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The Floral Biology of Banksias in Relation to Crop Production and Management

By

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Thesis submitted for the Degree of Doctor of Philosophy

December, 1990

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APPENDIX 2. Sedgley, M., Sharman, K.V. and Fuss, A.M. (1989). An overview of research into banksias, native daisies, eucalypts and acacias at the Waite Research Institue. *In:* 'The Production and Marketing of Australian Flora (Proceedings)", The University of Western Australia and The Western Australian Department of Agriculture, Perth, Western Australia.

APPENDIX 3. List of oral presentations and seminars.

The Floral Biology of Banksias in Relation to Crop Production and Management

SUMMARY

Shoot growth in relation to the flowering of *Banksia coccinea* and *B. menziesii* was investigated to develop plantation management strategies for cut flower production. In both species there was a positive relationship between shoot length, shoot diameter and number of leaves, and the probability of a shoot producing an inflorescence. The majority of blooms of both species were initiated on shoots in their second year of growth, although some shoots flowered in their first year, and others did not produce an inflorescence within the 17 month observation period. The data was used to develop proposed criteria for the pruning of *B. coccinea* and *B. menziesii* bushes based on the retention of shoots of minimum basal diameter of 4.5mm and 6mm respectively under the environmental and cultural conditions of the experiments.

Floral initiation and development in both species were investigated using scanning electron microscopy. Floral initiation in both species occurred in late spring. Subsequent floral development was slow, and the sequence of development was similar for both species. The timing of events differed, however, as floral development in *B. menziesii* took 6 to 8 months from floral initiation to peak anthesis between April and July. In *B. coccinea* macroscopic inflorescences were not observed until May, with peak anthesis occurring between August and November, 9 to 12 months after initiation.

Cut flower production from seedling plants was investigated on commercial plantations in southern Australia. Both species exhibited a high degree of plant to plant variability in the total number of blooms produced, bloom quality, stem and inflorescence length of the harvested bloom and the time of production within the flowering season. Inflorescence colour also showed a high degree of variability, with red and pink being the dominant colours in *B. coccinea* and *B. menziesii* respectively. Some blooms of each species showed abnormal floret development. Production of *B. coccinea* showed significant

between site variability in the number of blooms produced and the proportion which were abnormal. This was attributed largely to differences in environmental conditions.

Techniques for controlled hand pollination of *Banksia* were developed. Florets of known age were identified by removing all open florets and bagging the inflorescence for 24 hours. After this period all unopened florets were removed, leaving a ring of florets which had undergone anthesis. Self-pollen was removed by moving a looped, synthetic pipe cleaner over the pollen presenter segment of the style. Pollen transfer was achieved by rubbing a pollen-laden pollen presenter from the designated male parent over the stigmatic region of the recipient pistil.

Peak stigma receptivity of *B. menziesii* was recorded at 3 days post-anthesis by observing pollen germination with fluorescence microscopy, and by scanning electron microscopy of changes in the width of the stigmatic groove. Pollen tubes reached the base of the style 6 days after pollination at peak receptivity, and subsequently 11.1% of the hand pollinated florets set follicles. This was considerably higher than the natural level of fruit set of open-pollinated populations.

B. coccinea showed both protandry and partial self-incompatibility. Peak stigma receptivity as measured by pollen germination was recorded at 3 days after anthesis and maximum production of stigmatic exudate at 6 days. Pollen tubes reached the base of the style by 6 days after pollination. A 5 X 5 diallel experiment was conducted and the results measured by pollen tube growth. Self-pollinations generally resulted in poorer pollen tube growth than crosses and there was significant specific and general combining ability as well as reciprocal effects. Cross-pollination resulted in improved fruit set and seed to flower ratio over both selfing and open pollination.

DECLARATION

I HEREBY DECLARE that the work presented in this thesis has been carried out by myself and does not incorporate any material previously submitted for another degree in any university. To the best of my knowledge and belief, it does not contain any material previously written or published by another person, except where due reference is made in the text. I am willing to make the thesis available for photocopy and loan if it is accepted for the award of the degree.

A. M. Fuss

ACKNOWLEDGEMENTS

My sincere thanks go to

- Dr Margaret Sedgley for the guidance and enthusiastic encouragement she offered so willingly throughout the project;

- Dr Don Aspinall for his advice and helpful comments during the course of preparing this thesis;

- the banksia growers in South Australia and Western Australia who allowed access to their plantations and so openly shared their knowledge. In particular, I am indebted to Alan Keith, Bill Bagshaw and their families, of Blewitt Springs, S.A.;

- Geoff Watton and the Engineering and Water Supply Department for access to the plantation at Happy Valley;

- Stuart McClure for assistance with scanning electron microscopy;

- Sandra Pattison and Lynne Giles for statistical advice and their friendship;

- Andrew Dunbar and Jennie Groom for photographic services;

- the members of the Department of Plant Physiology for support and friendship.

A special thank you to my parents, John and Janice, for the interest they have taken and encouragement and support they have given during this project.

This work was supported by the Playford Memorial Trust Horticultural Research Scholarship, and I am extremely grateful to the Trustees for the honour of being the first recipient of this Scholarship. Support provided by the Doreen McCarthy Bursury from the Australian Federation of University Women, South Australia, Inc., the D.R. Stranks Travelling Fellowship from the University of Adelaide and the Rural Credits Development Fund of the Reserve Bank is also gratefully acknowledged.

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1. INTRODUCTION

FAMILY: **PROTEACEAE** R.Br.

SUB-FAMILY: **Grevilleoideae** Engl.

TRIBE: **Banksieae** Reichb.

SUBTRIBE: **Banksiinae** L. Johnson and B. Briggs

GENUS: ***Banksia*** L.f.

1.1 THE FAMILY PROTEACEAE

The family, Proteaceae, was named by Scottish botanist Robert Brown in 1809 in his paper "On the natural order of plants called Proteaceae" to the Linnean Society of London (Brown, 1810), after the type genus from South Africa, *Protea*.

The Proteaceae is an ancient family, and findings of fossilised pollen and plant parts suggest that it existed during the late Cretaceous period. A recent find of a fossilised fruiting cone of *Banksia archaeocarpa* at Kennedy Range, Western Australia, is very similar to that of *Banksia attenuata* and dates the genus *Banksia* to the late Eocene (McNamara and Scott, 1983). There are 73 genera of Proteaceae, confined predominantly to the Southern Hemisphere but with some genera occurring in China, southern India, central America and central Africa. Of these, 42 genera are known to occur in Australia, and 31 are found only in Australia.

Macadamia is the most commercialised member of the family, with extensive plantings in Hawaii, Australia, South Africa, Zimbabwe, Malawi, Kenya, California and Central America. Both *M. integrifolia* and *M. tetraphylla* have been developed for commercial nut production, leading to a range of cultivars to suit differing growing conditions. The cut flower industry exploits the greatest number of genera, however, including *Adenanthos, Banskia, Conospermum, Dryandra, Isopogon, Leucadendron, Leucospermum, Protea, Serruria* and *Stirlingia*. These and several other genera, including *Hakea* and *Grevillea*, are utilised by the nursery trade. Other commercial uses

for Proteaceae include honey production, timber and possibly pharmacology, bonsai, and interior plantscapes.

1.2 THE GENUS BANKSIA

Banksias were first collected by Sir Joseph Banks and Dr. Daniel Solander, along the east coast of Australia, as they accompanied Captain James Cook on the Endeavour in 1770. It was, however, Carl Linnaeus fil. who described the genus in 1782 and honoured its discoverer by calling it *Banksia*. In 1891, a German botanist, Otto Kuntze, proposed that the genus be named *Sirmuella*, though this name never became widely accepted.

There are 75 named species of *Banksia* (George, 1987) with the genus subdivided into two subgenera. The subgenera are *Banskia*, which includes the 72 species with long to rounded inflorescences, and *Isostylis*, those with flowers in a short-head like inflorescence. The large subgenus *Banksia* is further subdivided into two sections; *Banksia* and *Oncostylis*. The section *Banksia* includes those species which have straight style ends, and is composed of ten series; *Banksiae*, *Bauerinae*, *Coccineae*, *Crocinae*, *Cyrtostylis*, *Grandes*, *Orthostylis*, *Prostratae*, *Quercinae* and *Tetragonae*. *Oncostylis* includes three series; *Abietinae*, *Dryandriodeae* and *Spicigerae*, all of which have hooked style-ends.

By far the greatest concentration of species occurs in the south-west corner of Western Australia, where 60 species have been recorded. The remainder are distributed in the south-eastern and eastern regions of the Australian mainland, with the exception of two species native to Tasmania, and a tropical species which is found in parts of tropical northern Australia, Papua New Guinea, Irian Jaya and the Aru Islands (George, 1984).

Banksias are woody evergreens, ranging in habit from prostrate forms to trees up to 25 metres in height, with a majority as shrubs and bushes (George, 1984). They are generally very hardy plants and have several anatomical and physiological features adapted to the harsh environment in which they live. Such features include hard and tough leaves, woody seed follicles which may stay closed until activated by extreme heat, and in

some cases thick bark and fire tolerant lignotubers. They require a well drained soil, and seem to be well adapted possibly via their characteristic proteoid roots to the low soil fertility which is common throughout Australia. Many *Banksia* species prefer to grow in a slightly acidic, well drained soil (Holliday and Watton, 1975), although there are others which can tolerate alkaline conditions (Holliday and Watton, 1975; Gotham and Butler, 1977; Webb, 1977), particularly in a heavier soil type.

Perhaps the most stunning feature of the genus *Banksia*, as with the rest of the Proteaceae family, is the floral structure. The flowers are densely arranged around a central woody axis to give a large conspicuous inflorescence which may vary in shape from spherical to cylindrical. Pairs of flowers occur in vertical rows, spiralling around the axis to form an attractive pattern. Anthesis is marked by the extension of the wiry, often brightly coloured style. In most species anthesis occurs in an acropetal direction along the length of the inflorescence, although in some, the reverse is true.

While many species are well suited for use in amenity horticulture, the terminal inflorescences and attractive foliage give several species considerable potential for the cut flower trade. In addition banksias provide a valuable source of nectar and pollen to sustain honeybee colonies during the winter months. Other economic uses involve cottage industries, such as the turning of *B. grandis* infructescences to produce ornaments and coasters, and the extraction of dyes (George, 1987). The timber of *B. serrata* has been used to make bullock tokes, boat knees, etc. (Venkata Rao, 1971), and that of *B. verticillata* and *B. seminuda* have been used in furniture making (Mueller, 1879; Venkata Rao, 1971).

1.3 BANKSIA COCCINEA

Banksia coccinea (Plate 1.1) was named by Robert Brown in 1810, and is synonomous with *B. purpurea* Schnitzl. and *Siemuellera coccinea* (R.Br.) Kuntze. This name derives from *coccineus* which is latin for scarlet, and refers to the brilliant colour of the styles. In addition, the colour is reflected in its common name the Scarlet Banksia, although it is also referred to as the Waratah Banksia and the Albany Banksia. *B. coccinea* is the sole member, and therefore the type species, of the series *Coccineae*, characterised by short, broad leaves, red styles in vertical rows, and very small beaked follicles (George 1987).

B. coccinea is native to the southwest corner of Western Australia around the Stirling Ranges area. Its limits are bounded to the south and west by the Hay River northeast of Denmark, by the Fitzgerald National Park to the north and to the east by the Stokes National Park, south east of Munglinup (Taylor and Hopper, 1988).

B. coccinea grows as a shrub or small tree up to 8 metres, and does not develop a lignotuber. As mentioned previously the inflorescences of this species are characteristicly scarlet although some colour variation has been reported. The blooms are wider than they are high, terminally positioned on the stem and produced from June to January. The flowers are arranged in vertical rows, have straight styles and do not persist on the developing infructescence.

It is these striking blooms which give this species potential for use as cut flowers and in amenity horticulture. Their value as cut flowers was reflected in 1980-81 when over 500,000 stems were recorded to have been picked under licence by bush pickers, making it the sixth most heavily picked species in Western Australia at that time (Wrigley and Fagg, 1989). This potential was recognised by George (1987),

'a striking plant needing selection and development for horticulture.'

Plate 1.1 Inflorescence of *Banksia coccinea* R.Br.

"Scarlet Banksia". (x1.5).

1.4 BANKSIA MENZIESII

Banksia menziesii (Plate 1.2), was named by Robert Brown (1830) after Archibald Menzies, surgeon-naturalist on the Discovery expedition with English navigator, George Vancouver. *B. menziesii* is commonly known as Menzies' Banksia or the Firewood Banksia, and is synonomous with *Sirmuellera menziesii* (R.Br.) Kuntze. This species is placed in the series *Orthostylis* along with *B. aemula*, *B. baxteri*, *B. candolleana*, *B. ornata*, *B. sceptrum*, *B. serrata* and *B. speciosa*. The series is characterised by robust flowers with either hairy or papillose styles and beaked follicles (George, 1981; George, 1987).

Indigenous to Western Australia, *B. menziesii* is limited to a fairly narrow coastal strip of deep sand from Kalbarri in the north to Waroona in the South. The heavy soils of the Darling Scarp mark the eastern extension of the species although there are some isolated populations further east in pockets of sand, occurring near Beverley, Toodyay and Wongan Hills (Taylor and Hopper, 1988).

This species forms a lignotuber and usually grows into a tree, up to 10 metres tall, particularly in the southern part of its range. However towards its northern limit it tends to be smaller and shrubbier. The terminal inflorescences produced from February through to August, are ovoid to cylindrical with florets are arranged such that they form vertical rows (George, 1987). The florets curve upwards upon anthesis but they are not persistent, falling to reveal an ornately patterned infructescence on which large beaked follicles develop. Inflorescence colour is highly variable. Wrigley and Fagg (1989) describe the species as having yellow styles and a pink perianth, with golden yellow, brown and coppery forms also in existence; George (1978) mentions flowers being pale to deep pink or reddish, sometimes cream, chocolate or rusty-brown; while bronze, yellow and greenish variants were recorded during the preparation of the Banskia Atlas (Taylor and Hopper, 1988).

Plate 1.2 Inflorescence of *Banksia menziesii* R.Br.

"Menzies' Banksia". (x1.25).

1.5 BANKSIAS IN THE CUT FLOWER INDUSTRY

The unique blooms of banksias and other Proteaceae are presently showing considerable potential for the floricultural industry, both in Australia and overseas (Parvin *et al.*, 1973; George, 1984; Barth, 1986; Joyce and Burton, 1989). The interest generated by these markets has led to commercial cultivation of banksias in various regions of southern Australia, California, Hawaii, Israel and South Africa (Elphick, 1985). In addition there are trial plantings of *B. ashbyi*, *B. prionotes* and *B. speciosa* in Tenerife, Spain (Rodriguez Pérez, 1989).

In Western Australia, production is confined to the south west region, where at the beginning of 1989 it was estimated that over 223,200 *Banksia* plants were under cultivation (Pegrum and Webb, 1990). Of the 139 hectares of Proteaceous crops under cultivation in South Australia in 1985, most were in the Adelaide Hills area and in the Lower South East. Of this, 56 hectares were planted to *Banksia* (Barth, 1986).

It is difficult to determine the exact value of *Banksia* production, as most growers also produce other flower crops, particularly from the Proteaceae family, and are unable to separate their incomes (Kernick, 1986). In 1987-88, export of flowers and foliage from Australia was valued at A$10.6 million[1] (Pegrum, 1988). Of this, nearly A$6 million[1] was exported from Western Australia alone, and it is estimated that over A$5 million[1] was contributed by Australian native wildflowers and South African proteas (Pegrum and Webb, 1990). In Western Australia a large proportion of stems of the native wildflowers are harvested from the wild. In 1980-81 nearly 1.3 million stems of some 29 *Banksia* species, including over 0.5 million stems of *B. coccinea*, and about 0.2 million of each *B. baxteri* and *B. hookeriana*, were cut by lisenced bush pickers (George, 1984). In South Australia the total wholesale value in 1984 of the combined protea and *Banksia* production amounted to A$200,000[1] (Barth, 1986).

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[1] A$ Australian dollar

The opportunity exists to increase cut flower sales of banksias, particularly to overseas countries such as Japan and the United States (Lamont, 1986). Australia has the advantage of being able to supply flowers to northern hemispere markets during their winter months when production is low. Banksias offer considerable flexibility in marketing. As there are a number of species in flower at any given time, they are able to satisfy a year-round market, with opportunity to sell them fresh, dried or dyed (George, 1984; Windle *et al.*, 1990). However to supply export markets it will be essential to increase production and quality. This can be achieved either by increasing plantings, increasing the productivity of individual plants or a combination of both. At present production is restricted to species in their wild form, and only those having brightly-coloured, conspicuous flowers on suitable stems are accepted in the market place. In addition there is no clonal propagation of banksias, so growers are disadvantaged by the variability of seedling plants, as well as by the lack of information regarding cultivation and management.

1.6 PROJECT AIMS

This research aims to contribute to the improvement of banksias for cut flower production by investigating flowering in relation to management, and by developing selection and breeding methods for cultivar production. This is achieved via the following steps:

1. Investigation of shoot growth of *B. coccinea* and *B. menziesii*.
2. Examination of the morphological development of the apical meristem in *B. coccinea* and *B. menziesii* from floral initiation to anthesis by scanning electron microscopy.
3. Study of the variability that exists between seedling plants of *B. coccinea* and *B. menziesii* grown in southern Australian plantations.
4. Development of techniques for hybridisation of banksias, whilst investigating the breeding system of *B. menziesii*.
5. Investigation of the breeding system of *B. coccinea*.

2. LITERATURE REVIEW

2.1 INTRODUCTION

This review deals with reproductive biology and its importance in the development of plants for commercial horticulture. Reproductive development is discussed, with reference to its relationship to vegetative growth, and breeding systems in woody perennials, and, where possible, concentrates on banksias and other members of the Proteaceae. An understanding of growth cycles is essential in developing plantation management practices and in manipulating flowering for commercial production, while a knowledge of the breeding system is fundamental for crop improvement through breeding, and for species conservation.

2.2 RELATIONSHIP BETWEEN VEGETATIVE AND REPRODUCTIVE GROWTH CYCLES

2.2.1 Juvenility

Juvenility is a physiological state exhibited by seedling plants of most woody perennials, and describes their inability to produce flowers even if the plant is subjected to environmental conditions which are known to be inductive (Sedgley and Griffin, 1989). Many plant species have distinctive morphology associated with this phase of growth.

The transition to a reproductively mature adult plant is gradual, yet often marked by the loss of, or a change in, the juvenile morphological characteristics. In the Proteaceae such morphological changes include a change in leaf form, from dissected to simple (eg. *Buckinghamia*, *Opistholepis*; Venkata Rao, 1971) or simple to compound (eg. *Cardwellia sublimis*; Johnson and Briggs, 1963); a change in phyllotaxis, as in *Macadamia*, from opposite to whorls of 3 or 4 leaves in *M. integrifolia* and *M. tetraphylla*, respectively (Storey, 1985). In *Citrus*, a change in branching habit and the loss of thorniness marks the transition (Furr *et al.*, 1947), while in other plants there is a gradual loss of the ability of cuttings to root (Gardner, 1929; Paton *et al.*, 1970).

The juvenile phase may be as short as 3 weeks in some roses, or up to 40 years in some forest tree species (Matthews, 1963; Hackett, 1985). The juvenile phase of seedling plants of *Macadamia* lasts from 3 to 7 years (Storey, 1985) and that of *Telopea*, 2 to 3 years (Worrall, 1983). Similarly in *Banksia leptophylla*, it is 3-4 years, and 4-5 years in *Banksia burdettii* and *Banksia prionotes* (Lamont and Barker, 1988), while in *Banksia grandis*, a plant may take 10 or more years to flower from seed (George, 1987). The transition is generally believed to be associated with the attainment of a minimum size, and is influenced by both environmental and genetic factors (Matthews, 1963; Visser, 1965).

The duration of the juvenile period and the morphological changes associated with its completion have a very real influence both on commercial production and on plant breeding programmes. From the view of commercial production of fruit, nut and ornamental crops, a juvenile period means low returns on investment until the crop comes into flowering. Vegetative growth is more rapid during the juvenile period than following the onset of reproductive development, and therefore extension of the juvenile phase is favoured to minimise flowering in tree species grown for timber (Greenwood, 1987). Juvenility also has benefits to the nursery industry. Cuttings of juvenile material propagate readily, allowing rapid multiplication of clonal material. However, with regard to plant breeding and selection, the juvenile phase leads to a long generation time and therefore slow generation turnover. This reduces the efficiency of programmes aiming to produce improved cultivars of woody perennials.

A plant growing under environmental conditions which promote rapid and continuous growth will more rapidly attain the required size to come into flowering. Rapid growth can be promoted by placing the plant in optimal temperature, day length and light intensity regimes, with adequate water and nutrients (Doorenbos, 1955; Longman and Wareing, 1959; Holst, 1961; Zimmerman, 1971). In addition, conditions which avoid or break dormancy will encourage continuous growth (Hackett, 1985). Grafting of juvenile seedlings onto mature rootstocks has been effective in increasing the likelihood

of flowering of the scion material (Visser, 1973). Among the plant growth hormones which have been tested, gibberellic acid has been successful in causing precocious flowering in some species of conifers (Pharis and Morf, 1967; Pharis *et al.*, 1976). However, it does not bring about a stable conversion to the mature state, and reversion to the juvenile state may occur if application of the hormone is not continued (Hackett, 1985).

These methods of reducing the length of the juvenile period are effective in the short term. In the long term the problem can only be overcome through selection and breeding and an understanding of the genetic controls. This has already been achieved in some apple varieties.

2.2.2 Temporal relationship between growth cycles

After the completion of the juvenile phase, flowering is integrated with flushes of vegetative growth. The timing of the reproductive and vegetative phases of growth vary with species and are often dependant on prevailing environmental conditions. Under constant environmental conditions, many tropical plants, including *Papaya* and *Guava*, grow, flower and fruit continuously (Chin and Yong, 1980). However, in a tropical environment with wet and dry seasons, growth is often reduced and no fruit is set during the "dry". This situation of cyclic growth, rather than continuous growth, is more common among woody perennials than that of continuous growth. In the tropical species *Mangifera indica* L. cv. Kensington grown in northern Australia, four vegetative growth flushes occurred each year, with flowering extending from May to August (Scholefield *et al.*, 1986). The first of the two main vegetative flushes preceeded flowering, occurring between March and May, and the other occurred during flowering and early fruit development, in July and August. *Macadamia* species usually produce about three flushes, each followed by a short resting period, and flowering coincides with the first spring flush (Storey, 1985). In *Leucospermum*, vegetative growth occurs during the spring and summer months, with shoots exhibiting strong apical dominance. At the completion of the shoot extension phase, reproductive development commences and culminates with flowering in early spring (Jacobs, 1985). A similar growth cycle is reported for

Telopea (Faragher, 1989). In contrast, maximum vegetative growth in the fynbos shrub *Protea neriifolia*, cultivated under the summer rainfall conditions of the eastern Transvaal highlands of South Africa, was recorded in September (early spring) (Heinsohn and Pammenter, 1988). Flowering in this apecies extended from January to July, peaking in April and May. Vegetative shoots continued to grow during the flowering period, while extension growth of lateral shoots showed two distinct growth flushes in spring and in summer.

In many of the temperate tree species there is a distinct dormant period during the winter months. The dormant period is characterised by the loss of leaves in deciduous species and is considered an adaptive mechanism to the environmental conditions of the place of origin (Vegis, 1964). Resumption of growth in spring is marked by leafy growth in apples, with full bloom following 4 to 5 weeks later (Forshey and Elfving, 1983). This situation is characteristic of pome fruits and soft fruits, and also occurs in *Tilia americana*, *Nyssa sylcatica* and *Robinia psuedoacacia* (Kozlowski, 1971). Most stone fruits, however, reach full bloom before vegetative buds burst (Barnard and Read, 1933). In these species there may also be a second growth flush later in the season, from lateral buds on the present season's growth. Leafing also follows flowering in species of *Populus* and *Fraxinus*, and in *Ulmus americana*, while in *Betula lenata*, *B. alleghaniensis*, and *Salix nigra* flowers open at approximately the same time as the leaves begin expanding (Kozlowski, 1971). Additional vegetative growth flushes may occur later in the season depending on environmental conditions.

2.2.3 Physical relationship between growth cycles

Flowering is often considered to be related to the age and size of the shoots on which they form. Mango shows a tendency to produce most inflorescences on older shoots (Scholefield *et al.*, 1986) and it is thought that this relates to the higher starch reserves in the older shoots which are necessary prior to the transition to flowering (Singh, 1960; Suryanarayana, 1978). However, in the three ericaceous shrubs studied by Cooper and McGraw (1988), *Rhododendron maximum*, *R. nudiflorum* and *Kalmia latifolia*, shoot size was shown to be a better predictor of reproductive potential than shoot age. The

absence of flowers on shoots in the smallest class size suggested that a threshold size must be reached before flowering can occur and that this may relate to internal resource limitations.

The production of different sized shoots, which are functionally different, is well illustrated in apple trees. Three types of shoots are produced; (1) terminal shoots, from the terminal buds of the previous season's growth, (2) lateral shoots, from the lateral buds of the previous season's growth, and (3) bourse shoots, from the base of flower clusters (Forshey and Marmo, 1985; Forshey *et al.*, 1987). The bourse shoots or spurs, which bear the majority of the flowers, are short, mostly 6 to 10cm long. The lateral shoots also tend to be short, while the terminal shoots are generally long, with a modal shoot length of 26 to 30cm (Forshey and Marmo, 1985). Avocado also produces both long and short shoots, and although they appear to have no functional difference, it was suggested that the function of the short shoots may be to provide a temporary increase in leaf area before they are shaded and die (Scholefield *et al.*, 1985).

In the proteaceous genus *Telopea*, Faragher (1989) reports that thin, weakly growing shoots do not produce inflorescences, whereas thicker, stronger shoots do. Similarly, blooms produced on long, thick stems on commercial *Leucospermum* cultivars are of bettter quality than those on short, thin stems (Jacobs and Minnaar, 1980; Jacobs, 1983). Napier *et al.* (1986) found that application of benzyladenine to *Leucospermum* 'Red Sunset' in March had the effect of increasing shoot diameter and improving bloom quality.

2.3 FLORAL INITIATION

Floral initiation is defined as the first detectable morphological change which occurs in a bud as the meristem ceases to produce leaves and commences the production of floral appendages (Sedgley and Griffin, 1989). It is preceded by floral induction, the programming of the vegetative meristem to the reproductive state. The change in the apical meristematic cells is a result of environmental triggers which activate the genes responsible for sexual reproductive development in the plant. These triggers, day-length

and temperature, may act independently or in combination. Detectable cytochemical changes in apical meristematic cells, particularly increases in the levels of nucleic acids and histones, are associated with floral induction (Buban and Faust, 1982).

2.3.1 Morphological changes

Morpological changes in the apical meristem, at the time of floral initiation, are the result of cell division and cell expansion. Perhaps the most striking change which occurs in woody plants is the broadening of the apical meristem. This is particularly so in pear, where the diameter of the meristem undergoes a one and a half times increase in five days (Banno *et al.*, 1986). While broadening of the apex is also characteristic of herbaceous plants, they frequently exhibit a marked increase in the height of the meristem, or 'doming', during or just prior to floral initiation (Bernier *et al.*, 1981). This is not the case in woody perennials, and in contrast a flattening of the meristem is often associated with the increase in the diameter of the region in apricot and peach (Barnard and Read, 1933), Douglas fir (Owens and Pharis, 1967), sour cherry (Diaz *et al.*, 1981) and Washington Navel orange (Lord and Eckard, 1985).

Bud scales and bracts are the first primordia to be initiated, and form the outer protective sheath of the developing floral bud (Jackson and Sweet, 1972). Within the bud, floral primordia are initiated acropetally. However if the flowers are produced in an inflorescence, the branches of the inflorescence must first be formed, followed by the initiation of secondary and tertiary floral meristems along the branches (Sedgley and Griffin, 1989), as in *Acacia pycnantha* (Sedgley, 1985), avocado (Scholefield *et al.*, 1985) and *Pyrus* (Banno *et al.*, 1986).

Phyllotactic changes may be associated with the transition to producing floral parts from (Bernier *et al.*, 1981), usually towards a more complex arrangement of appendages. In *Citrus*, sepals and petals are initiated in the same phyllotactic spiral as leaves, although it is more condensed and approximates to a whorl (Lord and Eckard, 1985). The stamens and the carpels are then initiated in whorls.

Much of the literature pertaining to the timing of floral initiation is misleading, as often the time of macroscopic appearance of floral buds is reported as the time of floral initiation. However, the initial morphological changes are occurring in the bud, and therefore require microscopic observation to accurately determine the time of floral initiation. In the last 15 years, scanning electron microscopy has revolutionised such investigations, providing a three-dimensional view of the apex and thus superseding the traditional methods of microtomed sections of buds and compound microscopy.

2.3.2 Initiation in the Proteaceae

Investigations into the time of floral initiation in the Proteaceae are limited. Floral initiation in *Macadamia integrifolia* occurs during May (late autumn) under Australian conditions when minimum temperatures are between 11 and 15°C and the daylength 10 h 40-50 min (Moncur *et al.*, 1985; Stephenson and Gallagher, 1986). Although there are no reports on the influence of photoperiod on floral initiation, Storey (1985) suggests that *Macadamia* species appear to be day neutral plants and that floral initiation is influenced mainly by temperature. Scanning electron micrographs of terminal buds of *Leucadendron discolor* (Ben-Jaacov *et al.*, 1986) also showed that floral initiation had taken place by late autumn (November) in Israel. Floral initiation in *Leucospermum* cv. Red Sunset grown in South Africa was investigated by taking longitudinal sections of shoot apices fixed and embedded in paraffin wax (Napier *et al.*, 1986). Involucral bracts were formed during the prefloral state in April, followed by floret initiation during May and June (late autumn). Jacobs (1983) reports that *Leucospermum* cv. Red Sunset is in an induced state from April through until June after which it declines gradually to the vegetative state in October, but that subsequent initiation may be influenced by light intensity. Light intensity and daylength also influence floral initiation in *Telopea speciosissima* (Faragher, 1989). Scanning electron microscopy revealed that floral initiation in this species occurs in mid-summer, a time of high light intensity and long days. Extending the daylength by supplementary lighting with high intensity sodium lamps has the effect of increasing flowering. There has been

no documentation of either the time of floral initiation or the factors which influence it in *Banksia*.

2.4 FLORAL DEVELOPMENT

2.4.1 Floral bud morphology

The morphology of floral buds is well summarised by Jackson and Sweet (1972). Floral buds may be produced either terminally on a shoot, or in lateral positions, in leaf axils. They may contain only floral parts, or a mixture of leaves as well as floral parts. The latter provides two alternatives at bud burst; a determinate shoot, which produces leaves and terminates florally, or an indeterminate shoot, where the apex remains vegetative and flowers are produced in the axils of leaves and bracts.

In general, plants which produce flowers in the terminal position, either from a floral bud or a mixed bud, are favoured in floriculture, and are used to provide the focal point in floral art displays. Bud structure, together with the age of wood on which flowers are initiated, are important considerations in the development of horticultural crop management practices.

2.4.2 Inflorescence morphology of the Proteaceae

Within the Proteaceae family inflorescence morphology is extremely diverse (Venkata Rao, 1971; Wrigley and Fagg, 1989). Flowers may be displayed either as a solitary flower, as in *Adenanthos* spp, *Persoonia juniperina*, *Lambertia uniflora* and *Strangea cynanchicarpa*, or more commonly as an inflorescence. Inflorescence structure is extremely diverse amongst angiosperms, yet is often characteristic of a botanical family, for example the capitulum of the Asteraceae. However in the Proteaceae nearly every form of inflorescence is represented. The raceme (*Macadamia, Grevillea*) and panicle (*Stirlingia*, some species of *Conospermum*) type inflorescences are the most common forms. The floral head, characteristic of the Asteraceae, with individual florets being borne on an enlarged receptacle surrounded by involucral bracts and so giving the appearance of a single large flower (Blackmore and Toothill, 1984), characterises some proteaceous genera, including *Dryandra*, *Lambertia* and *Protea*. The three monotypic

Australian genera, *Agastachys*, *Cenarrhenes* and *Symphionema*, display their flowers in spikes, while some species of *Conospermum* and *Stenocarpus* provide examples of corymbs and umbels, repectively.

2.4.3 Floret morphology of the Proteaceae

Although inflorescence morphology amongst the members of the Proteaceae is extremely diverse, the structure of the individual flowers is more uniform and quite simple (Venkata Rao, 1971). They have three whorls of floral parts, including a perianth, an androecium and a gynoecium. Several genera also have a whorl of nectiferous appendages. There are four members in each of the perianth, nectary and androecious whorls, and a single pistil. The flowers range from being quite symmetrical to zygomorphic, however, this may alter as the flower develops, particularly at anthesis. In all but one species of the entire family the stamens are fused to the members of the perianth, the tepals. Only in *Bellendena montana* are the stamens free. The degree of fusion is variable, with the anthers becoming sessile or nearly so in a number of genera. There may be one, two or several ovules within the unilocular ovary, depending on taxa. In most cases, the perigynous flowers are hermaphrodite, although several genera (eg. *Leucadendron*) are dioecious.

In *Banksia*, each floret has a single elongated pistil with two ovules housed in the unilocular ovary. Anthers are attached near the tip of each of the four perianth parts, or tepals, by short filaments. This region of the perianth is called the perianth limb, and encloses the distal portion of the style and the terminal stigma before anthesis. The distal portion of the style is modified for the special function of pollen presentation. Prior to anthesis the anthers dehisce, depositing the pollen onto the pollen presenter (Venkata Rao, 1971). At anthesis the style is released from the perianth, presenting the pollen to foraging insects (Lamont and Collins, 1988), birds (Whelan and Burbridge, 1980; Collins and Spice, 1986) and small mammals (Carpenter, 1978; Paton and Turner, 1985).

2.4.4 Timing of floral development in the Proteaceae

As there has been no documentation of the time of floral initiation in *Banksia*, the total duration of floral development cannot be determined, despite the numerous reports on the time of flowering of all the species. Several investigations have considered the rate of development from macroscopic appearance of the floral bud to anthesis and found it to be highly variable, and not necessarily related to the size of the inflorescence. The inflorescence of *B. grandis* takes 4 weeks to reach anthesis (Scott, 1982) while *B. spinulosa* var. *collina* may take up to 32 weeks (McFarland, 1985).

The racemous inflorescences of *Macadamia* are borne in leaf axils (Storey, 1985). Under Australian conditions floral development takes 145 days from initiation to anthesis in late September-October, and includes a dormant period during winter of 50-96 days immediately after initiation (Moncur *et al.*, 1985). Floral buds on cuttings of *Leucadendron discolor* developed rapidly from initiation to macroscopic bud appearance in December and reached anthesis by April (spring) (Ben-Jaacov *et al.*, 1986). *Leucospermum* cv. Red Sunset took 7 months to reach peak anthesis in October (spring) from floral initiation in late-March (autumn) (Napier *et al.*, 1986), and although rate of development and time of flowering were not affected by shading, the resulting inflorescence size was decreased (Jacobs and Minnaar, 1980).

2.5 BREEDING SYSTEMS

2.5.1 Dichogamy

Dichogamy is the temporal separation of sexes due to differences in the timing of maturation of male and female organs within an individual plant (Lloyd and Webb, 1986). Thus there are two possibilities;

(1) female organs mature before male organs - Protogyny

(2) male structures mature before female organs - Protandry.

Such mechanisms reduce the interference between the presentation of pollen and stigmas, and promotes outcrossing. The success of the mechanism depends on the interval between or degree of separation of presentation, and the degree of synchrony of blossoms within a

plant (Lloyd and Webb, 1986). These are important considerations in the development of plantation management strategies and plant breeding programmes.

Protandry has been reported in the Proteaceae (Carolin, 1961; Venkata Rao, 1971; Johnson and Briggs, 1975), but there have been few confirmations of the timing between the maturity or availability of pollen for pollination and stigma receptivity. In several genera (*Persoonia*, *Symphyonema*, *Conospermum*) anther dehiscence is stimulated by insects foraging for nectar at the base of the open flower (Carolin, 1961). However many other genera have a specialized pollen presentation mechanism, whereby the anthers dehisce prior to anthesis, depositing pollen onto the distal end of the style. This region, known as the pollen presenter, is often modified to perform this function (Carolin, 1961; Venkata Rao, 1971). At anthesis the pollen presenter is released from the perianth and pollen is available to foraging animals which act as pollen vectors. However at this time the stigma may not be receptive, as has been demonstrated in *Macadamia* (Sedgley *et al.*, 1985). Anther dehiscence in *Macadamia* occurs 1-2 days pre-anthesis, depositing pollen onto the upper style. However even if the pollen is not removed, it does not germinate until 2 days after anthesis, which coincides with the production of extracellular secretion. Stigma exudate has also been observed in *Grevillea* (Lamont, 1982; Herscovitch and Martin, 1989). Peak esterase production in the stigmatic region of *Banksia prionotes* was recorded 40 hours after anthesis (Collins and Spice, 1986). *B. prionotes* also showed opening and closing of the stigmatic groove after anthesis. Similar stigmatic movements were observed for *Leucospermum cordifolium*, with the groove opening within 24 hours of anthesis and attaining maximum receptivity 2-5 days after anthesis (Brits and van den Berg, 1990). Brits and van den Berg (1990) also reported that *Protea* appears to follw a similar pattern to *Leucospermum*.

