The development of the graft union. *Journal of Experimental Botany* **12(35)**, 294-302.

Rogers, W. S., Beakbane, A. B. (1957). Stock and scion relations. *Annual Review of Plant Physiology* **8**, 217-236.

Roh, M. S., Lawson, R.H. (1996) Requirements for new floral crops – perspectives for the United States of America. *Acta Horticulturae* **454**, 29-38

Ruiz-Sifre, G., Dole, J. M., Kahn, B. A., Richardson, P. E., Ledford, J. (1997). Correlation of poinsettia graft union development with transmission of the free-branching characteristic. *Scientia Horticulturae* **69**, 135-143.

Salinger, J. P. (1985). Commercial Flower Growing. Butterworth Horticultural Books, Wellington. 269 pages.

Salomon, E. S., Reuveni, O. (1994). Effect of paclobutrazol treatment on the growth and first flowering of intact and autografted seedlings of mango. *Scientia Horticulturae* **60**, 81-87.

Santamour, F. S. (1988a). Graft compatibility in woody plants: an extended perspective. *Journal of Environmental Horticulture* **6**(March), 27-32.

Santamour, F. S. (1988b). Graft compatibility related to cambial peroxidase isozymes in Chinese chestnut. *Journal of Environmental Horticulture* **6**, 33-39.

Schneider, J. H. M., Jacob, J. J., van de Pol, P. A. (1995). *Rosa multiflora* 'Ludiek', a roostock with resistant features to the root lesion nematode *Pratylenchus vulnus*. *Scientia Horticulturae* **63**, 37-45.

Scott, J. K. (1980). Estimation of the outcrossing rate for *Banksia attenuata* R.Br and *Banksia menziesii* R.Br. (Proteaceae). *Australian Journal of Botany* **28**, 53-59.

Sedgley, M. (1991). Banksia (*Banksia hookeriana*) 'Waite Orange'. *Plant Varieties Journal* **4**(2), 9-11.

Sedgley, M. (1995a). Banksia variety. *Plant Varieties Journal* **8**(2), 8-9, 18-19.

Sedgley, M. (1995b). Cloning and selection of banksias. Australian Flora and Protea Growers Association Conference proceedings, Barossa Valley, SA.

Sedgley, M. (1996). *Banksia*, Family Proteaceae. Ch. 3. In 'Native Australian Plants - Horticulture and Use'. 1st ed. (Eds: Johnson, K. A., Burchett, M.),, pp. 18-35.

Sedgley, M., Fuss, A. (1992). Correct pruning lifts *Banksia* yields. *Australian Horticulture* **90**(7), 50-53.

Sedgley, M., Sierp, M. G., Maguire, T. L. (1994). Interspecific hybridisation involving *Banksia prionotes* Lind. and *Banksia menziesii* R. Br. (Proteaceae). *International Journal of Plant Science* **155**, 755-762.

Sedgley, M., Wirthensohn, M. G., Delaporte, K. (1996). Interspecific hybridisation between *Banksia hookeriana* Meisn. and *Banksia prionotes* Lindl. (Proteaceae). *International Journal of Plant Science* **157**, 638-643.

Shemi, N. (1996). Artificial media, grafted plants and rootstocks in field trials of *Leucadendron* at Kibbutz Hagoshrim. Abstract in Proceedings of the International Protea Association, 8th Biennial conference, 4th International Protea Working Group Symposium, March 17-21, Israel.

Siniscalco, C., Pavolettoni, L. (1988). Rejuvenation of *Eucalyptus* x *Trabutii* by successive grafting. *Acta Horticulturae* **227**, 98-100.

Skene, D. S., Shepherd, H. R., Howard, B. H. (1983). Characteristic anatomy of union formation in T- and chip budded fruit and ornamental trees. *Journal of Horticultural Science* **58(3)**, 295-299.x

So werden sie richtig behandelt (1995). Markt Blatt, BGM Hamburg, No.102, December 1995.

Stephenson, R. A. (1980). Grafting macadamias. Department of Primary Industry Bulletin, Nambour, Queensland, 9 pages.

