PROCEEDINGS

OF THE

ROYAL SOCIETY OF VICTORIA

INCLUDING

TRANSACTIONS OF MEETINGS

Volume 108

NUMBER 1

ROYAL SOCIETY'S HALL
9 VICTORIA STREET, MELBOURNE, VICTORIA 3000

POPULATION ECOLOGY OF *BANKSIA SAXICOLA* (PROTEACEAE)

N. D. MIDDLETON[A], P. Y. LADIGES[A] & N. J. ENRIGHT[B]

[A]School of Botany, The University of Melbourne, Parkville, Victoria 3052, Australia
[B]Department of Geography and Environmental Studies, The University of Melbourne, Parkville, Victoria 3052, Australia.

MIDDLETON, N. D., LADIGES, P. Y. & ENRIGHT, N. J. 1996:09:30. Population ecology of *Banksia saxicola* (Proteaceae). *Proceedings of the Royal Society of Victoria* 108 (1): 43–56. ISSN 0035-9211.

Banksia saxicola A. S. George is a rare species with a disjunct distribution, at Wilsons Promontory National Park and the Grampians National Park, Victoria. Phenetic classification and ordination analyses using 13 leaf, inflorescence and infructescence characters, indicated that population differences are present but individual plants do not exclusively group into geographically isolated populations. Plants from Mt William (Grampians) are different from those at Wilsons Promontory, but those from Mt Rosea (Grampians) overlap with both of these populations. Leaf flavonoid analysis revealed minor differences between Grampians and Wilsons Promontory populations, with the latter being intermediate between *B. integrifolia* and Grampians *B. saxicola*.

Germination of seed extracted from 1, 2–3 and >3 year old infructescences varied from 15–35% at 50 days. Seed from Wilsons Promontory had the highest germination rate. Cold stratification of seed only promoted germination of seed >3 years of age from Mt William. *Banksia saxicola*, from Wilsons Promontory, had the highest level of serotiny, although more seed was stored per plant at Mt William due to greater fecundity. Granivory of canopy stored seed was highest at Wilsons Promontory.

At the Grampians, *B. saxicola* is locally common, plants are generally healthy, and spontaneous seed release together with favourable environmental conditions, have allowed populations to regenerate in the absence of fire. At Wilsons Promontory, however, plants are less abundant, and with no regeneration in the last 19 years the population is aging. For continued existence of *B. saxicola* at Wilsons Promontory, conservation management strategies need to include burning.

BANKSIA SAXICOLA A. S. George is the only rare species of *Banksia* found in Victoria (Gullan et al. 1990). A conservation rating of 3RC (George 1987) is designated to *B. saxicola* because populations of the species are small, occur in National Parks and have a total range covering more than 100 km. The species has a disjunct distribution, being found only at the Wilsons Promontory and Grampians (Gariwerd) National Parks (Fig. 1). Within the Grampians, *B. saxicola* grows predominantly on rocky mountain summits as shrubs and small trees up to 6 m in height. At Wilsons Promontory, *B. saxicola* is found as an understorey tree, up to 15 m in height, in wet *Eucalyptus* forest.

The type specimen of *B. saxicola* was collected from Mt William, Grampians, by George (1981). *B. saxicola* is distinguished from *B. integrifolia* L. f. and *B. canei* J. H. Willis, to which it is closely related, by absence of a lignotuber, grey-yellow coloured inflorescences, flowering time (January to March) and whorls of relatively large (40–100 × 10–35 mm) and often serrate leaves (George 1981; Thiele 1993).

The disjunct distribution, differences in habit and habitat between geographically isolated populations, and a general lack of knowledge of this rare species, prompted this investigation. The aims were to identify differences between populations relating to genetic isolation and to document regeneration strategies and demography of the populations. Management practices for conservation are suggested.

MATERIALS AND METHODS

Study sites and sampling

Three populations of *B. saxicola* were sampled at: Mt Rosea (37°18′S,142°30′E; 680–885 m asl) and the summit of Mt William (37°18′S,142°36′E; 1167 m asl) within the Grampians National Park, and at Wilsons Promontory National Park (39°2′S, 146°23′E; 150–280 m asl).

At Mt William (MW), *B. saxicola* and *Eucalyptus baxteri* (Benth.) Maiden & Blakely ex J. Black dominate the vegetation, forming a subalpine shrubland (3.5 m). Annual rainfall for the

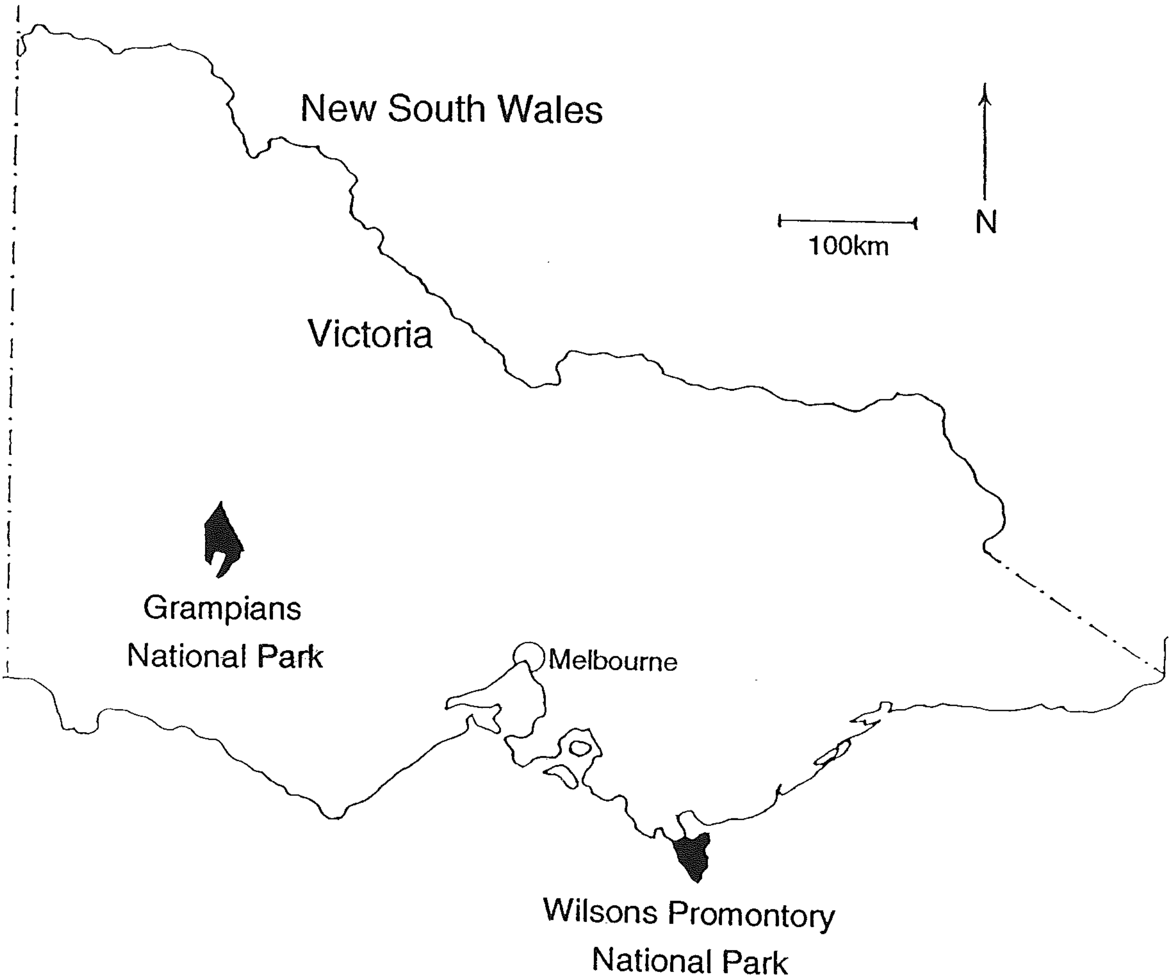

Fig. 1. Geographic distribution of National Parks within which *B. saxicola* is confined.