2.5.2 Monoecy and dioecy

Monoecy and dioecy refer to the spatial separation of sexes. In monoecious plants the male and female reproductive structures are produced in different flowers on the same plant, while in dioecious species the different sexual organs are borne in different flowers on different plants. Therefore outcrossing is mandatory in dioeceous species, as

pollen is produced by staminate flowers on androecious plants, and the female reproductive organs develop in pistillate flowers on gynoecious plants. Thus in the case of a dioecious fruit or nut crop, the breeding system of the species is an essential consideration in plantation layout and management in order to maximise yields. It is important to create a balance between an adequate pollen supply to produce an economic yield whilst trying to reduce the numbers of unproductive male trees.

Both forms of spatial sex separation also occur in conjunction with the production of hermaphrodite flowers, resulting in a range of sexual systems. The hermaphrodite is the usual form in the Proteaceae family, however several genera exhibit different sexual systems. *Conospermum*, *Synaphea*, species of the *Eurylaema* section of *Adenanthos*, and some species of *Protea* show partial male sterility, with one of the four anthers aborting during development (Johnson and Briggs, 1963; Venkata Rao, 1971; Wrigley and Fagg, 1989). In the monotypic genus *Placospermum*, each flower has three infertile staminodes and only one fertile stamen (Venkata Rao, 1971; Wrigley and Fagg, 1989). Venkata Rao (1971) also reports that the flowers towards the top of the inflorescence of *P. coriaceum* may become male by abortion, thus creating a situation of andromonoecy, while gynomonoecy, the presence of some female as well as hermaphrodite flowers, was reported by Johnson and Briggs (1963). Andromonoecy has also been observed in *Sphalmium* (Briggs *et al.*, 1975; Wrigley and Fagg, 1989), *Stirlingia* (Venkata Rao, 1971; Wrigley and Fagg 1989) and *Xylomelum* (Johnson and Briggs, 1963; Wrigley and Fagg, 1989), and strict monoecy in *Dilobeia* (Venkata Rao, 1971; Johnson and Briggs, 1963). *Aulax*, *Heliciopsis* and *Leucadendron*, are dioecious genera, producing female and male flowers on different plants (Venkata Rao, 1971; Johnson and Briggs, 1963). *Bellendena montana* exhibits gynomonoecy-androdioecy, where the 'male' plants produce only staminate flowers, and the 'female' plants have both pistillate and hermaphrodite flowers (Venkata Rao, 1971). The sexual characteristics described here, of unisexuality, dioecy, monoecy, partial male sterility and gynodioecy, are considered to be advanced evolutionary trends (Venkata Rao, 1971).

2.5.3 Self-incompatibility

Self-incompatibility is the inability of fertile plants to reproduce after self-pollination and is a genetically controlled outbreeding mechanism (de Nettancourt, 1977). Sedgley and Griffin (1989) treat self-incompatibility in its broadest sense, to include both pre- and post-zygotic mechanisms, and suggest that the distinction between the two in terms of genetic control may be more apparent than real.

Prezygotic self-incompatibility operates by inhibiting pollen tube growth in the pistil, and so preventing fertilisation. The genetic control is based on one or two polyallelic loci involved in a pollen-pistil interaction (Heslop-Harrison, 1975). Two different genetically controlled systems operate in plants; gametophytic self-incompatibility and sporophytic self-incompatibility. Gametophytic self-incompatibilty is controlled by the haploid genome of each pollen grain interacting with the diploid genome of the pistil, such that pollen tube growth from grains with a common S-allele with the pistil is inhibited in the style (Fig 2.1). In contrast, sporophytic self-incompatibility is controlled by the interaction of the diploid genotype of the sporophyte, the pollen producing plant, which is imparted to the pollen grains during development, and the diploid genome of the pistil. In this situation a pollen grain carrying one of the same S-alleles as the pistil will be inhibited from germinating on the stigma (Fig. 2.2). Pollen grains are believed to attain the diploid phenotype, responsible for the sporophytic self-incompatibility reaction, in the final stages of maturation by transfer of substances to the pollen exine from the breakdown of the tapetum (Heslop-Harrison, 1975).

The mechanism involved in producing the self-incompatible response is related to the site of pollen inhibition, and is also correlated with characteristics of the pollen and the pistil. However, it should be pointed out that there are exceptions to the following generalisations, and that research surrounding self-incompatibility systems has been mainly confined to herbaceous species (de Nettancourt, 1977; Sedgley and Griffin, 1989). Gametophytic self-incompatibility, where pollen tube growth is inhibited in either the style or the ovary, is often associated with wet stigmas and binucleate pollen, which retains viability in storage and germinates readily *in vitro* (Brewbaker, 1967;

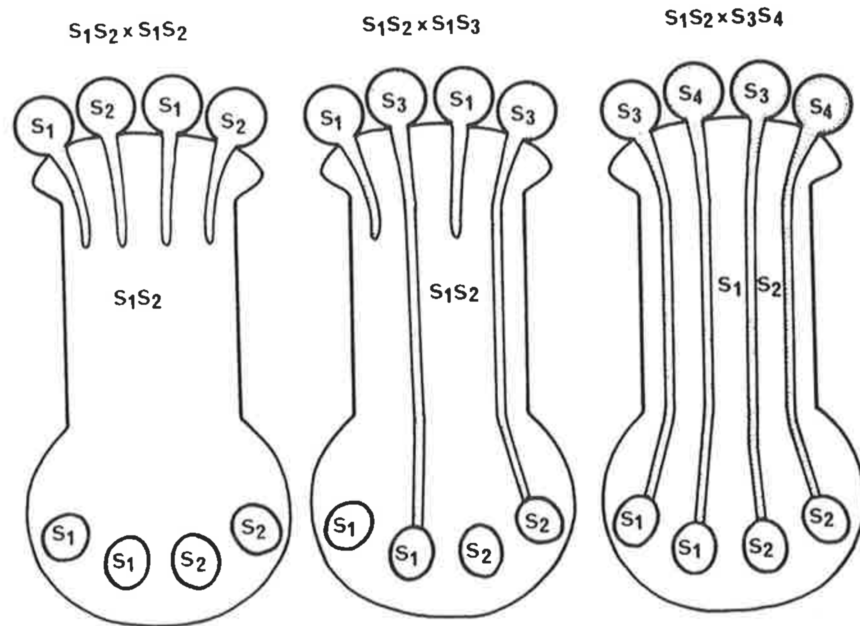


Fig. 2.1 Gametophytic self-incompatibility system. Pollen grains germinate on the stigma, however growth of pollen tubes from grains with a common S-allele with the pistil is inhibited in the upper style. (From Sedgley and Griffin, 1989).

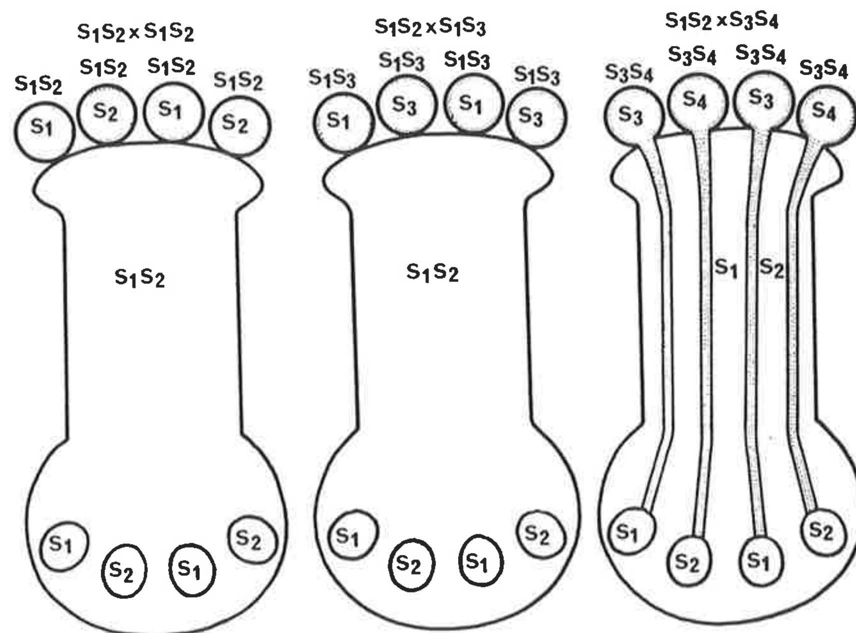


Fig. 2.2 Sporophytic self-incompatibility system. Pollen grains whose parent carries a common S-allele with the pistil are inhibited from germinating on the stigma. This example shows independent action of the alleles in both the pistil and the pollen. (From Sedgley and Griffin, 1989).

Heslop-Harrison and Shivanna, 1977). Wet stigmas produce a surface secretion creating a free fluid surface (Heslop-Harrison and Shivanna, 1977). Sporophytic self-incompatibility is characterised by stigmatic inhibition of pollen tube growth, as in *Ulmus americana* (Ager and Guries, 1982). This type of self-incompatibility has been broadly correlated with dry stigmas and the release of trinucleate pollen from the anther, which germinates poorly *in vitro* and rapidly loses viability during storage (Brewbaker, 1967). Dry stigmas have little or no surface secretion at maturity (Heslop-Harrison and Shivanna, 1977).

A study on pollen cytology has shown that species of *Leucadendron*, *Grevillea* and *Macadamia* have binucleate pollen grains (Brewbaker, 1967). Heslop-Harrison and Shivanna (1977) suggest that the stigma type is generally uniform within a family and, from their examination of *Embothrium* and *Grevillea* species, described the Proteaceae as having a dry stigma with unicellular surface papillae. However, more recent studies on *Grevillea* (Lamont, 1982; Martin and Herscovitch, 1989) and *Macadamia* (Sedgley *et al.*, 1985) have recorded the production of extracellular stigmatic exudate. Binucleate pollen, together with a wet stigma would suggest that gametophytic self-incompatibility would be the most likely system to operate in species of Proteaceae. This has been supported by observations of stylar inhibition of pollen tube growth in *Macadamia* (Sedgley 1983). However, the mechanism operating does not cause complete inhibition of pollen tube growth following selfing, as shown by reduced levels of seed set in *Macadamia* (Urata, 1954; Sedgley, 1983; Sedgley *et al.*, 1990) and *Grevillea* (Brough, 1933).

Stylar inhibition appears to be most common in tree species, such as *Citrus* (Ton and Krezdorn, 1967) and *Prunus* (Crane and Brown, 1938). However Seavy and Bawa (1986) believe that pre-zygotic late-acting self-incompatibility in angiosperms may not be as rare as previously considered. Sedgley and Smith (1989) have shown that in *Eucalyptus woodwardii* fewer ovules were penetrated following self- than cross-pollination due to inhibition of pollen tubes in the ovary. In *Acacia retinodes*, pollen tube growth is arrested in the nucellus (Kernick *et al.*, 1986). Inhibition in chestnut and *Theobroma cacao* may occur at an even later stage, with release of the male gametes into

the embryo sac but failure to fertilise the egg and central cell (McKay, 1942; Cope, 1962).

The inhibition reaction can manifest itself in several ways which can be readily identified by fluorescence microscopy (Martin, 1959). In sporophytic systems the cell wall component, ß-1,3-glucan or callose, is deposited in the pollen grains and stigmatic cells, whereas deposition occurs in the inhibited pollen tubes following a gametophytic self-incompatibility reaction (Knox, 1984). Inhibited pollen tubes may also show growth abnormalities. In self-incompatible crosses in *Macadamia* cultivars, pollen tube growth was arrested in the style, with the formation of swollen tips, and the discharge contents through a sub-terminal pore (Sedgley, 1983). Pollen tube growth following self-pollinations of cherry and plum was also arrested in the stylar tissue and tube tips were swollen (Roy, 1938). Branching of pollen tubes has also been described as an incompatible reaction in *Rhododendron* (Williams *et al.*, 1982), however it has also been observed in compatible crosses, suggesting that there may be a haustorial function (Sedgley, 1979; Tilquin *et al.*, 1983; Martin and Herscovitch, 1989).

As there have been few genetical studies on post-zygotic self-incompatibility, it is difficult to distinguish it from inbreeding depression, which is proposed to result from the presence of many deleterious recessive alleles throughout the genome. Abortion may occur before the zygote divides, as in *Rhododendron* (Williams *et al.*, 1984), or after only a few cell divisions (Schmitt and Perry, 1964). However more work is required in this area to determine the genetic control of the mechanism.

2.5.4 Breeding system of *Banksia*

Banksias are protandrous (Carolin, 1961; Venkata Rao, 1971; Johnson and Briggs, 1975), but there has been no work on the precise timing of stigma receptivity. In *B. prionotes* movement of the stigmatic groove was observed after flower opening and showed peak esterase production at 40 hours post-anthesis (Collins and Spice, 1986). Lamont and Collins (1988) observed changes in the flower colour of *Banksia ilicifolia*

which were considered to be age-dependent. It is likely that these observations may be related to stigma receptivity.

In banksias, anther dehiscence prior to anthesis deposits pollen onto the pollen presenter and near the stigma. Dispite this banksias do not appear to be self pollinating (Paton and Turner, 1985; Whelan and Goldingay, 1986). This suggests that the pollen presentation mechanism together with the protandrous nature of the flower may promote outcrossing. Indeed it seems that outcrossing is essential for seed set in *B. attenuata, B. menziesii* (Scott, 1980), *B. prionotes* (Collins and Spice, 1986), *B. ericifolia* (Paton and Turner, 1985), *B. paludosa, B. spinulosa* (Carthew *et al.*, 1988; Goldingay and Whelan, 1990) and all species which are able to resprout after being damaged by fire (Carpenter and Recher, 1979). However, *B. ericifolia* (Blake, 1971; Carpenter and Recher, 1979), *B. spinulosa* var. *cunninghammii*, *B. serrata* (Salkin, 1987), *B. marginata* and *B. oblongifolia* (Blake, 1971) have all been reported to be self-compatible.

2.6 POLLINATION IN THE PROTEACEAE

Pollination in angiosperms involves the transfer of pollen from the anthers, the male reproductive organs, to the stigma, the receptive region of the pistil, the female reproductive structure. In some plants this happens automatically when the anthers dehisce, and pollen becomes deposited onto the stigma of the same flower. This type of pollen transfer (autogamy) occurs in the Proteaceae, for example *Grevillea robusta* and *G. banksii* (Brough, 1933). More commonly the process of pollination is affected by abiotic factors, including wind and water, or by biotic agents, such as birds, insects or mammals. As a result, self-pollination can occur by transfer of pollen either within a flower (autogamy) or between flowers on the same plant (geitogamy), while in cross or xenogamous pollinations, pollen is transferred to stigmas of flowers on another plant. In the Proteaceae, pollination is performed primarily by biotic vectors, and there exists a close association between floral characteristics and the class of vector. Such associations

have received much attention by pollination ecologists (Proctor and Yeo, 1973; Faegri and van der Pijl, 1979; Meeuse and Morris, 1984).

2.6.1 Ornithophily

Bird pollinated flowers tend to be conspicuous and vividly coloured, and are often red (Faegri and van der Pijl, 1979). The individual florets tend to be tubular, produce copious quantities of nectar at the base of the tube and emmit no odour. Species of the Australian genera *Banksia, Dryandra, Grevillea, Adenanthos, Hakea, Telopea* and *Lambertia* produce flowers or inflorescences exhibiting these characteristics, and are visited and pollinated predominantly by honeyeaters (Ford *et al.*, 1977; Paton and Ford, 1977; Hopper, 1980; Wooller *et al.*, 1983; Collins and Rebelo, 1987; Pyke 1987). When the bird probes the flower foraging for nectar, the pollen, which was deposited on the pollen presenter prior to anthesis, is dusted onto its forehead, throat or beak (Paton and Ford, 1977). This pollen may then be involuntarily transferred to other flowers during subsequent nectar-seeking visits. The probing of florets during foraging also effects floret opening, as shown in *Banksia menziesii* (Ramsey, 1988). White-eyes, lorikeets and other birds also frequent blooms, but are unlikely to be as efficient pollinators as the long-beaked honeyeaters. Bird pollination of South African Proteaceae is restricted to *Leucospermum*, *Mimetes* and *Protea* and is performed predominantly by sugarbird and sunbirds (Collins and Rebelo, 1987).

2.6.2 Entomophily

Most insect pollinated flowers tend to be blue, purple, white or yellow, as insects perceive a different colour range from birds and humans (Faegri and van der Pijl, 1979). In addition, these flowers may produce a sweet perfume and nectar which is easily accessed.

Bees frequent *Persoonia* to forage for nectar produced at the base of the flowers (Carolin, 1961). By pushing its proboscis between the style and the encircling anthers, pollen is deposited on the bee's body and proboscis. This pollen is then transferred during

subsequent visits to older flowers, where the perianth segments and the anthers have curled backwards exposing the sticky, receptive stigma.

Isopogon and *Petrophile* are both insect pollinated although they have a pollen presentation mechanism similar to *Banksia* (Carolin, 1961; Wrigley and Fagg, 1989). *Banksia attenuata* was visited by a range of honeyeaters, however exclusion experiments suggest that insects may play a more important role as pollinators in this species (Whelan and Burbridge, 1980). Honey bees foraging on *Banksia* species are considered to be acting as pollen vectors, while visits by native bees, nocturnal moths and ants did not appear to be transferring pollen (Paton and Turner, 1985). However honey bees did not appear to influence floret opening, making them less effective pollinators than the avian vectors studied (Ramsey, 1988). Bees collecting pollen from *Telopea* flowers were not considered to be pollinators because of the protandrous nature of the florets (Pyke, 1987).

Other insect pollinated members of the Proteaceae have more active methods of ensuring that insect visitors transfer pollen. In the genus *Conospermum* the style is held under tension until the flower is visited by an insect pollinator, usually a fly. This causes the anthers to dehisce producing a cloud of pollen, some of which will land on the insect, and the style to be released forcing the stigma to contact the body of the potential pollinator (Carolin, 1961; Wrigley and Fagg, 1989). Similar trigger mechanisms operate in *Synaphea*, *Symphionema*, and the monotypic genus *Cenarrhenes* (Carolin, 1961; Wrigley and Fagg, 1989). Moths are thought to be the usual pollinator of *Symphionema*.

Beetles and other insects found on flower clusters are considered as potential pollinators of *Hakea microcarpa* (Webb, 1985). Small, non-specialized beetles were found to be the important pollinators of *Protea repens* (Coetzee and Giliomee, 1985) and the dioecious genus *Leucadendron* (Hattingh and Giliomee, 1989). It has even been suggested that phoresic *Proctolaelaps* mites may play an essential role in pollinating

'bearded' proteas by transferring pollen from large vectors to the small, dry stigmatic groove of the flowers (Rebelo, 1985).

2.6.3 Therophily

Some members of the Proteaceae are pollinated by small mammals (Collins and Rebelo, 1987). These therophilous flowers are grouped into robust inflorescences which are often dull in colour, produce a copious supply of nectar and have a strong odour to attract pollinators (Faegri and van der Pijl, 1979). Proteaceous inflorescences visited by mammals are usually close to the ground or concealed by surrounding foliage.

This pollination syndrome is particularly prevalent in *Protea*, where rodents are frequent visitors to the geoflorous species (Rouke and Weins, 1977; Weins and Rouke, 1978; Rouke, 1980). In Australia, rodents and small marsupials are reported to effect pollination by foraging for nectar on inflorescences of some *Banksia* and *Dryandra* species (Carpenter, 1978; Hopper, 1980; Wooller *et al.*, 1983). Indeed the hooked style ends of *Banksia* species including *B. ericifolia*, *B. spinulosa* and *B. occidentalis* are considered to be specialised for transferring pollen to mammalian vectors (Carpenter, 1978; Hopper, 1980). There is also evidence that these small animals also regularly ingest large quantites of *Banksia* pollen (Turner, 1984; Goldingay *et al.*, 1987).

2.7 SEED SET IN THE PROTEACEAE

The level of seed set in the Proteaceae is low relative to the number of flowers produced with the exception of the genera *Aulax*, *Leucodendron* and *Orothamnus* and the *Protea* species, *P. mellifera* (Tables 2.1 and 2.2). In the southern African genera there is only one ovule per ovary, while in the Australian genera the ovary usually contains two ovules, and thus has the potential to develop two seeds per fruit (Vogts, 1982; Collins and Rebelo, 1987).

Table 2.1 Mean number of flowers per inflorescence and natural fruit set in some members of the Proteaceae.

	Number of florets per inflorescence	Percentage of barren infructescence	Mean number of fruits per fertile infructescence	Fruit to flower ratio for fertile infructescences	Fruit to flower ratio for all infructescence	References
Aulax						
A. cancellata	15	0	13.0	86.7	86.7	Collins and Rebelo (1987)
A. umbellata	12	0	12.0	100.0	100.0	Collins and Rebelo (1987)
Banksia						
B. asplenifolia	-	80	11	-	-	Carpenter and Recher (1979)
B. attenuata	1933	51	3.6	0.2	0.1	Cowling *et al.* (1987)
	2629	-	13.3	0.5	-	Lewis and Bell (1981)
	2689	-	8.7	0.3	-	Scott (1982)
	1350	51	7.9	0.6	0.3	Wooller *et al.* (1983)
	1306	11	11.3	0.9	0.8	Whelan and Burbridge (1980)
B. baxteri	273	-	2.1	0.8	-	Hopper (1980)
	250	13	1.8	0.7	0.6	Wooller *et al.* (1983)
B. burdettii	972	14	8.0	0.8	0.7	Lamont and Barker (1988)
B. coccinea	275	3	14.4	5.2	5.1	Wooller *et al.* (1983)
B. elegans	393	94	2.8	0.7	0.04	Lamont and Barrett (1988)
B. ericifolia	1500	49	42	2.8	1.4	Zammitt and Westoby (1987)
	-	49	24	-	-	Carpenter and Recher (1979)
	949	37	48	5.1	3.2	Paton and Turner (1985)
	1500	62	39.5	2.6	1.0	Zammit and Hood (1986)
B. grandis	2888	85	50	1.7	0.3	Abbott (1985)
	3372	-	88	2.6	-	Scott (1982)
	3750	51	50.2	1.3	0.7	Wooller *et al.* (1983)
	2709	64	92.4	3.4	1.2	Whelan and Burbridge (1980)
B. ilicifolia	64	-	1.3	2.0	-	Scott (1982)
	69	71	1.1	1.6	0.5	Whelan and Burbridge (1980)
B. integrifolia	1020	3	-	-	-	McFarland (1985)
B. leptophylla	350	28	25.2	7.2	5.2	Cowling *et al.* (1987)
B. littoralis	1410	-	98	7.0	-	Lewis and Bell (1981)
	1410	-	56	4.0	-	Scott (1982)
	1039	39	67.7	6.5	4.0	Whelan and Burbridge (1980)
B. menziesii	1002	62	2.9	0.3	0.1	Cowling *et al.* (1987)
	930	-	7.5	0.8	-	Lewis and Bell (1981)
	930	-	4.2	0.5	-	Scott (1982)
	1044	71	3.9	0.4	0.1	Whelan and Burbridge (1980)
B. nutans	300	68	7.3	2.4	0.8	Wooller *et al.* (1983)
B. oblongifolia	1500	56	50.7	3.4	1.5	Zammitt and Hood (1986)
	1500	72	39	2.6	0.7	Zammitt and Westoby (1987)
B. occidentalis	1131	-	58.1	5.1	-	Hopper (1980)
B. paludosa	-	77	24.8	-	-	Whelan and Goldingay (1986)
B. prionotes	1431	5	32.2	2.3	2.1	Cowling *et al.* (1987)
B. spinulosa	-	88	6	-	-	Carpenter and Recher (1979)
	792	35	-	-	-	McFarland (1985)
	-	78	80.7	-	-	Whelan and Goldingay (1986)
	665	31	55.9	8.4	5.8	Vaughton (1988)

Table 2.1 Continued.

	Number of florets per inflorescence	Percentage of barren infructescence	Mean number of fruits per fertile infructescence	Fruit to flower ratio for fertile infructescences	Fruit to flower ratio for all infructescence	References
Banksia continued						
B. telmatiaea	846	-	56	6.7	-	Lewis and Bell (1981)
	688	-	29.5	4.3	-	Scott (1982)
B. tricuspis	1200	69	24	2.0	0.6	Lamont and Van Leeuwen (1988)
Grevillea						
G. leucopteris	100	-	6.3	6.3	-	Lamont (1982)
Lambertia						
L. formosa	7	90	1.2	17.1	1.7	Pyke (1982)
Leucospermum						
L. attenuatum	109	-	1.3	1.2	-	Horn (1962)
L. bolusii	157	-	3.8	2.4	-	Horn (1962)
	38	45	0.5	1.3	0.7	Collins and Rebelo (1987)
L. conocarpum	104	-	4.9	4.7	-	Horn (1962)
	87	1	12.8	14.7	14.6	Collins and Rebelo (1987)
L. cordifolium	163	-	10.7	6.6	-	Lamont (1985)
	154	38	3.7	2.4	1.5	Collins and Rebelo (1987)
L. cuneiforme	79	-	10.6	13.4	-	Lamont (1985)
	69	25	3.4	4.9	3.7	Collins and Rebelo (1987)
L. erubescens	56	-	3.7	6.6	-	Lamont (1985)
	68	-	2.1	3.1	-	Collins and Rebelo (1987)
L. glabrum	66	29	5.9	8.9	9.6	Collins and Rebelo (1987)
L. oleifolium	99	33	0.7	0.7	0.5	Collins and Rebelo (1987)
L. nutans	148	-	3.2	2.2	-	Horn (1962)
L. reflexum	161	-	3.7	2.3	-	Horn (1962)
	119	34	2.2	1.8	1.2	Collins and Rebelo (1987)
L. prostratum	76	-	1.0	1.3	-	Collins and Rebelo (1987)
L. vestitum	111	21	2.6	2.3	1.9	Collins and Rebelo (1987)
Protea						
P. acuminata	112	50	4.6	4.1	2.1	Collins and Rebelo (1987)
P. amplexicaulis	161	60	7.9	4.9	2.0	Collins and Rebelo (1987)
P. aristata	379	10	16.3	4.1	3.7	Collins and Rebelo (1987)
P. aurea aurea	73	0	19.1	26.2	26.2	Collins and Rebelo (1987)
P. aurea potbergensis	75	30	4.2	5.6	3.9	Collins and Rebelo (1987)
P. barbigera	430	-	12.0	2.8	-	Horn (1962)
P. burchellii	142	29	6.6	4.6	3.3	Collins and Rebelo (1987)
P. compacta	90	-	15.1	16.8	-	Horn (1962)
P. compacta hybrid	295	-	9.8	3.3	-	Horn (1962)
P. coronata	309	10	7.4	2.4	2.2	Collins and Rebelo (1987)
P. cynaroides	379	0	13.5	3.6	3.6	Collins and Rebelo (1987)
P. grandiceps	464	20	25.3	5.5	4.4	Collins and Rebelo (1987)
P. incompta	289	-	10.1	3.5	-	Horn (1962)

Table 2.1 Continued.

	Number of florets per inflorescence	Percentage of barren infructescence	Mean number of fruits per fertile infructescence	Fruit to flower ratio for fertile infructescences	Fruit to flower ratio for all infructescence	References
Protea continued						
P. lacticolor	94	-	4.9	5.2	-	Horn (1962)
	172	10	20.7	12.0	10.8	Collins and Rebelo (1987)
P. lanceolata	57	-	6.5	11.4	-	Horn (1962)
	65	20	9.6	14.8	11.8	Collins and Rebelo (1987)
P. latifolia	144	-	34.8	24.1	-	Horn (1962)
P. laurifolia	364	-	4.8	1.3	-	Horn (1962)
P. lepidocarpendron	138	20	1.6	1.2	0.9	Collins and Rebelo (1987)
P. longiflora	256	-	1.8	0.7	-	Horn (1962)
	178	20	15.8	8.9	7.1	Collins and Rebelo (1987)
P. longiflora hybrid	170	-	10.9	6.4	-	Horn (1962)
P. mellifera	78	-	55.2	70.3	-	Horn (1962)
P. minor	249	-	4.3	1.2	-	Horn (1962)
P. mundii	96	-	3.3	3.4	-	Horn (1962)
	158	20	11.0	7.0	5.6	Collins and Rebelo (1987)
P. neriifolia	297	-	10.7	3.6	-	Horn (1962)
	270	0	17.4	6.4	6.4	Collins and Rebelo (1987)
P. neriifolia hybrid	338	-	19.0	5.6	-	Horn (1962)
P. nitida	273	-	5.5	2.0	-	Collins and Rebelo (1987)
P. obtusifolia	284	-	3.5	1.2	-	Horn (1962)
	235	60	2.5	1.1	0.4	Collins and Rebelo (1987)
P. odorata	45	44	2.6	5.8	3.2	Collins and Rebelo (1987)
P. pulchella	149	-	14.5	9.7	-	Horn (1962)
P. punctata	151	0	28.3	18.7	18.7	Collins and Rebelo (1987)
P. repens	112	-	26.1	23.3	-	Coetzee and Giliomee (1985)
	102	0	30.6	30.0	30.0	Collins and Rebelo (1987)
P. scolymocephala	80	-	3.4	4.3	-	Horn (1962)
	113	50	1.9	1.7	0.8	Collins and Rebelo (1987)
P. speciosa	326	20	20.4	6.3	5.0	Collins and Rebelo (1987)
P. susannae	162	-	10.0	6.2	-	Horn (1962)
	150	0	21.5	14.3	14.3	Collins and Rebelo (1987)
Telopea						
T. speciosissima	160	48	3.0	1.9	1.0	Whelan and Goldingay (1989)
	80	16	2.5	3.1	2.6	Pyke (1981)

Table 2.2 Mean number of flowers per inflorescence and natural seed set in the genera of southern African Proteaceae. The genus *Leucospermum* is divided into 'a' (Sections *Crassicaudex, Conocarpodendron, Tumidifolium, Brevifilamentum* and *Cardinistyly*) and 'b' (Sections *Leucospermum, Diastelloides, Crinitinae* and *Hanatum*). (Adapted from Rouke and Rebelo, 1985).

Genus	Number of flowers per inflorescence	Number of seeds per infructescence	Percentage seed set
Aulax	20.3	20.3	100.0
Leucodendron	43.3	33.4	77.1
Orothamnus	29.9	15.0	50.1
Sorocephalus	40.8	10.6	26.0
Serruria	100.8	24.4	24.2
Leucospermum a	57.4	13.5	23.5
Spatella	37.7	8.1	21.5
Mimetes	319.7	68.1	21.3
Paranomus	85.4	10.5	12.3
Vexatorella	35.7	3.0	8.4
Protea	218.1	18.1	8.3
Leucospermum b	31.8	2.1	6.6
Diastella	15.4	1.0	6.5

In the Proteaceae, seeds develop in one of three different types of fruits; (1) fleshy drupes, (2) indehiscent achenes and nuts, and (3) dehiscent follicles (Venkata Rao, 1971). Fleshy drupes are adapted to dispersal by birds and develop on species of *Persoonia, Cenarrhenes* and *Beauprea*. Indehiscent fruits are characteristic of the subfamily Proteoideae, and dehiscent follicles, of the subfamily Grevilleoideae.

Banksias produce hard woody follicles which mature in one to two years (Elliot and Jones, 1980; George, 1987). The follicles of some species dehisce spontaneously, and will usually do so at a particular time of year, while others do so in response to fire. At dehiscence the follicle splits along a horizontal suture to release the separator and two winged seeds. The separator is a stiff woody structure, with two apical wings which control the release of the seeds from the follicle (George, 1987). It develops from the contact area of the outer integument of the two ovules, which grow together and thicken to form a septum between the two seeds (Venkata Rao, 1971). The shape and size of the seed and the seed wing are characteristic of each species (George, 1981). The terminal papery wing, which aids dispersal, develops from a membranous expansion of the outer integument or of the chalaza, into which the funicular bundles do not extend (Venkata Rao, 1971).

2.8 HYBRIDISATION IN THE PROTEACEAE

Hybridisation occurs readily in several genera of Proteaceae, including *Grevillea* (Butler, 1986; Wrigley and Fagg 1989), *Leucadendron* (Amos, 1987), *Leucospermum* (Ito *et al.*, 1978; Parvin, 1981; Brits, 1985a,b), *Macadamia* (Storey and Saleeb, 1966; Sedgley *et al.*, 1990) and *Protea* (Brits, 1988). Spontaneous hybridisation from chance cross-pollination between cultivated species of *Grevillea* has resulted in some 150 named hybrid cultivars (Butler, 1986; Wrigley and Fagg, 1989).

In contrast there are only five registered cultivars of *Banksia*, *B. canei* "Celia Rosser" (Salkin, 1979; Butler, 1986; Wrigley and Fagg, 1989), *B.* "Giant Candles" (Butler, 1986; Peach, 1989; Wrigley and Fagg, 1989), *B. serrata* "Austraflora Pygmy

Possum" (Austraflora Nurseries Pty. Ltd., 1989; Wrigley and Fagg, 1989), *B. spinulosa* var. *cunninghamii* "Lemon Glow" (Salkin, 1989; Wrigley and Fagg, 1989) and *Banksia spinulosa* var. *spinulosa* "Birthday Candles" (Molyneux, 1990). With the exception of *B.* "Giant Candles", these are all selected forms of their particular species. *B.* "Giant Candles" is believed to be a hybrid of *B. ericifolia* var. *ericifolia* and *B. spinulosa* var. *spinulosa* which arose spontaneously in a nursery in Brisbane, Queensland. Other presumed hybrids are reported to occur between *B. prionotes* and *B. hookeriana* (Keighery, 1985), *B. robur* and *B. oblongifolia*, *B. ericifolia* and *B. spinulosa* var. *cunninghamii*, *B. marginata* and *B. conferta* var. *penicillata*, *B. marginata* and *B. integrifolia* var. *integrifolia*, *B. paludosa* and *B. integrifolia* var. *integrifolia*, *B. aemula* and *B. serrata* (George, 1987), *B. hookeriana* and *B. menziesii* (Dixon, 1986; Taylor and Hopper, 1988) and *B. prionotes* and *B. lindleyana* (George, 1988). A pollen sterile *B. menziesii* X *B. hookeriana* hybrid was collected by G. Keighery, north of Badgingarra, W.A. (Taylor and Hopper, 1988).

2.9 COMMERCIAL PLANT BREEDING

The highly competitive and fashion conscious nature of cut flower marketing means that the industry relies heavily on plant breeding and selection programmes for the production of novelty products. The potential of such programmes to create new varieties is well illustrated by the traditional cut flower, the rose, with plant breeders introducing hundreds of new varieties since the 1930's (Hasek, 1980). These programmes are also essential for the development of improved plant material, and attempt to improve yield and bloom quality, introduce disease resistance, adapt plants to different environments or, in the case of woody perennials, reduce the juvenile phase.

Initial improvements can be made by the selection of superior individual plants from existing populations. Selection is currently under way on several Australian native species for cut flower production, including Geraldton wax (*Chamelaucium uncinatum*) and the waratah (*Telopea speciosissima*) (Lamont, 1986; Mullins, 1987). However, following selection, clonal propagation is essential to maintain and multiply those superior individuals for improved product uniformity. Further improvements in

quality, and the generation of novelty cultivars results from breeding programmes by controlled hand pollinations amongst superior and unusual individuals to produce hybrids of known parentage. The progeny from these crosses must then be evaluated on a range of selection criteria to choose those of superior quality, that is those which show some advantage over the previous generation. A knowledge of the floral anatomy and breeding system of a species is imperative to develop methods of emasculation and to isolate individual florets to avoid contamination with unwanted pollen, collecting and storing pollen, and performing artificial pollination.

An alternative method of generating variation is through the use of mutation breeding (Broertjes and van Harten, 1988). Genetic mutations arise spontaneously in nature although at the very low rate of approximately 1×10^{-6}-10^{-7} for a particular gene towards recessive in a single cell. Artificial treatments however can increase the rate of mutation a thousandfold. Artificial mutations may be induced by chemical or physical treatments which interfere with the natural replication of genetic material within a cell. Colchicine has been used since the 1940's to induce polypoidy. Ionizing radiation treatments involve the use of X-rays and gamma-rays, but there is no control over the change in genetic make-up induced. New developments in genetic engineering in plants therefore are destined to supersede the use of mutation breeding. Genetic engineering involves transforming plants with foreign pieces of genetic material which have been precisely characterised and are known to impart a particular character (Bliss, 1984), thus giving the breeder considerably more control over the outcome. Work on the floral pigments of petunia is showing this technique to be a very powerful tool in ornamental plant breeding (Mol *et al.*, 1989a,b), and the same technology is being used in Australia to develop the blue rose, which has eluded rose breeders for years.

Breeding in the Proteaceae is likely to be limited initially by the lack of knowledge of the breedings system, the low level of seed set and the long generation time. The effect of low seed set has already been acknowledged as a problem in hybridisation experiments on South African genera (Vogts, 1960; Brits, 1984). However since they have not been truly domesticated, there is considerable scope to increase the variety of hybrids which

exist and breed for particular improvements such as *Phytophthora* resistance (McCredie *et al.*, 1985).

3. EXPERIMENTAL SITES

3.1 MAIN EXPERIMENTAL SITE: BLEWITT SPRINGS, SOUTH AUSTRALIA

3.1.1 Plantation layout and management

Approximately 140 seedling plants of *B. coccinea* and 235 of *B. menziesii* were planted in 1983 on a commercial cut flower plantation at Blewitt Springs, South Australia (latitude 35°10'S, longitude 138°34'E) (Figs 3.1 and 3.2). The plantation is situated on the eastern slope of a steep hill which had previously been planted to grapevines. The plants are arranged in rows following the contour, with 3.0m between rows and 1.8m spacings between plants for *B. coccinea* and for *B. menziesii* 3.4m and 2.3m respectively. The soil type of the location is deep sand of pH 6.6.