Storey, W. B., Hamilton, R. A., Fukunaga, E. T. (1961). The relationship of nodal structure to training *Macadamia* trees. *Horticultural Science* **61**, 317-323.

Swenson, W. K., Dunn, J. E., Conn, E. E. (1989). Cyanogenesis in the Proteaceae. *Phytochemistry* **28**(3), 821-823.

Taylor, A., Hopper, S. D. (1988). The Banksia Atlas. Vol. 8. (Australian Flora and Fauna Series.) Australian Government Publishing Service, Canberra, Australia. 245 pages.

Thiele, K. R. (1993). A cladistic analysis of *Banksia* L. f. Doctoral Thesis, University of Melbourne, Australia. 226 p.

Thiele, K., Ladiges, P. Y. (1996). A cladistic analysis of *Banksia* (Proteaceae). *Australian Systematic Botany* **9**, 661-733.

Thomas, G. (1993). *Grevillea* lost from Lara. *Australian Horticulture* **91**(11), 70.

Tibbits, W. N., Bachelard, E. P. (1981). Effects of fertiliser and frequency of watering on the internal water relations of seedlings of *Angophora costata* and *Banksia serrata*. *Australian Forest Research* **111**, 23-34.

Treutter, D., Galensa, R., Feucht, W., Schmidt, P. P. S. (1985). Flavanone glucosides in callus and phloem of *Prunus avium*: identification and stimulation of their synthesis. *Physiologia Plantarum* **65**, 95-101.

Tubbs, F. R. (1973a). Research fields in the interaction of rootstocks and scions in woody perennials - Part 1. *Horticultural Abstracts* **43(5)**, 247-253.

Tubbs, F. R. (1973b). Research fields in the interaction of rootstocks and scion in woody perennials - Part 2. *Horticultural Abstracts* **43(6)**, 325-335.

Tynan, K., Scott, E. S., Sedgley, M., Dixon, K., Sivasithamparam, K. (1995). *Phytophthora* dieback in *Banksias*: screening for resistance. *Acta Horticulturae* **387**, 159-162.

Van Doorn, W. G. (1995). Water relations of cut flowers. *Horticultural Reviews* **18**, 1-30+. (N)

van Lieburg, R., Kendrick, R. E. (1996). A new source of daylight. *Chronica Horticulturae* **36**(3), 19-20.

Vithanage, V., Winks, C. W. (1992). Isozymes as genetic markers for *Macadamia*. *Scientia Horticulturae* **49**, 103-115.

Vivian-Smith, A. (1995). Cyanogenesis and the fruit growth of three *Macadamia* species. B.Sc. Honours Reseach Thesis, Department of Botany. 50 p.

Wang, Y., Kollman, R. (1996). Vascular differentiation in the graft union of *in-vitro* grafts with different compatibility - structural and functional aspects. *Journal of Plant Physiology* **147**, 521-533.

Warren Wilson, J., Warren Wilson, P. M. (1960). The position of regenerating cambia - a new hypothesis. *New Phytologist* , 63-73.

Weatherhead, I., Barnett, J. R. (1986). Development and structure of unusual xylem elements during graft union formation in *Picea sitchensis* L. *Annals of Botany* **57**, 593-598.

Webb, M. (1991). Banksias for cutflower production. Farmnote No. 23/96 Agriculture of Western Australia.

Weiss, D. (1994). Partial floral development in poinsettia. *Flowering Newsletter* **17**, 44-48.

Whelan, R. J., Goldingay, R. L. (1986). Do pollinators influence seed-set in *Banksia paludosa* Sm. and *Banksia spinulosa* R. Br.? *Australian Journal of Botany* **11**, 181-186.

Wiesman, Z. (1995). Rootstock and nitrate involvement in 'Ettinger' avocado response to chloride stress. *Scientia Horticulturae* **62**, 33-43.

Wills, R. T., Robinson, C. J. (1994). Threats to flora-based industries in Western Australia from plant disease. *Journal of the Royal Society of Western Australia* **77**, 159-162.

Wilson, C. J. (1975). Experimental techniques used in banksia grafting. *International Plant Propagators' Society Combined Proceedings* **25**, 246-248.