area averages 914 mm with snowfall occurring most years at the summit. Average daily temperatures vary from 19.9°C in summer to 8.4°C in winter. Wildfire is infrequent, last occurring in 1959. The soil is shallow and derived from medium to fine-grained quartzose sandstone. At Mt Rosea (MR) growing conditions are less severe than at MW. Snowfall is less frequent and large granite boulders provide sheltered microhabitats. Small trees and shrubs of *B. saxicola* grow up to 6.5 m in height, scattered amongst large sandstone boulders or sheltered beneath trees of *Eucalyptus serraensis* Ladiges & Whiffin. On the upper plateau of MR, fire has not occurred since 1939.

At Wilsons Promontory (WP), *B. saxicola* has a relatively restricted distribution compared with MW and MR. Only 166 *B. saxicola* individuals were located in an area of 80 × 3000 m, 2–5 km inland, along the walking track to Sealers Cove. The area was last burnt in 1951 (T. Willett, pers. comm.). Annual rainfall averages 1051 mm, with average temperatures varying from 16.2°C in summer to 11.3°C in winter. Within this area, *B. saxicola* grows to heights of 15 m in moist gullies and on sheltered slopes. Soils are derived from granitic rocks, which are exposed throughout the area.

A population of six putative *B. saxicola* × *B. marginata* Cav. hybrids was located along Redmans Road (37°13′S,142°35′E; 490 m asl), Grampians National Park. No *B. saxicola* plants were found in this area. The sclerophyllous woodland was dominated by *Eucalyptus obliqua* L'Hér. and *B. marginata* was abundant in the understorey.

Plants were aged by node counts (Lamont 1985) and only those plants (19–35 years old) with infructescences (cones) were included in analyses. The number of inflorescences, sterile (barren) infructescences and 1, 2–3 and >3 year old fertile infructescences were recorded per plant. Since inflorescence buds may remain dormant for an indeterminate period of time, infructescences were aged by colour and by degree of deterioration (Lamont 1985; Cowling et al. 1987). Where possible, six infructescences from each age group were collected per plant. Due to the rarity of *B. saxicola*, sampling was restricted to no more than 10% of the total fruit (follicles), which in some instances led to insufficient fruit and inflorescences available for collection. Samples of leaves, including five with undamaged mucronate tips and five one year of age, were collected from each plant and used for morphological and flavonoid analyses, respectively.

Morphological analysis

Twenty-nine plants (10 MW, 12 MR and 7 WP) were scored for 13 quantitative characters (Table 1) for a multivariate, phenetic analysis of geographic population variation. Five measurements of each character were obtained and means calculated for each plant. Single factor ANOVAs and Sheffé's test were used to identify significant differences between populations for each of these characters. Plants were compared in a multivariate analysis using mean values of all characters and the PATN computer package (Belbin 1987). Values were range standardised and dissimilarities between plants were calculated using the metric Manhattan distance (MM). Cluster analyses were based on flexible weighted and unweighted pair-group method using averages (WPGMA and UPGMA, respectively). Ordination analyses were performed using KYSP multidimensional scaling. KYSP was preferred to other ordination techniques because it is highly robust (Faith et al. 1987). Ordinations were viewed in the first two dimensions.

Leaf characters		
1.	ll	leaf length
2.	lw	leaf width
3.	ll/lw	leaf length to width
4.	mtl	mucronate tip length
Inflorescence characters		
5.	rl	rachis length
6.	yl	style length[A]
Fruit characters		
7.	fl	follicle length
8.	fw	follicle width
9.	ufl/lfl	uppermost to lowermost follicle length
Seed characters		
10.	el	endosperm length
11.	sl	seed length, including wing
12.	sw	seed width, including wing
13.	sl/el	seed to endosperm length

[A]Style length was measured at pollen presentation.

Table 1. Morphological characters used in analysis of geographic variation in *Banksia saxicola*.

Leaf flavonoid analysis

Mature leaves were collected from 35 *B. saxicola* (8 MW, 11 MR and 16 WP) and 10 *B. integrifolia* subsp. *integrifolia* individuals from Wilsons Promontory. Flavonoid extraction followed that of Mabry et al. (1970). Leaves were air-dried and ground to a powder. Samples of ground leaves (2.5 g per plant) were placed in clean 25 ml glass vials with 20 ml of 80% aqueous methanol. Vials were agitated and left to settle at room temperature for 36 hours. Methanol solutions were spotted onto Whatman 3MM chromatography paper (460 × 570 mm) and allowed to air-dry between each of five applications.

Flavonoids were separated using two dimensional chromatography. An initial run using TBA (tertiary butanol : glacial acetic acid : distilled water, 3 : 1 : 1 v = v) was followed by a second, perpendicular to the first, in HOAc (15% aqueous glacial acetic acid). Chromatography papers were air-dried after each solvent application. To observe flavonoids, each dried chromatogram was viewed on a light box equipped with 360 nm Black UV fluorescent tubes. Individual flavonoids were identified by their position and colour prior to, and following, ammonia fume application. Since the aim was to detect any genetic variation between populations, identification of flavonoids beyond separation of compounds by colour change and chromatographic position was not necessary (T. Whiffin, pers. comm.).

Demographic analyses

Observations of ecological traits such as plant health, death and age at reproductive maturity were noted. Age at reproductive maturity was determined by the age of trees with only one year old infructescences (i.e. plants in their first year of reproductive maturity). Because such plants totalled four, six and zero at MW, MR and WP, respectively, an additional population near Mafeking Road, Grampians National Park (37°22′S,142°34′E; 580 m asl) was included for this investigation.

For each infructescence collected (178 MW, 61 MR and 81 WP), the numbers of open and closed follicles were recorded to determine canopy seed store. Seeds were extracted by placing infructescences individually in paper bags and heating in an oven at 200°C for 5 minutes. Wet–dry cycles were occasionally required to open follicles sufficiently to remove the seed (Cowling & Lamont 1985). Once collected, seeds were categorised as firm (endosperm present and intact, presumably viable), aborted (thin, papery seed lacking endosperm) or predated (part or entire endosperm eaten by granivorous insects, leaving a pale brown granular powder; Lamont 1985; Lamont & Van Leeuwen 1988). The proportion of predated seed per infructescence was calculated and used to determine the level of granivory for infructescences of different ages within each population.