Prior to the experimental period all plants had been subjected to routine plantation management practices, including limited drip-irrigation to supplement rainfall, and annual fertilising in autumn (March to May) with Complete Mineral Mix (nitrogen (as ammonia) 10.5%, phosphorus (water soluble) 1.5%, phosphorus (citrate soluble) 0.2%, potassium (as sulphate) 5.0%, sulphur (as sulphate) 18.1%, calcium (as phosphate) 3.7%, iron (as sulphate) 2.2%, manganese (as sulphate) 0.6%, molybdenum (as molybdate) 0.005%, zinc (as sulphate) 0.35%) (Top Australia Ltd), and biannually with low phosphorus Osmocote Controlled Release Fertiliser (18:2.6:10, N:P:K) (Sierra). Harvesting of blooms served as the only form of pruning in previous years. Weeds were controlled by mowing between rows and by either whipper-snipping or spraying with Round-Up (glyphosate), at the minimum dosage, around the base of plants. These management practices were maintained throughout the experimental period. Meteorological data for the experimental period, March 1988 to November 1990, are presented in Table 3.1.

Fig. 3.1 7 year old *B. menziesii* bush growing in the experimental plantation at Blewitt Springs, S.A. Scale bar: 30cm.

Fig. 3.2 7 year old *B. coccinea* bush growing in the experimental plantation at Blewitt Springs, S.A. Scale bar: 30cm.

Table 3.1 Mean (± standard error) monthly maximum and minimum temperatures, daylength and sunshine hours, and total rainfall at nearest available locations to Blewitt Springs for the experimental period.

		Temperature (°C)[1] max	min	Daylength (hours)[2]	Sunshine Hours[3]	Rainfall (mm)[4]
1988	March	25.3 ±1.0	11.6 ±0.6	12.2 ±0.0	8.0 ±0.6	8
	April	19.6 ±0.5	8.7 ±0.5	11.1 ±0.0	7.8 ±0.5	24
	May	17.3 ±0.7	8.8 ±0.6	10.2 ±0.0	3.4 ±0.5	157
	June	14.0 ±0.4	5.6 ±0.6	9.5 ±0.0	3.8 ±0.5	97
	July	12.7 ±0.3	5.8 ±0.4	10.0 ±0.0	2.9 ±0.5	91
	August	14.1 ±0.7	5.4 ±0.6	10.5 ±0.0	4.5 ±0.5	68
	September	18.0 ±0.7	6.6 ±0.7	11.5 ±0.0	6.8 ±0.6	77
	October	20.5 ±1.0	8.2 ±0.7	13.0 ±0.0	8.0 ±0.5	24
	November	20.9 ±1.3	8.6 ±0.5	13.6 ±0.0	6.2 ±0.8	44
	December	24.7 ±1.1	12.0 ±0.6	14.3 ±0.0	8.2 ±0.8	29
1989	January	25.9 ±1.2	12.5 ±0.7	14.1 ±0.0	9.7 ±0.6	2
	February	25.8 ±1.1	12.1 ±0.6	13.2 ±0.0	10.3 ±0.3	6
	March	25.3 ±1.4	13.6 ±0.8	12.2 ±0.0	5.9 ±0.6	5
	April	20.2 ±0.8	9.1 ±0.9	11.2 ±0.0	6.2 ±0.6	60
	May	16.0 ±0.4	8.6 ±0.6	10.2 ±0.0	4.1 ±0.5	99
	June	11.4 ±0.4	4.7 ±0.7	9.5 ±0.0	2.9 ±0.4	122
	July	11.9 ±0.5	2.5 ±0.8	10.3 ±0.0	3.4 ±0.5	120
	August	11.7 ±0.4	4.6 ±0.6	10.5 ±0.0	4.6 ±0.6	87

Table 3.1 continued.

		Temperature (°C)[1] max	min	Daylength (hours)[2]	Sunshine Hours[3]	Rainfall (mm)[4]
1989	September	15.7 ±0.8	5.9 ±0.6	11.5 ±0.0	7.2 ±0.6	74
	October	18.0 ±0.8	7.1 ±0.7	12.6 ±0.0	6.7 ±0.7	57
	November	21.4 ±1.2	10.4 ±0.5	13.6 ±0.0	7.0 ±0.8	68
	December	25.0 ±1.1	11.7 ±0.6	14.3 ±0.0	9.0 ±0.7	9
1990	January	26.4 ±1.0	12.9 ±0.6	14.1 ±0.0	8.7 ±0.6	10
	February	24.3 ±1.2	12.1 ±0.6	13.2 ±0.0	6.7 ±0.7	23
	March	25.9 ±1.0	11.8 ±0.7	12.2 ±0.0	8.7 ±0.5	2
	April	19.6 ±0.7	8.8 ±0.7	11.1 ±0.0	7.4 ±0.5	35
	May	17.6 ±0.8	6.2 ±0.9	10.2 ±0.0	4.6 ±0.5	14
	June	13.9 ±0.5	4.6 ±0.7	9.5 ±0.0	3.3 ±0.5	117
	July	12.5 ±0.5	4.8 ±0.5	10.0 ±0.0	2.9 ±0.5	182
	August	12.6 ±0.3	5.0 ±0.5	10.5 ±0.0	3.9 ±0.6	94
	September	16.7 ±1.1	6.5 ±0.8	11.5 ±0.0	6.9 ±0.7	70
	October	19.7 ±1.1	8.0 ±0.6	12.6 ±0.0	7.2 ±0.7	49
	November	22.6 ±1.2	8.3 ±0.7	13.6 ±0.0	-	5

[1]Daily maximum and minimum temperatures recorded at Kuitpo Forest Reserve by the South Australian Woods and Forests Department.

[2]Calculated from the times of sunrise and sunset published quarterly in The South Australian Government Gazette.

[3]Recorded at the Waite Agricultural Research Institute, Urrbrae.

[4]Recorded at the experimental site at Blewitt Springs.

3.1.2 Experimental plants

All plants of *B. menziesii* and *B. coccinea* were assessed visually in March 1988 and April 1988 respectively, to select thirty plants of each species which were of similar vegetative size and form. The position of experimental plants within the species block on the plantations is shown in Figures 3.3 and 3.4. Upon selection each plant was numbered from 1 to 30 and all shoots longer than 10cm on *B. coccinea* and 5cm on *B. menziesii* were labelled. This represented approximately 90% of the total number of shoots per bush of *B. coccinea*, with a mean of 108.9 ± 4.3 (standard error), and 95% of the total number per bush of *B. menziesii*, with a mean of 69.7 ± 4.4 (standard error). The labels were made of coloured plastic tape marked with the plant and shoot number for future identification. Those shoots with a single growth flush were tagged at the base of the shoot, while those more than one year old were tagged at the most recent transition between the previous and the latest flush, as recognised by the presence of bud scales. Property owners continued to harvest blooms from plants 11-30 only.

3.2 OTHER EXPERIMENTAL SITES

3.2.1 South Australia

Experimental sites in South Australia, other than the main site at Blewitt Springs, included commercial plantations of seedling plants of *B. coccinea* at Millicent (latitude 37°36'S; longitude 140°21'E) and Mt. Gambier (latitude 37°50'S; longitude 140°47'E) and an untended plantation with seedling plants of both *B. coccinea* and *B. menziesii* at Happy Valley (latitude 35°04'S, longitude 138°34'E).

Meteorological data from the nearest available centre to the experimental sites are presented in Fig. 3.5. The nearest centre for Millicent is Mt. Gambier, and for Happy Valley the Blewitt Springs data is relevant.

Fig. 3.3 The layout of *B. coccinea* block, planted in 1983, with 3.0m between rows and 1.8m spacings within rows. Experimental plants were numbered from 1 to 30. (not to scale)

Fig. 3.4 The layout of *B. menziesii* block, planted in 1983, with 3.4m between rows and 2.3m spacings within rows. Experimental plants were numbered from 1 to 30. (not to scale)

Figure 3.3

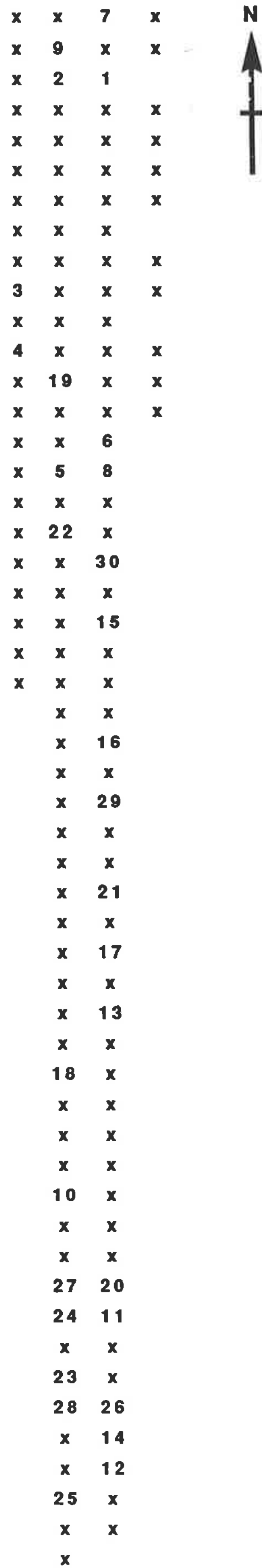
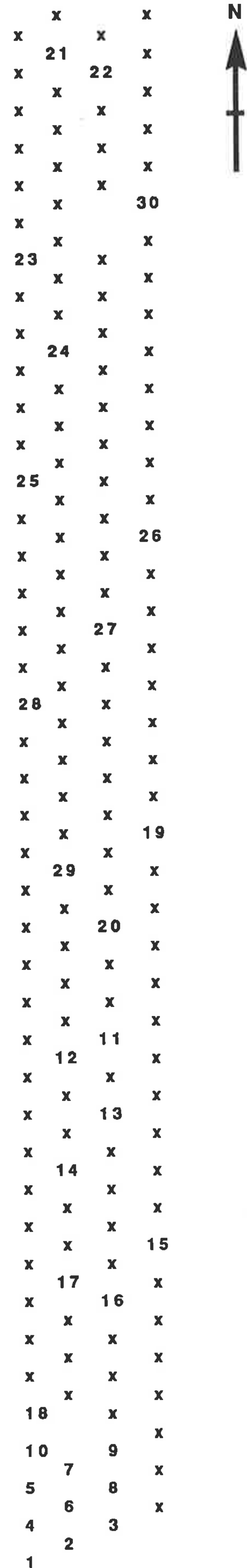


Figure 3.4



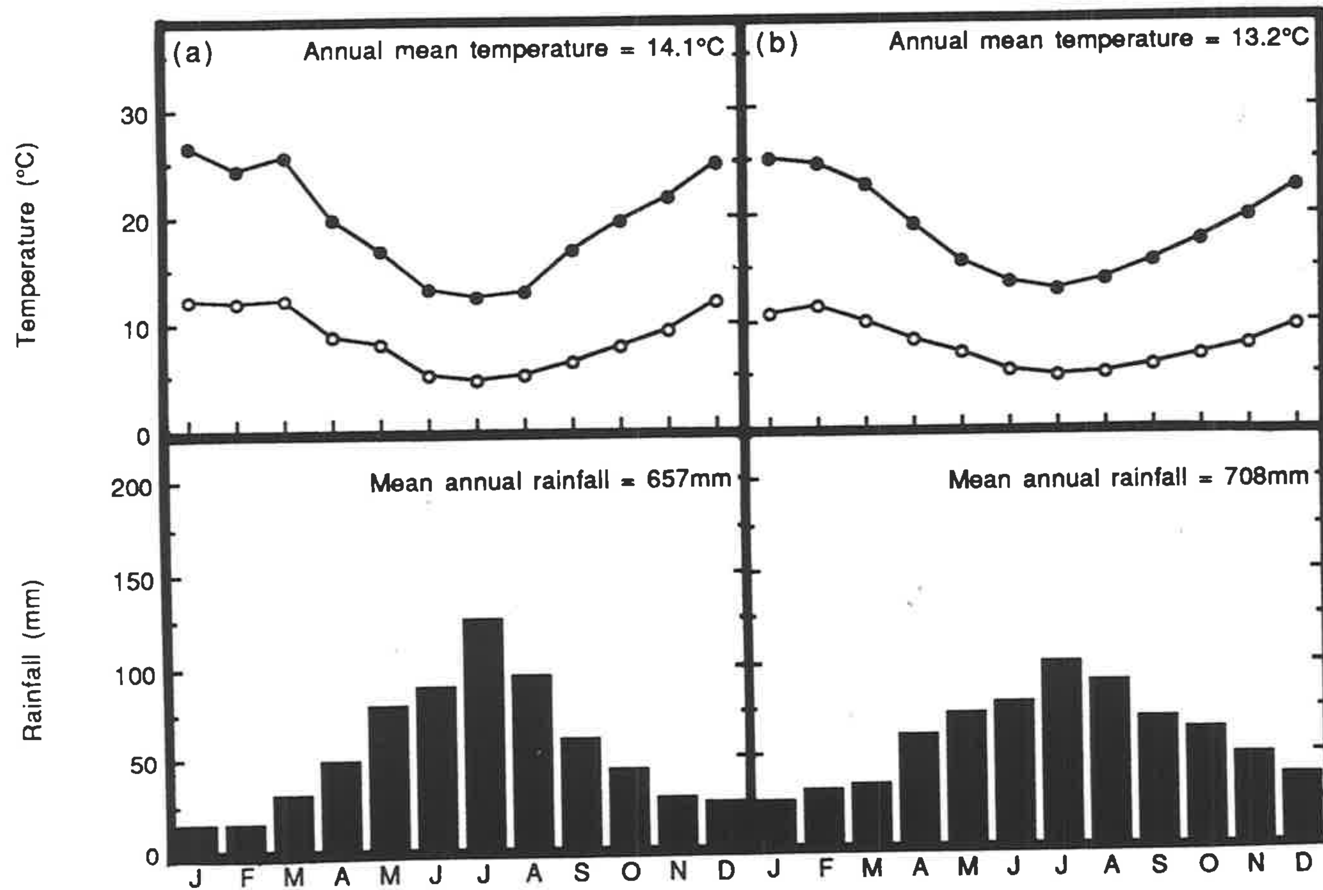


Fig. 3.5 Meteorological data for experimental sites in South Australia. Cumulative mean monthly maximum (●) and minimum (○) temperatures and rainfall for (a) Blewitt Springs and (b) Mt Gambier.

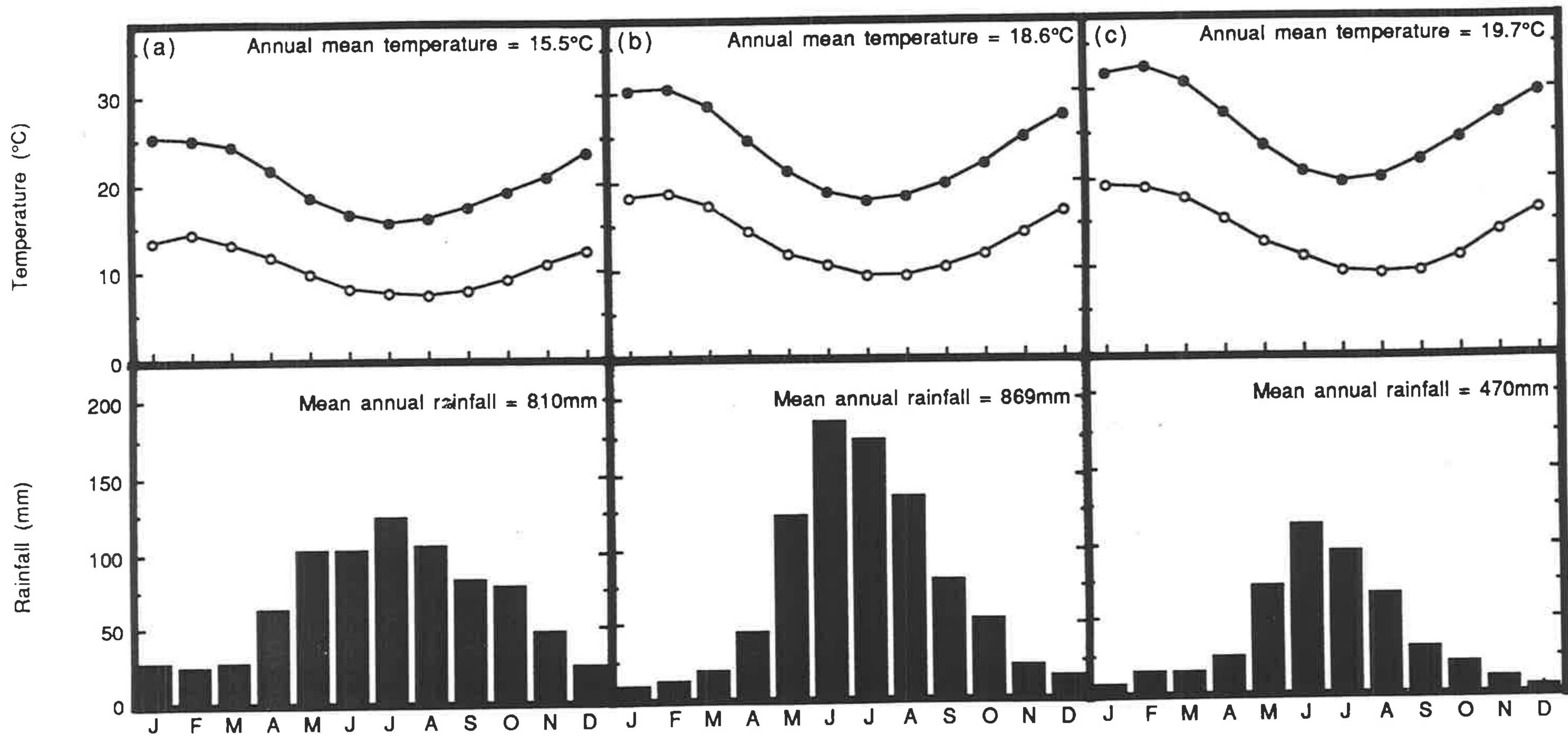


Fig. 3.6 Meteorological data for experimental sites in Western Australia. Cumulative mean monthly maximum (●) and minimum (○) temperatures and rainfall for (a) Albany, (b) Perth, and (c) Geraldton.

3.2.2 Western Australia

B. coccinea plants on three commercial properties in Western Australia, one at Muchea (latitude 31°35'S; longitude 115°58'E) and two near Albany (latitude 35°00'S; longitude 117°52'E) (designated as Albany-1 and Albany-2) were used, as was a natural population at King River (latitude 34°56'S, longitude 117°54'E). Experimental results were collected from wild populations of *B. menziesii* at King's Park and Botanic Garden, Perth (latitude 31°57'S, longitude 115°51'E), 7 kilometres south of Eneabba (latitude 29°49'S, longitude 115°16'E), Lake Indoon (latitude 29°49'S, longitude 115°16'E) and 40 kilometres south of Kalbarri (latitude 27°42'S, longitude 115°51'E).

Meteorological data from the nearest available centre to the experimental sites are presented in Fig. 3.6. The nearest centre for Muchea is Perth, for Eneabba, Lake Indoon and Kalbarri is Geraldton.

3.3 PLANTS FOR EXPERIMENTATION

The experimental plants are detailed in Table 3.2.

Table 3.2 Summary of plants and locations.

Chapter	Species	Experimental site	Number of plants used
4	*B. coccinea*	Blewitt Springs, S.A.	6 (No. 1-6)
	B. menziesii	Blewitt Springs, S.A.	6 (No. 1-6)
5	*B. coccinea*	Blewitt Springs, S.A.	30 (No. 1-30)
	B. menziesii	Blewitt Springs, S.A.	30 (No. 1-30)
6	*B. coccinea*	Albany-1, W.A.	30
		Albany-2, W.A.	15
		Blewitt Springs, S.A.	99 (including No. 1-30)
		Millicent, S.A.	20
		Mt. Gambier, S.A.	35
		Muchea, W.A.	30
	B. menziesii	Blewitt Springs, S.A.	106 (including No. 1-30)
7	*B. menziesii*	Blewitt Springs, S.A.	10 (No. 1-10)
		Eneabba, W.A.	10
		Happy Valley, S.A.	8
		Kalbarri, W.A.	10
		Kings Park, W.A.	10
		Lake Indoon, W.A.	10
8	*B. coccinea*	Blewitt Springs, S.A.	10 (No. 1-10)
		Happy Valley, S.A.	2
		King River, W.A.	10

4. SHOOT GROWTH IN *B. coccinea* AND *B. menziesii*

4.1 INTRODUCTION

To develop management strategies for the successful cultivation of any plant species, it is essential to have an understanding of the growth cycles of the plant. Growth in both *B. coccinea* and *B. menziesii* follows Leeuwenberg's model (Hallé *et al.*, 1978). Shoot extension growth occurs from the terminal bud during each active growth season until an inflorescence is initiated in the terminal position or the terminal bud is damaged. Upon termination of extension growth new lateral shoots arise from axillary buds. This chapter investigates shoot growth of bushes of *B. coccinea* and *B. menziesii* grown for cut flower production, with the aim of determining the relationship between the age and size of shoots and their likelihood of flowering. Such information will be beneficial in developing strategies for plantation management, particularly pruning, yield prediction and selection of superior plants for clonal propagation and use in breeding programmes.

4.2 MATERIALS AND METHODS

4.2.1 Experimental plants

The details of plants used in this chapter are presented in Chapter 3 (Table 3.2).

4.2.2 Measurements

Measurements of shoot growth were made at monthly intervals on all labelled shoots of *B. menziesii*, from March 1988, and on every second shoot of *B. coccinea*, from April 1988, until August 1989. The length of each growth flush was measured, to the nearest 0.5cm, from the point of attachment of the label, at the base of the shoot or at the most recent transition between the previous and the latest flush, to either the base of the terminal bud or the developing inflorescence, or a bud scar. The length of any previous growth flushes (1986 and 1987) was measured at the first recording time only. The basal diameter of each flush was taken 1cm above the base of the flush, at the point of attachment of the label, to the nearest 0.1mm using manual calipers. At the completion of extension growth of the flush in both years, in July for *B. coccinea* and March for *B.*

menziesii, the number of leaves per flush was recorded. In the first year only the length, including lamina and petiole, and width of the fifth leaf were recorded to the nearest 0.1cm. The vegetative/floral status of each shoot and the development of laterals was recorded throughout the experimental period. Flowering shoots were removed following anthesis.

4.2.3 Statistical analysis

Shoots were categorised depending on the number of growth flushes from the axillary origin of that shoot and the development status at the end of each flowering season. The categories of shoots were those which

- produced an inflorescence which bloomed in the first year of growth (1988)
- produced an inflorescence which aborted in the first year of growth (1988)
- produced an inflorescence which bloomed in the second year of growth (1988 or 1989)
- produced an inflorescence which aborted in the second year of growth (1988 or 1989)
- did not initiate an inflorescence within the first two years of growth (1988 or 1989).

The number of shoots in each category as a proportion of the total per tree were analysed as binomial data. This generated a chi-square test for differences between plants in the proportion of shoots per plant for a given shoot category.

Analysis of variance (ANOVA) was conducted to test for differences in shoot length and basal diameter due to shoot category, having first adjusted for a possible plant effect. Similar analyses were used to test for differences in shoot length and basal diameter on comparable growth flushes occurring in different years to investigate the effect of season.

The relationship in both years between shoot length and shoot diameter; number of leaves, and shoot length and shoot diameter; and length and width of the fifth leaf, were subjected to regression analysis, taking plant to plant variability into consideration.

Those shoots which did not produce a bloom within two years growth were excluded from the regression analysis.

The number of shoots producing laterals out of the total number of shoots for each shoot category and each plant was analysed as binomial data. This resulted in a chi-square test to determine if estimates of the probability of a shoot developing laterals were based simply on the total proportion of shoots producing laterals, or were affected by shoot category or plant to plant variability alone or by an additive affect of shoot category and plant to plant variability.

4.3 RESULTS

4.3.1 *B. coccinea*

Although 28% of the shoots flowered in their first year of growth, the majority of inflorescences were initiated on second year wood (Table 4.1). There were significant differences between trees in the proportions of shoots which produced blooms in their first year of growth ($p<0.01$) or in their second year of growth ($p<0.01$). 29% of the labelled shoots did not produce a bloom within two years of growth, and the proportion of non-productive shoots was similar on each of the six bushes sampled. Shoots which produced a bloom in their first year of growth were longer and thicker than those which did not (Table 4.1). After one year's growth there were significant differences in both shoot length ($p<0.01$) and basal shoot diameter ($p<0.01$) between shoots which had flowered in their first year, those which would go on to flower in their second year, and those which after a further year of growth would not produce a bloom. There were similar significant differences in the length ($p<0.01$) and diameter ($p<0.01$) of floral and non-floral shoots in the second year's growth. In any given year, shoots that were floral were longer and thicker than the non-floral shoots.

Active growth of *B. coccinea* shoots commenced in October and resulted in a rapid increase in the basal diameter of the previous year's growth, and extension growth from the terminal bud (Fig. 4.1). After February there was no further increase in shoot length for either floral or non-floral shoots, but basal diameter continued to increase. Both extension growth and shoot diameter were greater in the flowering than in the non-

flowering shoots. Some shoots of *B. coccinea* produced more than one growth flush within a season (Table 4.2). Within any growth season it was possible to distinguish between the different flushes. At the base of the first flush for the season, there was a ring of bud scars around the stem (Fig. 4.2a). Subsequent flushes within that season, were characterised by bud scars separated by extension growth (Fig. 4.2b). There was a tendency for shoots which flushed twice not to flower in that season, with the highest proportions of blooms being produced on shoots after a single growth flush (Table 4.2).

There was a positive relationship between shoot length and basal diameter of shoots which produced a bloom on first or second year wood based on the first year's data (Table 4.3). In the second year the relationship was much weaker. Data from both years showed between plant variation. There was also a strong positive correlation in both years between the number of leaves on a shoot and that shoot's length and basal diameter. There was variation between plants in both years, although the influence of shoot diameter on the number of leaves was less variable between plants in the first year. Plants of *B. coccinea* also acted independently with regard to the relationship between the length and width of the fifth leaf, indicating between plant variation in leaf shape.

The effect of season on shoot growth was considered by comparing retrospective observations of length and diameter of similar flushes on shoots which developed from axillary buds in 1986 and 1987, and flowered in their second year. There was no significant difference in the length of the first flush or the diameter of the second flush. However the second flush of the 1986 shoots was significantly longer than that of the 1987 shoots (35.4 ± 2.4cm and 28.5 ± 1.0cm respectively, $p<0.01$).

In both 1988 and 1989 there was an additive effect of plant and shoot category on the development of laterals on *B. coccinea* shoots (Table 4.4). Shoots which did not produce an inflorescence did not produce laterals either.

Table 4.1 Number of shoots per tree and the length and diameter of each flush in the first two years of growth from an axillary bud for *B. coccinea* and *B. menziesii*. (mean ± standard error). NB. Only every second shoot per bush was recorded for *B. coccinea*, but the sample was representative of the whole bush.

Description of shoot category	Number of shoots per tree	Proportion of total (%)	Length of growth in the xth year (cm)		Basal diameter of growth in the xth year measured in year y (mm)		
			x=1	x=2	x=1 in y=1	x=1 in y=2	x=2 in y=2
Banksia coccinea							
Produced an inflorescence in first year	14.3 ± 4.0	28	33.9 ± 1.7	-	6.8 ± 0.2	-	-
Produced an inflorescence in second year	21.3 ± 3.6	42	20.2 ± 1.0	30.7 ± 1.0	4.4 ± 0.2	9.1 ± 0.2	8.2 ± 0.2
Did not produce an inflorescence after 2 years	14.7 ± 5.0	29	17.0 ± 1.0	26.5 ± 2.0	4.2 ± 0.2	7.0 ± 0.2	5.8 ± 0.2
Banksia menziesii							
Produced an inflorescence in first year	6.0 ± 2.3	9	40.0 ± 2.5	-	8.8 ± 0.3	-	-
Produced an inflorescence which aborted in first year	6.0 ± 1.3	9	17.8 ± 2.2	-	4.7 ± 0.3	-	-
Produced an inflorescence in second year	31.5 ± 5.5	45	26.8 ± 0.8	20.9 ± 0.5	6.0 ± 0.1	12.6 ± 0.3	9.7 ± 0.1
Produced an inflorescence which aborted in second year	12.5 ± 1.2	18	19.0 ± 1.0	15.8 ± 0.9	4.5 ± 0.2	7.9 ± 0.3	6.6 ± 0.2
Did not produce an inflorescence after 2 years	13.7 ± 3.3	20	13.5 ± 0.7	14.7 ± 1.1	4.0 ± 0.1	6.4 ± 0.3	5.0 ± 0.2

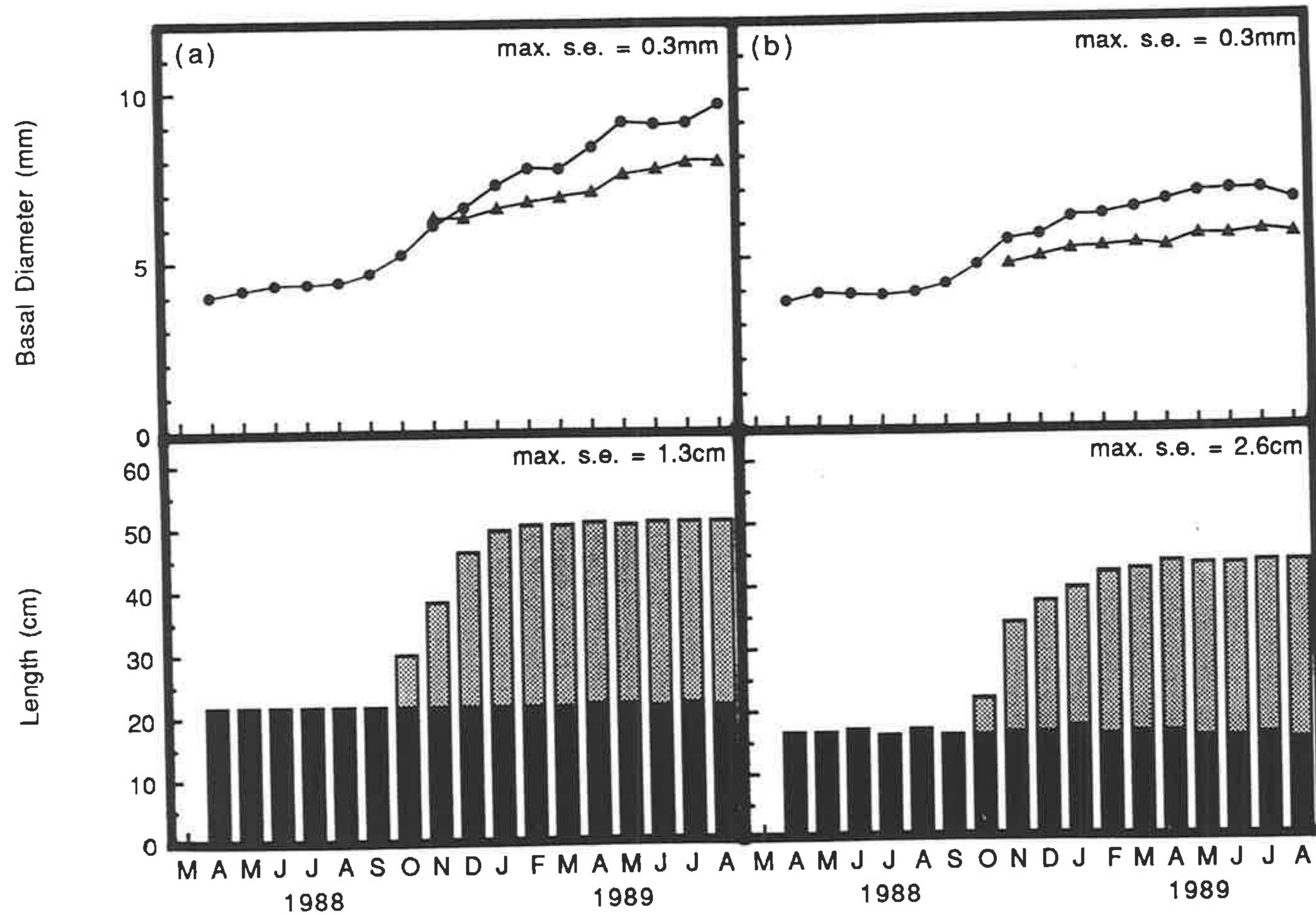


Fig. 4.1 Changes in basal shoot diameter and extension growth for *B. coccinea* shoots which (a) flowered in their second year of growth and (b) did not flower after two years of growth, from monthly observations from April 1988 to August 1989. Basal diameter and length of extension growth of first year's growth (●, ■) and second year's growth (▲, ▩).

Table 4.2 Number of axillary shoots of *B. coccinea* for one season's growth at commencement of the experiment, and a breakdown of the types of growth exhibited. (*, inflorescence; o, resting terminal bud produced at the end of an active growth season; >, semi-resting terminal bud produced during an active growth season).

Number of shoots and type of growth flush in the xth year x=1	x=2	Percentage of total number of shoots
60 ——— *		27%
109 ——— o	62 ——— *	28%
	34 ——— o	15%
	5 —— > —— *	2%
	8 —— > —— o	4%
26 —— > —— *		12%
28 —— > —— o	16 ——— *	7%
	5 ——— o	2%
	2 —— > —— *	1%
	5 —— > —— o	2%

Fig. 4.2 *B. coccinea* shoots which flushed (a) once in a given season showing a ring of bud scars (arrow) at the base of the flush, and (b) twice in a given season showing bud scars separated by extension growth at the base of the second flush (arrow) resulting in a region of stem devoid of leaves (]). Scale bar: 5cm.

2 a

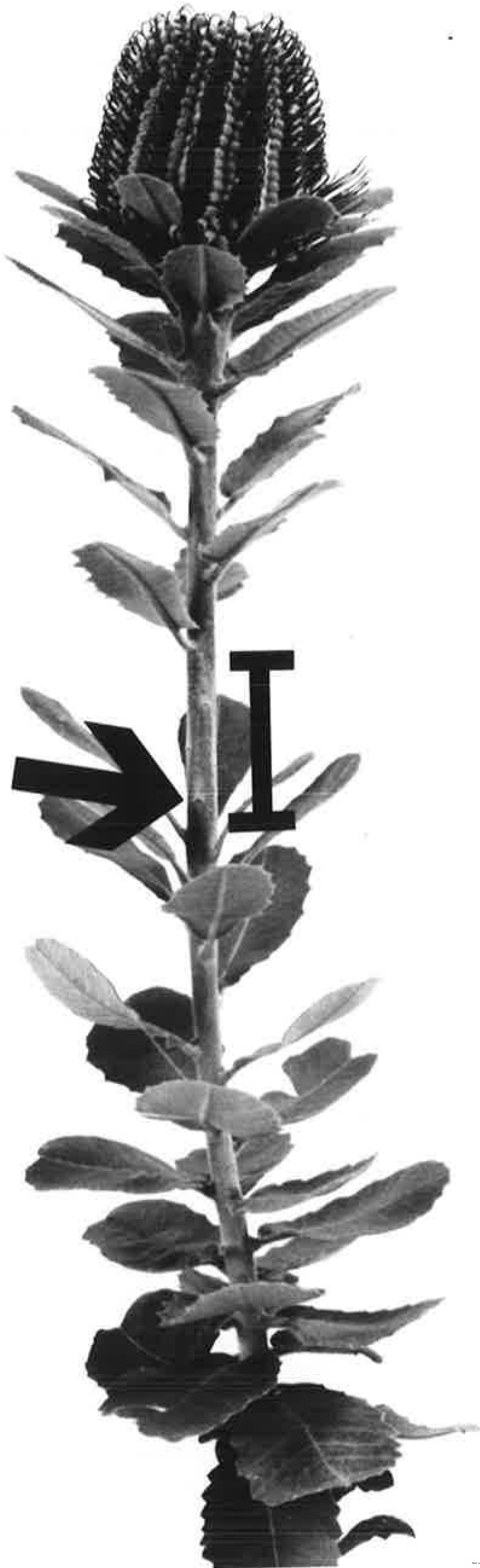

b

Table 4.3 Relationship between vegetative characters of shoots of *B. coccinea* and *B. menziesii*, excluding those shoots which did not produce an inflorescence in two years of growth.

	Characters		Year	R^2
B. coccinea				
shoot length	basal diameter		1988	0.75
			1989	0.48
shoot length	basal diameter	number of leaves	1988	0.82
			1989	0.83
leaf length	leaf width		1988	0.55
B. menziesii				
shoot length	basal diameter		1988	0.48
			1989	0.37
shoot length	basal diameter	number of leaves	1988	0.87
			1989	0.87
leaf length	leaf width		1988	0.81

Table 4.4 Estimates of the probability of a shoot producing laterals for each of the six *B. coccinea* plants in 1988 and 1989, based on an additive effect of plant and shoot category. Those shoots which did not produce an inflorescence in 1988 were followed through to 1989 as a) and b).