Windle, B. E., Barth, G. E., Lewis, I. R. (1990). Report of a working party to review the opportunities and constraints on growth of the floriculture industry in South Australia. Technical Report, No 170, SA Department of Agriculture for the Floriculture working party.

Witkowski, E. T. F., Lamont, B. B. (1995). Commercial picking of *Banksia hookeriana* from natural populations adversely affects shoot, flower and seed production and plant nutrient status. *Acta Horticulturae* **387**, 23-29.

Wojtusik, T., Felker, P. (1993). Interspecific graft incompatibility in *Prosopis*. *Forest Ecology and Management* **59**, 329-340.

Wolpert, J., Walker, A., Weber, E., Bettiga, L., Smith, R., Verdegaal, P. (1994). Use of *Phylloxera*-resistant rootstocks in California: past, present and future. *Grape Grower* **Part II**, 10-17.

Wood, B., Seaton, K., Grimm, M. (1996). Pests of export wildflower and proteas. Farmnote, No. 1/96 replaces 90/88, Agriculture Western Australia.

Wood, P. (1978). Propagation of South American plant species. *International Plant Propagators' Society Combined Proceedings* **28**, 468-472.

Wrigley, J. W. (1973). Grafting of Australian plants. *International Plant Propagators' Society Combined Proceedings* **23**, 423-426.

Wrigley, J. W., Fagg, M. (1989). Banksias, waratahs and grevilleas. Collins, Sydney, Australia.

Yeoman, M. M., Brown, R. (1976). Implications of the formation of the graft union for organisation in the intact plant. *Annals of Botany* **40**, 1265-76.

Yeoman, M. M., Kilpatrick, D. C., Miedzybrodzka, M. B., Gould, A. R. (1978). Cellular interactions during graft formation in plants, a recognition phenomenon? In: Cell-cell Recognition (Ed. ASG Curtis), Symposia for the Society for Experimental Biology, **32**, 139-161.

Zammit, C. (1988). Dynamics of resprouting in the lignotuber shrub *Banksia oblongifolia*. *Australian Journal of Ecology* **13**, 311-320.

Zhen-Xiang Lu, Reighard, G. L., Baird, W. V. (1996). Identification of peach rootstock cultivars by RAPD markers. *HortScience* **31**, 127-129.

Appendix 1 *VideoPro 32* Programs

1. Gross tissue types image analysis set-up program

```
' wood data program to set up data variables and conditions for
' gross tissue type (gtt) measurements in um - including cork length
' formulae calculations at end of program
'
data close
data units pixel
data scale 1
data aspect 1.000
data column clear
data column 1 identifier              ' image no
data column 2 code                    ' scale factor
data column 3 Area              '  pith area
data column 4 Area              ' vb area
data column 5 count     ' vb count
data column 6 Area      ' cortex (-/+ cuticle) area
data column 7 Area              ' leaf trace area
data column 8 count     ' leaf trace count
data column 9 Area              ' cork + cuticle area
'
data column 10 length   ' cambium to cuticle or cork - pixel
data column 11 length   ' cork thickness - pixel
data column 12 length   ' cuticle thickness - pixel - LAST!
'
data formula c3+c4+c6+c7+c9 totarea
data column 13 formula          ' total area
data formula 100*c3/c13 pctpith
data column 14 formula          ' % pith
data formula 100*c4/c13 pctvbs
data column 15 formula          ' % vbs
data formula 100*c6/c13 pctcortex
data column 16 formula          ' % cortex/cuticle
data formula 100*c7/c13 pctlt
data column 17 formula          ' % leaf traces
data formula 100*c9/c13 pctcork
data column 18 formula          ' % cork+cuticle
'
'       FORMULAE FOR LENGTHS
data formula c2*c10/1000 camb>c/um
data column 19 formula          ' c to c  - um
'
data formula c2*c11/1000 cork/um
data column 20 formula          ' cork thickness - um
'
data formula c2*c12/1000 cuticle/um
data column 21 formula          ' cuticle thickness - um
'
data formula c19+c20+c21 camb>surface
data column 22 formula          ' total distance cambium to surface
data column * record
program comment "Press OK to append to data file. PAUSE if new file"
data append
program run wd-meas.prg
'
'       END OF PROGRAM
```