Although previous reports indicate that seed of *B. saxicola* requires a cold stratification period to break dormancy (Salkin & Hallam 1978), the altitudinal range of *B. saxicola* suggests that stratification may not be necessary for all populations. To test this, where available two samples of 10 firm seeds from each age class (1, 2–3 and >3 years) per plant were germinated. One sample was stratified at 4°C for 30 days prior to moistening, while the control sample was not treated prior to germination. For germination, seeds were placed in petri dishes on filter paper moistened with dilute Thirum fungicide (1.5 g/1000 ml distilled water). Petri dishes were placed in a growth cabinet at 15°C with a light (13 hours) and dark (9 hours) cycle, although this cycle was not expected to have any effect on germination (Sonia & Heslehurst 1978). Seeds were kept moist and germination was recorded over a 50 day period.

Statistical analysis

Mean values of the number of fertile and sterile infructescences per plant, total number of inflorescences per plant, flowers per inflorescence, follicles per infructescence, firm, aborted and predated seed per infructescence, and percentage seed germination at various time intervals, were calculated.

Percentage values for predation, serotiny and seed germination were arcsine transformed to normalise the data (Sokal & Rohlf 1987). Two-way analysis of variance (ANOVA) and Scheffé's test were used to determine the significance of differences between means ($P \leqslant 0.05$).

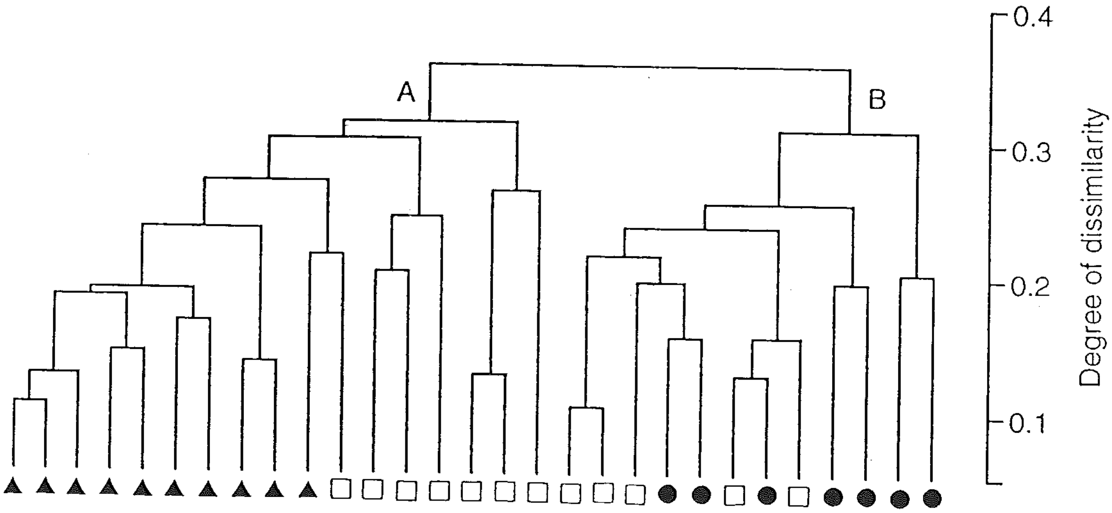

Fig. 2. Cluster analysis (MM, WPGMA) of 10 Mt William, 12 Mt Rosea and 7 Wilsons Promontory *B. saxicola* plants based on leaf, inflorescence and infructescence characters. (Black triangle = Mt William; white square = Mt Rosea; black circle = Wilsons Promontory.)

RESULTS

Morphological analysis

Cluster analyses using either WPGMA and UPGMA gave similar results, thus the WPGMA classification only is shown (Fig. 2). All MW individuals clustered into group A, all WP individuals clustered into group B, while plants from MR were more variable and were split between these two groups. The seven MR plants that clustered in group A with those from MW were collected at higher altitude than the remaining five plants from MR, which clustered in group B with plants from WP. Plants in group A had, for example, smaller leaves than plants in group B, which may be explained by plant exposure at higher altitude.

Ordination confirmed the pattern in the cluster analysis. A plot of axes 1 × 2 (Fig. 3) shows plants from WP and MW separate from one another. Seven plants from MR formed a cluster, but five overlapped in the ordination space with WP and MW. In particular, plant 16 from MR is an outlier and clustered near MW in the ordination. Plant 16 was atypical in having smaller leaves than other plants from MR.

Mean values (± standard deviation) were calculated for each morphological character for each population and one way ANOVAs and Scheffé's test indicated significance between populations for these measures (Table 2). While plants from MW have smaller leaves (ll, lw) and larger upper follicles (ufl/lfl) than those from MR and WP, plants from MW and MR have larger seeds (sw, sl, el) than those from WP.