Description of shoot category		Probability of a shoot producing laterals on plant number: 1	2	3	4	5	6
1988							
Produced an inflorescence in second year of growth		0.35	0.63	0.90	0.29	0.90	0.83
Produced an inflorescence in first year of growth		0.02	0.07	0.28	0.02	0.28	0.17
Did not produce an inflorescence in first year of growth,	a)	0.00	0.00	0.00	0.00	0.00	0.00
	b)	0.00	0.00	0.00	0.00	0.00	0.00
1989							
a) Produced an inflorescence in second year of growth		0.24	0.70	0.23	0.20	0.89	0.54
b) Did not produce an inflorescence within two years		0.00	0.00	0.00	0.00	0.00	0.00

4.3.2 *B. menziesii*

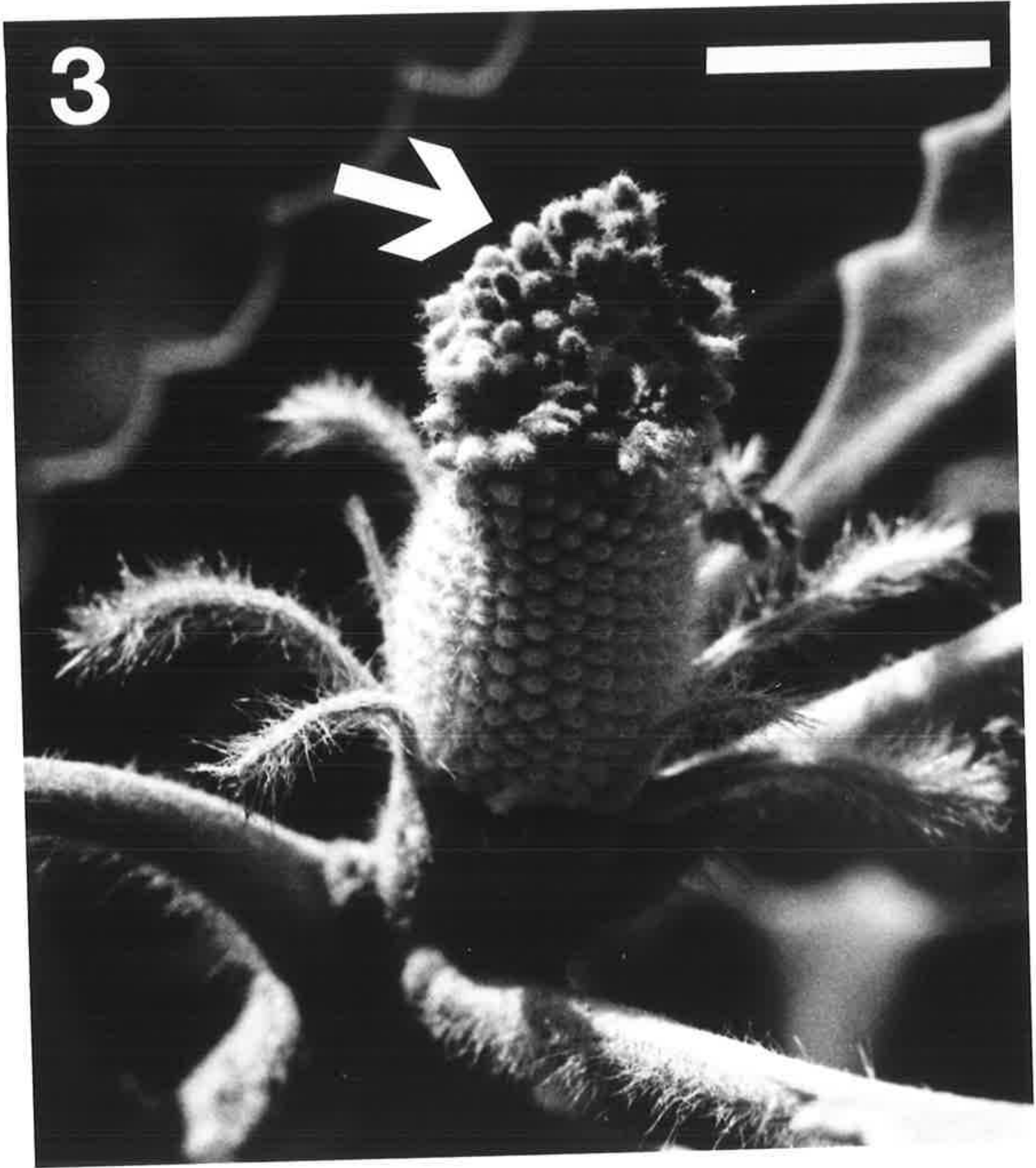
A proportion of the inflorescences initiated on both first and second year wood aborted in the early stages of development (Table 4.1). Aborted blooms were recognised by the shedding of the common bracts from the central woody core of the developing inflorescence (Fig. 4.3). Abortion at earlier stages also occurred but was not visible until later in the flowering season when the involucral bracts were shed to reveal a small woody spike at the tip of the shoot, the axis of the aborted inflorescence.

The majority of shoots of *B. menziesii* (Table 4.1) initiated blooms in their second year of growth, with no significant difference between plants in the proportion of shoots which initiated inflorescences. There was, however, a significant difference between trees in the initiation of blooms on first year wood irrespective of whether they developed through to anthesis ($p<0.5$) or aborted during development ($p<0.01$). Two trees in particular contributed to this difference between trees, having a much higher proportion of shoots in these categories than the other four trees. There was a relationship between the likelihood of a shoot flowering and its basal diameter and shoot length. There were significant differences in both shoot length ($p<0.01$) and diameter ($p<0.01$) between shoot categories after the first year's growth. Those shoots which produced a bloom were considerably thicker than those which set a vegetative terminal bud or initiated an inflorescence which subsequently aborted. Similar differences were observed with the second year's growth.

Extension growth from terminal buds of *B. menziesii* occurred from October to January, but growth ceased in December on shoots that did not produce a bloom in their second year (Fig. 4.4). The increase in shoot diameter was greatest in flowering shoots and parallelled the increase in extension growth. Those shoots which initiated inflorescences that subsequently aborted were intermediate between the floral and the non-floral shoots for both length of extension growth and basal diameter.

In *B. menziesii* there was a poor correlation between shoot length and basal diameter in both years of the study (Table 4.3). There was a strong relationship between the number of leaves on a flush and the length and diameter of the flush in both years.

Fig. 4.3 Inflorescence of *B. menziesii* aborted in the early stages of development showing the shedding of common bracts from the central core of the inflorescence (arrow). Scale bar: 1cm.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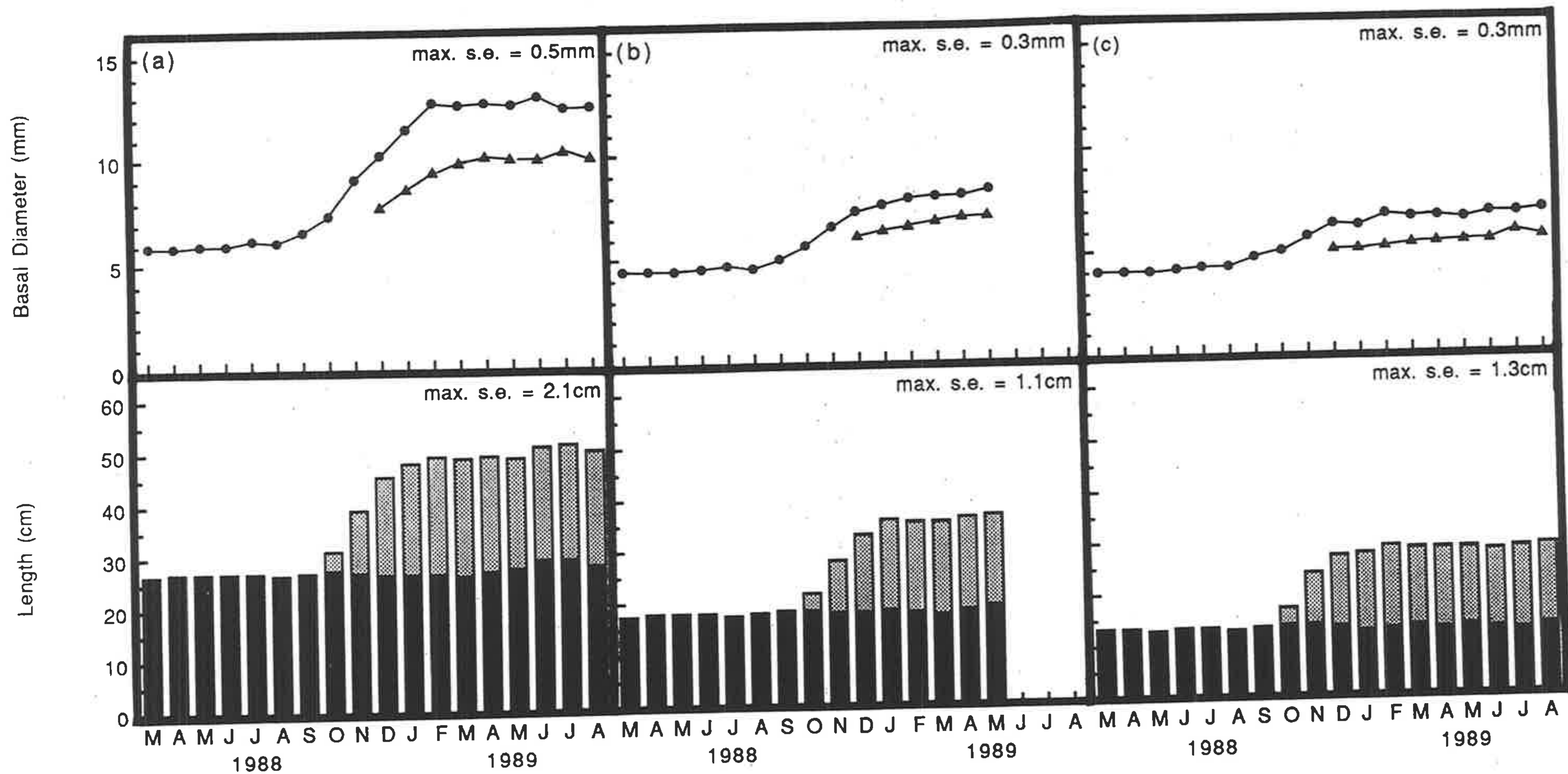

Fig. 4.4 Changes in basal shoot diameter and extension growth for *B. menziesii* shoots which (a) flowered in their second year of growth, (b) initiated an inflorescence in their second year of growth which subsequently aborted in the early stages of development and (c) did not flower after two years of growth, from monthly observations from March 1988 to August 1989. Measurement of shoots with aborted inflorescences ceased in May 1989. Basal diameter and length of extension growth of first year's growth (●, ■) and second year's growth (▲, ▨).

Table 4.5 Estimates of the probability of a shoot producing laterals for each of the six *B. menziesii* plants in 1988, based on shoot category only (with no plant to plant variation), and in 1989, based on the proportion of shoots producing laterals. Those shoots which did not produce an inflorescence in 1988 were followed through to 1989 as a), b) and c).

Description of shoot category		Probability of producing laterals
1988		
Produced an inflorescence in second year of growth		0.32
Produced an inflorescence which aborted in second year of growth		*
Produced an inflorescence in first year of growth		0.06
Produced an inflorescence which aborted in first year of growth		0.06
Did not produce an inflorescence in first year of growth,	a)	0.00
	b)	0.00
	c)	0.00

	Probability of a shoot producing laterals on plant number:					
	1	2	3	4	5	6
1989						
a) Produced an inflorescence in second year of growth	0.41	0.50	0.60	0.95	0.40	1.00
b) Produced an inflorescence which aborted in second year of growth	0.25	0.11	0.00	0.27	0.00	0.60
c) Did not produce an inflorescence within two years	0.14	0.00	0.50	0.00	0.00	0.00

* Too few observations to be included in analysis.

Similarly the relationship between the length and the width of the fifth leaf was strong, with each of the six plants acting independently.

There were no significant differences in length or diameter of comparable flushes produced in different seasons for those shoots which produced inflorescences on second year wood.

For *B. menziesii* there were differences in the probability of a shoot producing laterals between shoot categories in 1988 but no between plant variation as there was in *B. coccinea* (Table 4.5). In 1989 there was variability between plants as well as between shoot categories in the production of laterals (Table 4.5).

4.4 CONCLUSIONS

The propensity of a shoot to flower has been shown to be influenced by both the age of the shoot and its size. Shoots with a minimum basal diameter of 4.5mm for *B. coccinea* and 6mm for *B. menziesii* have the highest probability of producing an inflorescence. In both species the majority of blooms were initiated on shoots in their second year of growth, although some shoots produced an inflorescence in the first year. Shoots of *B. menziesii* which flowered in their first year were extremely vigorous and attained a length in one year comparable to two years' growth of those flowering in the second year. However, *B. coccinea* blooms which formed on first year wood had very short stems, while shoots flowering in their second year attained a much longer total stem length. These criteria can be used by growers to assess which shoots to retain and which to remove during pruning.

In addition there was a high degree of between plant variation in the relationships between physical shoot parameters, such as probability of flowering, shoot length, shoot diameter, number of leaves and lateral shoot development. This variation has important implications for crop improvement through breeding and selection programmes.

Abortion of inflorescences in the early stages of development was a consistent feature of *B. menziesii*, and represents a significant reduction in potential returns to the grower. The reason for this is not clear, but may relate to differences in environmental conditions between South Australia and the native habitat in Western Australia. The

annual mean temperature at Blewitt Springs is 14.1°C, which is less than that over the natural range of *B. menziesii* from Perth, W.A. (18.7°C) to Geraldton, W.A. (19.7°C). Some plant to plant variation was observed for this character so it may be possible to select cultivars more suited for the South Australian environment.

The double flushing observed in some of the shoots of *B. coccinea* is similar to that which occurs in many tropical woody plant species, including avocado (Scholefield *et al.*, 1985) and mango (Scholefield *et al.*, 1986). This flushing pattern has not previously been reported for any *Banksia* species. However, in the context of producing a high quality cut flower it is undesirable, as an unattractive region of bare stem occurs at the base of the second growth flush. Leaf size, shape and density on a shoot were highly variable in both species. These may become important selection criteria as the market for banksia blooms becomes more sophisticated and leaf characters assume greater importance.

The development of lateral shoots also needs consideration. In both species shoots tend to develop laterals when terminal growth ceases, due to either initiation of an inflorescence or to damage to the terminal bud. This development of lateral shoots around the base of the inflorescence interferes with the floral display and reduces the appeal of the bloom (Fig. 4.5). In *B. coccinea* lateral shoots grow at an acute angle to the stem on which the inflorescence is developing. As the inflorescence increases in width during development, the rows of florets are forced to part around adjacent laterals causing permanent distortion of the symmetry of the inflorescence. Laterals on *B. menziesii* tend to grow out at a much wider angle and therefore do not detract from the bloom to the same extent. However, they do interfere with floral appearance and must be removed when the blooms are graded after harvest. It is possible that removal of laterals may reduce the vase life of the bloom as occurs in other Proteaceous species, due to the production of ethylene from the wounds (Harre, 1988). Therefore selection of plants with a low probability of lateral shoots developing around an inflorescence is desirable for the production of standard cut flowers.

Alternatively, a specialist market could be developed for spray blooms by selecting for the development of flowering lateral shoots. Since laterals tend to arise

when growth from the terminal bud ceases, the removal of the terminal bud soon after shoot elongation would result in the development of several lateral shoots around the shoot apex, all of similar length and morphology. Such shoots will often flower synchroncusly, to give a multiple headed stem (Fig. 4.6). These "multiheads" could attract high prices in the market place. The fact that second year shoots have a higher probability of producing laterals may entail the sacrifice of early production, but the tree to tree variability in the production of lateral shoots may compensate via careful breeding and selection.

Fig. 4.5 The development of lateral shoots (arrows) around the base of a *B. coccinea* inflorescence, causing distortion to the symmetry of the inflorescence. Scale bar: 5cm.

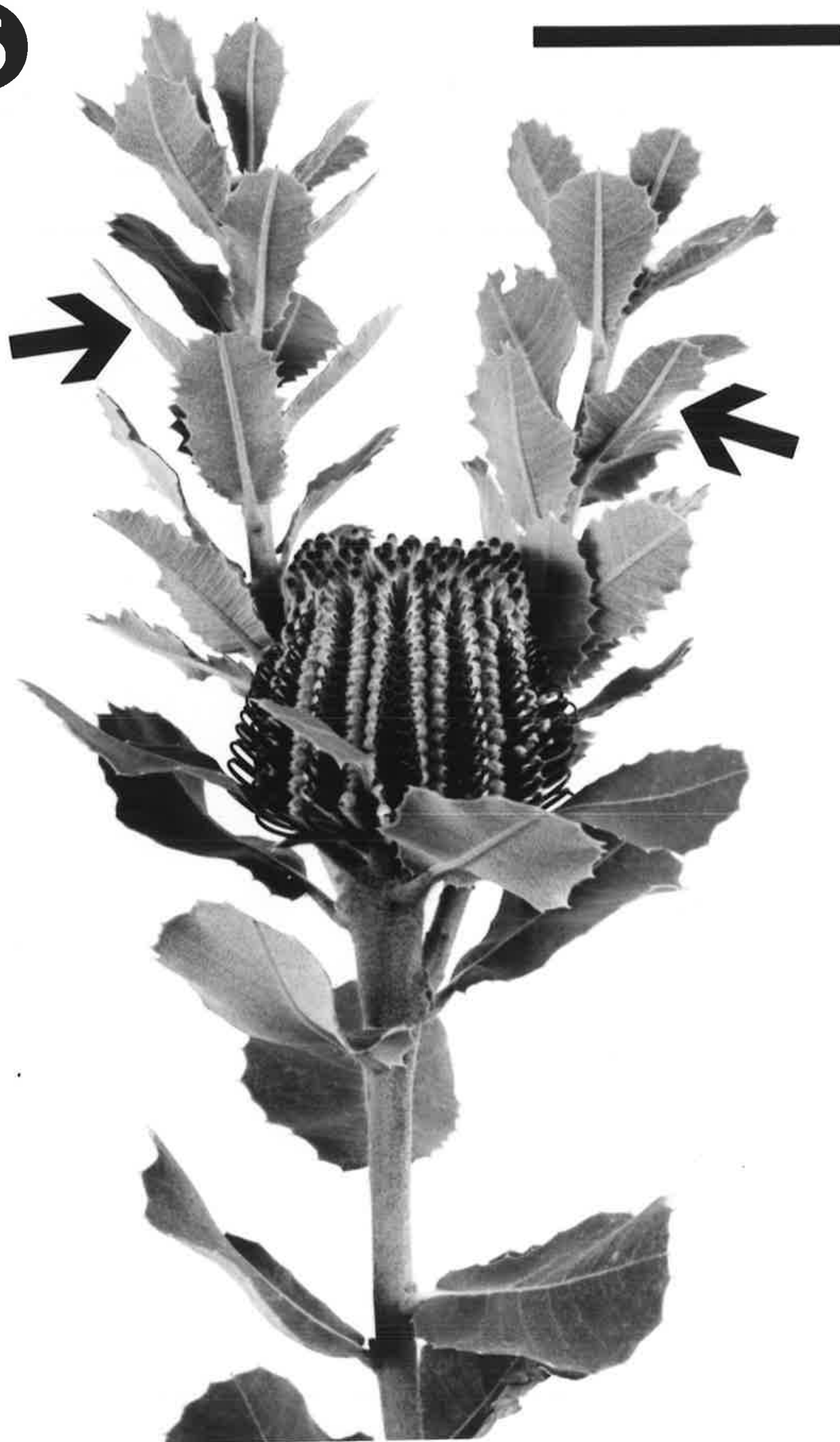
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Fig. 4.6 A multiple headed stem of *B. coccinea*, created by the development of lateral shoots which flower synchronously (arrows). Scale bar: 5cm.

6

5. FLORAL INITIATION AND DEVELOPMENT IN *B. coccinea* AND *B. menziesii*

5.1 INTRODUCTION

To achieve maximum productivity, it is important to gain an understanding of the time of floral initiation and development for appropriate scheduling of management practices, such as pruning and irrigating. There have been no microscopic studies of the transition of the apical meristem from the vegetative state to the reproductive state in any *Banksia* species. This chapter investigates this transition in *B. coccinea* and *B. menziesii* using scanning electron microscopy. The timing of subsequent floral development in these species is also studied.

5.2 MATERIALS AND METHODS

5.2.1 Experimental plants

The details of plants used in this chapter are presented in Chapter 3 (Table 3.2).

5.2.2 Sampling

Shoots were harvested from plants numbered 7 to 30 on the 12th (±1) day of each calender month for *B.menziesii* and the 21st (±1) day for *B. coccinea* from March and April 1988, respectively, until January 1990. A different plant was chosen at each sampling time, and every fourth shoot of *B. menziesii* and every sixth shoot of *B. coccinea* was harvested, excluding shoots which were obviously reproductive. This gave between four and twenty-four shoots per sample, depending on the total number of shoots per plant. The shoots were placed in a plastic bag and returned promptly to the laboratory, where they were transferred to water.

The terminal apices were dissected from the harvested shoots within 24 hours with the aid of a stereomicroscope, by removing leaves and bracts in order to facilitate viewing of the apex. The dissected apices were immediately fixed in FPA50 (5 parts formalin, 5 parts propionic acid, 90 parts 50% ethanol, 0.5% caffeine) for a minimum of 5 days, before transferring to 70% ethanol for storage. The tissue was dehydrated in a

graded ethanol series (70%, 100%, 100%), critical-point dried and mounted on stubs. Each sample was coated with gold to a thickness of 44 nm and examined with a Cambridge Stereoscan (250 MK3) scanning electron microscope operated at 20 kV. Some material was also observed fresh and uncoated, to minimise disruption of pollen during processing, and was observed at 5kV.

5.2.3 Measurements

Stages of apical development were recorded and dimensions of the apical meristem were measured for least two samples from each developmental stage. The diameter and height of each apex was measured. In the case of vegetative meristems the diameter was measured from the axil of the most recent primordium through the centre of the apex to a line joining the next two most recent primordia (Fig. 5.1). The height was taken along a line perpendicular to that of the diameter and passing through the summit of the apex (Fig. 5.2). For reproductive primordia, the diameter was measured at the base of the developing inflorescence, and the height from the first common bract of the immature inflorescence to the summit.

The macroscopic stages (visible to the eye) of inflorescence development were recorded at monthly intervals on all reproductive shoots of *B. coccinea* during the 1988 flowering season and during the 1989 season for *B. menziesii* on plants numbered 1 to 6. Measurements of inflorescence size were made on at least three blooms for each developmental stage. The diameter was measured at the widest point, including florets and extended styles where present, and the height was taken along the central axis of the flower spike from the first common bracts to the summit.

The phyllotaxy of the vegetative and floral shoots was determined from photomicrographs and from shoot and inflorescence material. The angle of divergence of vegetative material was calculated by $\frac{m}{n} \times 360°$ where m is the number of turns of the genetic spiral between leaves on the same orthostichy, separated by n internodes (Dormer, 1972). This method was also used to calculate the angular divergence within mature inflorescences, substituting common bracts for leaves.

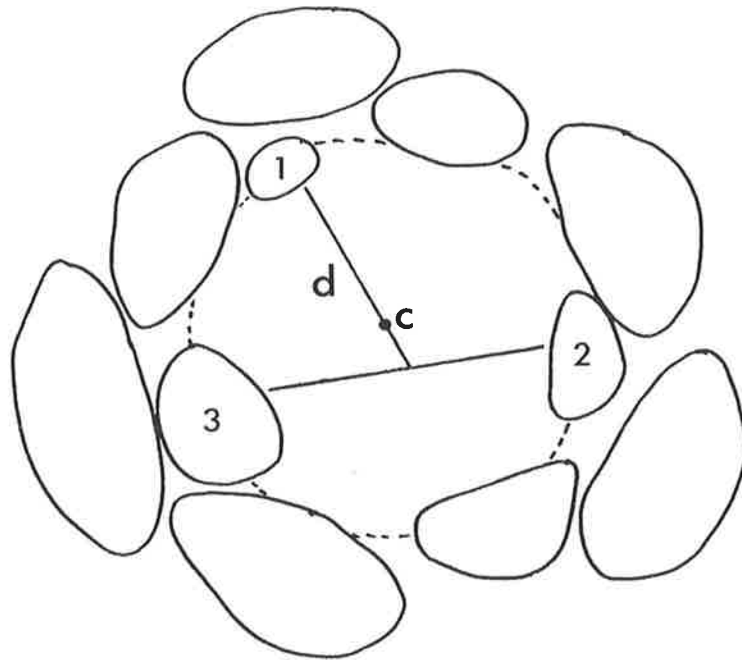


Fig. 5.1 Measurement of vegetative meristem diameter. The diameter (d) measured from the axil of the most recent primordia (1) through the centre of the apex (c) to the intersection with a line joining the two next older primordia (2 and 3).

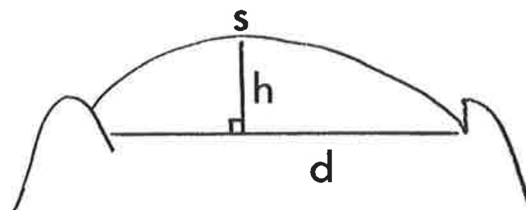


Fig. 5.2 Measurement of vegetative meristem height. The height (h) measured along the line perpendicular to that of the diameter (d) and passing through the summit of the apex (s).

5.3 RESULTS

The sequence of floral initiation and development was similar for the two species. Vegetative apices produced leaf primordia in a spiral sequence (Fig. 5.3). Hairs developed on the leaf primordia, and persisted until the leaves were fully expanded. The first evidence of reproductive development of the apex was indicated by a broadening of the apex and the initiation of the involucral bract primordia (Fig. 5.4). These were also produced in a spiral sequence and developed hairs at an early stage. This was followed by doming of the meristem and acropetal initiation of common bracts (Figs 5.5 and 5.6). These were arranged in 13 genetic spirals (Fig. 5.5) such that they appeared to form vertical rows on the long axis of the inflorescence, and like the leaf and involucral bract primordia rapidly became covered in hairs. The developing inflorescence was enclosed within hairy involucral bracts (Fig. 5.6). Subsequent stages of inflorescence development were also acropetal. Primordia were initiated in the axil of each common bract (Fig. 5.7), and developed into two floral bract primordia (Fig. 5.8). The individual floret primordia were initiated in the axils of the floral bracts (Fig. 5.9). They produced four perianth parts (Fig. 5.10) on which a mass of hairs developed, obscuring the view of subsequent development of floral parts.

At first macroscopic appearance of the inflorescence (Fig. 5.11) only the common bracts were visible above the hairy involucral bracts. Following elongation of the central core of the inflorescence, floral bracts were observed in *B. menziesii*, but were never visible in the macroscopic stages of development of *B. coccinea* due to the presence of hairs. Paired florets of *B. menziesii* emerged in rows from between the organised array of bracts (Fig. 5.12), each floret subtended by its floral bract, with the pair of florets and their floral bracts subtended by a common bract. Elongation of the florets (Fig. 5.13), resulted from an increase in length of the perianth tube and the style enclosed within it. However perianth elongation ceased prior to that of style elongation, thus causing the flexing style to split the perianth tube and protrude. The splitting of the tube always occurred along the suture in the perianth nearest the other floret in the pair. Perianth limbs of paired florets were thus forced apart (Figs 5.14 and 5.15) by the

arching of the styles. In *B. coccinea* the styles extended and arched beyond the limits of the perianth (Fig. 5.15). When styles of all florets on the inflorescence had extended and split the perianth, anthesis commenced with those florets at the base of the inflorescence and continued acropetally (Figs 5.14 and 5.15). The modified upper style, the pollen presenter, with the stigma at its tip was released from the perianth limb at anthesis (Fig. 5.16).

A single bilobed anther was attached by a short filament to the distal region of each of the four perianth parts (Fig. 5.17). Anther dehiscence occurred prior to anthesis, depositing the pollen onto the pollen presenter region of the style (Figs 5.18 and 5.19), but not in the region of the stigmatic groove (Fig. 5.18). In the field, the pollen was removed from the pollen presenter by foraging insects and birds within a day of anthesis (Fig. 5.20) The smooth surfaced pollen presenter of *B. coccinea* was more or less conical, while that of *B. menziesii* was cylindrical and ridged (Fig.5.20). In both species the stigmatic groove was apically located and extended a short distance longitudinally down one side of the pollen presenter (Fig. 5.20). The eight depressions between the ridges on the pollen presenter of *B. menziesii* corresponded to the positioning of the anther lobes.

The pollen was characteristic for each of the species (Figs 5.21 and 5.22). Both had two germination apertures. The porate grains of *B. coccinea* were longer ($83.0 \pm 1.4\mu m$) but narrower ($32.0 \pm 0.5\mu m$) than the colporate grains of *B. menziesii* ($57.8 \pm 1.0\mu m$; $40.8 \pm 0.8\mu m$). The surface of the *B. menziesii* grains was lightly patterned, while the exine of *B. coccinea* grains was more sculptured. Both species had orbicules associated with the pollen upon release from the anther, and these were larger in *B. coccinea* than in *B. menziesii*. The structure was similar in both the dehydrated and hydrated state.

Figures 5.3-5.10. Scanning electron micrographs of fixed material.

Fig. 5.3 *B. coccinea* vegetative meristem at stage 1, showing leaf primordia (l) and developing hairs (h). Scale bar: 100µm.

Fig. 5.4 *B. menziesii* apical meristem at stage 2, showing initiation of involucral bracts (ib). Scale bar: 100µm.

Fig. 5.5 *B. menziesii* apical meristem at stage 3, showing acropetal initiation of common bracts (cb) and developing hairs (h). Some common bracts have been removed to aid observation. Scale bar: 400µm.

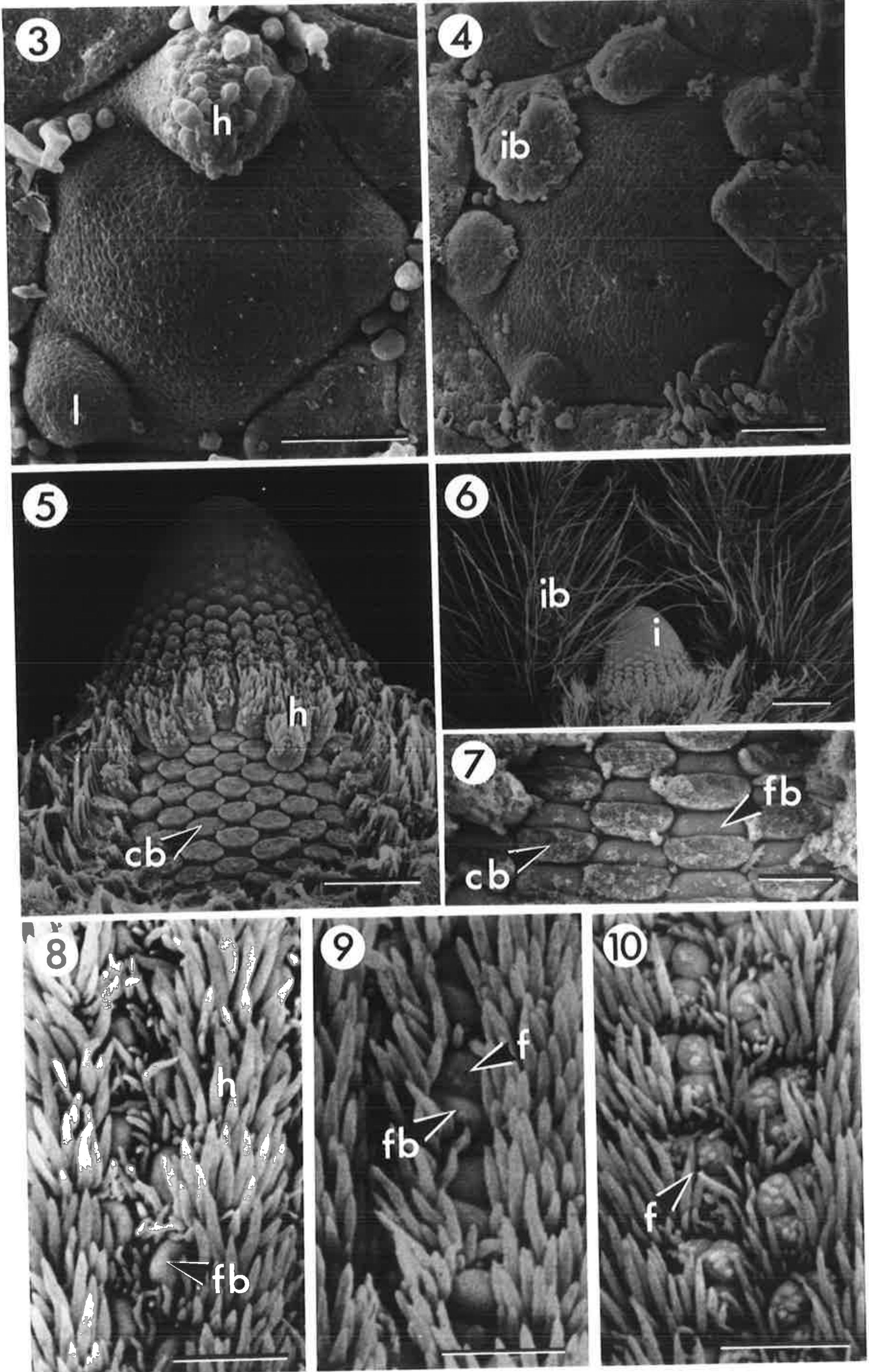
Fig. 5.6 *B. menziesii* apical meristem at stage 3, showing hairy involucral bracts (ib) enclosing developing inflorescence primordium (i). Scale bar: 800µm.

Fig. 5.7 *B. menziesii* apical meristem at stage 4, showing initiation of floral bract primordia (fb) in the axils of the common bracts (cb). The common bracts have been removed to aid observation. Scale bar: 200µm.

Fig. 5.8 *B. coccinea* apical meristem at stage 4, showing developing floral bract primordia (fb) obscured by hairs (h) on common bracts. Scale bar: 200µm.

Fig. 5.9 *B. coccinea* apical meristem at stage 5, showing initiation of floret (f) in the axil of the floral bract (fb). Scale bar: 200µm.

Fig. 5.10 *B. coccinea* apical meristem at stage 5, showing initiation of the four perianth members of the florets (f). Scale bar: 300µm.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ib
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f

Fig. 5.11 Macroscopic appearance of inflorescence of *B. menziesii* (stage 6), showing involucral bracts (ib) and common bracts (cb). Scale bar: 10mm.

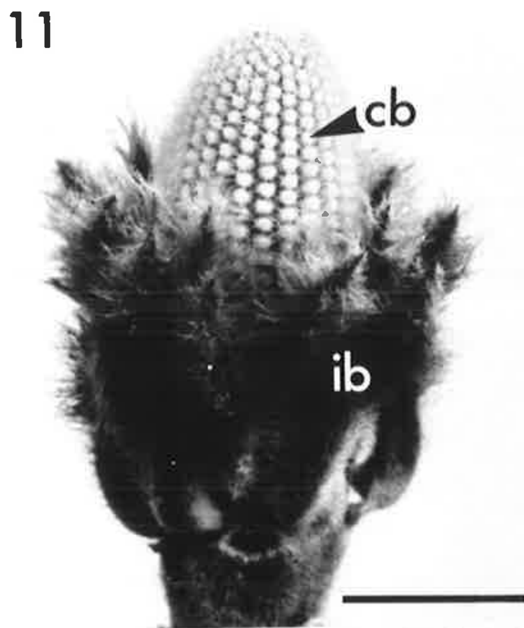
Fig. 5.12 Macroscopic appearance of floret pairs (f) of *B. menziesii* (stage 7), showing common bracts (cb) and floral bracts (fb). Scale bar: 5mm.

Fig. 5.13 Extension of florets (f) in pairs (stage 8) of *B. menziesii*. Scale bar: 10mm.

Fig. 5.14 *B. menziesii* inflorescence at anthesis, showing florets at anthesis (an) (stage 10) and florets at stage 9, style extension (se). Note that the extension of the styles has pushed apart the florets of the floret pairs so that the apparent rows consist of florets from two different common bracts. Scale bar: 20mm.

Fig. 5.15 *B. coccinea* inflorescence at anthesis, showing florets at anthesis (an) (stage 10) and florets at stage 9, style extension (se). Note that the styles extended beyond the perianth in this species, and that extension of the styles has pushed apart the florets of the floret pairs so that the apparent rows consist of florets from two different common bracts. Scale bar: 20mm.