2. Gross tissue types image analysis measurement programs

```
'measurement program for gross tissue types - with cork or without cork
'
program control close
data identifier                 ' image file number
data code                       ' enter scale factor
image read
program comment "Press OK to trace areas"
edit cover
edit size 3
edit line                       ' trace tissues
program pause
binary process outline
image store load
'
measure write disable
measure view disable
edit cover
edit fill
binary erode 2
binary dilate 2
```

```
measure field          ' pith 1
data column 3 protect
edit clear
'
binary store retrieve
edit cover
edit fill
binary erode 2
binary dilate 2
binary clean
measure field
data column 4 protect  ' vbs area 2.1
measure field
data column 5 protect  ' vb count 2.2
edit clear
'
binary store retrieve
edit cover
edit fill
binary erode 2
binary dilate 2
measure field          ' cortex 3
data column 6 protect
edit clear
'
binary store retrieve
edit cover
edit fill
binary erode 2
binary dilate 2
binary clean
measure field
data column 7 protect  ' leaf traces area 4.1
measure field
data column 8 protect  ' leaf trace count 4.2
edit clear
'
binary store retrieve
edit cover
edit fill
binary erode 2
binary dilate 2
measure field          ' cork area 5
data column 9 protect
edit clear
'
'measure field
measure line           ' cambium to cork or cuticle
data column 10  protect
'
measure field
measure line           ' cork thickness - RH click if not present
data column 11 protect
'
'measure field
measure write enable
measure view enable
measure line           '  cuticle thickness - HAS TO BE LAST MMT.
data column * record
data save
program comment "Press OK to store binary and continue to next image"
binary retrieve
binary write
program run wd-meas.prg
```

3. Vascular bundle image analysis set-up program

```
'       set up program VASCULAR BUNDLE TISSUE TYPE ANALYSIS
'
data close
data units pixel
data scale 1
data aspect 1.000
data column 1 identifier        ' image no.
data column 2 code              ' scale no.
data column 3 Area              ' fibre cap (pith)
data column 4 Area              ' xylem
data column 5 Area              ' cambium
data column 6 Area              ' phloem
data column 7 Area              ' fibre cap (cortex)
data column 8 Area              ' non interfascicular area b/n vb
data column 9 Area              ' interfascicular area
data column 10 length           'unseriate ray length
data column 11 length           'unseriate ray length
data column 12 length           'unseriate ray length
data column 13 length           'unseriate ray length
data column 14 length           'unseriate ray length
'
'       Calculations based on raw data from above
'
data formula c3+c4+c5+c6+c7 vbarea
data column 15 formula
data formula c8+c9+c15 totarea
data column 16 formula
data formula 100*c15/c16 pctvb
data column 17 formula
'
data formula 100*c3/c16 pctfbp
data column 18 formula
data formula 100*c4/c16 pctxyl
data column 19 formula
data formula 100*c5/c16 pctcmb
data column 20 formula
data formula 100*c6/c16 pctphl
data column 21 formula
data formula 100*c7/c16 pctfbc
data column 22 formula
data formula 100*(c3+c7)/c16 pctfbc+b
data column 23 formula
data formula 100*c9/c16 pctif
data column 24 formula
data column * record
program comment "Press OK to append to data file. PAUSE if new file"
data append
program run vb-meas.prg
'
'       END vb. set up program
```