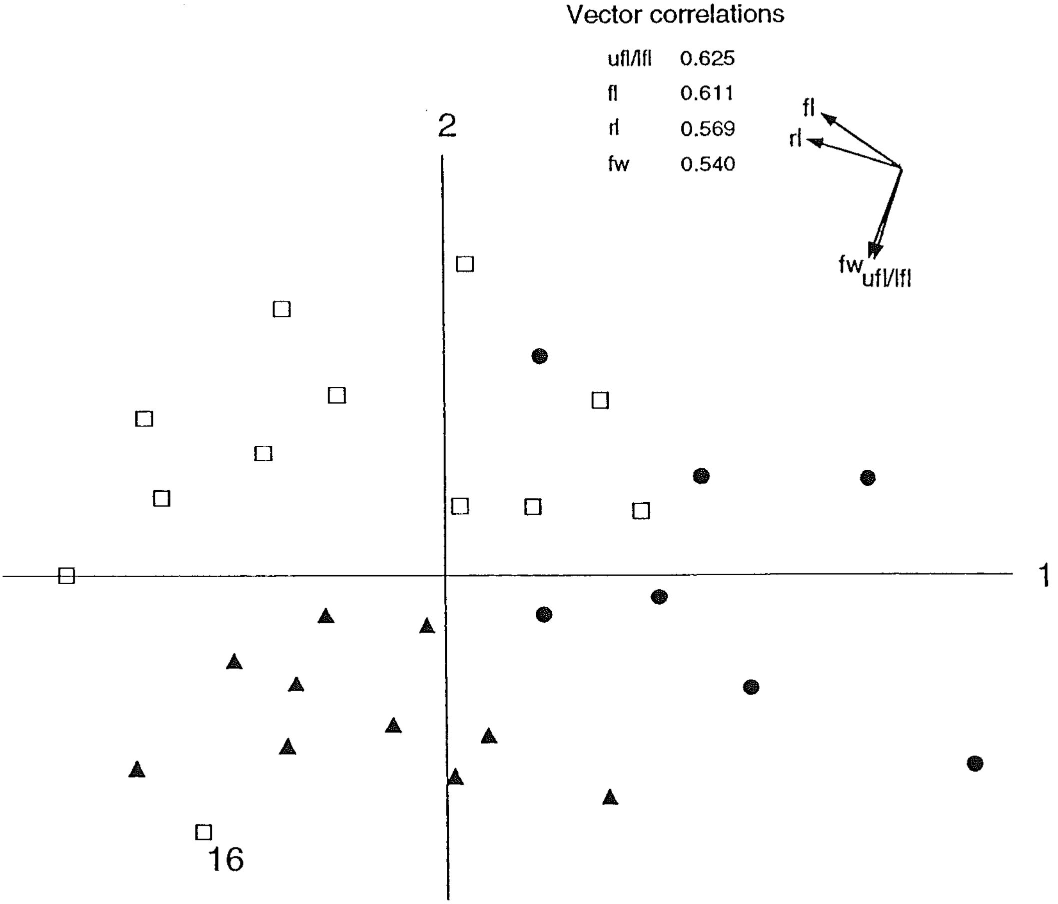

Fig. 3. Two dimensional ordination of *B. saxicola* individuals from three populations, based on morphological leaf, inflorescence and infructescence characters. (Black triangle = Mt William; white square = Mt Rosea; black circle = Wilsons Promontory; values indicate character correlation with each dimension.)

Leaf flavonoid analysis

Twenty-three leaf flavonoids were recorded for the two *Banksia* species analysed (Table 3). Flavonoid compositions of *B. saxicola* and *B. integrifolia* were very similar, with each of the 45 specimens analysed containing flavonoids 1–9. Flavonoids 10 and 11 were present in all *B. saxicola* and all but three *B. integrifolia* individuals. Variation between *B. integrifolia* and *B. saxicola* was evident by the presence of flavonoid 24. While this flavonoid was present in more than half of the *B. integrifolia* plants analysed, it was absent from all *B. saxicola* plants. Differentiation between *B. saxicola* populations was also evident. Flavonoids 22 and 23 were not observed in MW and MR populations, but were present in 88% and 38% of *B. saxicola* plants from WP, and 100% and 90% of *B. integrifolia* individuals, respectively. The higher frequency of occurrence of flavonoids 19–21 and lower occurrence of flavonoids 14–18 in *B. saxicola* from Wilsons Promontory, compared with the Grampians, indicates additional differences between these disjunct populations. Flavonoid patterns of the two Grampians populations were very similar.

Hybridisation

Observations of the roots of the six putative *B. saxicola* × *B. marginata* hybrid plants and the plants' close proximity indicate that they were probably produced by root-suckering from one individual. Infructescences of this clone had retained floral parts for some years and contained follicles that opened at maturity, similar to *B. marginata*. However, other attributes, such as arrangement, size and shape of the leaves and follicle length, indicate similarity to *B. saxicola* (Table 4).

Reproductive ecology

Although the overall germination percentage of the presumed viable seed was low (15–35%), significant differences were found between sites ($P \leqslant 0.05$). Seed collected from WP had a higher percentage germination than seed from MW and MR (Fig. 4). Contrary to a previous report (Salkin & Hallam 1978), seed of *B. saxicola* germinated without cold stratification. Overall, there was no significant difference in percentage germination between stratified and non-stratified seed when seed of different ages was pooled (Table 5a). However, when analysed according to seed age, stratification did promote germination of seed from MW that was greater than three years old ($P \leqslant 0.05$; Fig. 5). Although seed from MW showed a significant interaction effect between pre-germination treatment and seed age (Table 5b), due to lack of seed this was not tested for the other sites.

Despite the higher germination percentage of seed from WP compared to MW and MR, no recent regeneration was recorded in the field at WP. The youngest plant observed at WP was 19 years of age, the last fire being in 1951.

Character	Mt William	Mt Rosea	Wilsons Promontory	
Leaf length (mm)	56.6±6.5a	78.5±15.5b	86.5±12.7b	***
Leaf width (mm)	21.8±4.2a	23.4±3.3b	28.5±4.8b	**
Leaf length to width	2.7±0.4a	3.5±0.5ab	3.1±0.5b	**
Mucronate tip length (mm)	3.3±0.3	3.4±1.0	2.0±0.3	–[A]
Rachis length (mm)	66.9±5.9	67.2±12.6	58.2±9.6	NS
Style length (mm)	26.5±2.3	28.8±1.8	27.9±2.3	–[A]
Follicle length (mm)	15.1±1.0	15.6±1.4	14.4±1.5	NS
Follicle width (mm)	5.9±0.5	5.6±0.6	5.4±0.7	NS
Upper to lower follicle length	1.2±0.1a	1.0±0.1b	1.0±0.1b	***
Endosperm length (mm)	9.3±0.8a	9.3±1.2a	7.8±0.8b	**
Seed length, including wing (mm)	15.9±1.2a	16.2±1.8a	13.3±1.3b	***
Seed width, including wing (mm)	7.4±0.5a	8.2±0.8a	6.3±0.8b	***
Seed/endosperm length	1.7±0.1	1.7±0.1	1.7±0.1	NS

[A]Missing data for WP rendered sample size too small for statistical comparison.

Table 2. Mean values ± standard deviation for morphological characters used in phenetic analysis. Different letters denote differences between populations at the significance level indicated (*=$P \leqslant 0.05$, **=$P \leqslant 0.01$, ***=$P \leqslant 0.001$, NS = not significant).

	Flavonoid																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
B. saxicola																								
Mt William	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Grampians	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+		+			
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+			
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+				
Mt Rosea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+			
Grampians	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+					
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+			
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+					
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+				
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+								
Wilsons	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+	+		+		
Promontory	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+		+		+	+	+	
	+	+	+	+	+	+	+	+	+	+	+	+	+							+		+		
	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+		+		+	+		
	+	+	+	+	+	+	+	+	+	+	+	+	+	+					+	+	+	+		
	+	+	+	+	+	+	+	+	+	+	+		+	+					+		+	+		
	+	+	+	+	+	+	+	+	+	+	+	+		+	+		+	+	+	+		+		
	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+	+	+		+	+		
	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+	+	+	+	+	+	
	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+			+			+	+	
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+			+	
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+	+		+	+	
	+	+	+	+	+	+	+	+	+	+	+	+		+					+	+				
	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+		+	+		+	+	
	+	+	+	+	+	+	+	+	+	+	+	+	+		+				+	+		+		
	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+		+	+		+		
B. integrifolia																								
subsp. *integrifolia*																								
Wilsons	+	+	+	+	+	+	+	+	+	+		+	+	+	+		+	+				+		
Promontory	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+			+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	
	+	+	+	+	+	+	+	+	+	+	+	+		+	+		+	+		+		+	+	
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	
	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3. Presence of leaf flavonoids in three populations of *B. saxicola* and a single population of *B. integrifolia* subsp. *integrifolia*.

Attribute	*B. marginata*	Hybrid	*B. saxicola*
Habit[A]	shrub	tree	tree
Root-sucker	variable	most probable	unknown
Leaf length (mm)	15–60[B]	79 (66–93)[C]	40–100[B]
Leaf width (mm)	3–13[B]	13 (9–16)[C]	10–35[B]
Leaf shape	serrate towards tip oblanceolate	entire oblanceolate	entire obovate
Leaf arrangement	scattered	whorled	whorled
Serotiny level	low	low	medium
Follicle length (mm)	7–17[B]	20 (15–22)[C]	12–20[B]

[A]Although tree forms of *B. marginata* exist, only shrub forms were present in the area. In an equivalent woodland, *B. saxicola* forms trees.
[B]Range of values obtained from George (1981).
[C]Mean (range) from 24 samples.