Fig. 5.16 *B. menziesii* florets at stage 9, style extension (left) and at stage 10, anthesis (right), showing perianth limb (pl), style (s) ovary (o), tepals (t), and pollen presenter (pp). Scale bar: 10mm.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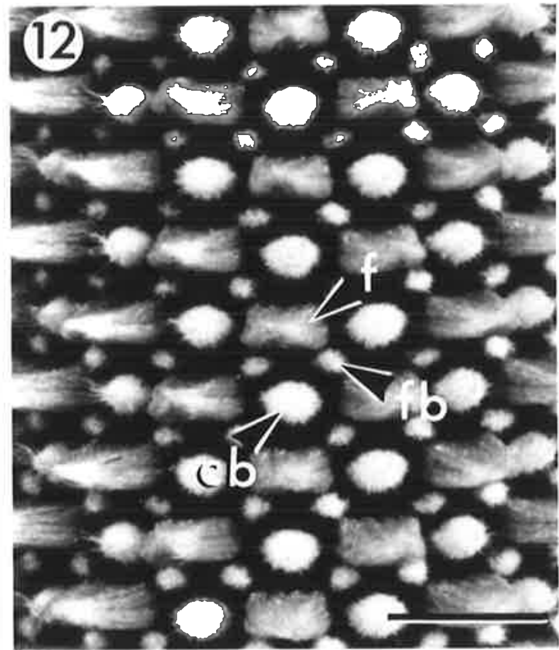
cb
ib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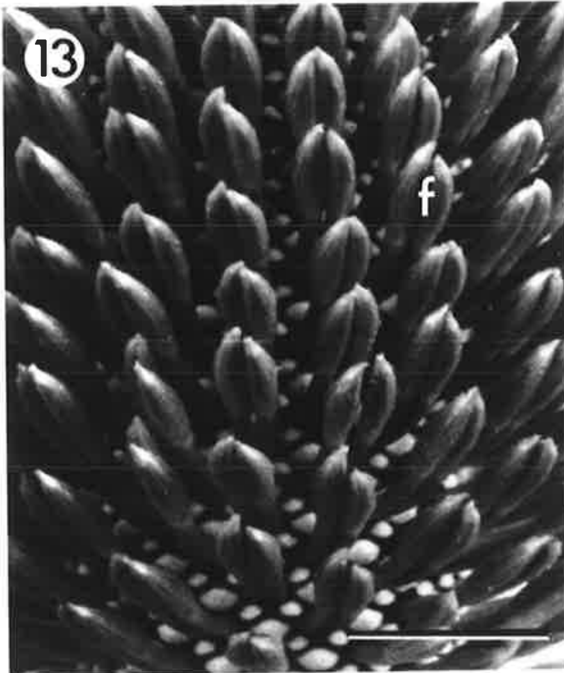
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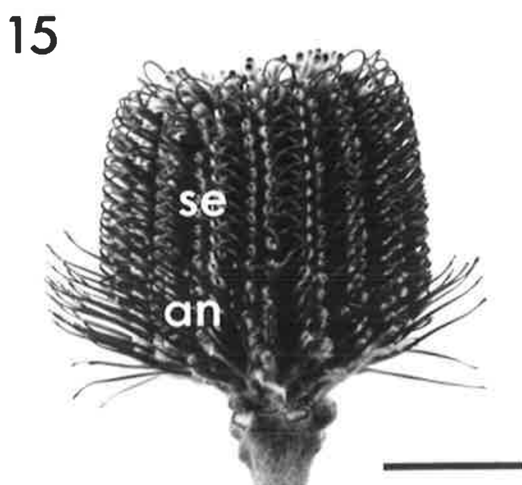
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se
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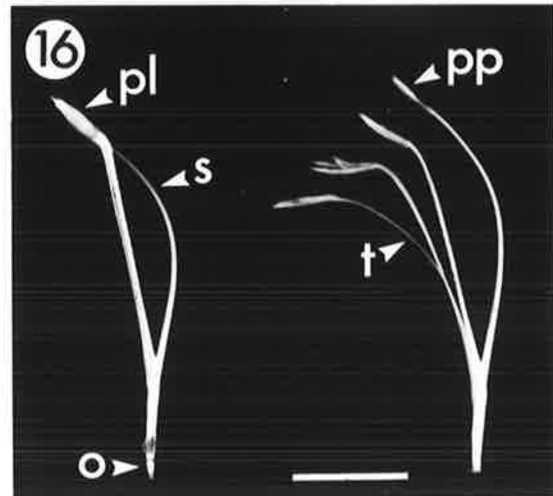
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Figures 5.17-5.22 Scanning electron micrographs of fixed material except where stated.

Fig. 5.17 Distal region of *B. coccinea* tepal at anthesis with dehisced anther (a) on short filament (fi). Scale bar: 800μm.

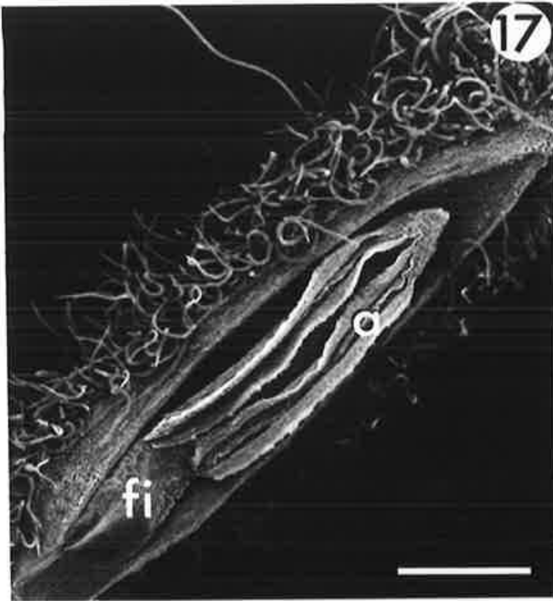
Fig. 5.18 *B. menziesii* pollen presenter at anthesis, showing stigmatic groove (g) and pollen grains (p) deposited below the groove. Fresh, uncoated sample. Scale bar: 100μm.

Fig. 5.19 *B. coccinea* pollen presenter at anthesis, showing deposited pollen grains (p). Scale bar: 200μm.

Fig. 5.20 *B. menziesii* pollen presenter at one day post-anthesis, showing ridged surface and position of stigmatic groove (g). Fresh, uncoated sample. Note that the pollen has been removed by foraging fauna. Scale bar: 200μm.

Fig. 5.21 *B. coccinea*, biporate pollen grain showing sculptured exine (e), germination pores (gp) and associated orbicules (or). Scale bar: 20μm.

Fig. 5.22 *B. menziesii*, bicolporate pollen grain showing lightly pitted exine (e), germination pores (gp) and associated orbicules (or). Scale bar: 20μm.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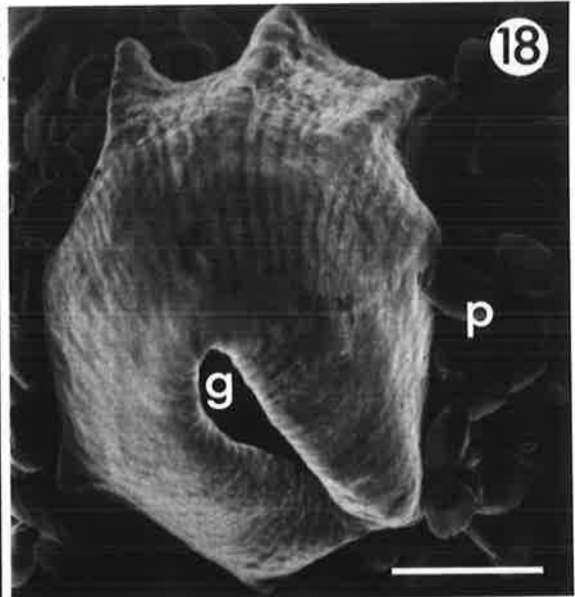
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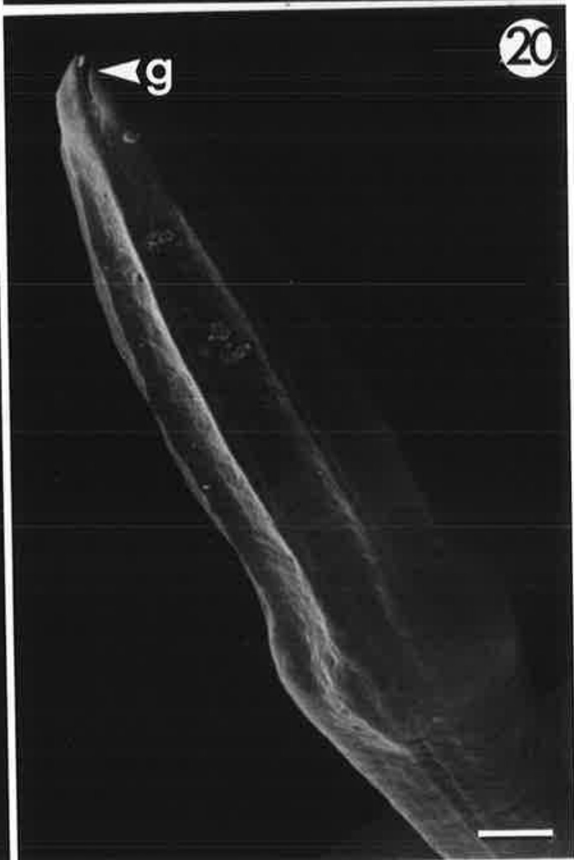
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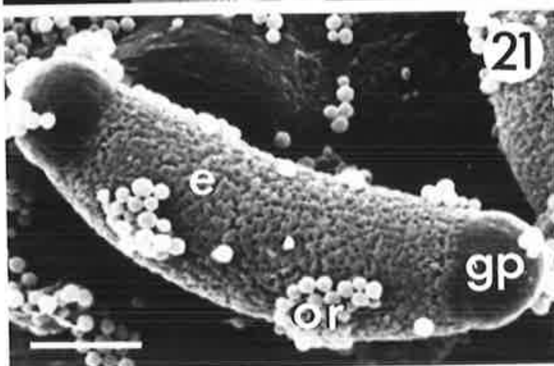
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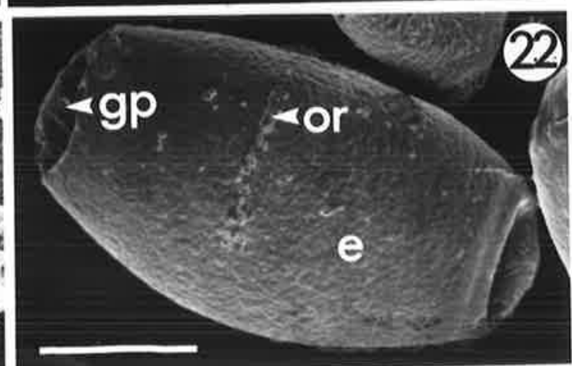
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The inflorescence of both species consisted of 13 genetic spirals with successive primordia of each being initiated simultaneously during microscopic development. However in the *B. coccinea* inflorescence the angle of divergence (Dormer, 1972) was 27.7°, thus creating a much steeper spiral than in the inflorescence of *B. menziesii* (14.6°). In contrast, there was less variation in the angular divergence between the two species in the vegetative state. In *B. coccinea* leaves on the same orthostichy were separated by eight internodes and three turns of the genetic spiral, thus the angle of divergence was 135°. Separation by thirteen internodes and five turns between leaves in *B. menziesii* created an angle of 138.5°. Thus there was a marked change in the phyllotaxy of both species at the transition to the reproductive phase of growth. This change occurred rapidly and was mediated by initiation of the involucral bracts. There was a mean of 352 ± 12 florets per inflorescence in *B. coccinea*, as compared with 723 ± 100 for *B. menziesii*.

A proportion of the shoots of both *B. coccinea* and *B. menziesii* were vegetative at most sampling times (Table 5.1). The highest proportion of microscopic floral apices was observed between November and May for *B. coccinea* and between October and February for *B. menziesii*. The low proportion of vegetative shoots at the final two sampling periods resulted because most of the labelled shoots had flowered by that stage of the experiment. Ten stages of development were identified in the transition from the vegetative meristem to the inflorescence at anthesis which were characterised both morphologically and by their dimensions (Table 5.2). The first stage was a vegetative meristem which was similar in size for the two species. The transition to the reproductive state was identified by the broadening of the apical meristem and the initiation of involucral bracts (stage 2). Stage 3 was characterised by the doming of the meristem and initiation of common bract primordia. Floral initiation occurred during late spring in both species (Tables 5.1 and 5.2), but subsequent inflorescence development was more rapid in *B. menziesii*. When the floret bracts were initiated (stage 4) the height of the meristem had doubled over that observed for stage 3 in *B. menziesii*, and quadrupled in *B. coccinea*. Floret initiation (stage 5) commenced in January for

Table 5.1 Number of vegetative and floral apices detected by scanning electron microscopy on shoots sampled at monthly intervals from *B. coccinea* and *B. menziesii* plants. Shoots with macroscopic inflorescences were excluded. (- : not available).

		B. coccinea			*B. menziesii*		
		Vegetative	Floral	Percentage of floral shoots	Vegetative	Floral	Percentage of floral shoots
1988	March	-	-	-	7	0	0
	April	4	8	67	14	2	13
	May	23	1	4	8	0	0
	June	16	1	6	12	0	0
	July	13	0	0	16	0	0
	August	12	0	0	15	0	0
	September	17	0	0	18	1	6
	October	16	0	0	13	1	7
	November	16	6	27	19	2	5
	December	19	0	0	3	10	77
1989	January	14	7	33	2	4	67
	February	5	3	27	7	1	14
	March	11	4	38	8	0	0
	April	8	3	37	7	0	0
	May	9	3	25	5	0	0
	June	18	2	10	2	0	0
	July	15	0	0	5	0	0
	August	17	1	6	1	0	0
	September	4	0	0	-	-	-
	October	5	0	0	2	2	50
	November	2	3	60	2	3	60
	December	0	5	100	0	5	100
1990	January	0	5	100	1	4	80

Table 5.2 Stages of development and mean dimensions (± standard error) of the apical meristem of *B. coccinea* and *B. menziesii*.

Developmental Stage	*B. coccinea*				*B. menziesii*			
	Peak[1] (month)	Range (month)	Height (mm)	Diameter (mm)	Peak[1] (month)	Range (month)	Height (mm)	Diameter (mm)
1 Vegetative meristem	Jul.-Oct.	Jan.-Dec.	0.1 ± 0.0	0.2 ± 0.0	May-Aug.	Jan.-Dec.	0.1 ± 0.0	0.2 ± 0.0
2 Involucral bract initiation	Nov.	Nov.	0.2 ± 0.1	0.5 ± 0.0	Oct.	Oct.-Nov.	0.2 ± 0.0	0.5 ± 0.0
3 Common bract initiation	Nov.	Nov.-Apr.	0.8 ± 0.1	0.6 ±0.1	Dec.	Oct.-Dec.	1.9 ± 0.3	1.7 ± 0.2
4 Floral bract initiation	Jan.	Jan.-Apr.	3.2 ± 0.2	1.8 ± 0.3	Dec.	Nov.-Dec.	3.6 ± 0.2	3.6 ± 0.4
5 Floret initiation	Apr.	Jan.-Apr.	4.2 ± 0.5	2.1 ± 0.3	Jan.	Jan.-Apr.	5.8 ± 0.8	4.5 ± 0.5
6 Macroscopic appearance of inflorescence	May	May -Sep.	11.0 ± 1.2	9.3 ± 1.3	Feb.	Nov.-Jul.	26.8 ± 1.9	19.5 ± 1.5
7 Macroscopic appearance of florets	Jul.	May-Oct.	29.0 ± 1.3	17.0 ± 0.7	Feb.	Jan.-Aug	47.3 ± 2.2	18.7 ± 0.9
8 Floret extension	Aug.	Jul.-Oct.	37.5 ± 1.8	35.3 ± 2.5	Mar.	Jan.-Aug.	69.6 ± 3.3	28.7 ± 1.8
9 Style extension	Sep.	Jul.-Oct.	36.9 ± 2.0	53.3 ± 0.9	Apr.	Feb.-Sep.	86.9 ± 4.5	61.0 ± 3.2
10 Anthesis	Oct.	Jul.-Nov.	41.1 ± 1.9	80.7 ± 2.0	May	Feb.-Sep.	80.4 ± 7.3	88.2 ± 1.9

[1]Month during which the highest proportion of shoots were observed for each stage.

B. menziesii and in April for *B. coccinea*. Macroscopic appearance of the inflorescence (stage 6) occurred earlier in *B. menziesii* than in *B. coccinea*, and the dimensions of the inflorescence of the former were twice those of the latter. Stage 7 was characterised by the appearance of floret pairs. From stage 8 onwards increases in the width of the inflorescence was attributed to lengthening of the perianth tube and the style within, followed by protrusion of the style beyond the perianth (stage 9). At anthesis (stage 10) the increase in the diameter of the inflorescence was due to the release of the style from the perianth limb. Floral development in *B. menziesii* took 8 months from the time of floral initiation to peak anthesis in May. Development was slower in *B. coccinea*, requiring 12 months to reach the stage of peak anthesis in October.

5.4 CONCLUSIONS

This is the first study to investigate the detailed timing of floral initiation and development for any species of *Banksia*. The time of floral initiation and the stages of floral development were similar for both *B. coccinea* and *B. menziesii*, whereas the timing of floral development differed. Floral initiation in both species occurred during late spring, suggesting that they may be responding to conditions of increasing daylength, increasing temperature or water stress. The floral development of *Banksia* is relatively slow. *B. coccinea* remains in the microscopic stages of development for a longer period than *B. menziesii* and has a slower rate of macroscopic development, resulting in a delay in peak anthesis of five months.

The banksia inflorescence is a condensed panicle and each floral pair is a reduced branch subtended by a bract, the common bract. Comparative studies (Venkata Rao, 1971) of proteaceous inflorescences suggest that the characteristic pairing of florets, in all but one genus of the tribe Grevilleoideae, is a result of the suppression of all but two flowers on the lateral branch of a panicle.

The floral structure of *B. coccinea* and *B. menziesii* is similar, hermaphrodite and perigynous, but there are distinct taxonomic differences between the two species. In *B.*

menziesii the pollen presenter is similar to that of *B. prionotes* (Collins and Spice, 1986), cylindrical and ridged with the depressions corresponding to the position of the anther lobes, whereas that of *B. coccinea* is conical with a smooth surface and basal ridge.

Pollen presentation is not unique to *Banksia*, and occurs in several other genera of Proteaceae (Venkata Rao, 1971; Wrigley and Fagg, 1989) and in some genera of the Asteraceae (Small, 1915; Sharman and Sedgley, 1988). In *B. coccinea* and *B. menziesii* the pollen grains are deposited below the stigmatic groove. The pollen presentation mechanism and the structure and size of the stigmatic groove in relation to pollen grain size and shape, may have important implications for the breeding system in *Banksia* species.

6. VARIABILITY IN *B. coccinea* AND *B. menziesii*

6.1 INTRODUCTION

Most *Banksia* plantations have been established using plants raised from seed collected from the wild, and therefore show a high degree of between plant variability. This chapter investigates this variability in relation to cut flower production of *B. coccinea* and *B. menziesii* cultivated in southern Australian plantations, and considers the variability in yield and the development of quality criteria in relation to climate and management.

6.2 MATERIALS AND METHODS

6.2.1 Experimental plants

The details of plants used in this chapter are presented in Chapter 3 (Table 3.2).

6.2.2 Within site variation

During the harvest period of 1988 the details of the blooms cut from *B. coccinea* and *B. menziesii* bushes numbered 11 to 30 were recorded. This information included harvest date, stem length (excluding inflorescence), inflorescence length, offset angle of inflorescence, and the percentage of leaf damage and abnormal floret development. Objective quality criteria were derived from these data. Inflorescence colour (Horticultural Colour Chart; British Colour Council, 1938-1941) was recorded for 99 plants of *B. coccinea* in November 1989 and 106 plants of *B. menziesii* in May 1989.

A Poisson model was fitted to the total number of blooms produced per plant, as well as the number of blooms produced per plant per month and bloom quality, and a chi-square test for variability between plants was performed. The variability in stem length and inflorescence length between bushes was analysed using a one way analysis of variance.

6.2.3 Between site variation of *B. coccinea*

Seedling plants at three South Australian locations were assessed in September 1988, and plants on three properties in Western Australia were assessed in September

1989. Details on plantation management and layout, and bush age, were recorded, and counts made of the total number of blooms per bush and the number of blooms showing abnormal floret development.

6.3 RESULTS

6.3.1 Within site variation

The total number of blooms produced per *B. coccinea* plant differed significantly between plants ($p<0.001$), with an average of 20.9 blooms per plant, but ranging from 0 to 52 (Table 6.1) (Plate 6.2). The average stem length (excluding inflorescence) was 55.4cm and the average inflorescence length 5.4cm, both of which showed significant plant to plant variation ($p<0.001$ for both). Peak production of blooms in September was recorded for 50% of the plants, while 12% and 38% of the plants produced most of their blooms in August and October respectively.

There was a significant difference between the *B. menziesii* plants in the total number of blooms produced ($p<0.001$), which ranged from 1 to 22, with a mean of 9.6 (Table 6.2). The number of blooms produced per tree per month differed between trees ($p<0.001$), except in July and October. Both stem length and inflorescence length showed plant to plant variation ($p<0.001$ for both), with the mean stem and inflorescence length recorded 37.4cm and 9.6cm respectively.

Three grades of bloom were identified based on stem and inflorescence length, offset angle of inflorescence and the percentage of leaf damage and abnormal floret development (Table 6.3). Only grades 1 and 2 would normally be marketed. Abnormal floret development varied from a complete lack of floret extension over part or all of the central axis (Figs 6.1 and 6.2) to reduced or uneven floret extension, particularly toward the apex of the axis (Fig. 6.3).

Plate 6.1 Colour variation in *B. menziesii* blooms, (a) yellow, (b) pink and (c) deep red. (x0.5).

Plate 6.2 Between bush variation in bush size and number of blooms produced for *B. coccinea*. Both bushes were planted in 1983, and are 6 years old. (x0.035).

Table 6.1 Yield and time of peak production of *B. coccinea* blooms from 20 bushes, and the mean stem length (excluding inflorescence) and inflorescence length of harvested blooms. (± standard error).

Tree number	Number of blooms	Stem length (cm)	Inflorescence length (cm)	Month of peak production
11	36	49.3 ± 3.1	6.3 ± 0.3	October
12	0	-	-	-
13	24	50.1 ± 3.3	4.6 ± 4.6	October
14	25	49.9 ± 3.3	4.9 ± 0.2	September
15	13	60.1 ± 4.2	6.9 ± 0.1	September
16	25	44.7 ± 3.0	4.6 ± 0.6	September
17	13	59.0 ± 4.6	4.3 ± 1.0	October
18	8	84.3 ± 7.4	5.8 ± 5.8	October
19	21	63.1 ± 3.6	6.2 ± 0.1	August
20	52	42.4 ± 1.6	5.7 ± 0.2	August
21	30	39.4 ± 2.3	4.1 ± 0.3	September
22	6	51.8 ± 6.3	4.7 ± 0.7	October
23	33	53.5 ± 3.0	5.7 ± 0.3	September
24	29	54.5 ± 2.6	4.3 ± 0.3	September
25	24	61.6 ± 2.7	7.5 ± 0.3	August
26	16	55.5 ± 3.2	5.3 ± 0.9	September
27	21	63.3 ± 4.1	5.6 ± 0.6	September
28	0	-	-	-
29	17	58.8 ± 3.8	-	September
30	25	55.4 ± 3.4	-	October

Table 6.2 Yield and time of peak production of *B. menziesii* blooms from 20 bushes, and the mean stem length (excluding inflorescence) and inflorescence length of harvested blooms. (± standard error).

Tree number	Number of blooms	Stem length (cm)	Inflorescence length (cm)	Period of peak production
11	8	43.3 ± 5.1	9.4 ± 0.7	April-September
12	9	31.7 ± 1.6	9.6 ± 0.6	August
13	8	37.3 ± 2.1	10.3 ± 0.3	June-September
14	13	37.2 ± 1.9	10.8 ± 0.6	April
15	13	38.2 ± 1.5	10.7 ± 0.5	March
16	15	39.5 ± 1.8	10.4 ± 0.6	April
17	6	37.5 ± 2.7	9.6 ± 0.4	May-June
18	5	33.4 ± 3.6	12.0 ± 12.0	May
19	22	32.4 ± 1.9	9.2 ± 0.4	May
20	10	44.3 ± 1.0	8.4 ± 0.4	May
21	14	34.1 ± 2.2	9.8 ± 0.6	May
22	1	42.0 ± 42.0	7.5 ± 7.5	June
23	14	41.2 ± 1.5	10.5 ± 0.4	April
24	11	32.5 ± 2.2	7.0 ± 0.4	Aug.
25	13	41.4 ± 1.6	8.0 ± 0.3	March
26	3	38.3 ± 3.3	10.2 ± 0.7	May
27	4	26.8 ± 4.4	7.8 ± 0.4	March-April
28	7	38.3 ± 2.2	10.7 ± 0.5	April
29	10	42.7 ± 1.6	9.8 ± 0.5	August
30	5	35.6 ± 4.9	10.1 ± 0.9	April-October

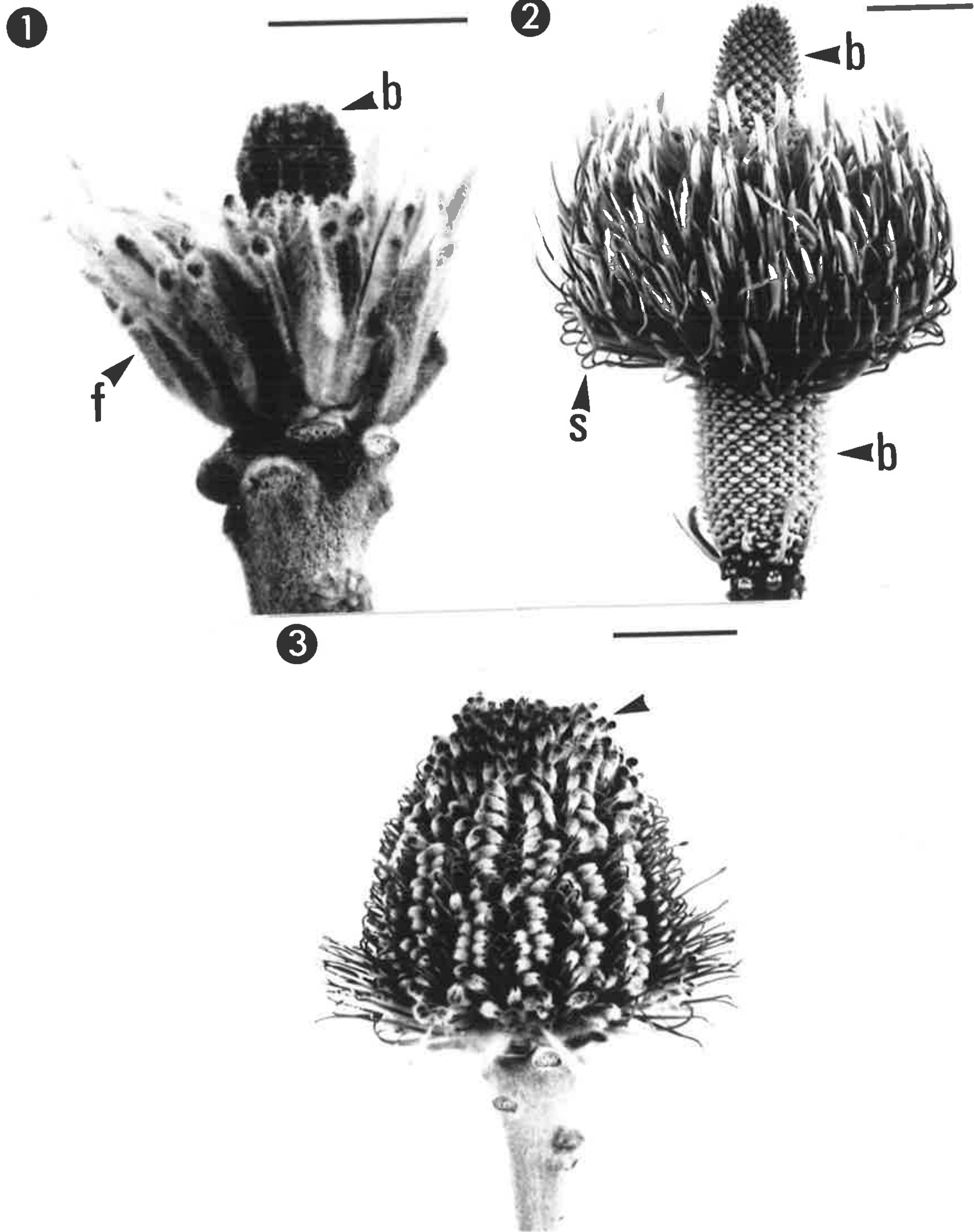
Table 6.3 Quality criteria for cut blooms of *B. coccinea* and *B. menziesii*.

		Grade 1	Grade 2	Grade 3
Length of stem including inflorescence (cm)				
	-*B. coccinea*	>60	>40	<40
	-*B. menziesii*	>50	>40	<40
Length of inflorescence (cm)	-*B. coccinea*	>6	>5	<5
	-*B. menziesii*	>10	>8	<8
Leaf damage (%)		0	<20	>20
Offset angle of inflorescence (° from vertical)		<15	<45	>45
Abnormal floret development (%)		<5	<15	>15

Fig. 6.1 Abnormal floret development in *B. coccinea* inflorescence with few extended florets (f) and an area of exposed common and floral bracts where no florets have developed (b). Scale bar: 2cm.

Fig. 6.2 Abnormal floret development in *B. menziesii* inflorescence showing area of exposed common and floral bracts at the top and bottom of the inflorescence where no florets have developed (b), and the uneven arrangement of florets which have extended. Note in the lower florets the uncharacteristic looping of the style (s). Scale bar: 2cm.

Fig. 6.3 Abnormal floret development in *B. coccinea* inflorescence showing uneven floret extension, and the lack of style extension in florets towards the apex of the inflorescence (arrow). Scale bar: 2cm.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3

The harvest period for *B. coccinea* extended from July to October, with peak bloom production occurring in September (Table 6.4). There was a decline in the percentage of first grade blooms produced over this time, with an increase in the proportion of seconds. Significant differences were recorded between trees in the total number of blooms graded as one ($p<0.001$), two ($p<0.001$) and three ($p<0.001$). There was also significant variation in the time of production for the different trees ($p<0.001$).

B. menziesii blooms were produced over an 8 month period extending from March through to October (Table 6.5). Peak production occurred in March/April, with a high proportion of marketable inflorescences and few as grade 3. There was between plant variation in the number of blooms graded as one ($p<0.05$), two ($p<0.05$) and three ($p<0.01$).

Variation in inflorescence colour (Table 6.6) was apparent during the later stages of floral development, when the styles were visible (see 5.3). In both species style colour played the major role in the overall colour display. There was little variation in perianth colour in *B. coccinea*. The overall grey colour was imparted by a mixture of black, white and tan hairs, with a predominance of white. The most frequent style colour observed was the characteristic scarlet red with a minority of dark red, orange and pink. The perianth of *B. menziesii* was covered with very fine hairs through which the surface colour was visible, thus imparting a greater influence on the colour display of the inflorescence of this species than for *B. coccinea*. While pink was the overall dominant colour, others observed were deep red, yellow and apricot, produced by varying combinations of style and perianth colour (Plate 6.1).

6.3.2 Between site variation of *B. coccinea*

There was a significant difference in the number of blooms produced per bush at each of the six experimental sites ($p<0.001$), and also a great deal of variation between sites ($p<0.001$) (Table 6.7). The highest level of production per bush was recorded at Albany-2 and the lowest at Muchea, north of Perth. The proportion of abnormal blooms produced per plant showed a highly significant difference between the six sites ($p<0.001$). At Muchea and Albany-1 there was no between plant variation in the

Table 6.4 Distribution of production and quality grading of harvested blooms from 20 trees of *B. coccinea.*

		July	August	September	October
Percentage of total blooms		4	23	45	28
Percentage	Grade 1	62	37	31	21
	Grade 2	19	46	45	69
	Grade 3	19	17	24	9

Table 6.5 Distribution of production and quality grading of harvested blooms from 20 trees of *B. menziesii.*

		March/April	May/June	July/August	September/October
Percentage of total blooms		35	31	24	10
Percentage	Grade 1	43	30	14	15
	Grade 2	46	46	33	30
	Grade 3	11	24	53	55

Table 6.6 Inflorescence colour variants in *B. coccinea* and *B. menziesii*. Colour names and codes from British Colour Council 1938-1941.

B.coccinea						
Inflorescence colour		Dark Red	Red	Orange	Pink	
Style colour	-name	Oxblood Red	Blood Red	Nasturtium Red	Blood Red	
	-code	00823	820	14	$820/_3$	
Perianth colour	-name	Sung Green	Sung Green	Sung Green	Sung Green	
	-code	$000658/_3$	$000658/_3$	$000658/_3$	$000658/_3$	
Number of plants		4	80	14	1	
B. menziesii						
Inflorescence colour		Deep Red	Pink	Yellow	Apricot	
Style colour	-name	Cardinal Red	Scarlet	Canary Yellow	Aureolin	Delft Rose
	-code	822	$19/_2$	$2/_2$	$3/_1$	$020/_1$
Perianth colour	-name	Rhodonite Red	Orient Pink	Empire Yellow	Rhodonite Red	Orient Pink
	-code	$0023/_3$	$416/_2$	$603/_3$	$0022/_2$	$416/_3$
Number of plants		30	60	1	2	13

Table 6.7 Production and management of *B. coccinea* in South Australia and Western Australia.

Location	Age of bushes (years)	Number of bushes assessed	Total number of blooms per bush (mean ± s.e.)	Percentage of blooms with abnormal floret development	Pruned	Spacing between bushes X rows
Blewitt Springs, S.A.	5	90	23.1 ± 1.9	5.4	yes	1.8m X 3.0m[A]
Millicent, S.A.	5	20	58.8 ± 5.8	11.6	yes	1.8m X 1.8m[A]
Mt. Gambier, S.A.	6	35	33.9 ± 5.4	5.7	yes	1.8m X 1.8m[A]
Albany, W.A. -1	4	30	16.4 ± 2.0	8.6	no	1.0m X (1.0m x 3.0m)[B]
Albany, W.A. -2	7	15	75.3 ± 11.7	1.7	no	1.0m X (1.0m x 2.0m)[C]
Muchae, W.A.	6-7	30	3.2 ± 0.8	5.3	no	2.9m X (2.4m x 3.4)[C]

[A]Arranged in single rows.
[B]Arranged in triple rows.
[C]Arranged in double rows.

proportion of abnormal blooms, while at the other four sites the variation was highly significant ($p<0.001$). The highest proportion of blooms showing abnormalities was at Millicent, in the lower south east of South Australia, where 55% of the abnormal blooms exhibited uneven floret extension. In contrast very few blooms at Albany-2 were deformed in any way. There were major differences in the management of the plantations between South Australia and Western Australia. The lack of pruning together with compact arrangement of plants into double and triple rows in the Western Australian plantations made weed control and harvesting difficult, particularly as many of the plants were above 3m tall.

6.4 CONCLUSIONS

This study has demonstrated that both *B. coccinea* and *B. menziesii* show significant plant to plant variablilty in yield, quality and time of production, and that there is ample opportunity for selection of superior genotypes. If banksias are going to compete successfully with the traditional cut flower crops, such as roses, carnations and chrysanthemums, then growers will have to ensure continued supply of quality product. To achieve this goal it will be essential to develop clonal material which yields a high number of top quality blooms. To assist this development, bloom quality criteria have been identified, in collaboration with the growers and the market, so that each tree in a selection programme can be objectively assessed. Applying these criteria to the 20 plants assessed, only three trees of *B. coccinea* (trees 15, 19 and 15; Table 6.1) and one of *B. menziesii* (tree 23; Table 6.2) could be considered as producing grade 1 blooms based on the combination of average stem and inflorescence lengths. The criterion of stem length presented in Table 6.3 includes the inflorescence, as this measurement has now been accepted by the industry.

While variability within sites is due largely to genetic variation between the seedling plants, variability between sites is due to a combination of genetic variability, climate and management. Although plantation management varied between sites, particularly between South Australia and Western Australia, climate is considered to be

the major factor influencing the yield differences. The difference in bloom number per bush between Albany-2 and the South Australian sites may be more apparent than real, however, as the unpruned plants in Western Australia are much larger than those in South Australia. The higher temperatures at Muchea may inhibit floral initiation, resulting in lower yields. This emphasises the importance of understanding the control of flowering in relation to site selection.

The relatively low yield and high proportion of abnormal blooms at Albany-1 may be due to the age of the bushes. Banksias, like most woody perennials grown from seed, undergo a juvenile phase during which the plants will not flower, and early crops are often poor and erratic. Selection for precocious flowering in fruit trees has been successful in bringing rapid economic returns to growers (Hackett, 1985), and the same could be achieved for cut flower production of banksias.

The high proportion of abnormal blooms in *B. coccinea*, particularly at Millicent is of concern. The cause of these abnormalities is unknown, although it may be significant that the lowest incidence of abnormalities was recorded at Albany-2, within the native range of the species. Relatively few abnormalities wer observed at nearby Mt. Gambier, however, and it is also possible that some of the abnormalities may be due to insect attack.

7. DEVELOPMENT OF HYBRIDISATION TECHNIQUES FOR *Banksia* AND THE BREEDING SYSTEM OF *B. menziesii*

7.1 INTRODUCTION

The principles of hybridisation methodology including emasculation to prevent self pollination and isolation or bagging to prevent unwanted insect or wind pollination are well established, but require adaptation to each new species of interest (Sedgley and Griffin, 1989). The aim of this chapter is to develop techniques for controlled hand pollination of *Banksia* to be used in investigations of the breeding system and in breeding programmes. These techniques are examined in an investigation of the breeding system of *B. menziesii*, to determine the time of stigma receptivity, rate of pollen tube growth and self-incompatibility. Follicle development following controlled cross pollination is compared with fruit set in open pollinated populations to give an indication of the level of fertility which can be expected within the species.

7.2 MATERIALS AND METHODS

7.2.1 Experimental plants

The details of plants used in this chapter are presented in Chapter 3 (Table 3.2). The controlled hand pollination experiments were conducted between 1988 and 1990 on *B. menziesii* plants numbered 1 to 10 at the experimental site at Blewitt Springs, S.A. The observations on fruit set were conducted in Western Australia on wild populations at King's Park, Perth, Eneabba, Lake Indoon and Kalbarri, and on plantations at Happy Valley and Blewitt Springs, S.A.