4. Vascular bundle image analysis measurement program

```
'       Program for measuring VB (not VICTORIA BITTER - vascular bundle!) component
tissues
'
program control close
data identifier                 ' image no. + vb. no
data code                       ' scale factor
image read
edit cover
edit size 3
edit line               'trace vb + RHS area to next vb.; trace interfasc. area
program pause
binary process outline
binary store load
'
measure write disable
measure view disable
edit cover
edit fill
binary erode 2
binary dilate 2
measure field
data column 3 protect           ' fibre cap (pith) 1
edit clear
'
binary store retrieve
edit cover
edit fill
binary erode 2
binary dilate 2
measure field
```

```
data column 4 protect          ' xylem 2
edit clear
'
binary store retrieve
edit cover
edit fill
binary erode 2
binary dilate 2
measure field
data column 5 protect          ' cambium 3
edit clear
'
binary store retrieve
edit cover
edit fill
binary erode 2
binary dilate 2
measure field
data column 6 protect          ' phloem 4
edit clear
'
binary store retrieve
edit cover
edit fill
binary erode 2
binary dilate 2
measure field
data column 7 protect          ' fibre cap (cortex) 5
edit clear
'
binary store retrieve
edit cover
edit fill
binary erode 2
binary dilate 2
measure field
data column 8 protect  ' non-interfascicular cell area  b/n vb 6
'
binary store retrieve
edit cover
edit fill
binary erode 2
binary dilate 2
measure field
data column 9 protect  ' interfascicular cell area 7
edit clear
'
measure field          ' null data into column 11
measure line           ' seriate ray length 1
data column 10 protect
measure field          ' null data into column 12
measure line           ' seriate ray length 2
data column 11 protect
measure field          ' null data into column 13
measure line           ' seriate ray length 3
data column 12 protect
measure field          ' null data into column 14
measure line           ' seriate ray length 4
data column 13 protect
measure field          ' null data into column 15
measure line           ' seriate ray length 5
data column 14 protect
measure write enable
measure view enable
measure field
data column * record
data save
binary retrieve
binary write
program run vb-meas.prg
```

Appendix 2 Raw data of conjoint analysis

A. Ranked card orders of respondents, n = 30

Respondent no., n = 30	Ranked card order (most to least preferred) location									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	
1	6	7	5	2	1	4	3	8	9	IPM
2	6	7	5	4	2	9	1	3	8	"
3	9	5	6	3	2	8	1	7	4	"
4	2	3	7	1	9	5	6	4	8	"
5	6	5	7	2	3	1	9	8	4	"
6	6	4	1	3	7	2	9	5	8	"
7	6	5	1	7	9	3	4	2	8	"
8	5	1	6	9	8	2	7	3	4	"
9	9	1	5	6	2	7	3	4	8	"
10	5	9	1	4	7	6	2	8	3	"
11	2	6	1	7	5	9	8	3	4	"
12	5	9	6	2	4	3	8	7	1	"
13	9	5	6	2	8	3	4	1	7	"
14	1	5	9	6	7	2	3	4	8	note: 2nd. card changed from 6>7
15	1	5	7	4	2	9	6	8	3	Bonn
16	7	9	2	5	1	6	4	3	8	Marbach
17	9	1	5	7	2	6	8	4	3	Hoenes, Ludwigsburg
18	6	5	3	2	8	9	4	1	7	Krugele, Stuttgart
19	9	8	2	3	6	5	1	4	7	Appich, Stuttgart
20	6	3	5	2	1	7	4	8	9	Liebchen, Ludwigsburg
21	4	1	7	5	2	8	6	3	9	
22	5	1	2	7	8	4	9	6	3	Iris, Bonn
23	6	5	1	2	4	7	8	3	9	C. Koch, Berlin
24	5	2	9	6	1	7	8	3	4	F. Bremer, Hannover
25	9	5	1	6	7	2	3	8	4	Munich
26	8	9	5	1	6	2	7	4	3	"
27	1	7	9	5	2	6	3	8	4	"
28	2	1	3	8	7	9	5	4	6	Ludwigsburg
29	1	5	7	9	2	6	4	8	3	"
30	3	1	9	6	7	4	5	2	8	"

B. Frequency and cumulative percentage of card sorting raw data

Preference 1

Bin	Freq.	Cum. %	Bin	Freq.	Cum. %
1	4	13.33%	6	8	26.67%
2	3	23.33%	9	6	46.67%
3	1	26.67%	5	5	63.33%
4	1	30.00%	1	4	76.67%
5	5	46.67%	2	3	86.67%
6	8	73.33%	3	1	90.00%
7	1	76.67%	4	1	93.33%
8	1	80.00%	7	1	96.67%
9	6	100.00%	8	1	100.00%