Table 4. Attributes distinguishing a possible *B. saxicola*×*B. marginata* hybrid found near Redmonds Road, Grampians National Park.

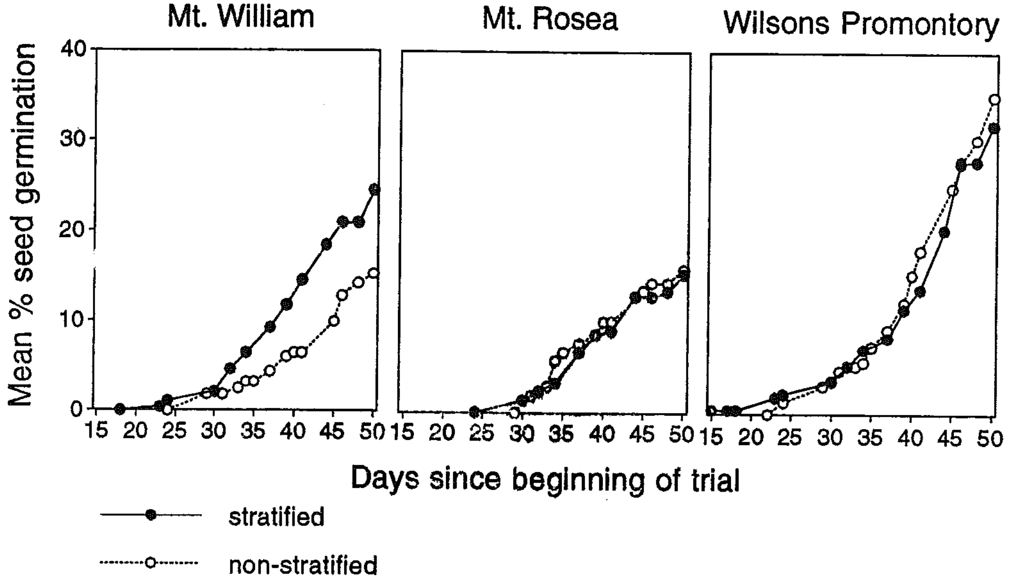

Fig. 4. Mean percentage germination over 50 days of stratified and control seed from Mt William, Mt Rosea and Wilsons Promontory. All seeds were firm and presumed viable.

Interfire regeneration is abundant at MW and MR and at an additional dense *B. saxicola* population near Mafeking, Grampians. At MW, MR and Mafeking, plants as young as 8 years of age were producing inflorescences, however, fertile infructescences were not seen until plants were 14–21 years of age. Data on age to reproductive maturity could not be obtained from WP, because all plants examined were reproductively mature with infructescences >3 years of age. Of the 166 *B. saxicola* individuals counted at WP, 31 (16%) had fallen or were standing but lacked foliage and were presumed dead. No dead individuals were seen at MW or MR, although at MR many plants appeared old, with spindly prostrate branches and low fruit production. Compared to MR and WP, plants of an equivalent age (>33 years) at MW were highly fecund.

Variation, both between and within populations, was generally large for the reproductive attributes measured (Table 6). The number of flowers per inflorescence and follicles per infructescence did not vary noticeably between populations. At MW *B. saxicola* plants produced more inflorescences

Source	SS	df	MS	F-ratio
(a) Germination of seed from different sites and pregermination treatments				
Treatment	79.029	1	79.029	0.255
Site	4827.479	2	2413.739	7.800 *
Treatment × Site	1020.065	2	510.032	1.648
Residual	36207.088	117	309.462	
Total	42133.661	122	3312.262	
(b) Germination of seed from MW of different ages and pregermination treatments				
Treatment	949.597	1	949.597	4.216 *
Age	538.295	2	269.147	1.195
Treatment × Age	1797.601	2	898.801	3.991 *
Residual	10811.178	48	225.233	
Total	14096.671	53	2342.778	
(c) Seed retention in infructescences of different ages and sites				
Site	617.221	2	308.611	2.596
Age	16257.006	2	8128.503	68.369***
Site × Age	1875.176	4	468.794	3.943 *
Residual	6420.106	54	118.891	
Total	25169.509	62	9024.799	
(d) Predation of seed of different ages and sites				
Site	660.906	2	330.453	4.163 *
Age	3801.322	2	1900.661	23.946***
Site × Age	1323.505	4	330.876	4.169 **
Residual	4206.673	53	79.371	
Total	9992.406	61	2641.361	

Table 5. Analysis of variance with replication using arcsine transformed data for germination, seed retention and seed predation (significance levels: *=P⩽0.05; **=P⩽0.01; ***=P⩽0.001).

per year than at other sites, resulting in a greater total number of fertile (and infertile) infructescences, and therefore a greater seed store per plant than at MR and WP. The percentage of firm seeds per infructescence decreased with age of infructescence at all sites. One year old infructescences from MR contained more viable

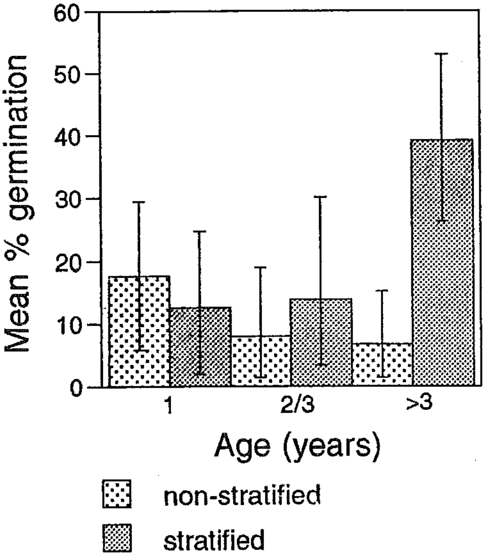

Fig. 5. Mean percentage germination of stratified and control seed of 1, 2–3 and >3 years of age from Mt William. Bars represent back-transformed upper and lower 95% confidence limits. All seeds were firm and presumed viable.

Attribute	Population MW	MR	WP
Plant age (years)	(19–>32)	25 (20–44)	(19–>30)
Inflorescences/plant	38 (15–91)	16 (0–75)	11 (0–33)
Flowers/inflorescence	917±61	965±52	863±50
Fertile infructescences/plant	(28–>300)	9 (1–36)	48 (10–114)
1 year fertile infructescences/plant	23 (4–75)	9 (0–13)	9 (3–16)
2-3 year fertile infructescences/plant	25 (6–75)	2 (0–8)	16 (3–50)
>3 year fertile infructescences/plant	(11–>150)	5 (0–25)	16 (0–50)
Sterile infructescences/plant	(26–>100)	28 (1–100)	43 (11–80)
Follicles/fertile infructescence	40±11	38±13	32±9
% aborted seed/infructescence	35±12	15±8	47±7
% firm seed/1 year infructescence	66±15	76±12	51±7
% firm seed/2-3 year infructescence	46±13	71±11	52±20
% firm seed/>3 year infructescence	30±14	33±24	25±22

Table 6. Reproductive attributes *B. saxicola* plants from Mt William, Mt Rosea and Wilsons Promontory populations. Values are presented as mean ± standard deviation or mean (range). Ten, twelve and eight plants were sampled from each population respectively.

seed and fewer aborted seed than those of the same age from WP and MW. At MR the proportion of sterile infructescences was higher than at MW or WP.