7.2.2 Experiment 1: Emasculation and pollen transfer

A number of emasculation techniques were assessed. The removal of the distal portion of the corolla bearing the anthers prior to anthesis resulted in excessive damage to the flowers, spontaneous dehiscence of the anthers, and deposition of pollen in the stigmatic groove. This approach was abandoned in favour of pollen removal from the pollen presenter after anthesis. The following methods involving removal of pollen from

the pollen presenter at anthesis were assessed visually; washing with distilled water, with distilled water plus surfactant, or with 100% ethanol dispensed from a 250ml plastic squeeze bottle; brushing with a cotton bud, a cotton pipe cleaner, or a synthetic pipe cleaner. Pistils emasculated with the synthetic pipe cleaner were examined fresh and uncoated with a Cambridge Stereoscan (250 MK3) scanning electron microscope (SEM) operated at 10 kV to assess the efficiency of pollen removal.

The pollen transfer method chosen, which consisted of rubbing a pollen-laden pollen presenter from a floret which had just undergone anthesis, on the tip of the pistil to be pollinated, was assessed by viewing the stigmatic region of fresh, pollinated pistils by SEM.

7.2.3 Experiment 2: Time of stigma receptivity

7.2.3.1 Pollen germination

Fourteen *B. menziesii* inflorescences, in which approximately one-quarter of the florets had undergone anthesis, were labelled on 22 February 1989, and all open florets were removed (Fig. 7.1). The entire inflorescence was surrounded by a wire spiral to prevent contact between the flowers and the pollination bag (van Wyk, 1977). The spiral was attached to the stem just below the inflorescence (Fig. 7.2) and covered with a glassine bag secured with a twist-tie. After 24 hours the bag was opened and all remaining unopened florets were removed, leaving a ring of between 14 and 23 open florets (Fig. 7.3). The pollen was removed and the inflorescences rebagged until pollination at 0, 1, 3, 6, 9 and 12 days after anthesis. Two inflorescences per treatment were pollinated with fresh pollen from two other plants and the bag replaced. 24 hours after pollination the pistils were harvested. The pistils of the two control inflorescences (pollen removed, but not pollinated) were collected at day 6.

Harvested pistils were fixed in Carnoy's solution (6 parts ethanol, 3 parts chloroform, 1 part acetic acid) for 2 days before transferring to 70% ethanol for storage. The pistils were then hydrated through a series of alcohols to distilled water (70%, 30%, distilled water x2) before softening for 4 hours in 0.8N sodium hydroxide at 75°C. Decolourised aqueous aniline blue (0.1% water soluble aniline blue in

0.1N K_3PO_4, stirred overnight, filtered through Whatman No. 1 filter paper and made up to pH 9 with KOH) was used to stain for β-1,3-glucan present in the pollen tube wall. Pistils were bisected longitudinally for ease of observation, and squashed in 80% glycerol for fluorescence microscopy (Martin, 1959). Slides were sealed with clear nail varnish. Routine observations were conducted on an Olympus BHA microscope fitted with a reflective light fluorescence attachment and the appropriate excitation and barrier filters for 290μm. The number of pollen tubes in the pollen presenter segment of the style was recorded.

7.2.3.2 Changes in stigmatic groove

The stigmatic groove of unpollinated pistils at 0, 1, 3, 6, 9 and 12 days after anthesis, from which the pollen had been removed, were collected on 21 April 1989, and examined fresh and uncoated with the SEM operated at 5kV. The width of the stigmatic groove at its widest point, half way along its length, was measured from micrographs of three to five samples per treatment.

7.2.4 Experiment 3: Pollen tube growth

Twelve inflorescences of *B. menziesii* were prepared for controlled hand pollination on 30 March 1989, as in 7.2.3.1. Florets were pollinated three days after anthesis with fresh pollen from two other plants and the inflorescences rebagged. Pistils were harvested 1, 3, 6, 9, and 12 days after pollination, from two inflorescences at each sampling time, fixed and prepared for fluorescence microscopy (see 7.2.3.1). Pistils on the control treatment inflorescences (pollen removed, but not pollinated) were collected and fixed 15 days after anthesis. The number of pollen tubes in the upper and lower halves of the style were recorded.

7.2.5 Experiment 4: Seed set

Forty seven inflorescences of *B. menziesii* were prepared for controlled hand pollination between 18 April and 22 May 1989, as in 7.2.3.1. Florets were pollinated at day 3 with pollen from another plant and rebagged. The bags were removed when flower parts began to fall and the developing infructescences were left on the bush to mature.

The number of follicles per infructescence was recorded and seeds extracted following opening of the follicles by heating over a Bunsen burner to simulate the natural mechanism of seed release following fire (George, 1987; Wrigley and Fagg, 1989).

7.2.6 Experiment 5: Self-incompatibility -fruit set

Eight inflorescences of *B. menziesii* were labelled, the number of florets per inflorescence recorded, and the inflorescence bagged before any florets opened. From the commencement of anthesis, all newly opened florets were hand pollinated at 3 day intervals, without emasculation, until the entire inflorescence had been pollinated. The inflorescences were rebagged after each treatment. 5 infloresences received pollen from two other plants, and 3 were selfed, during the period from 9 July and 17 August 1990. Bags were removed when flower parts began to fall and the developing infructescences left on the bushes until maturity. The number of developing follicles per infructescence was recorded.

7.2.7 Experiment 6: Natural fruit set

7.2.7.1 Measurements

The level of fruit set of *B. menziesii* was assessed by scoring the number of open-pollinated follicles on every infructescence on eight to ten plants in populations at each of the experimental locations, during September 1989 (see Table 3.2).

The relationship between inflorescence length and number of florets, as estimated by the number of rows of flowers multiplied by the number of flowers in a row, was assessed on 17 inflorescences at anthesis at Blewitt Springs. The mean inflorescence length was compared with that of 14 mature open pollinated infructescences, and the maximum number of potential follicles per infructescence was calculated.

7.2.7.2 Statistical analysis

A Poisson model was fitted and a chi-square analysis used to test for differences within and between sites for both the number of follicles per infructescence and for the number of follicles per fertile infructescence. To test for differences within and between sites in the proportion of barren infructescences per plant, a binomial model was fitted

and chi-square test used. The relationship between inflorescence length and the number of florets was determined by a regression analysis, using an estimate of the number of florets present on the inflorescence. From the regression analysis, the quadratic equation

$$y = 418x - 20x^2 - 1155$$

where y is the number of florets on an inflorescence of x centimetres long, provided the best estimate of the number of florets (R^2=0.95). A Mann-Whitney test was used to compare the inflorescence and infructescence measurements. The mean inflorescence length was 7.3 ± 0.3cm and did not differ significantly from the length of the mature infructescence (T=111, p=0.75).

7.3 RESULTS

7.3.1 Experiment 1: Emasculation and pollen transfer

Pollen removal with a synthetic pipe cleaner was the most effective method. Washing pollen from the pollen presenter at anthesis was ineffective, while brushing with a cotton bud deposited cotton fibres on the pollen presenter and stigmatic region. The cotton pipe cleaner was efficient at removing pollen but was more difficult to manipulate than the looped synthetic pipe cleaner (Fig. 7.4). This removed most of the pollen when carefully moved once down and up over the pollen presenter. No pollen was observed by SEM in the stigmatic groove, and a mean of 36.5 ± 21.5, of an original number of approximately 2800, grains remained on the basal area of the pollen presenter. Rinsing in alcohol proved a suitable method of cleaning the pipe cleaner for repeated use. This method of pollen removal was applied in all subsequent controlled pollination experiments. Rubbing a pollen-laden pollen presenter against the tip of the pistil (Fig. 7.5) deposited 5.6 ± 1.6 pollen grains in the vicinity of the stigmatic groove, some of which were lodged in the groove itself (Fig. 7.6).

Figures 7.1-7.6. Controlled hand pollination technique for *Banksia*.

Fig. 7.1 Preparation of a *B. menziesii* inflorescence for controlled pollination by removal of all open florets. Scale bar: 35mm.

Fig. 7.2 *B. menziesii* inflorescence surrounded by a wire spiral to prevent contact between the flowers and the pollination bag. Scale bar: 35mm.

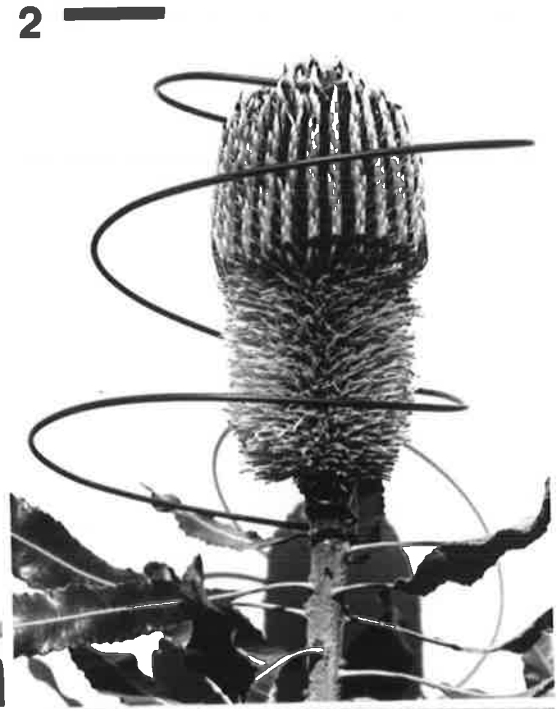
Fig. 7.3 *B. menziesii* inflorescence showing a ring of open florets at anthesis (arrowheads). Scale bar: 20mm.

Fig. 7.4 Removal of self-pollen using a looped synthetic pipe cleaner. Scale bar: 15mm.

Fig. 7.5 Hand pollination using a pollen-laden pollen presenter (pp), rubbed against the tip of pistil (pi). Scale bar: 20mm.

Fig. 7.6 Scanning electron micrograph of pollen (p) in the stigmatic groove (s) following hand pollination of *B. menziesii*. Scale bar: 150µm.

1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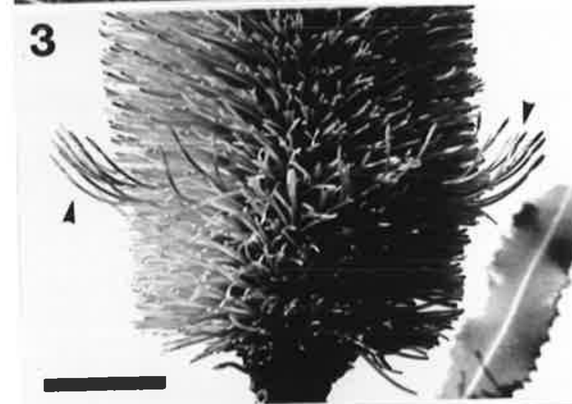
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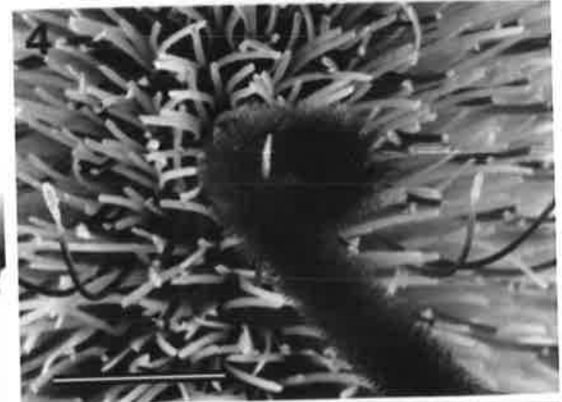
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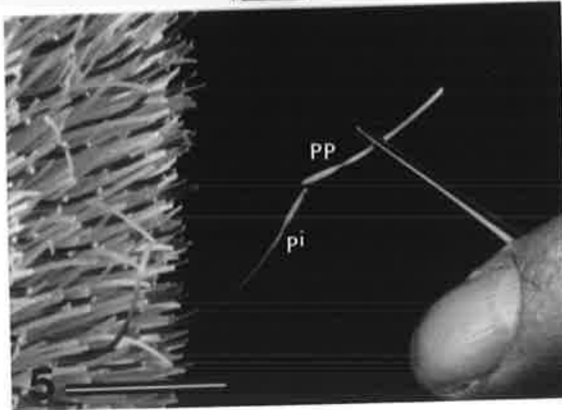

PP
Pi
5

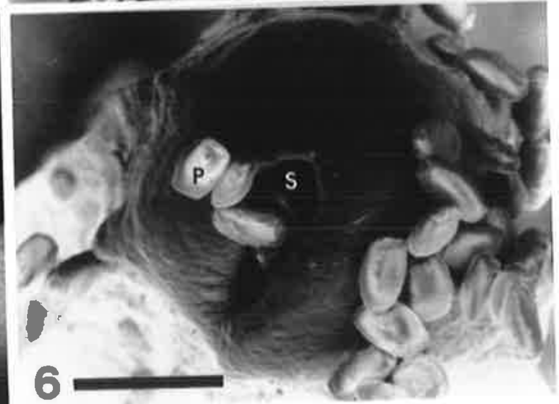
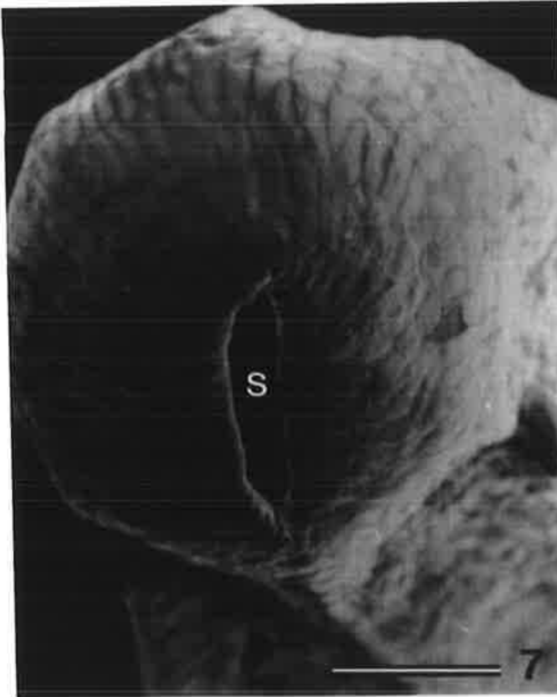

P
S
6

Fig. 7.7 Scanning electron micrograph of the stigmatic groove (s) of *B. menziesii* at anthesis. Scale bar: 100μm.

Fig. 7.8 Scanning electron micrograph of stigmatic groove (s) of *B. menziesii* 3 days post-anthesis. Scale bar: 100μm.

Fig. 7.9 Hand pollinated infructescence of *B. menziesii*, showing follicles developing from open pollination (o) prior to preparation of the inflorescence for controlled pollination and those resulting from controlled hand pollination (h). Scale bar: 30mm.

Fig. 7.10 Barren (a) and fertile (b) infructescences of *B. menziesii*. Scale bar: 30mm.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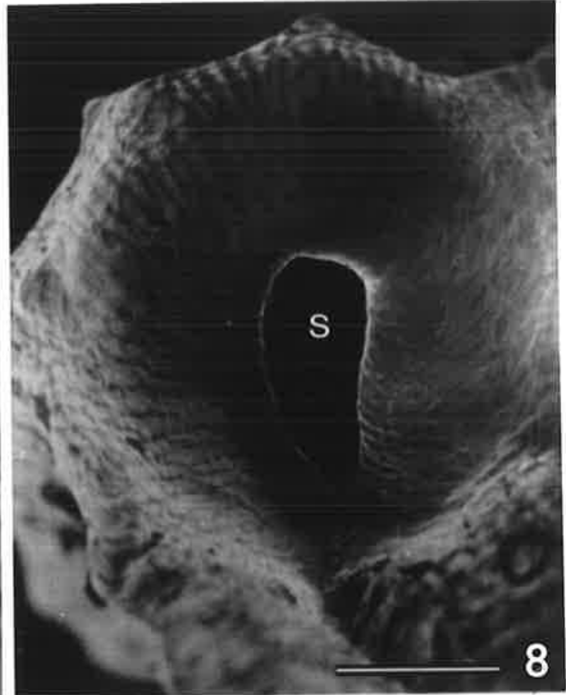
7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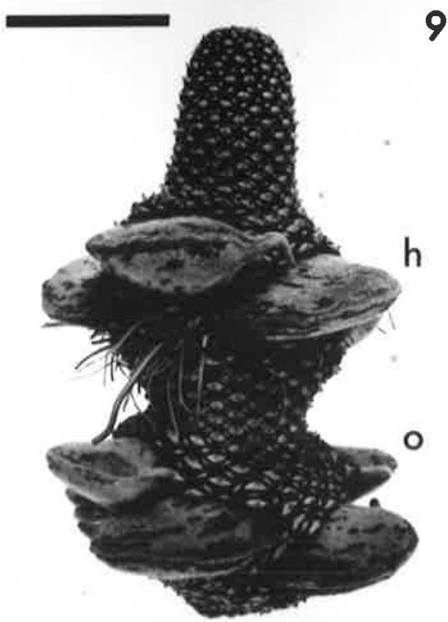
9
h
o

10

a

b

7.3.2 Experiment 2: Time of stigma receptivity

The width of the stigmatic groove increased from anthesis (Fig. 7.7) to a maximum at 3 days post-anthesis (Fig. 7.8). Pollen germination on the stigma declined from 3 days after anthesis (Table 7.1). From these two observations it was concluded that peak stigma receptivity in *B. menziesii* occurs at 3 days after anthesis. A mean of 0.03 ± 0.03 pollen grains germinated on the stigma of the control treatment.

7.3.3 Experiment 3: Pollen tube growth

Following pollination, pollen grains germinated and began to grow in the transmitting tissue. Pollen tube numbers in the style were extremely low, with rarely more than one pollen tube per style. Maximum pollen tube growth was observed at 6 days when pollen tubes had reached the base of the style in 41% of the pistils observed (Table 7.2). Significantly fewer pollen tubes were recorded following 6 days due to difficulties in observation upon degeneration of the style tissue. Control pistils had no pollen tubes.

7.3.4 Experiment 4: Seed set

Follicles developed from 11.1% of the florets which were hand pollinated and left for seed set. These follicles developed in a distinct band around the infructescence, due to the removal of adjacent flowers during the hand pollination treatment (Fig. 7.9). Thus the seeds of known parentage were readily distinguished from those produced previously following open pollination. Extraction of the seeds showed that not all ovules developed into a mature seed. The mean number of seeds per follicle was 0.94 ± 0.08.

7.3.5 Experiment 5: Self incompatibility -fruit set

Follicles developed on 80% of the infructescences following controlled cross pollination and on 33% following selfing. The mean number of follicles per infrutescence and the fruit to flower ratio overall were 6.0 ± 2.8 and 0.01 for the crosses, and 1.3 ± 1.3 and 0.002 for the selfs.

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Table 7.1 Stigma receptivity of *B. menziesii* as measured by the number of pollen tubes in the pollen presenter at 24 hours after pollination and the width of the stigmatic groove. (mean ± standard error).

	Day of Pollination (number of days after anthesis)					
	0	1	3	6	9	12
Width of stigmatic groove (µm)	34.7 ±1.5	37.9 ±1.7	46.5 ±3.4	26.1 ±0.8	22.7 ±2.9	15.4 ±1.4
Number of pollen tubes	1.4 ±0.2	1.6 ±0.3	1.8 ±0.3	1.1 ±0.3	0.3 ±0.2	0.3 ±0.1

Table 7.2 Number of pollen tubes per pistil in the upper and lower halves of the style of *B. menziesii* following pollination 3 days after anthesis. (mean ± standard error).

Number of pollen tubes in:	Day of Harvest (number of days after pollination)				
	1	3	6	9	12
upper style	0.13 ±0.05	0.18 ±0.06	0.43 ±0.08	0.38 ±0.08	0.21 ±0.07
lower style	0.00 ±0.00	0.10 ±0.05	0.41 ±0.08	0.14 ±0.06	0.05 ±0.04

Table 7.3 Fruit set of *B. menziesii* following controlled hand pollination and open-pollination. (mean ± standard error).

Location	Percentage of barren infructescences per tree[3]	Mean number of follicles per infructescence[3]	Mean number of follicles per fertile infructescence[3]	Maximum Number of follicles per infructescence	Percentage follicle set per inflorescence
Controlled hand pollination[1]					
Blewitt Springs, S.A.	-	1.5 ±0.3	3.3 ±0.4	9	11.08% ±2.19%
Open pollination[2]					
Blewitt Springs, S.A.	24.8% ±5.9%	7.6 ±0.7	9.6 ±0.7	37	0.91% ±0.08%
Eneabba, W.A.	72.7% ±8.0%	0.9 ±0.1	3.4 ±0.2	12	0.11% ±0.01%
Happy Valley, S.A.	73.8% ±3.4%	1.2 ±0.1	4.0 ±0.2	16	0.14% ±0.01%
Kalbarri, W.A.	22.6% ±4.7%	3.5 ±0.2	4.6 ±0.2	14	0.42% ±0.02%
King's Park, W.A.	60.2% ±7.0%	1.4 ±0.1	3.6 ±0.2	16	0.17% ±0.01%
Lake Indoon, W.A.	42.3% ±9.3%	3.7 ±0.4	6.1 ±0.5	20	0.44% ±0.05%

[1]Based on hand pollinating 14.0 ± 0.9 florets per inflorescence. Of the 47 inflorescences pollinted over 10 trees, 44.9% set fruit.

[2]Based on open pollination of the whole inflorescence (700-900 flowers).

[3]Significant differences between open pollinated sites, $\chi^2_{5,\ (0.001)} = 20.5$.

7.3.6 Experiment 6: Natural fruit set

The natural percentage fruit set of fertility of *B. menziesii* following open pollination of the entire inflorescence was considerably lower than that which resulted from controlled hand pollination (Table 7.3). This difference may be due to the low number of hand pollinated flowers but there were also significant differences between the six open pollination sites for all parameters used to indicate the fertility level ($p<0.001$), and there was also a high degree of variability within sites ($p<0.001$). In most locations there was a high percentage of infructescences which produced no follicles (Table 7.3, Fig. 7.10a), and only at Blewitt Springs was there no significant difference between trees. Those inflorescences which did produce follicles (Fig. 7.10b), produced very few relative to the original number of florets (Table 7.3).

7.4 CONCLUSIONS

This is the first detailed investigation of the reproductive biology of a *Banksia* species with a view to artificial hybridisation. The techniques of pollen removal, pollen transfer and inflorescence isolation can now be used in controlled breeding programs for the development of improved cultivars for the cut flower market. These methods are similar to those under development by Brits and van den Berg (1990) for hybridisation of South African members of the Proteaceae.

The delay of stigma receptivity in *B. menziesii* until 3 days post-anthesis is consistent with the protandrous nature of other members of the Proteaceae (Venkata Rao, 1971). For successful pollination it appears that the pollen must be placed within the stigmatic groove. This can only occur when the groove attains its widest dimension of 46μm diameter at 3 days after anthesis. Thus to maximise the likelihood of pollen germination, and subsequent seed set, hand pollinations should be conducted on the day of peak stigma receptivity.

The lower percentage follicle set following self pollination of the entire inflorescence rather than crossing suggests that there may be a self-incompatibility

mechanism operating in *B. menziesii*. This could now be thoroughly investigated utilising the techniques developed in this study.

The major factor likely to limit the success of controlled breeding of banksias is the low inherent fertility within the genus. *B. menziesii* is no exception to this trend within the genus and it was for this reason that the fertility was investigated in the native habitat (Table 7.3). There was a significant difference in the fruit to flower ratio between the six populations observed, possibly due to differences in soil moisture and nutrition, and efficiency of pollinators at the different localities. The highest level of fruit set following open pollination was recorded at Blewitt Springs. This may reflect the cultural environment of these plants, including the annual application of fertiliser, irrigation to supplement rainfall during the summer months, weed control, and the continual removal of blooms, preventing the build up of a seed bank. However this was considerably lower than that following controlled cross pollination under cultivated conditions. This demonstrates the benefit of hand pollination for maximising seed set, and also of efficient management of the crop in terms of nutrition, irrigation and weed control.

There is a further limitation to the fertility of a banksia inflorescence which relates to the spatial conformation of the infructescence. There is no increase in length of the infructescence following flowering, and the mature follicle is a large, woody structure. Comparison of the area of a mature follicle with the surface area of a mature infructescence shows that a maximum of 35 follicles can be accommodated on the mature infructescence (Table 7.4). This spatial limitation may also explain the observed difference between 41% of hand pollinated pistils with a pollen tube in the base of the style and 11.1% follicle set. Those follicles which develop first and most rapidly are likely to have an advantage with regard to resource allocation. Follicles appeared to be randomly scattered over the developing infructescence, although in other species a skewed distribution is apparent. For example, in *B. ericifolia* and *B. oblongifolia* more follicles developed in the middle third than either the top and bottom thirds of the inflorescence, which is probably related to pollinator efficiency (Zammitt and Hood, 1986). Given that

each follicle develops a mean of only 0.9 seeds, the potential seed set per infructescence of 33 seeds (Table 7.4). Thirty five follicles represents a fruit set of 4%, which is considerably less than the achieved fruit set following controlled hand pollination of 11.1%. Thus, by repeated hand pollination of an inflorescence, as successive new flowers open, it is theoretically possible to achieve the predicted maximum of 33 seeds per infructescence.

Table 7.4 Potential number of follicles and seeds per infructescence of *B. menziesii.*

Mean infructescence length (cm)	7.7 ± 0.4
Mean infructescence diameter (cm)	4.5 ± 0.1
Average surface area of fertile infructescence (cm^2)	108.2
Mean follicle length (cm)	2.7 ± 0.0
Mean follicle width (cm)	1.1 ± 0.0
Average area of follicle[1] (cm^2)	3.06
Number of florets per inflorescence[2]	876
Potential number of follicles	876
Potential maximum number of seeds	1752
Potential number of follicles which can be accommodated	35
Percentage of potential number of follicles	4.0%
Potential maximum number of seeds which can be accommodated	70
Number of seeds which develop per follicle	0.9 ± 0.1
Potential number of seeds	33

[1]Calculated as a rectangle

[2]Calculated using regression equation

8. INVESTIGATION OF THE BREEDING SYSTEM OF *B. coccinea*

8.1 INTRODUCTION

This chapter investigates various aspects of the reproductive biology of *B. coccinea*, including the time of stigma receptivity, pollen tube growth, self-incompatibility and seed set. This is achieved by conducting controlled hand pollinations using the techniques developed and described for *B. menziesii* in the previous chapter, and studying the level of fruit set from open pollination.

8.2 MATERIALS AND METHODS

8.2.1 Experimental plants

The details of plants used in this chapter are presented in Chapter 3 (Table 3.2). The controlled hand pollination experiments were conducted in 1988 and 1989 on *B. coccinea* plants numbered 1 to 10 at the experimental site at Blewitt Springs, S.A. The observations on fruit set were conducted in Western Australia on a natural population at King River and in South Australia on plantations at Happy Valley and Blewitt Springs.

8.2.2 Experiment 1: Time of stigma receptivity

8.2.2.1 Pollen germination

Twenty *B. coccinea* inflorescences were prepared for experimentation as described in 7.2.3.1. Between 11 and 21 florets were available for experimentation per inflorescence. Florets were pollinated at 0, 1, 3, 6, 9 and 12 days after anthesis with fresh pollen from two other plants and the inflorescences were rebagged. Two inflorescences were used per treatment. Pistils were collected 24 hours after pollination and processed for fluorescence microscopy (see 7.2.3.1). Counts were made of the number of pollen tubes in the pollen presenter segment of the style. Fluorescence micrographs were taken on a Zeiss Axiophot Photomicroscope with Ilford HP5 400 ASA black and white negative film.

8.2.2.2 Changes in stigmatic groove

Five to 7 pistils were harvested on 2 November 1989 at each of 0, 1, 3, 6, 9, and 12 days after anthesis, and the stigmatic groove examined, fresh and uncoated with a Cambridge Stereoscan (250 MK3) scanning electron microscope (SEM) operated at 5kV.

8.2.3 Experiment 2: Pollen tube growth

Florets of ten inflorescences which had been prepared for experimentation (see 7.2.3.1) were pollinated on 2 October 1989, three days after anthesis, with fresh pollen from two other *B. coccinea* plants. The pistils of two inflorescences were harvested 1, 3, 6, 9 and 12 days after pollination. The pistils of the two control treatments (emasculated but not pollinated; neither emasculated nor pollinated) were harvested at 9 days post-anthesis. All pistils were prepared for fluorescence microscopy (see 7.2.3.1). The number of pollen tubes in the pollen presenter and the upper and lower style were counted.

8.2.4 Experiment 3: Self-incompatibility

8.2.4.1 Pollen tube growth

A 5 x 5 diallel experiment was conducted using single-tree plots. Inflorescences were prepared for experimentation during the period from 7 September to 10 November 1989, and pollinated at 3 days post-anthesis. The florets were left for 9 days before fixation and preparation for fluorescence microscopy (see 7.2.3.1). The number of pollen tubes was counted as described previously. Two inflorescences were used per cross, to give an average of 41.8 florets per cross.

The following binomial model was fitted to each of the data sets:

$$y_{ijk} \sim B(n_{ijk}, p_{ij})$$

where $i = 1, 2, \ldots, 5$; $j = 1, 2, \ldots, 5$; $k = 1, 2, \ldots, s_{ij}$; s_{ij} is the number of styles for female i and male j; y_{ijk} is the number of pollen tubes in the given style section for female i, male j and style k; n_{ijk} is the number of pollen tubes in the pollen presenter segment of the style for female i, male j and style k; p_{ij} is the probability that a pollen

tube reaches the given style section for female i and male j. The data was analysed according to Sedgley *et al.* (1990). The model was fitted using Glim 3.77 (Payne, 1987) to find estimates of p_{ij} ($\hat{p}_{ij}$) to provide information on which crosses were the most successful. y_{ijk} and n_{ijk} were also expressed as a ratio, x_{ijk}. The data were then considered as a diallel cross where all possible crosses between c plants (c=5 in this case) were represented, that is, c2 combinations consisting of c selfings and c(c-1) crosses, where both reciprocal crosses were separately recognised. The analysis followed the method described by Griffing (1956) which allows the variation to be divided into that due to the population mean, general combining ability, specific combining ability, reciprocal effects and experimental error. This information was then displayed both as an analysis of variance table and as a table of estimated variance components. The analysis ignored the c possible self-pollinations as they would have introduced bias (Griffing, 1956). For the full analysis of the c(c-1) crosses the linear model was

$$x_{ijk} = u + gca_i + sca_i + r_{ij} + errror_{ijk}$$

where i = 1, 2, . . ., c; j = 1, 2, . . ., c; u = population mean; gca_i = general combining ability of the ith plant; sca_{ij} = specific combining ability of the cross i x j ($r_{ij} = -r_{ij}$). The diallel analysis was implemented using the programme DIALL developed by Schaffer and Usanis (1969).

8.2.4.2 Seed set

During October and November 1989, 28 inflorescences were labelled, bagged and pollinated as in 7.2.6. Fourteen inflorescences were self pollinated and 14 cross pollinated with pollen from two other trees, one from that population and one from Happy Valley. Bags were removed when florets began to fall. Infructescences were harvested approximately seven months later, and the number of follicles per infructescence and the number of seeds per follicle recorded.

8.2.5 Experiment 4: Natural fruit set

The level of fruit set of *B. coccinea* was assessed by scoring the number of follicles resulting from open pollination of every infructescence on plants at King River in

September 1990, Happy Valley in September 1989 and Blewitt Springs in June 1990 (see Table 3.2). A Poisson model was fitted to the data and chi-square analysis used to test for differences in the number of follicles per infructescence both within and between sites.

Inflorescence length was compared with that of mature infructescences by the Mann-Whitney test and the number of florets regressed against inflorescence and infructescence length. During follicle development inflorescence length was significantly reduced from 4.3cm at anthesis to 4.0cm at infructescence maturity ($T=4321.5$, $p=0.0266$), due to the loss of hairy bracts at the top of the central core. The linear equation derived from the regression analysis

$$y = 95.4\,x - 20.3 \qquad (R^2=0.49)$$

where y is the number of florets which were present on an infructescence of x cm length, provides an estimate of the potential number of follicles on a mature infructescence. The flower to fruit set ratio in the open pollinated populations was estimated and follicle size determined. The potential number of follicles was calculated according to the number of florets and the spatial limitations of the infructescence.

8.3 RESULTS

8.3.1 Experiment 1: Time of receptivity

Peak stigma receptivity, based on the number of germinated pollen grains, was recorded at 3 days after anthesis and declined rapidly thereafter (Table 8.1). Secretion was observed on a proportion of stigmas at all stages after anthesis and increased from little or no secretion at anthesis (Fig. 8.1) to moderate amounts at 3 days post-anthesis (Fig. 8.2), and copious secretion by 6 days after anthesis (Fig. 8.3).

Table 8.1 Stigma receptivity of *B. coccinea* as measured by the number of pollen tubes in the pollen presenter at 24 hours after pollination (mean ± standard error), and the percentage of pistils with secretion on the stigmatic groove.

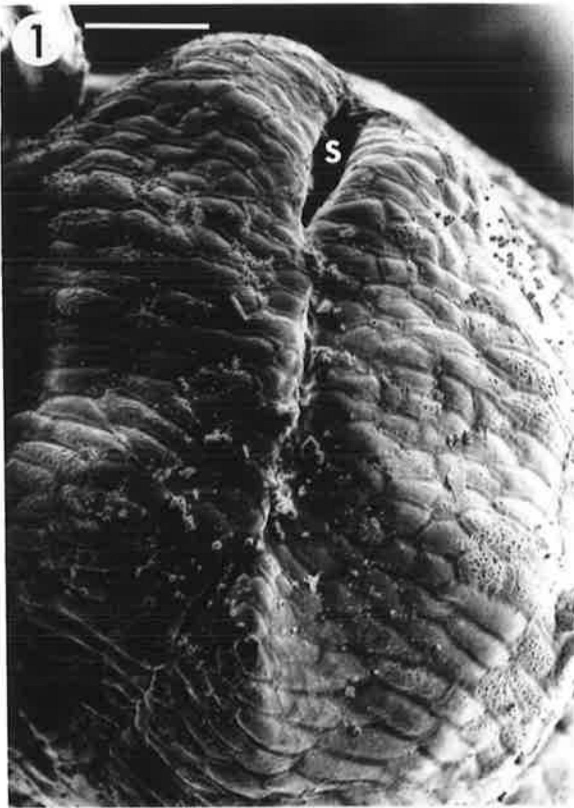
	Day of Pollination (days after anthesis)					
	0	1	3	6	9	12
Number of pollen tubes	0.4 ±0.1	4.9 ±0.6	6.3 ±0.5	0.8 ±0.3	1.7 ±0.3	0.0 ±0.0
Percentage of pistils with secretion	40%	20%	80%	100%	100%	100%

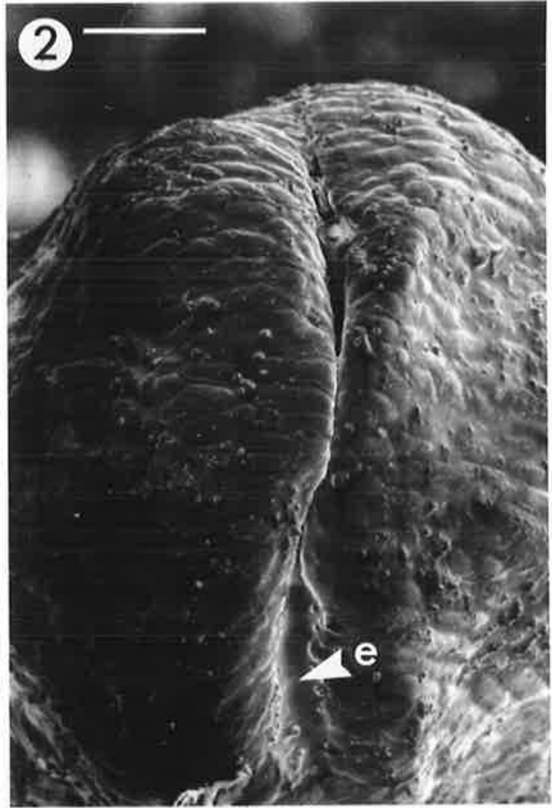
Fig. 8.1 Scanning electron micrograph of the stigmatic groove (s) of *B. coccinea* at anthesis. Scale bar: 50μm.

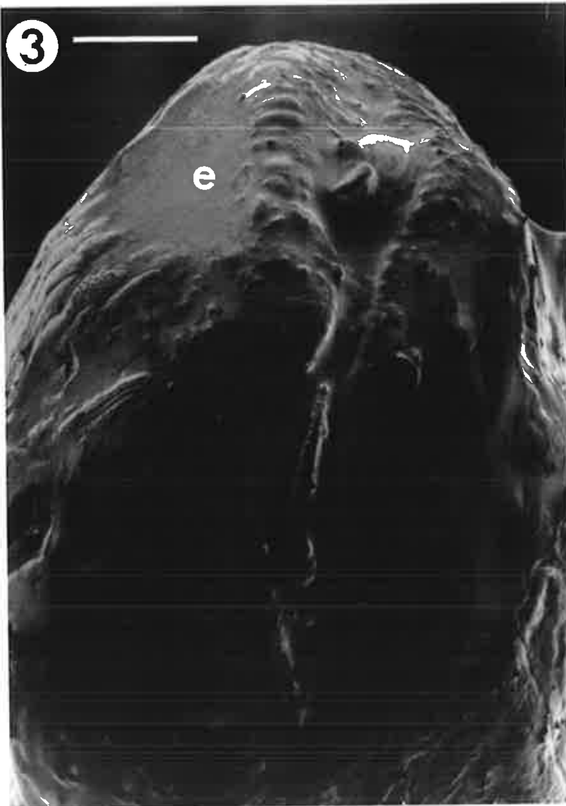
Fig. 8.2 Scanning electron micrograph of the stigmatic groove of *B. coccinea* 3 days after anthesis, showing a small amount of exudate (e). Scale bar: 50μm.