Preference 2

Bin	Freq.	Cum. %	Bin	Freq.	Cum. %
1	7	23.33%	5	10	33.33%
2	1	26.67%	1	7	56.67%
3	2	33.33%	9	4	70.00%
4	1	36.67%	7	3	80.00%
5	10	70.00%	3	2	86.67%
6	1	73.33%	2	1	90.00%
7	3	83.33%	4	1	93.33%
8	1	86.67%	6	1	96.67%
9	4	100.00%	8	1	100.00%

Preference 3

Bin	Freq.	Cum. %	Bin	Freq.	Cum. %
1	6	20.00%	1	6	20.00%
2	3	30.00%	5	6	40.00%
3	2	36.67%	7	5	56.67%
4	0	36.67%	6	4	70.00%
5	6	56.67%	9	4	83.33%
6	4	70.00%	2	3	93.33%
7	5	86.67%	3	2	100.00%
8	0	86.67%	4	0	100.00%
9	4	100.00%	8	0	100.00%

Preference 4

Bin	Freq.	Cum. %	Bin	Freq.	Cum. %
1	2	6.67%	2	7	23.33%
2	7	30.00%	6	5	40.00%
3	3	40.00%	7	4	53.33%
4	3	50.00%	3	3	63.33%
5	3	60.00%	4	3	73.33%
6	5	76.67%	5	3	83.33%
7	4	90.00%	1	2	90.00%
8	1	93.33%	9	2	96.67%
9	2	100.00%	8	1	100.00%

Preference 5

Bin	Freq.	Cum. %	Bin	Freq.	Cum. %
1	4	13.33%	2	8	26.67%
2	8	40.00%	7	6	46.67%
3	1	43.33%	1	4	60.00%
4	2	50.00%	8	4	73.33%
5	1	53.33%	4	2	80.00%
6	2	60.00%	6	2	86.67%
7	6	80.00%	9	2	93.33%
8	4	93.33%	3	1	96.67%
9	2	100.00%	5	1	100.00%

Preference 6

Bin	Freq.	Cum. %	Bin	Freq.	Cum. %
1	1	3.33%	2	5	16.67%
2	5	20.00%	6	5	33.33%
3	3	30.00%	9	5	50.00%
4	3	40.00%	7	4	63.33%
5	2	46.67%	3	3	73.33%
6	5	63.33%	4	3	83.33%
7	4	76.67%	5	2	90.00%
8	2	83.33%	8	2	96.67%
9	5	100.00%	1	1	100.00%

Preference 7

Bin	Freq.	Cum. %	Bin	Freq.	Cum. %
1	3	10.00%	4	6	20.00%
2	1	13.33%	3	5	36.67%
3	5	30.00%	8	5	53.33%
4	6	50.00%	1	3	63.33%
5	2	56.67%	6	3	73.33%
6	3	66.67%	9	3	83.33%
7	2	73.33%	5	2	90.00%
8	5	90.00%	7	2	96.67%
9	3	100.00%	2	1	100.00%

Preference 8

Bin	Freq.	Cum. %	Bin	Freq.	Cum. %
1	2	6.67%	8	8	26.67%
2	2	13.33%	3	7	50.00%
3	7	36.67%	4	7	73.33%
4	7	60.00%	1	2	80.00%
5	1	63.33%	2	2	86.67%
6	1	66.67%	7	2	93.33%
7	2	73.33%	5	1	96.67%
8	8	100.00%	6	1	100.00%
9	0	100.00%	9	0	100.00%

Preference 9

Bin	Freq.	Cum. %	Bin	Freq.	Cum. %
1	1	3.33%	8	8	26.67%
2	0	3.33%	4	7	50.00%
3	6	23.33%	3	6	70.00%
4	7	46.67%	9	4	83.33%
5	0	46.67%	7	3	93.33%
6	1	50.00%	1	1	96.67%
7	3	60.00%	6	1	100.00%
8	8	86.67%	2	0	100.00%
9	4	100.00%	5	0	100.00%

Note: bin = card number; cards are ordered first by their number, and then by from the highest to the lowest frequency within each preference

Appendix 3 RIRDC Project Final Report

Germany – a major export market for Australian wild flower - *Banksia*

Mibus, R. Final report: RIRDC project, 1997. *Germany - a major export market for Australian wild flower - banksia.* Adelaide: University of Adelaide.