An ANOVA of the percentage closed follicles per infructescence for ages 1, 2–3 or >3 years at different sites showed significant differences (Table 5c) between the three age groups ($P \leqslant 0.001$). The interaction factor between infructescence age and population was also significant ($P \leqslant 0.05$; Table 5c). The percentage closed follicles decreased more rapidly with age at MW and MR, than at WP (Fig. 6). Infructescences older than three years from MW and MR had spontaneously released 54% and 62% of their seed, respectively, while only 17% of seed had been released from plants at WP. *B. saxicola* plants from WP are therefore more serotinous than those from the Grampians.

Predation

Granivorous insects are well documented to cause reproductive losses in *Banksia* (Scott 1982; Vaughton 1990; Zammit & Hood 1986; Zammit & Westoby 1988). Larvae found in *B. saxicola* follicles were tentatively identified as *Arotrophora* (Lepidoptera:Tortricidae) or *Xyloryctis* (Lepidoptera:Xyloryctidae) using the descriptions provided by Scott (1982). *Arotrophora aucuatalis* is the most widespread predatory moth larva of *Banksia* and is capable of infecting both inflorescences and infructescences. Weevils (Curculioinidae:Coleoptera) were also found within the *B. saxicola* follicles. The level of predation varied significantly with site ($P \leqslant 0.05$) and age ($P \leqslant 0.001$). The interaction factor between site and age was also significant ($P \leqslant 0.01$; Table 5d). Predation of 1 year old seed was low for all sites (0.04–2.59%) and increased to the greatest level in infructescences >3 years of age collected from WP (Fig. 7).

In addition to seed loss due to insect damage, infructescences that had been attacked by birds were found on the ground at WP and MR. Loss of reproductive effort due to birds was not assessed because the plants from which infructescences originated could not be determined, however, cockatoos are known to cause heavy inflorescence and infructescence loss in some *Banksia* species (Witkowski et al. 1991).

Predation of *B. saxicola* leaves by leafminers was also observed. This was abundant at WP, less frequent at MR, and not observed at MW. Miners primarily attacked 1 year old leaves prior to February and the infected leaves were

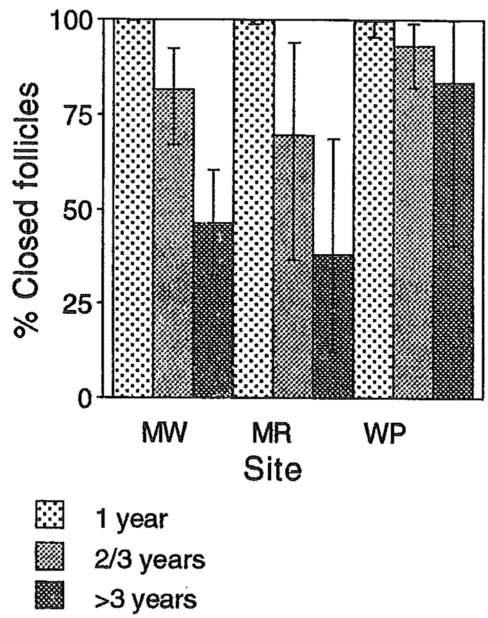

Fig. 6. Serotiny of three *B. saxicola* populations as indicated by percentage closed follicles per infructescence by infructescence age. Bars represent back-transformed upper and lower 95% confidence limits.

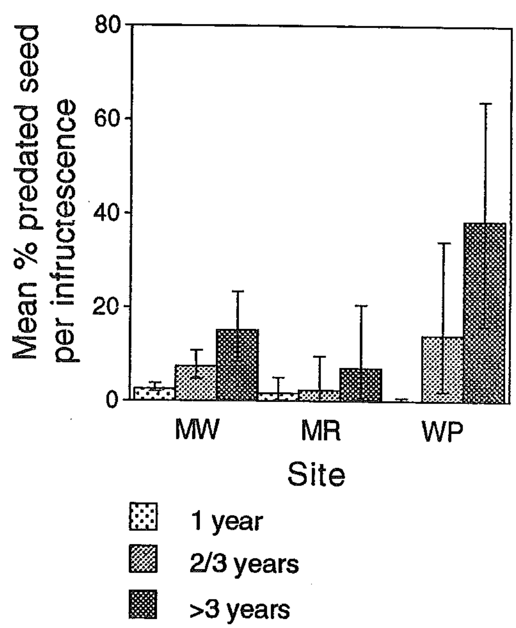

Fig. 7. Predation of *B. saxicola* seed with increase in infructescence age. Bars represent back-transformed upper and lower 95% confidence limits.

shed between March and April. Leafminers (Lepidoptera) were not identified due to their metamorphosis prior to sampling, however, the linear and blotch mines observed are comparable to mines made by Nepticulidae (Hering 1951).

DISCUSSION

Morphological analysis

Cluster analysis and ordination showed that plants from MW in the Grampians (shrub form) are different in morphology from plants from WP (tree form). However, plants from MR (Grampians) showed considerable variation, overlapping both these populations. Thus, no discrete groups correlate with the geographic disjunction of the Grampians and Wilsons Promontory (Fig. 2). Some morphological differences, such as leaf size, are probably phenotypic rather than genotypic, and hence reflect environmental differences between sites, such as altitude and exposure. In the sub-alpine environment at MW, for example, plants have the smallest leaves and the shortest stature. At MR, plants with the smallest leaves and stature were at the highest altitude and plants with the largest leaves and tallest stature were growing down slope in more sheltered habitats.

Although there is insufficient morphological disjunction within *B. saxicola* to warrant taxonomic recognition, such as Grampians and WP sub-species, the variation noted is relevant for conservation of the species' total gene pool.

Leaf flavonoid analysis

Flavonoid composition is considered stable over a wide range of environmental conditions (Leach & Whiffin 1989) and has been used as evidence of speciation, hybridisation and gene flow between plants (Mabry et al. 1970; Leach & Whiffin 1989; Whiffin & Ladiges 1992). Thiele & Ladiges (1994) identified 25 leaf flavonoids in *B. integrifolia* subsp. *integrifolia* from Victoria, whereas in the present study only 24 were found. This difference may be due to different flavonoid concentrations from different methodologies or from some flavonoids degrading over time to produce a number of breakdown products (B. Meurer-Grimes, pers. comm.). Variation in the abundance of specific flavonoids in each population, together with the absence of flavonoids 22 and 23 from MW and MR, indicate genetic differentiation between *B. saxicola* populations. Similarity in flavonoid composition between *B. saxicola* and *B. integrifolia* at WP indicates possible hybridisation between these two populations or retention of plesiomorphic (primitive) characters in *B. saxicola* at Wilsons Promontory.