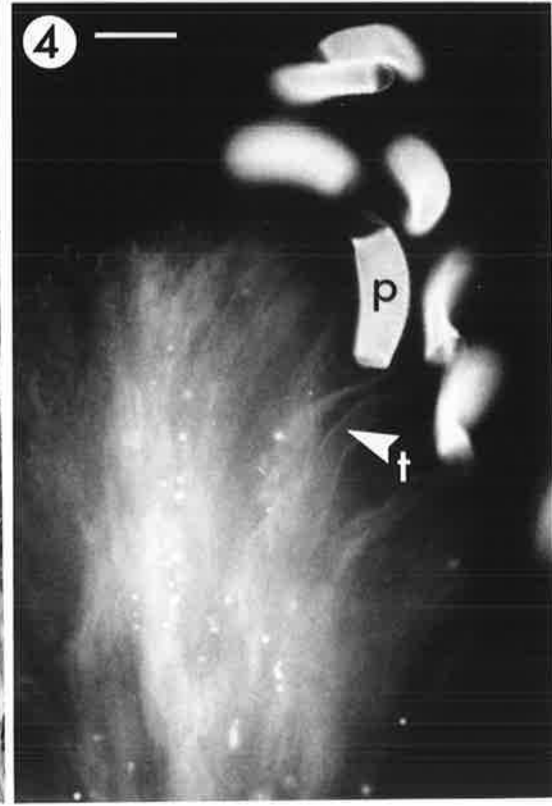
Fig. 8.3 Scanning electron micrograph of the stigmatic groove of a *B. coccinea* pistil 6 days after anthesis, showing copious exudate. Scale bar: 50μm.

Fig. 8.4 Fluorescence micrograph of germinating pollen grains (p) of *B. coccinea* with pollen tubes (t) penetrating the transmitting tissue of the pollen presenter 9 days after pollination. Scale bar: 50μm.


1
s


2
e


3
e


4
p
t

8.3.2 Experiment 2: Pollen tube growth

Pollen grains germinated on the stigma (Fig. 8.4) but very low numbers of pollen tubes grew down the style towards the ovary (Table 8.2). The maximum number of tubes in the lower style was recorded 6 days after pollination and pollen tubes were seen penetrating the ovules (Figs 8.5 and 8.6). It was not possible to consistantly recover ovaries from between the bracts on the central core of the inflorescence. A decline in the number of pollen tubes observed was recorded at 9 and 12 days after pollination due to difficulties in observation with the degradation of stylar tissue. Very few pollen tubes were observed in either control treatments.

8.3.3 Experiment 3: Self-incompatibility

8.3.3.1 Pollen tube growth

There was a tendency toward poorer pollen tube growth following selfing than following cross-pollination (Tables 8.3 and 8.4), and significant specific and general combining ability was demonstrated amongst the five *B. coccinea* plants, as well as significant reciprocal effects (Table 8.5). The variance components are presented in Table 8.6.

The crosses were ranked in order of fertility according to the probability of a pollen tube in the pollen presenter reaching the lower style (Table 8.4). Overall plant B performed poorly, particularly as a female parent, while A proved to be a consistently fertile female. The self-pollinations were generally poor as were the combinations of C x B and D x B, while E x C was a fertile combination. Marked reciprocal effects were exhibited by combinations involving A with B, D and E, and C with D. The percentage of pistils with pollen tubes in the final segment of the style varied between crosses from 6.3 to 76.9 (mean = 43.8%).

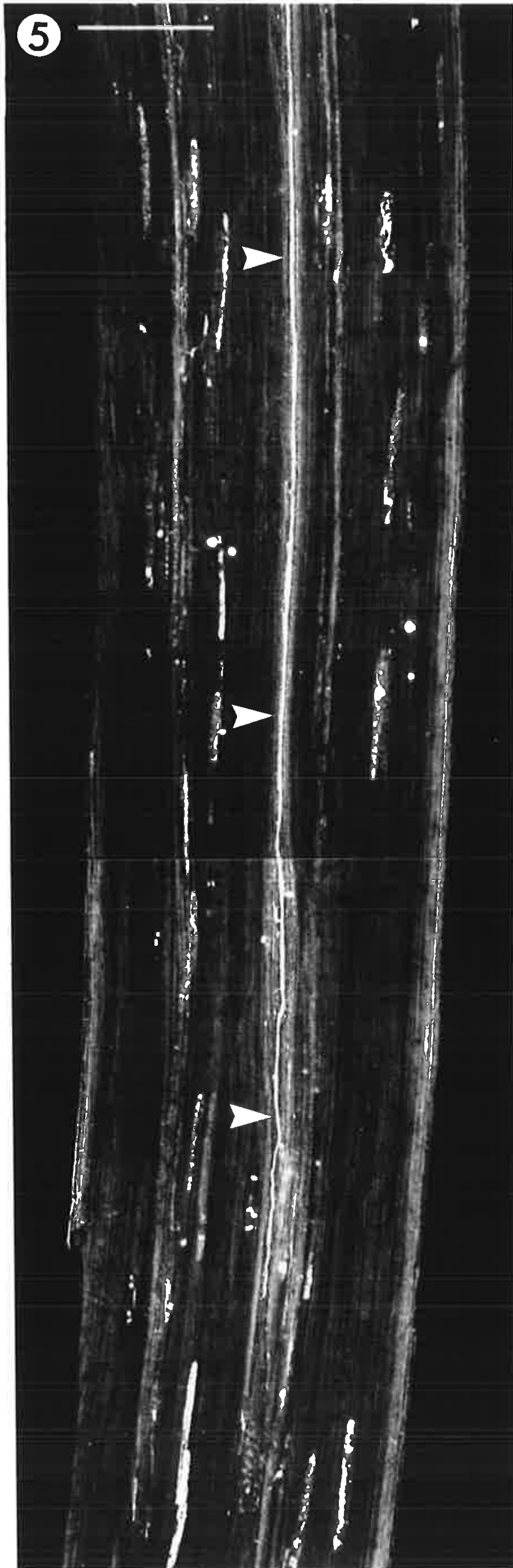
Table 8.2 Pollen tube growth of *B. coccinea* up to 12 days after pollination. (mean ± standard error).

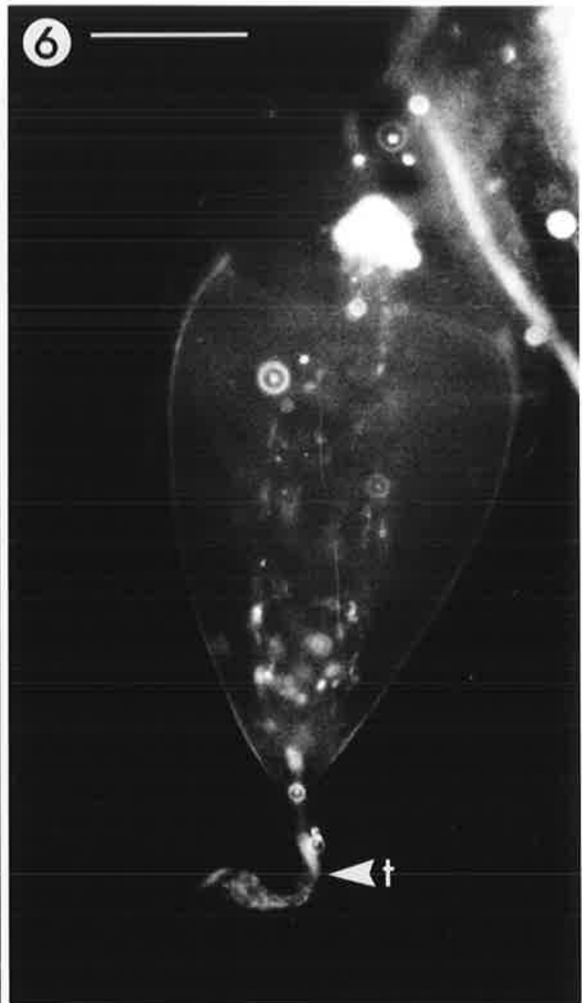
Days after pollination	Number of pollen tubes in the: pollen presenter	upper style	lower style
1	5.43 ±0.53	0.44 ±0.11	- -
3	7.43 ±0.80	2.38 ±0.28	0.24 ±0.07
6	7.35 ±0.51	3.27 ±0.21	0.85 ±0.06
9	4.64 ±0.57	1.49 ±0.21	0.48 ±0.08
12	4.13 ±0.61	0.57 ±0.13	0.15 ±0.06
Controls			
Emasculated, not pollinated Harvested 9 days after anthesis	0.19 ±0.08	0.13 ±0.07	0.01 ±0.01
Not emasculated, not pollinated Harvested 9 days after anthesis	2.70 ±0.37	0.38 ±0.17	0.11 ±0.09

Fig. 8.5 Fluorescence micrograph of pollen tube (arrows) in lower style of *B. coccinea* 9 days after pollination. Scale bar:100µm.

Fig. 8.6 Fluorescence micrograph of pollen tube (t) penetrating the micropyle of a *B. coccinea* ovule 9 days after pollination. Scale bar: 50µm.

Fig. 8.7 Follicles (arrows) on mature infructescence of *B. coccinea*. Scale bar: 10mm.


5


6
t

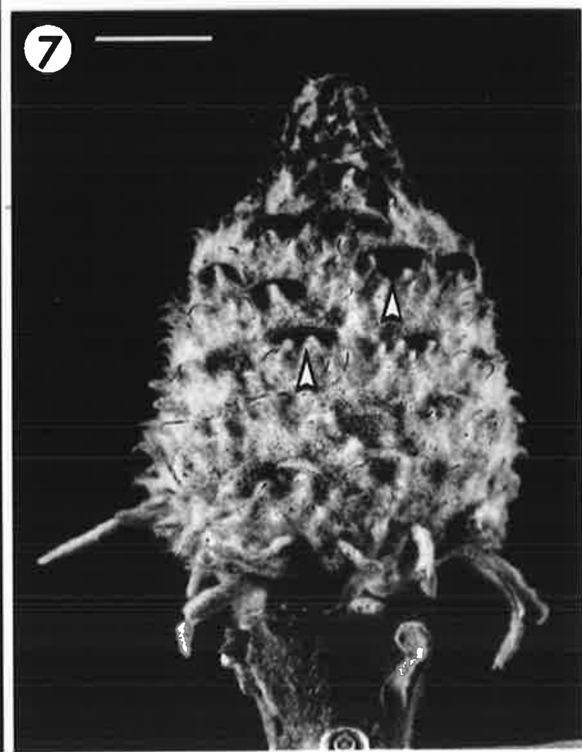

7

Table 8.3 Pollen tube growth following self- and cross-pollination of five *B. coccinea* plants. s_{ij} is the number of styles for female i and male j; p_{ij} is the probability that a pollen tube in the pollen presenter segment of the style reaches the given style section for female i and male j.

			Male				
			A	B	C	D	E
Female	A	s_{ij} =	41	35	44	36	39
		p_{ij} (upper style) =	0·589	0·590	0·652	0·598	0·662
		p_{ij} (lower style) =	0·130	0·220	0·261	0·213	0·349
	B		49	32	47	38	41
			0·135	0·071	0·035	0·046	0·116
			0·093	0·020	0·014	0·035	0·065
	C		37	39	49	38	54
			0·439	0·267	0·586	0·316	0·318
			0·167	0·046	0·085	0·194	0·148
	D		50	30	25	41	34
			0·421	0·129	0·157	0·217	0·227
			0·108	0·046	0·090	0·109	0·102
	E		90	43	35	33	45
			0·202	0·357	0·390	0·270	0·226
			0·106	0·152	0·220	0·135	0·079

Table 8.4 *B. coccinea* crosses in order of decreasing fertility based on $p_{ij(lower\ style)}$ values presented in Table 8.3.

Female parent X Male parent
A X E
A X C
E X C
A X B
A X D
C X D
C X A
E X B
C X E
E X D
A X A
D X D
D X A
E X A
D X E
B X A
D X C
C X C
E X E
B X E
C X B ≡ D X B
B X D
B X B
B X C

Table 8.5 Analysis of variance of *B. coccinea* cross-pollination experiment as measured by pollen tube growth.

Source of variation	Degrees of freedom	Mean squares	Significance
Mean	1	30·6	P<0·001
General combining ability	4	0·9	P<0·001
Specific combining ability	5	0·2	P=0·05
Reciprocal	10	0·4	P<0·001
Error	817	0·8	
Total	837		

Table 8.6 Variance components for *B. coccinea* cross-pollination experiment as measured by pollen tube growth.

Source of variation	Value	Standard deviation	Percentage of total variance
General combining ability	0·00272	0·00215	2·96
Specific combining ability	0·00150	0·00143	1·63
Reciprocal	0·00418	0·00213	4·54
Error	0·08357	0·00413	90·86

8.3.3.2 Seed set

Follicles developed on all inflorescences following hand pollination (Fig. 8.7). Cross-pollinated inflorescences produced an average of 40.7 ± 4.0 follicles from 307 florets, while 27.9 ± 2.8 follicles developed from selfing. In 75.4% of the follicles resulting from cross pollination and 62.2% from selfing, one seed developed per follicle, and no mature seeds were observed in the remaining follicles. This represents a fruit set of 10% following crossing and 5.6% following selfing.

8.3.4 Experiment 4: Natural fruit set

There were significant differences between trees in the number of follicles per infructescence at both King River ($p<0.01$) and Blewitt Springs ($p<0.01$), and highly significant differences between the three sites ($p<0.001$) (Table 8.7). Few inflorescences set no follicles.

8.4 CONCLUSIONS

The flowers of *B. coccinea* are protandrous. The stigma surface is dry at anthesis, but peak receptivity at 3 days post-anthesis coincides with an increase in the production of exudate.

B. coccinea showed partial stylar self-incompatibility with pollen tube growth inhibited in the upper region of the style, however there were no observed abnormalities in pollen tube growth. Self-incompatibility acting at the pre-zygotic level can act in the ovary, as well as the style, by preventing fertilization (Seavey and Bawa, 1986). Post-zygotic mechanisms have also been reported, resulting in selective abortion of immature seeds. Either of these mechanisms could contribute to the reduced seed production in *B. coccinea* following selfing, although the latter is more likely as remnants of seeds were often observed. Genetic control of fertility is suggested in *B. coccinea* by the observation of variable fertility, specific and general combining ability and reciprocal effects between plants.

In addition to the protandrous nature of the florets and a partial self-incompatibility mechanism, it is likely that the spatial limitation of the infructescence of *B. coccinea* may pose a constraint on the level of fruit set. *Banksia* seeds are produced in large woody follicles (George, 1987), and the dimensions of the infructescence pose a spatial limitation on the number that can ultimately develop to maturity (Table 8.8). A maximum of 148 mature follicles can be accommodated on a *B. coccinea* infructescence, thus giving a maximum potential fruit to flower ratio of 48·2%. However even following controlled cross pollination inflorescences produced on average only 40.7 follicles, a fruit to flower ratio of 13.3% and a seed to flower ratio of 10%, while pollen tubes were observed in the final segment of 85% of styles. The highest fruit to flower ratio for *B. coccinea* was observed in the commercial plantation situation where the plants received irrigation, fertiliser and weed control, suggesting that the availability and allocation of resources may contribute to fruit set to flower ratios.

Table 8.7 Fruit set of *B. coccinea* following open-pollination. (mean ± standard error).

Location	Percentage of barren infructescences per tree	Mean number of follicles per infructescence[1]	Maximum number of follicles per infructescence	Percentage of florets which set follicles per infructescence
Blewitt Springs, S.A.	0.0%	27.3	43	8.1%
	±0.0%	±2.0		±0.7%
Happy Valley, S.A.	0.0%	17.2	25	5.6%
	±0.0%	±0.8		±0.3%
King River, W.A.	1.3%	15.2	30	5.0%
	±1.3%	±0.6		±0.2%

[1]Significant differences between sites, $\chi^2_{2,\ (0{\cdot}001)} = 13.8$.

Table 8.8 Potential number of follicles and seeds per infructescence of *B. coccinea*.

Mean infructescence length (cm)	3·4 ± 0·2
Mean infructescence diameter (cm)	2·9 ± 0·1
Average surface area of fertile infructescence (cm^2)	31·1
Mean follicle length (cm)	0·7 ± 0·0
Mean follicle width (cm)	0·3 ± 0·0
Average area of follicle[1] (cm^2)	0·2
Number of florets per inflorescence[2]	307
Potential number of follicles	307
Potential maximum number of seeds	614
Potential number of follicles which can be accommodated	148
Percentage of potential number of follicles	48·2%
Potential maximum number of seeds which can be accommodated	298
Number of seeds which develop per follicle	0·75 ± 0·02
Potential number of seeds	111

[1]Calculated as a rectangle.

[2]Calculated using regression equation.

9. GENERAL DISCUSSION

For banksias to compete successfully in the market place with traditional cut flower crops, such as roses, carnations and chrysanthemums, a continued supply of a quality product is essential. This project has emphasised the importance of crop improvement in achieving this goal, and has made progress towards improving two Western Australian species, *B. coccinea* and *B. menziesii*, for cut flower production. This has been achieved by following three main objectives. The first was to ascertain the growth cycles of these species so that plantation management practices and strategies can be established on a sound basis. The second was to investigate the degree of variability which exists in seedling populations of these species and to use this to develop criteria for selection programmes. The third was to develop hybridisation techniques to be used in breeding programmes, and to investigate the breeding system of these species to delineate the limitations which may be faced in such programmes.

The development of suitable management practices and strategies will lead to improved production in the near future. However, to develop practices such as pruning, irrigating and fertiliser application for any cut flower crop, a knowledge of the relationship between the vegetative and reproductive growth cycles, and in particular the timing of floral initiation, is required. The benefits derived from pruning woody perennial crops have long been known to the horticultural industry (Mika, 1986). These benefits include maximising productivity, creating a desired plant shape and size, prolonging the productive life of the plant and controlling disease. Brits *et al.* (1986) made a distinction between two types of pruning to be used on proteas; heading back and thinning out. Heading back is aimed at stimulating new growth by cutting shoots to leave several basal nodes with healthy green leaves, from which new shoots can emerge. Thinning out refers to the removal of shoots at the base, flush with the branch from which they arise, to reduce the complexity of shoot arrangement. Based on the results of this study we are now able to quantify pruning requirements for *B. coccinea* and *B. menziesii*. Shoots with a minimum basal diameter of 4.5mm for *B. coccinea* and 6mm for *B. menziesii* have the highest probability of producing an inflorescence, and these should be

encouraged by the removal of narrower shoots from the bush. Basal shoot diameter is not a convenient measurement to be routinely taken in the plantation, hence the attempt to correlate this parameter with other more practical measurements such as shoot length and number of leaves. Although many of these correlations were highly positive, the between plant and between year variation rendered them of limited use in this seedling-based plantation. Such characters would be of more use in a clonal plantation when commercial vegetative propagation techniques have been developed for banksias. It should be stressed that these figures relate specifically to *B. coccinea* and *B. menziesii* under the cultural and environmental conditions of Blewitt Springs, and that absolute values will vary under other circumstances, including location and species. In addition these methods need to be tested for each species to determine the number of nodes to be left when heading back shoots, to create a balance of vegetative and potentially reproductive growth to maximise the production of quality blooms.

Floral initiation in *B. coccinea* and *B. menziesii* cultivated under South Australian conditions occurs in late spring. In *B. menziesii* there are several months between peak bloom production in May, and floral initiation and the commencement of new growth in October. However, in *B. coccinea*, the flowering season extends from July to November, with the peak in October, thus new growth commences and inflorescences are initiated while the bushes are flowering. For this reason, it is important that pruning of *B. coccinea* is conducted in conjunction with the harvesting of blooms. Even so, some late blooms may need to be sacrificed to ensure good production in the following year. Delaying pruning until the completion of flowering may result in the removal of potential flowering shoots or in lateral buds insufficiently developed to produce an inflorescence in the following season.

The timing of floral initiation in both species suggests that they may respond to conditions of increasing daylength and increasing temperature. This is also likely for *B. baxteri* and *B. hookeriana* cultivated at Muchea, W.A., where the transition to flowering was observed in spring and early summer respectively (L. Rohl, unpublished data). This contrasts with studies on other genera of Proteaceae which have all indicated short day

conditions to be inductive (Moncur *et al.*, 1985; Ben-Jaacov and Kadman-Zahaui, 1988; Malan and Jacobs, 1988; Wallerstein, 1989), while Storey (1985) suggests that *Macadamia* is a day-neutral plant and that lower temperatures may play a more important role. It is possible, however, that floral induction in *Banksia* may be occurring under conditions of shorter daylength and lower temperatures, but that the first anatomical manifestation of initiation is delayed.

In its natural environment *B. coccinea* is confined to a small region centred around Albany, W.A. Production of inflorescences on plants of this species under cultivation north of Perth, W.A., has proved difficult (Windle *et al.*, 1990), suggesting that *B. coccinea* may have fairly rigid requirements for floral initiation and development and that the higher temperatures at Muchea may inhibit floral initiation, resulting in lower yields. Above optimal temperatures are known to inhibit floral initiation in *Chrysanthemum* (Cathey, 1954; Whealy *et al.*, 1987) and in the Australian natives, *Helipterum roseum* (Sharman *et al.*, 1989a, b) and *Acacia pycnantha* (Sedgley, 1985). At Blewitt Springs the lower temperatures, and the low rainfall in the summer months, which contrast with conditions in the natural habitat near Albany, may have the effect of retarding microscopic development of *B. coccinea* inflorescences or of imposing an eco-dormancy (Lang *et al.*, 1987), as macroscopic appearance of the blooms coincides with a marked increase in the amount of precipitation. Similarly, lower temperatures at Millicent are suggested to affect floral development resulting in the high incidence of abnormality.

B. menziesii grows naturally over a wide area in Western Australia, and also crops successfully away from its natural habitat. This greater tolerance of climatic variability may reflect less stringent requirements for floral initiation than those of *B. coccinea*. However, abortion of *B. menziesii* inflorescences at Blewitt Springs during the early stages of development may also be a temperature effect. The annual mean temperature at Blewitt Springs is less than that over the natural range of *B. menziesii* in Western Australia. Abortion has been reported to occur in *Telopea* (Faragher, 1989), and also in *Leucospermum patersonii* under a low temperature regime (17/12°C) plus

long days (Wallerstein, 1989). Sub-optimal temperatures also caused flower bud atrophy in 'Baccara' roses (Zieslin and Halevy, 1975) and terminal flower bud abortion in *Paeonia lactiflora* (Evans *et al.*, 1990).

These findings emphasise the importance of understanding the control of flowering in relation to site selection. Such information will also be essential for the development of management techniques to manipulate the growth cycles of *Banksia* plants to extend their natural flowering period. This has been achieved for the traditional cut flower crops, and growers are able to control environmental factors such as daylength and temperature to produce flowers on a year round basis (Larson, 1980). In addition they also have the flexibility to target specific marketing periods such as Mothers' day and Christmas, when demand for cut flowers is high. Thus an important area for future research is investigation of the environmental control of the flowering of banksias.

Considerable improvements in crop productivity and quality can also be gained in the near future by selection of superior genotypes and clonal propagation. This study has illustrated the plant to plant variability in time of production, yield, bloom quality and bloom colour which exists in seedling plantations of *B. coccinea* and *B. menziesii*. The range in production times between trees offers the opportunity to select for early and late blooming cultivars to extend the production season to the maximum. Care must be taken, however, to ensure that late blooming cultivars are also selected for high bloom quality, as there is a tendency for a reduction in the quality of blooms of both species as the season progresses.

Selection criteria for *B. coccinea* and *B. menziesii* based on bloom quality have been identified in this study. However the attraction of a cut flower are greatly influenced by its associated vegetative growth, and it is desirable to select for vegetative characters which will enhance the appearance of the overall floral display (Wilfret, 1987). For this reason it is proposed that *B. coccinea* plants should be selected to minimise probability of double flushing, as the unattractive region of bare stem which occurs at the base of the second flush is undesirable in the production of a high quality cut flower. Leaf

size, shape and density on the shoot were highly variable in both *B. coccinea* and *B. menziesii*. This provides ample opportunity for selection if these become of importance as the market for banksia blooms becomes more sophisticated and leaf characters assume greater importance.

Since between plant variation is considered to be primarily genetic, there is also the opportunity to combine several desirable characters through carefully controlled breeding and selection programmes. However the success of a breeding programme can be limited by a number of factors pertaining to the breeding system of a species. The low natural percentage seed set in both *B. coccinea* and *B. menziesii* suggests that such factors may influene the fruit to flower ratio following open pollination and are likely to be encountered in controlled breeding programmes. Several of these factors have been identified in this study.

One such limiting factor may be the timing of stigma receptivity. This study has established that peak stigma receptivity of both species occurs 3 days after anthesis and that hand pollinations should be conducted on that day to maximise the likelihood of pollen germination and subsequent seed set. In *B. coccinea* this coincides with the production of stigma exudate, a phenomonon also recorded in *Macadamia* (Sedgley *et al.*, 1985) and *Grevillea* (Lamont, 1982; Herscovitch and Martin, 1989). These findings are not in accordance with those of Heslop-Harrison and Shivanna (1977), who classified Proteaceae flowers in the dry papillate stigma group. However observations of the stigmatic groove of *B. menziesii* and *B. prionotes* (Collins and Spice, 1986) also showed no visible secretion, suggesting there may be heterogeneity of stigma type within the family. In the latter two species, the stigmatic groove displays an opening and closing mechanism following anthesis. For successful pollination, pollen must be placed within the groove, and in *B. menziesii* this can only occur when the groove attains its widest dimension. Hence it is likely that this mechanism reduces self pollination, as self pollen is usually removed by foraging fauna from the pollen presenter within hours of anthesis (Collins and Spice, 1986). Further research is required to determine whether secretion is produced within the depths of the groove, or if in fact it is truly a dry stigma.

A further limitation to the breeding system of many plants is a self-incompatibility mechanism (Knox, 1984). In many species of *Banksia* it appears outcrossing is essential for seed set (Scott, 1980; Collins and Spice, 1986; Carthew *et al.*, 1988), whereas in other species there is also evidence of seed set following self pollination (Carpenter and Recher, 1979; Paton and Turner, 1985). Cross pollination of *B. spinulosa* (Vaughton, 1988), *B. paludosa* (Goldingay and Whelan, 1990) and the two species in this study did, however, increase the number of follicles per infructescence over that of selfed inflorescences. In *B. coccinea* this partial self-incompatibility was found to be the result of pollen tube growth being inhibited in the upper style and appears to be under genetic control. *Macadamia* similarly shows a degree of self-incompatibility with improved pollen tube growth and initial seed set following cross pollination (Sedgley *et al.*, 1990).

The low level of fruit set observed in both species is also of concern. Woody perennials producing hermaphrodite flowers which exhibit self-incompatibility are characterised by having some of the lowest fruit to flower ratios in the plant kingdom (Sutherland, 1986), and low fertility is extremely common amongst members of the Proteaceae (Collins and Rebelo, 1987). However protandry and partial self-incompatibility alone cannot account for the low fruit to flower ratios observed in *Banksia*. It is proposed that the dimensions of the infructescence and the follicles pose a spatial limitation to the number of follicles which can ultimately develop to maturity. However in the two species studied, the number of follicles which developed per infructescence, even following controlled cross-pollination at peak receptivity, was far short of the potential which could be accommodated.

There are several hypotheses relating the apparent over-production of flowers to the low reproductive success in mass flowering species such as *Banksia*: (1) pollinator limitation; (2) pollinator attraction; (3) bet hedging; (4) selective abortion; and (5) pollen donation (Stephenson, 1981; Sutherland and Delph, 1984). These hypotheses are

not mutually exclusive but will be discussed individually in relation to the low reproductive success of banksias, and in the light of this study.

Pollinator limitation suggests that low fruit set is due to either low pollinator densities and/or infrequent visits of potential pollinators, and that if either of these problems were rectified fruit set would increase (Sutherland and Delph, 1984). However studies on the role of honeyeaters, small mammals and insects as pollinators have suggested that they are not a limiting factor in fruit set in *B. ericifolia*, *B. prionotes*, *B. menziesii* and *B. spinulosa*, and repeated addition of cross pollen to inflorescences of *B. ericifolia*, *B. paludosa* and *B. spinulosa* did not result in increased follicle production over open pollination (Paton and Turner, 1985; Collins and Spice, 1986; Whelan and Goldingay, 1986; Ramsey, 1989; Vaughton, 1990). Similarly in this study, controlled cross pollination of entire inflorescences of *B. coccinea* and *B. menziesii* did not markedly improve fruit set, although pollination of only a few florets on inflorescences of *B. menziesii* resulted in a high proportion of the florets setting fruit.

The pollinator attraction theory states that a large floral display is likely to attract potential pollinators and thus result in effective pollination (Meeuse and Morris, 1984). Paton and Turner (1985) observed that smaller inflorescences of *B. ericifolia* (≤16cm) were less likely to produce fertile infructescences than larger inflorescences (>16cm) but there was no significant relationship between inflorescence size and the number of follicles produced in those which did set seed. Both *B. coccinea* and *B. menziesii* display their flowers in conspicuous terminal inflorescences consisting of hundreds of individual florets clustered together around a central axis. Inflorescences of these species are visited by nectar feeding marsupials and honeyeaters (Wooller *et al.*, 1983; Ramsey, 1989). However these species, along with many other members of the Proteaceae, which produce large inflorescences, set only a few fruit (Collins and Rebelo, 1987). Assuming, however, that there is no differential fertility of florets based on inflorescence size, inflorescence size is not an issue with regard to controlled hand pollinations.

The concept of bet hedging suggests that plants produce large numbers of flowers to compensate for variations in resource availability for fruit maturation and in pollination success (Stephenson, 1980). Pollination success has effectively been dealt with under pollinator limitation and pollinator attraction, and has been shown to be an unlikely cause of low seed set in *Banksia*. Resource availability has frequently been suggested to be the cause of low fruit set in *Banksia* (Abbott, 1985; Paton and Turner, 1985; Whelan and Goldingay, 1986; Lamont and Barrett, 1988; Ramsey, 1989; Vaughton, 1990). It is interesting to note that in this study the highest levels of fruit set following open pollination of *B. coccinea* and *B. menziesii* were recorded for plants under cultivation at Blewitt Springs. This may reflect the annual application of fertiliser, irrigation to supplement rainfall during the summer months, weed control, and the continual removal of blooms. However the application of low phosphorus fertiliser to natural stands of *B. spinulosa*, while increasing the concentration of nitrogen, phosphorus and potassium in the leaves, did not significantly increase natural fruit set over that of untreated control plants (Wallace and O'Dowd, 1989). Further research is required to determine whether the availability of resources is limiting fruit set.

The bet hedging theory could also be extended to include compensation for predation of flowers and fruits. In *Banksia* there is a delay of several months between floral initiation, anthesis and subsequent seed maturation. During this period, reproductive success may be reduced by insect and bird damage to the inflorescence and developing seeds (Scott and Black, 1981; Scott, 1982; Zammitt and Hood, 1986; Lamont and van Leeuwen, 1988; Vaughton 1988; Vaughton, 1990), and this study has indicated that insect attack may be associated with some of the floral abnormalities observed near Mt. Gambier, S.A. Therefore, the more flowers produced, the greater the potential of providing seeds for the next generation.

Selective abortion of seed and/or fruits is likely to occur in plants which produce many flowers and have a high pollination success rate, and thus initiate more seeds or fruits than can possibly be matured (Stephenson, 1981). Abortion of seeds within a

fruit, or fruits within an infructescence, can be dependent on postion, order of pollination, number of developing seeds or source of pollen.

Position-dependent abortion in wild radish is based on a resource gradient from the basal to the stylar end of the fruit (Marshall and Ellstrand, 1988). Given the structure of a *Banksia* inflorescence, a resource gradient would exist along the length of the inflorescence from the base to the apex and could lead to a skewed distribution of follicles along the axis. In *B. ericifolia*, *B. oblongifolia* and *B. spinulosa* fewer follicles developed in the apical third of infructescences (Zammitt and Hood, 1986; Vaughton, 1988). In *B. coccinea*, in this study the top of the infructescences tended to develop no follicles, in keeping with this theory, whereas *B. menziesii* did not appear to produce a skewed distribution of follicles along the central axis.

Fruits which develop from flowers pollinated early are more likely to mature than those developing from later pollinations (Stephenson, 1980, 1981). The ordered sequence of flowering, and hence order of pollination of flowers, along a *Banksia* inflorescence suggests the likelihood of selective abortion based on temporal differences in pollination. The acropetal sequence of flowering in *B. coccinea* and *B. oblongifolia* could explain the predominantly basal distribution of follicles based on selective abortion of later pollinated flowers. However, based on this theory, the follicle distribution and the basipetal flowering in *B. spinulosa* and *B. ericifolia* are contradictory. In fact a higher proportion of seed aborted in the apical third of *B. spinulosa* inflorescences (Vaughton, 1988), suggesting that resource gradients may have a greater influence on which florets set seed than the time of pollination. This has implications on which florets are likely to be most profitable for hand pollination in breeding programmes, and is an area requiring further research.

Selective abortion of seeds and fruits based on pollen source may occur in favour of retaining those of higher genetic quality (Stephenson, 1981), and may result from the accumulation of lethal and sub-lethal genes (Weins, 1984). This happens particularly following self-pollination, and fruits developing from self-pollinated flowers are often

retained only in the absence of fruits from cross-pollination (Bushnell, 1920; Haber, 1928) or when total fruit set is low (Urata, 1954). This would effectively give the maternal parent some control over choice of mate. The results of this study show that in *B. coccinea* genetic interactions are very important, with pre-zygotic reduction in success following self-pollination and significant general and specific combing ability and reciprocal effects following cross-pollination.

Finally it is possible that not all flowers in a population are "equisexual", despite appearing morphologically hermaphrodite (Horovitz, 1978), and it has been suggested that many perfect flowers may function only as pollen donors (Sutherland and Delph, 1984). Johnson and Briggs (1963) reported that in some species of *Banksia* many apparently perfect flowers did not contain ovules, but provided no indication regarding the proportion of total flower numbers or which species. Such a phenomenon may be an evolutionary trend towards dioecy. If this theory holds, it would be expected that fruit to flower ratios would be lower for hermaphrodite species than for monoecious and dioecious species, as they are based on total number of flowers rather than the number of female flowers. This has been demonstrated in the Proteaceae, with the two dioecious genera, *Aulax* and Leucodendron, having a much higher percentage fruit set than hermaphrodite genera (Rouke and Rebelo, 1985; Collins and Rebelo, 1987). Detailed investigations of floral ontogeny in *Banksia* are required to determine if flowers show differential fertility, as this would have a very real effect on the rate in success of a breeding programme.

10. CONCLUSIONS

This study has contributed to the improvement of banksias for cut flower production by considering the current system of cut flower production based on plants raised from seed collected from the wild. We now know when floral initiation occurs in relation to the seasonal progression and the growth cycle of *B. coccinea* and *B. menziesii* plants under cultivation at Blewitt Springs, S.A., and this information has been used to develop pruning criteria. The between plant variability which exists in seedling based plantations of *B. coccinea* and *B. menziesii* has been studied and criteria for selecting superior plants of these species established. These criteria will aid in cultivar selection, but there is an urgent need to develop suitable clonal propagation techniques. Hybridisation techniques have been developed and can be used in breeding programmes to combine the characteristics of superior plants.

RECOMMENDATIONS FOR COMMERCIAL *BANKSIA* PRODUCTION

Pruning

It is recommended that shoots producing blooms be headed back at harvest, to leave several basal nodes with healthy green leaves from which regrowth can occur. Additional pruning should be conducted after the flowering period but before the commencement of the vegetative growth flush and floral initiation. Therefore pruning should be conducted during July and August for *B. menziesii* and in early November for *B. coccinea*. At pruning shoots with a basal diameter of less than 6.0mm for *B. menziesii* and 4.5mm for *B. coccinea* should be removed, while thicker shoots which are bent or more than two years old should be headed back.

Selection

Growers should attempt to identify plants which are high yielding and produce a high proportion of top quality blooms from which to propagate clonal material. Selection should also be for early and late flowering varieties.

Future research work

Determine the environmental controls of flowering, including temperature and daylength.

Develop clonal propagation techniques.

Select superior genotypes based on the time of production, yield, bloom quality and bloom colour.

Establish controlled breeding programmes to produce improved cultivars and novelty products for the cutflower trade.

11. REFERENCES

Abbott, I. (1985). Rate of growth of *Banksia grandis* Willd. (Proteaceae) in Western Australian forest. *Australian Journal of Botany*, 33: 381-391.

Ager, A.A. and Guries, R.P. (1982). Barriers to interspecific hybridization in *Ulmus americana*. *Euphytica*, 31: 909-920.

Amos, J. (1987). Leucadendron breeding. *Journal of the International Protea Association*, 13: 23-4.

Austraflora Nurseries Pty. Ltd. (1989). *Banksia serrata* 'Austraflora Pygmy Possum'. *Australian Plants*, 15: 200.