Reproductive ecology

Cold stratification is a prerequisite of seed germination for many montane plants (Tran & Cavanagh 1984). In montane environments, seed release followed by immediate germination in late summer or autumn, is non-adaptive as seedlings would be vulnerable to winter frosts and snowfall. In such environments, seed dormancy is selectively preferred, delaying seed germination until spring, when conditions for seedling growth are more favourable. In montane *Banksia* species, such as *B. canei*, *B. marginata* and *B. saxicola*, a period of cold is a suggested requirement to break seed dormancy (George 1981).

A previous study on the effect of pre-germination, cold stratification on *B. saxicola*, then referred to as 'Grampians *B. integrifolia*' (Salkin & Hallam 1978) found that a minimum stratification period of 43 days at 5°C was required for seed germination. In the present study, seed was stratified for 30 days at 4°C. There was no difference in the percentage germination between nonstratified and stratified seed except for enhanced germination in seed >3 years of age collected from MW, a sub-alpine environment and the highest altitude where *B. saxicola* is found. Because of this it seems that the germination behaviour of *B. saxicola* varies with altitude, similar to *B. marginata* (Salkin & Hallam 1978). Why stratification only enhanced germination of old seed is perplexing.

Many of the reproductive traits measured for *B. saxicola* varied greatly between plants at each site. This may be due to the range in age (19–35 years) of plants included in the analysis. Although all plants were producing seed, some may have been reproductively mature for only a few years. In such cases, accumulation of fruit would be significantly lower than for plants 10 to 15 years older. Another factor that would result in variation between plants, particularly at the Grampians, is habitat variation within each site. At MW and MR, scattered boulders produce non-uniform habitats. Plants in sheltered situations between large boulders were taller and more fecund than plants in exposed situations (pers. observ.). In these rocky environments, soil depth also varies, leading to additional differences in conditions for plant growth.

Seed abortion within *Banksia* has been calculated at 22–74% depending on the species (Cowling et al. 1987; Lamont & Barrett 1988; Lamont & van Leeuwen 1988). With 15%, 35% and 47% of seed aborted at MW, MR and WP respectively, *B. saxicola* has comparatively mid to low seed abortion rates for the genus. Lamont & Barrett (1988) suggest that seed abortion can indicate nutrient conservation by plants and may, therefore, indicate low nutrient supply. Seed abortion was lower at MR than MW or WP, however, plants at MR produced fewer fertile infructescences and a higher proportion of sterile infructescences than these two sites, therefore high nutrient supply at MR seems unlikely. Cowling et al. (1987) found that more serotinous *Banksia* species produced lower proportions of aborted seed. For *B. saxicola*, however, this relationship is not evident as plants from WP had the highest level of serotiny and the highest level of seed abortion. At MW, plants produce more inflorescences each year, resulting in a greater annual addition to the canopy seed store. The number of follicles per infructescence did not vary between localities, suggesting no difference in pollinator availability between sites.

Percentage firm seed per infructescence (an estimate of percentage viable canopy-stored seed) decreased with infructescence age due to spontaneous seed release, predation and decomposition. *B. saxicola* was found to have 66% (MW), 76% (MR) and 51% (WP) firm seed (for 1 year-old infructescences). In comparison with other species in the genus, *B. saxicola* may be considered to have medium percentage viable seed (see Cowling et al. 1987; Lamont & Barrett 1988; Lamont & van Leeuwen 1988).

Although *B. saxicola* plants may be seen flowering at eight years of age (Taylor & Hopper 1988), they do not produce viable seed until they are 14–21 years old. For the first few years of flowering only sterile infructescences are produced. This and the delay between first flower and first fruit production have been noted in other *Banksia* species (Lamont 1985; Gill & McMahon 1986; Salkin 1986; Whelan & Goldingay 1986; Lamont & Barret 1988; Witkowski et al. 1991). Reasons for this phenomenon remain unknown. Within *Banksia* flower to fruit ratios are typically low. Although not well understood, this could be due to spacial constraints, lack of nutrients, unavailability of pollinators, or mechanisms by which plants can selectively favour development of seeds produced by outcrossing (Salkin 1986; Whelan & Goldingay 1986; Fuss & Sedgley 1991).

Serotiny is well developed in Australian sclerophyllous vegetation. *B. saxicola* is often characterised as spontaneously releasing the majority of its seed within three years of production (George 1987; Wrigley 1989). In the present study, however, rarely were infructescences found with all seeds released (<1% of infructescences collected). In *Banksia*, 76% of taxa store seed within the canopy for release when infructescences dry or are burnt (Cowling & Lamont 1985; Gill 1976). Variation in the degree of serotiny (the rate of seed release) occurs both within and between *Banksia* species (Lamont 1985). Due to the difficulty in accurately aging *B. saxicola* seed, a value for the degree of serotiny following the method of Lamont (1991), could not be calculated. A qualitative estimate obtained from Fig. 6 indicates that plants from WP, the most moist site, were more serotinous than those from the Grampians.

Predation

Several species of insect are known to reduce available seed levels in *Banksia* (Scott 1982). These may or may not be species specific predators and are capable of destroying flowers and/or seeds. Scott (1982) concluded that insects infest inflorescences, and remain in the rachis until the seeds develop, such that seed predation occurs primarily in the first year of fruit formation. In such situations, granivory should not increase with infructescence age, as was found here (Fig. 7). A number of other studies have also found a higher level of granivory in older infructescences (Cowling et al. 1987; Zammit & Westoby 1988; Witowski et al. 1991; N. Enright, unpubl. data). Despite large variation in the amount of *B. saxicola* seed predation at each site (11–39%), average values are comparable to other *Banksia* species (negligible to 40%; Zammit & Hood 1986; Zammit & Westoby 1988; Witkowski et al. 1991).

Response to fire, regeneration and conservation

The effect of fire on *B. saxicola* is poorly understood. Taylor & Hopper (1988) list 'respouting', 'plant death with no seedling regeneration' and 'unspecified' as responses of *B. saxicola* to fire, which is relatively ambiguous. Despite this, *B. saxicola* is generally thought to be killed by fire with regeneration occurring from seed released from the canopy (George 1981; D. Cheal, pers. comm.). Evidence for this includes thin bark (2–4 mm) and the absence of a lignotuber (George 1981).

Species in which follicles open spontaneously (without fire) usually form uneven-aged stands (Lamont 1985). Such interfire regeneration is occurring at MW and MR. At WP, despite an uneven aged population and some seed release (17%), no *B. saxicola* plants under 19 years of age were found. Factors preventing regeneration at WP remain largely untested. Seed viability does not appear to be limiting as seed from WP was found to germinate at a higher rate than MW or MR when germinated under laboratory conditions (Fig. 4). Possible reasons for lack of recruitment at WP, aside from low seed release, could include an increased incidence of post-dispersal fungal attack and unsuitable germination/growing conditions. The moister environment at the WP site would enhance decomposition and fungal attack by *Rhizoctonia* and *Pythium*, which commonly cause damping-off in cultivated *Banksia* seedlings (McLean 1993). At WP the lower level of light beneath the canopy (pers. observ.) than at MR and MW may also result in conditions unfavourable for seedling establishment. Fire is, therefore, likely to be important for the survival of *B. saxicola* at Wilsons Promontory National Park not only to release seed from the canopy but to create favourable conditions for germination and seedling growth.