Banno, K., Hayashi, S. and Tanabe, K. (1986). Morphological and histological studies on flower bud differentiation and development in Japanese pear (*Pyrus serotina* Rehd.). *Journal of the Japanese Society for Horticultural Science*, 55: 258-265.

Barnard, C. and Read, F.M. (1933). Studies of growth and fruit bud formation. IV. A year's obsevartions on growth and fruit bud formation in the apricot and peach. *Journal of Agriculture, Victoria*, 31: 44-52.

Barth, G.E. (1986). Cut flowers - the industry in review - in South Australia. *Australian Horticulture*, 84: 32, 34-5.

Ben-Jaacov, J. and Kadman-Zahavi, A. (1988). Preliminary observations on bud and flower differentiation in *Leucadendron discolor*. *Journal of the International Protea Association*, 16: 44.

Ben-Jaacov, J., Shillo, R. and Avishai, M. (1986). Technology for rapid production of flowering pot-plants of *Leucadendron discolor* E. Phillips, S. Hutch. *Scientia Horticulturae*, 28: 379-387.

Bernier, G., Kinet, J-M. and Sachs, R.M. (1981). 'The Physiology of Flowering.' Vols 1, 2 and 3. (C.R.C. Press: Boca Raton, Florida, USA.)

Blackmore, S. and Toothill, E. (Eds). (1984). 'The Penguin Dictionary of Botany.' (Penguin Books: Middlesex, England.)

Blake, S.T. (1971). Flowering and seeding habits in some species of *Banskia*. *Queensland Naturalist*, 20: 21-24.

Bliss, F.A. (1984). The application of new biotechnology to crop improvement. *HortScience*, 19: 43-48.

Brewbaker, J.L. (1967). The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the angiosperms. *American Journal of Botany*, 54: 1069-1067.

Briggs, B.G., Hyland, B.P.M. and Johnson, L.A.S. (1975). *Sphalmium*, a distinctive new genus of Proteaceae from north Queensland. *Australian Journal of Botany*, 23: 165-172.

British Colour Council and The Royal Horticultural Society. (1938-1941). 'Horticultural Colour Chart.'

Brits, G.J (1984). Production research on South African proteas - current trends. *Veld and Flora*, 70: 103-105.

Brits, G.J. (1985a). Scarlet Ribbon - the first pastel coloured pincushion cultivar. *Horticultural Science*, 3: 31.

Brits, G.J. (1985b). Sunrise - a new early-flowering red pincushion cultivar. *Horticultural Science*, 3: 32.

Brits, G.J. (1988). Cultivar registration - international protea cultivar registration. *Protea News*, 7: 3-10.

Brits, G.J. and van den Berg, G.C. (1990). Interspecific hybridisation in *Protea*, *Leucospermum* and *Leucadendron* (Proteaceae). *In:* 'Prodeedings of the XXIII International Horticultural Congress'. (p. 1222.)

Brits, G.J., Jacobs, G. and Steenkamp, J.C. (1986). The pruning of proteas for cut flower production. Farming in South Africa: flowers and ornamental shrubs, B.15.

Broertjes, C. and van Harten, A.M. (1988). 'Applied Mutation Breeding for Vegetatively Propagated Plants.' (Elsevier Science Publishers: Amsterdam, The Netherlands.)

Brough, P. (1933). The life history of *Grevillea robusta* Cunn. *Proceedings of the Linnean Society of New South Wales*, 58: 33-73.

Brown, R. (1810). On the natural order of plants called Proteaceae. *Transactions of the Linnean Society of London*, 10: 67-226.

Brown, R. (1830). 'Supplementum Primum Prodromi Florae Novae Hollandiae'. (Taylor: London, England.)

Buban, T. and Faust, M. (1982). Flower bud induction in apple trees: internal control and differentiation. *Horticultural Reviews*, 4: 174-195.

Bushnell, J.W. (1920). The fertility and fruiting habit in *Cucurbita*. *Proceedings of the American Society for Horticultural Science*, 15: 47-51.

Butler, G. (1986). A record of registered cultivars. *Australian Horticulture*, 84: 18-21.

Carolin, R. (1961). Pollination of Proteaceae. *Australian Journal of Natural History* (formerly *Australian Museum Magazine*), 13: 371-374.

Carpenter, F.L. (1978). Hooks for mammal pollination? *Oecologia*, 35: 123-132.

Carpenter, F.L. and Recher, H.F. (1979). Pollination, reproduction, and fire. *American Naturalist*, 113: 871-879.

Carthew, S.M., Ayre, D.J. and 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.

Cathey, H.M. (1954). Chrysanthemum temperature study. C. The effect of night, day and mean temperature upon the flowering of *Chrysanthemum morifolium*. *Proceedings of the American Society for Horticultural Science*, 64: 499-502.

Chin, H.F. and Yong, H.S. (1980). 'Malaysian Fruits in Colour'. (Tropical Press: Kuala Lumpar, Malaysia.)

Coetzee, J.H. and Giliomee, J.H. (1985). Insects in association with the inflorescence of *Protea repens* (L.) (Proteaceae) and their role in pollination. *Journal of the Entomological Society of South Africa*, 48: 303-314.

Collins, B.G. and Rebelo, T. (1987). Pollination biology of the Proteaceae in Australia and southern Africa. *Australian Journal of Ecology*, 12: 387-421.

Collins, B.G. and Spice, J. (1986). Honeyeaters and the pollination biology of *Banksia prionotes* (Proteaceae). *Australian Journal of Botany*, 34: 175-185.

Cooper, S.D. and McGraw, J.B. (1988). Constraints on reproductive potential at the level of the shoot module in three ericaceous shrubs. *Functional Ecology*, 2: 97-108.

Cope, F.W. (1962). The mechanism of pollen incompatibility in *Theobroma cacao*. *Heredity*, 117: 157-182.

Cowling, R.M., Lamont, B.B. and Pierce, S.M. (1987). Seed bank dynamics of four co-occurring *Banksia* species. *Journal of Ecology*, 75: 289-302.

Crane, M.B. and Brown, A.G. (1937). Incompatibility and sterility in sweet cherry, *Prunus avium* L. *Journal of Pomology and Horticultural Science*, 15: 86-116.

de Nettancourt, D. (1977). 'Incompatibility in Angiosperms'. (Springer-Verlag: Berlin, West Germany.)

Diaz, D.H., Rasmussen, H.P. and Dennis, F.G. (Jr.). (1981). Scanning electron microscope examination of flower bud differentiation in sour cherry. *Journal of the American Society for Horticultural Science,* 106: 513-515.

Dixon, I.R. (1986). First hybrid Banksia from Western Australia, *B. hookeriana* x *B. menziesii* (dwarf form). *Protea News,* 4: 35.

Doorenbos, J. (1955). Shortening the breeding cycle of rhododendron. *Euphytica,* 4: 141-146.

Dormer, K.J. (1972). 'Shoot Organization in Vascular Plants.' (Chapman and Hall: London, England.)

Elliot, W.R. and Jones, D.L. (1980). 'Encyclopedia of Australian Plants'. (Lothian Publishing Company: Melbourne, Australia.)

Elphick, P. (1985). Commercial production of banksias. *In* 'Horticulture of Australian Plants.' (Lamont, B.B. and Watkins, P.A., Eds) (Western Australian Department of Agriculture: Perth, Australia.) (pp. 86-91.)

Evans, R.M., Anderson, N.O. and Wilkins, H.F. (1990). Temperature and GA_3 effects on emergence and flowering of potted *Paeonia lactiflora. HortScience,* 25: 923-924.

Faegri, K. and van der Pijl, L. (1979). 'The Principles of Pollination Ecology'. (Pergamon Press: Oxford, England.)

Faragher, J.D. (1989). *Telopea. In:* 'CRC Handbook of Flowering'. Vol. 6. (Halevy, A.H., Ed.) (CRC Press: Boca Raton, Florida, USA.) (pp. 593-597.)

Ford, H.A., Paton, D.C. and Forde, N. (1977). Birds as pollinators of Australian plants. *New Zealand Journal of Botany,* 17: 509-519.

Forshey, C.G. and Elfving, D.C. (1983). The relationship between vegetative growth and fruiting in apple trees. *Horticultural Reviews*, 11: 229-287.

Forshey, C.G. and Marmo, C.A. (1985). Pruning and deblossoming effects on shoot growth and leaf area of 'McIntosh' apple trees. *Journal of the American Society for Horticultural Science*, 110: 128-132.

Forshey, C.G., Weires, R.W. and van Kirk, J.R. (1987). Seasonal development of the leaf canopy of 'Mcspur McIntosh' apple trees. *HortScience*, 20: 881-883.

Furr, J.R., Cooper, W.C. and Reece, P.C. (1947). Flower formation in citrus trees. *American Journal of Botany*, 34: 1-8.

Gardner, F.E. (1929). The relationship between tree age and rooting of cuttings. *Proceedings of the American Society for Horticultural Science*, 26: 101.

George, A. S. (1984). The remarkable banksias - wildflowers of great potential. *Australian Horticulture*, 82: 16-8, 21.

George, A.S. (1981). The genus *Banksia*. *Nuytsia*, 3: 239-474.

George, A.S. (1987). 'The Banksia Book.' (Kangaroo Press: Sydney, Australia.)

George, A.S. (1988). New taxa and notes on *Banksia* L.f. (Proteaceae). *Nuytsia*, 6: 309-317.

Goldingay, R.L. and Whelan, R.J. (1990). Breeding system and tests for pollen-limitation in two species of *Banksia*. *Australian Journal of Botany*, 38: 63-71.

Goldingay, R.L., Carthew, S.M. and Whelan, R.J. (1987). Transfer of *Banksia spinulosa* pollen by mammals: implications for pollination. *Australian Journal of Zoology*, 35: 319-325.

Gotham, J. and Butler, G. (1977). Western Australian banksias in Canberra. *Australian Plants*, 9: 113-114.

Greenwood, M.S. (1987). Rejuvenation of forest trees. *Plant Growth Regulation*, 6: 1-12.

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Sciences*, 9: 463-493.

Haber, E.S. (1928). Inbreeding the table green (Des Moines) squash. *Proceedings of the American Society for Horticultural Science*, 25: 111-114.

Hackett, W.P. (1985). Juvenility, maturation and rejuvenation in woody plants. *Horticultural Reviews*, 7: 109-155.

Hallé, F., Oldeman, R.A.A. and Tomlinson, P.B. (1978). 'Tropical Trees and Forests, an architectural analysis'. (Springer-Verlag: Berlin/Heidelberg, West Germany.)

Harre, J. (1988). 'Proteas: the Propagation and Production of Proteaceae'. (Fisher: Fielding, New Zealand.)

Hasek, R.F. (1980). Roses. *In:* 'Introduction to Floriculture'. (Larson, R.A., Ed.) (Academic Press: Orlando, Florida, USA.) (pp. 81-105.)

Hattingh, V. and Giliomee, J.H. (1989). Pollination of certain *Leucadendron* species (Proteaceae). *South African Journal of Botany*, 55: 387-393.

Heinsohn, R-D. and Pammenter, N.W. (1988). Seasonality of shoot growth and flowering in the fynbos shrub *Protea neriifolia* cultivated in a summer rainfall area. *South African Journal of Botany*, 54: 440-444.

Herscovitch, J.C. and Martin, A.R.H. (1989). Pollen-pistil interactions in *Grevillea banksii* - the pollen grain, stigma, transmitting tisue and *in vitro* pollinations. *Grana*, 28: 69-84.

Heslop-Harrison, J. (1975). Incompatibility and the pollen-stigma interaction. *Annual Review of Plant Physiology*, 26: 403-425.

Heslop-Harrison, Y. and Shivanna, K.R. (1977). The receptive surface of the angiosperm stigma. *Annals of Botany,* 41: 1233-1258.

Holliday, I. and Watton, G. (1975). 'A Field Guide to Banksias.' (Rigby: Adelaide, Australia.)

Holst, M.J. (1961). Experiments with flower promotion in *Picea glauca* (Moench) Voss. and *Pinus resinosa* Ait. *Recent Advances in Botany,* 2: 1654-1658.

Hopper, S.D. (1980). Bird and mammal pollen vectors on *Banksia* communities at Cheyne Beach, Western Australia. *Australian Journal of Botany,* 28: 61-75.

Horn, W. (1962). Breeding research on South African plants: II. Fertility of Proteaceae. *South African Journal of Botany,* 28: 259-268.

Horovitz, A. (1978). Is the hermaphrodite flowering plant equisexual? *American Journal of Botany,* 65: 485-486.

Ito, P.J., Murakami, T. and Parvin, P.E. (1978). Protea improvement by breeding. *Journal of the International Protea Association,* 13: 20-22.

Jackson, D.J. and Sweet, G.B. (1972). Flower initiation in temperate woody plants. (A review). *Horticultural Abstracts,* 42: 9-24.

Jacobs, G. (1983). Flower initiation and development in *Leucospermum* cv. Red Sunset. *Journal of the American Society for Horticultural Science,* 108: 32-35.

Jacobs, G. (1985). *Leucospermum. In:* 'CRC Handbook of Flowering'. Vol. 3. (Halevy, A.H., Ed.) (CRC Press: Boca Raton, Florida, USA.) (pp. 283-286.)

Jacobs, G. and Minnaar, H.R. (1980). Light intensity and flower development of *Leucospermum cordifolium. HortScience,* 15: 644-645.

Johnson, L.A.S. and Briggs, B.G. (1963). Evolution in the Proteaceae. *Australian Journal of Botany,* 11: 21-61.

Johnson, L.A.S. and Briggs, B.G. (1975). On the Proteaceae - the evolution and classification of a southern family. *Botanical Journal of the Linnean Society*, 70: 83-182.

Joyce, D.C. and Burton, N.W. (1989). Australian floriculture - a blooming field. *HortScience*, 24: 410, 531.

Keighery, G. (1985). An enigmatic *Banksia* population. *Banksia Atlas Newsletter*, 3: 13.

Kernick, C. (1986). Banksia cutflower production and an assessment of its potential. Thesis for B. Appl. Sci. Agric., Roseworthy Agricultual College, South Australia.

Kernick, J., Kaul, V. and Williams, E.G. (1986). Self-incompatibility in *Acacia retinodes*: site of pollen-tube arrest is the nucellus. *Planta*, 169: 245-250.

Knox, R.B. (1984). Pollen-pistil interactions. *In:* 'Encyclopedia of Plant Physiology'. Vol. 17. (Linskens, H-F., and Heslop-Harrison, J., Eds) (Springer-Verlag: Berlin, West Germany.) (pp. 508-608.)

Kozlowski, T.T. (1971). 'Growth and Development of Trees'. Vols 1 and 2. (Academic Press: New York, USA.)

Kuntze, O. (1891). 'Revisio Generum Plantarum'. (Felix: Leipzig, East Germany.)

Lamont, B.B. (1982). The reproductive biology of *Grevillea leucopteris* (Proteaceae), including reference to its glandular hairs and colonizing potential. *Flora*, 172: 1-20.

Lamont, B.B. and Barker, M.J. (1988). Seed bank dynamics of a serotinous, fire-sensitive *Banksia* species. *Australian Journal of Botany*, 36: 193-203.

Lamont, B.B. and Barrett, G.J. (1988). Constraints on seed production and storage in a root-suckering *Banksia*. *Australian Journal of Ecology*, 76: 1069-1082.

Lamont, B.B. and Collins, B.G. (1988). Flower colour change in *Banksia ilicifolia*: a signal for pollinators. *Australian Journal of Ecology*, 13: 129-135.

Lamont, B.B. and van Leeuwen, S.J. (1988). Seed production and mortality in a rare *Banksia* species. *Journal of Applied Ecology*, 25: 551-559.

Lamont, G.P. (1986). Australian native plants as cut flowers - report on study tour of the nursery and flower industries of Japan and U.S.A. *New South Wales Department of Agriculture Miscellaneous Bulletin*, 22: 29-33.

Lang, G.A., Early, J.D., Martin, G.C. and Darnell, R.L. (1987). Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. *HortScience*, 22: 371-377.

Larson, R.A. (Ed.) (1980). 'Introduction to Floriculture'. (Academic Press: New York, USA.)

Lewis, J. and Bell, D.T. (1981). Reproductive isolation of co-occurring *Banksia* species at Yule Brook Botany Reserve, Western Australia. *Australian Journal of Botany*, 29: 665-674.

Lloyd, D.G. and Webb, C.J. (1986). The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. *New Zealand Journal of Botany*, 24: 135-162.

Longman, K.A. and Wareing, P.F. (1959). Early induction of flowering in birch seedlings. *Nature*, 184: 2037-2038.

Lord, E.M. and Eckard, K.J. (1985). Shoot development in *Citrus sinensis* L. (Washington Navel orange). I. Floral and inflorescence ontogeny. *Botanical Gazette*, 146: 320-326.

Malan, D.G. and Jacobs, G. (1988). The influence of photoperiod on flower induction of *Leucospermum* cv. Red Sunset. *Protea News*, 8: 3-4.

Marshall, D.L. and Ellstrand, N.C. (1988). Effective mate choice in wild radish: evidence for selective seed abortion and its mechanism. *The American Naturalist*, 131: 739-756.

Martin, A.R.H. and Herscovitch, J.C. (1989). Fertilizing pollen tubes parasitise ovary wall in *Grevillea*. *Annals of Botany*, 64: 163-165.

Martin, F. W. (1959). Staining and observing pollen tubes in the style by means of fluorescence. Stain Technology, 34: 125-128.

Matthews, J.D. (1963). Factors affecting the production of seed by forest trees. *Forestry Absracts*, 24: 1-13.

McCredie, T.A., Dixon, K.W. and Sivasithamparam, K. (1985). Variability in the resistance of *Banksia* L.f. species to *Phytophthora cinnamomi* Rands. *Australian Journal of Botany*, 33: 629-637.

McFarland, D.C. (1985). Flowering biology and phenology of *Banksia integrifolia* and *B. spinulosa* (Proteaceae) in New England National Park, N.S.W. *Australian Journal of Botany*, 33: 705-714.

McKay, J.W. (1942). Self-sterility in the Chinese chestnut (*Castanea mollissima*). *Proceedings of the American Society for Horticultural Science*, 41: 156-160.

McNamara, K.J. and Scott, J.K. (1983). A new species of *Bnaksia* (Proteaceae) from Eocence Merlinleigh sandstone of the Kennedy Range, Western Australia. *Alcheringa*, 7: 185-193.

Meeuse, B. and Morris, S. (1984). 'The sex life of flowers'. (Facts of Life: New York, USA.)

Mika, A. (1986). Physiological responses of fruit trees to pruning. *Horticultural Reviews*, 8: 337-378.

Mol, J.M.N., Stuitje, A.R. and van der Krol, A. (1989a). Genetic manipulation of floral pigmentation genes. *Plant Molecular Biology,* 13: 287-294.

Mol, J.M.N., Stuitje, A.R., Gerats, A., van der Krol, A. and Jorgensen, R. (1989b). Saying it with genes: molecular flower breeding. *Trends in Biotechnology,* 7: 148-153.

Molyneux, W. M. (1990). *Banksia spinulosa* var. *spinulosa* 'Birthday Candles'. *Plant Varieties Journal,* 3(1): 5-6.

Moncur, M.W., Stephenson, R.A. and Trochoulias, T. (1985). Floral development of *Macadamia integrifolia* Maiden & Betche under Australian conditions. *Scientia Horticulturae,* 27: 87-96.

Mueller, F. (1879). 'Report on the Forest Resources of Western Australia'. (Reeve: London, England.)

Mullins, M. (1987). Towards the perfect waratah. *Australian Horticulture,* 85: 21-25.

Napier, D.R., Jacobs, G., Van Standen, J. and Forsyth, C. (1986). Cytokinins and flower development in *Leucospermum. Journal of the American Society for Horticultural Science,* 111: 776-780.

Owens, J.N. and Pharis, R.P. (1967). Initiation and ontogeny of the microsporangate cone in *Cupressus arizonica* in response to gibberellin. *American Journal of Botany,* 54: 1260-1272.

Parvin, P. (1981). Hybridization of Proteas in Hawaii. *In:* 'The growing and marketing of Proteas, report of the first international conference of Protea Growers' (Mathews, P., Ed.) (Proteaflora Enterprises: Melbourne, Australia.) (pp. 65-67.)

Parvin, P.E., Criley, R.A. and Bullock, R.M. (1973). Proteas: developmental research for a new cut flower crop. *HortScience*, 8: 299-303.

Paton, D.C. and Ford, H.A. (1977). Pollination by birds of native plants in South Australia. *Emu*, 77: 73-85.

Paton, D.C. and Turner, V. (1985). Pollination of *Banksia ericifolia* Smith: birds, mammals and insects as pollen vectors. *Australian Journal of Botany*, 33: 271-286.

Paton, D.M., Willing, R.R., Nicholls, W. and Pryor, L.D. (1970). Rooting of stem cuttings of *Eucalyptus*: a rooting inhibitor in adult tissues. *Australian Journal of Botany*, 18: 175-183.

Payne, C.D. (Ed.) (1987). "Genstat 5 Reference Manual'. (Oxford University Press: Oxford, England.)

Peach, S.E. (1989). *Banksia* 'Giant Candles'. *Australian Plants*, 15: 200.

Pegrum, J. (1988). Making the most of our floral resources. *Western Australian Journal of Agriculture*, 29: 115-118.

Pegrum, J., and Webb, M. (1990). An overview of the Western Australian native plant and protea industries. *Journal of the International Protea Association*, 19: 30-33.

Pharis, R.P. and Morf, W. (1967). Experiments on the precocious flowering of western red cedar and four species of *Cupressus* with gibberellins A_3 and A_4/A_7 mixture. *Canadian Journal of Botany*, 45: 1519-1524.

Pharis, R.P., Ross, S.D., Wample, R.L. and Owens, J.N. (1976). Promotion of flowering in conifers of *Pinaceae* by certain of the gibberellins. *Acta Horticulturae*, 56: 155-162.

Proctor, M. and Yeo, P. (1973). 'The Pollination of Flowers'. (Collins: London, England.)

Pyke, G.H. (1982). Fruit set in *Lambertia formosa* Sm. (Proteaceae). *Australian Journal of Botany,* 30: 39-45.

Pyke, G.H. (1987). Pollination biology of *Telopea speciosissima. In:* 'Waratahs - Their Biology, Cultivation and Conservation'. (Armstrong, J.A., Ed.) Australian National Botanic Gardens Occassional Publication No. 9: 35-43.

Ramsey, M.W. (1988). Floret opening in *Banksia menziesii* R.Br.; the importance of nectivorous birds. *Australian Journal of Botany,* 36: 225-232.

Ramsey, M.W. (1989). The seasonal abumdance and foraging behaviour of honeyeaters and their potential role in the pollination of *Banksia menziesii. Australian Journal of Ecology,* 14: 33-40.

Rebelo, A.G. (1985). Might mites pollinate proteas? South African Journal of Science, 81: 694.

Rodriguez Pérez, J.A. (1989). Introduction of proteas for cut flower and foliage in Tenerife. *Acta Horticulturae,* 246: 265-267.

Rouke, A.G. and Rebelo, J.P. (1985). Seed germination and seed se in southern African Proteaceae: ecological determinants and horticultural problems. *Acta Horticulturae,* 185: 75-88.

Rouke, J. and Weins, D. (1977). Convergent floral evolution in South African and Australian Proteaceae and its possible bearing on pollination by nonflying mammals. *Annals of the Missouri Botanical Garden,* 64: 1-17.

Rouke, J.P. (1980). Under cover agents - rodents as pollinators of dwarf proteas. *Veld and Flora,* 66: 54-56.

Roy, B. (1938). Studies on pollen tube growth in *Prunus*. *Journal of Pomology and Horticultural Science*, 16: 320-328.

Salkin, A. (1979). *Banksia canei* 'Celia Rosser'. *Australian Plants*, 10: 171.

Salkin, A. (1987). Breeding systems in Eastern Australian Banksias. *Victorian Naturalist*, 103: 43-47.

Salkin, A. (1989). *Banksia spinulosa* var. *cunninghamii* 'Lemon Glow'. *Australian Plants*, 15: 200.

Schaffer, H.E. and Usanis, R.A. (1969). General least squares analysis of diallel experiments. A computer program DIALL. Research Report No. 1 Genetics Department, North Carolina State University.

Schmitt, D. and Perry, T.O. (1964). Self-sterility in sweetgum. *Forest Science*, 10: 302-305.

Scholefield, P.B., Oag, D.R. and Sedgley, M. (1986). The relationship between vegetative and reproductive development in the mango in northern Australia. *Australian Journal of Agricultural Research*, 37: 425-433.

Scholefield, P.B., Sedgley, M. and Alexander, D.McE. (1985). Carbohydrate cycling in relation to shoot growth, floral initiation and development and yield in the avocado. *Scientia Horticulturae*, 25: 99-110.

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.

Scott, J.K. (1982). The impact of destructive insects on reproduction in six species of *Banksia* L.f. (Proteaceae). *Australian Journal of Zoology*, 30: 901-921.

Scott, J.K. and Black, R. (1981). Selective predation by white-tailed black cockatoos on fruit of *Banksia attenuata* containing the seed-eating weevil *Alphitopis nivea*. *Australian Wildlife Research*, 8: 421-430.

Seavey, S.R. and Bawa, K.S. (1986). Late-acting self-incompatibility in angiosperms. *Botanical Review*, 52: 195-219.

Sedgley, M. (1979). Inter-varietal pollen tube growth and ovule penetration in the avocado. *Euphytica*, 28: 25-35.

Sedgley, M. (1983). Pollen tube growth in macadamia. *Scientia Horticulturae*, 18: 333-341.

Sedgley, M. (1985). Some effects of temperature and light on floral initiation and development in *Acacia pycnantha*. *Australian Journal of Plant Physiology*, 12: 109-118.

Sedgley, M. and Griffin, A.R. (1989). 'Sexual Reproduction of Tree Crops.' (Academic Press: London, England.)

Sedgley, M. and Smith, R.M. (1989). Pistil receptivity and pollen tube growth in relation to the breeding system of *Eucalyptus woodwardii* (*Symphyomyrtus*: Myrtaceae). *Annals of Botany*, 64: 21-31.

Sedgley, M., Bell, D.H., Bell, D., Winks, C.W., Pattison, S.J. and Hancock, T.W. (1990). Self- and cross-compatibility of macadamia cultivars. *Journal of Horticultural Science*, 65: 205-213.

Sedgley, M., Blesing, M.A. and Vithanage, H.I.M.V. (1985). A developmental study of the structure and pollen receptivity of the *Macadamia* pistil in relation to protandry and self-incompatibility. *Botanical Gazette*, 146: 6-14.

Sharman, K.V. and Sedgley, M. (1988). Floral initiation and development in *Helipterum roseum* (Hook.) Benth. and *Helichrysum bracteatum* (Vent.) Andrews (Asteraceae). *Australian Journal of Botany*, 36: 575-587.

Sharman, K.V., Sedgley, M., and Aspinall, D. (1989a). Effects of photoperiod, temperature and plant age on floral initiation and inflorescence quality in the Australian native daisies *Helipterum roseum* and *Helichrysum bracteatum* in relation to cut-flower production. *Journal of Horticultural Science*, 64: 351-359.

Sharman, K.V., Sedgley, M., and Aspinall, D. (1989b). Production of the Australian native daisies (*Helipterum roseum* and *Helichrysum bracteatum*) for the cut flower market. *Australian Journal of Experimental Agriculture*, 29: 445-453.

Singh, R.N. (1960). Studies in the differentiation and development of fruit buds in mango (*Mangifera indica* L.). IV. Periodical changes in the chemical composition of shoots and their relation with fruit-bud differentiation. *Horticultural Advance*, 4: 48-60.

Small, J. (1915). The pollen-presentation mechanism in the Compositae. *Annals of Botany*, 29: 457-470.

Stephenson, A.G. (1980). Fruit set, Herbivory, fruit reduction, and the fruiting strategy of *Catalpa speciosa* (Bignoniaceae). *Ecology*, 61: 57-64.

Stephenson, A.G. (1981). Flower and fruit abortion: proximate causes and ultimate functions. *Annual Review of Ecology and Systematics*, 12: 253-279.

Stephenson, R.A. and Gallagher, E.C. (1986). Effects of night temperature on floral initiation and raceme development in macadamia. *Scientia Horticulturae*, 30: 213-218.

Storey, W.B. (1985). *Macadamia. In:* 'CRC Handbook of Flowering'. Vol. 3. (Halevy, A.H., Ed.) (CRC Press: Boca Raton, Florida, USA.) (pp. 347-355.)

Storey, W.B. and Saleeb, W.F. (1966). Genetics of four vegetative characteristics in an interspecific macadamia hybrid. *California Macadamia Society Yearbook*, 12: 77-88.

Suryanarayana, V. (1978). Seasonal changes in sugars, starch, nitrogen and C:N ratio in relation to flowering in mango. *Plant Biochemistry Journal*, 5: 108-117.

Sutherland, S. (1986). Patterns of fruit-set: what controls fruit-flower ratios in plants? *Evolution*, 40: 117-128.

Sutherland, S. and Delph, L.F. (1984). On the importance of male fitness in plants: patterns of fruit-set. *Ecology*, 65: 1093-1104.

Taylor, A. and Hopper, S. (1988). 'The Banksia Atlas'. (Australian Government Publishing service: Canberra, Australia.)

Tilquin, J.P., de Brouwer, K., Mathieu, A. and Calier, M. (1983). Haustorial pollen tubes in *Fuchsia boliviana*. *Annals of Botany*, 52: 425-428.

Ton, L.D. and Krezdorn, A.H. (1967). Growth of pollen tubes in three incompatible varieties of *Citrus*. *Proceedings of the American Society for Horticultural Science*, 89: 211-215.

Turner, V. (1984). *Banksia* pollen as a source of protein in the diet of two Australian marsupials *Cerartetus nanus* and *Tarsipes rostratus*. *Oikos*, 43: 53-61.

Urata, U. (1954). Pollination requirements of Macadamia. *Hawaii Agricultural Experimentation Station Technical Bulletin*, 2: 1-40.

van Wyk, G. (1977). Pollen handling, controlled pollination and grafting of *Eucalyptus grandis*. *South African Forestry Journal*, 101: 47-53.

Vaughton, G. (1988). Pollination and seed set of *Banksia spinulosa*: evidence for autogamy. *Australian Journal of Botany,* 36: 633-342.

Vaughton, G. (1990). Predation by insects limits seed production in *Banksia spinulosa* var. *neoanglica* (Proteaceae). *Australian Journal of Botany,* 38: 335-340.

Vegis, A. (1964). Dormancy in higher plants. *Annual Review of Plant Physiology,* 15: 185-224.

Venkata Rao, C. (1971). 'Proteaceae'. (CSIR: New Delhi, India.)

Visser, T. (1965). On the inheritance of the juvenile period in apple. *Euphytica,* 14: 125-134.

Visser, T. (1973). The effect of rootstocks on growth and flowering of apple seedlings. *Journal of the American Society for Horticultural Science,* 98: 26-28.

Vogts, M. (1960). The South African Proteaceae: the need for more research. *South African Journal of Science,* 57: 297-305.

Vogts, M. (1982). 'South Africa's Proteaceae - know them and grow them'. (Proteaflora Enterprises Pty Ltd: Melbourne, Australia.)

Wallace, D.D. and O'Dowd, D.J. (1989). The effect of nutrients and inflorescence damage by insects on fruit-set by *Banksia spinulosa*. *Oecologia,* 79: 482-488.

Wallerstein, I. (1989). Sequential photoperiodic requirement for flower initiation and development of *Leucospermum patersonii* (Proteaceae). *Israel Journal of Botany,* 38: 25-34.

Webb, G. (1985). Insect pollinators of *Hakea microcarpa* (Proteaceae) at Bombala, New South Wales. *Victorian Naturalist,* 102: 204.

Webb, J. (1977). Why can't we grow all the Western Australian plants in the East? *Australian Plants,* 9: 109-112.

Weins, D. (1984). Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. *Oecologia*, 64: 47-53.

Weins, D. and Rouke, J.P. (1978). Rodent pollination in southern African *Protea* spp. *Nature*, 276: 71-73.

Whealy, C.A., Nell, T.A., and Barrett, J.E. (1987). High temperature effects on growth and floral development of chrysanthemum. *Journal of the American Society for Horticultural Science*, 112: 464-468.

Whelan, R.J. and Burbridge, A.H. (1980). Flowering phenology, seed set and bird pollination of five Western Australian *Banksia* species. *Australian Journal of Ecology*, 5: 1-7.

Whelan, R.J. and Goldingay, R.L. (1986). Do pollinators influence seed-set in *Banksia paludosa* Sm. and *Banksia spinulosa* R.Br.? *Australian Journal of Ecology*, 11: 181-186.

Wilfret, G.J. (1987). The role of the plant breeder in the eveluation and breeding of new floriculture crops. *Acta Horticulturae*, 205: 13-19.

Williams, E.G., Knox, R.B. and Rouse, J.L. (1982). Pollination sub-systems distinguished by pollen tube arrest after incompatibility interspecific crosses in *Rhododendron* (Ericaceae). *Journal of Cell Science*, 53: 255-277.

Williams, E.G., Ramm-Anderson, S., Dumas, C., Mau, S.L. and Clarke, A.E. (1984). The effect of isolated components of *Prunus avium* L. styles on *invitro* growth of pollen tubes. *Planta*, 156: 517-519.

Windle, B.E., Barth, G.E., and Lewis, I.R. (1990). Future of the floriculture industry of South Australia - report of a working party. *South Australian Department of Agriculture, Technical Report Number 170.*

Wooller, R.D., Russell, E.M., Renfree, M.B. and Towers, P.A. (1983). A comparison of seasonal changes in the pollen loads of nectarivorous marsupials and birds. *Australian Wildlife Research,* 10: 311-317.

Worrall, R.J. (1983). Growing waratahs commercially. *Australian Horticulture,* 81: 101-103.

Wrigley, J.W. and Fagg, M. (1989). 'Banksias, Waratahs and Grevilleas, and all other plants in the Australian Proteaceae Family.' (Collins: Sydney, Australia.)

Zammit, C. and Hood, C.W. (1986). Impact of flower and seed predators on seed-set on two *Banksia* shrubs. *Australian Journal of Ecology,* 11: 187-193.

Zammit, C. and Westoby, M. (1987). Population structure and reproduction status of two *Banksia* shrubs at various times after fire. *Vegetatio,* 70: 11-20.

Zieslin, N. and Halevy, A.H. (1975). Flower bud atrophy in 'Baccara' roses. II. The effect of environmental factors. *Scientia Horticulturae,* 3: 383-391.

Zimmerman, R.H. (1971). Juvenility and flowering of fruit trees. *Acta Horticulturae,* 34: 139-142.

APPENDIX 1. Fuss, A.M. and Sedgley, M. (1990). Floral initiation and development in relation to flowering of *Banksia coccinea* R.Br. and *B. menziesii* R.Br. (Proteaceae). Australian Journal of Botany, 38: 487-500.

Fuss, A. M. & Sedgley, M. (1990). Floral initiation and development in relation to the time of flowering in *Banksia coccinea* R.Br and *B. menziesii* R.Br (Proteaceae). *Australian Journal of Botany, 38*(5), 487-500.

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APPENDIX 2. Sedgley, M., Sharman, K.V. and Fuss, A.M. (1989). An overview of research into banksias, native daisies, eucalypts and acacias at the Waite Research Institue. *In:* 'The Production and Marketing of Australian Flora (Proceedings)", The University of Western Australia and The Western Australian Department of Agriculture, Perth, Western Australia.

Sedgley, M., Sharman, K. V. & Fuss, A. M. (1989, July). An overview of research into banksias, native daisies, eucalypts and acacias at the Waite Research Institute. *The production and marketing of Australian flora (proceedings),* The University of Western Australia and the Western Australian Department of Agriculture, Perth, WA.

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APPENDIX 3. List of oral presentations and seminars.

LIST OF ORAL PRESENTATIONS AND SEMINARS

May 1990	**Department of Entomology**, Waite Agricultural Research Institute, Adelaide, South Australia. Floral Biology of Banksias.
April 1990	**Nursery Industry Association of Australia 1990 Annual Conference**, The Beaufort Hotel, Darwin, Northern Territory. Presentation of NIAA Youth Award. Contributions of Scientific Research to the Nursery and Horticultural Industries of Australia.
April 1990	**Department of Plant Physiology**, Waite Agricultural Research Institute, Adelaide, South Australia. Floral Biology of Banksias.
April 1990	**Adelaide Hills Branch of the Australian Camellia Research Society**, General Meeting, Stirling, South Australia Banksias as Cut Flowers.
August 1989	**Australian Protea Growers Ass. Ltd. Annual Conference and General Meeting**, McLaren's on the Lake, McLaren Vale, South Australia. The Floral Biology of Banksias in relation to Crop Production and Management.
August 1989	**Protea Growers of South Australia Inc.**, General Meeting, Stirling, South Australia. The Floral Biology of Banksias in relation to Crop Production and Management.
September 1988	**Protea and Wildflower Conference**, Protea Producers of South Australia, Waite Agricultural Research Institute, Adelaide, South Australia. The Floral Biology of Banksias in relation to Crop Production and Management.
May 1988	**Australian Federation of University Women**, South Australia Inc., General Meeting, Adelaide, South Australia. Recipient of Doreen McCarthy Bursary. Banksias as Cut Flowers.
April 1988	**Booleroo and District Lions Club**, Melrose, South Australia. Banksias as Cut Flowers.