To determine a suitable fire management regime for conservation of *B. saxicola* at WP, plant age at reproductive maturity, and the level and rate of seed accumulation are required (Lamont 1985). Because the latter is unknown for *B. saxicola*, the fire interval required is best (and most conservatively) estimated as two times the plant age at reproductive maturity (A. Gill, pers. comm.). This corresponds to a 30–40 year fire interval for *B. saxicola.*

The use of fire as an ecological management tool within National Parks is a multifaceted and controversial topic. Fire regime (intensity, interval and season) and the response to fire by the community (flora and fauna) are the main ecological dilemmas. As *B. saxicola* predominantly flowers in late summer and seeds are mature after one year, late summer and autumn fires would maximise seed recruitment. This is important since *B. saxicola* is thought to be killed by fire and, therefore, relies on post-fire seed recruitment for survival.

CONCLUSION

Banksia saxicola occurs as geographically and hence genetically, isolated populations in the Grampians National Park and Wilsons Promontory National Park, Victoria. Results of morphological and flavonoid analysis indicate some degree of variation between Grampians and Wilsons Promontory populations. Because these population differences appear to be phenotypic as well as genotypic and are not clear-cut, taxonomic division of *B. saxicola* is not warranted. Genetic differences between populations require further study using molecular methods. Nevertheless, the degree of intraspecific variation noted here, the rarity of the species and its disjunct pattern of distribution, are sufficient reasons for devising a conservation management plan for *B. saxicola* at both WP and the Grampians.

At the Grampians National Park, *B. saxicola* is locally common and regeneration is occurring in the absence of fire. Fire management proposals include burning of communities containing *B. saxicola* at intervals of 20–30 years for rocky woodlands and >40 years for sub-alpine communities (Anon. 1985). Under these regimes continued existence of *B. saxicola* is likely to be assured.

At Wilsons Promontory, *B. saxicola* is less abundant than at the Grampians. No regeneration has occurred for 19 years and the population is at risk of extinction. Although a considerable quantity of relatively viable seed is retained within the canopy of this aging population, predation of seed is high and has been shown to increase with seed age. Because only 17% of seed is released spontaneously in the Wilsons Promontory population, a fire is required to release the bulk of seed and produce optimum conditions for germination (increase light incidence, decreased competiton, etc.). Burning only part of the *B. saxicola* stand at WP and growing plants from seed away from the site for transplanting would act as a safeguard, in case conditions for post-fire recruitment are sub-optimal.

A small area along the walking track to Sealers Cove at WP has been set aside as a Scientific Protection Area where off-track access is prohibited, primarily to protect *B. saxicola* (Anon. 1987). The species occurs both within and outside of this area. Within this zone, public use, safety and education are highly prioritised management objectives, therefore, the use of fire in ecological management is limited. Because the population of *B. saxicola* at WP has not been burnt since the 1951 wildfire (42 years ago), a burn in the near future is strongly advised.

ACKNOWLEDGEMENTS

We are grateful to Dr Trevor Whiffin for help with leaf flavonoid analyses, Marco Duretto for advice using PATN, and the Department of Conservation and Natural Resources for permission to study and collect plant material from Wilsons Promontory and Grampians National Parks.

REFERENCES

ANON., 1985. *Grampians National Park Plan of Management*, National Parks Service, Melbourne.

ANON., 1987. *Wilsons Promontory National Park Management Plan*, National Parks Service, Melbourne.

BELBIN, L., 1987. PATN. *Pattern Analysis Package Reference Manual*, CSIRO, Division of Wildlife and Rangelands Research, Canberra.

COWLING, R. M. & LAMONT, B. B., 1985. Seed release in *Banksia*: the role of wet-dry cycles. *Australian Journal of Ecology* 10: 169–171.

COWLING, R. M., LAMONT, B. B. & PIERCE, S. M., 1987. Seed bank dynamics of four co-occurring *Banksia* species. *Journal of Ecology* 75: 289–302.

FAITH, D. P., MINCHIN, P. R. & BELBIN, L., 1987. Compositional dissimilarity as a robust measure of ecological distance. *Vegetatio* 69: 57–68.

FUSS, A. M. & SEDGLEY, M., 1991. Pollen tube growth and seed set of *Banksia coccinea* R. Br. (Proteaceae). *Annals of Botany* 68: 377–384.

GEORGE, A. S., 1981. The genus *Banksia* L. f. (Proteaceae). *Nuytsia* 3: 239–473.

GEORGE, A. S., 1987. *The Banksia Book*, Kangaroo Press, Kenthurst.

GILL, A. M., 1976. Fire and the opening of *Banksia ornata* F. Muell. follicles. *Australian Journal of Botany* 24: 329–335.

GILL, A. M. & MCMAHON, A., 1986. A post-fire chronosequence of cone, follicle and seed production in *Banksia ornata*. *Australian Journal of Botany* 34: 425–433.

GULLAN, P. K., CHEAL, D. C. & WALSH, N. G., 1990. *Rare or Threatened Plants in Victoria*, Department of Conservation and Environment: Victoria.

HERING, E. M., 1951. *Biology of Leaf Miners*, W. Junk, Gravenhage.

LAMONT, B. B., 1985. Fire responses of sclerophyll shrublands: A population ecology approach, with particular reference to the genus *Banksia*. In *Symposium on Fire Ecology and Management in Western Australian Ecosystems*, J. R. Ford, ed., W.A. Institute of Technology, Perth, pp. 41–46.

LAMONT, B. B., 1991. Canopy seed storage and release—what's in a name? *Oikos* 60: 266–268.

LAMONT, B. B. & BARRETT, G. J., 1988. Constraints on seed production and storage in a root-suckering *Banksia*. *Journal of Ecology* 76: 1069–1082.

LAMONT, B. B. & VAN LEEUWEN, S. J., 1988. Seed production and mortality in a rare *Banksia* species. *Journal of Applied Ecology* 25: 551–559.

LEACH, G. J. & WHIFFIN, T. 1989. Ontogenetic, seasonal and diurnal variation in leaf volatile oils and leaf phenolics of *Angophora costata*. *Australian Systematic Botany* 2: 99–111.

MABRY, T. J., MARKHAM, K. R. & THOMAS, M. B., 1970. *The Systematic Identification of Flavonoids*, Springer-Verlag, New York, pp. 3–7.

MCLEAN, R., 1993. *Banksia* propagation. *Society for Growing Australian Plants Newsletter* 60: 6–8.

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.

SALKIN, A., 1986. Breeding systems in eastern Australian banksias. *Victorian Naturalist* 103: 43–47.

SALKIN, A. & HALLAM, N. D., 1978. The topodemes of *Banksia canei* J. H. Willis (Proteaceae). *Australian Journal of Botany* 26: 707–721.

SOKAL, R. R. & ROHLF, F. J., 1987. *Introduction to Biostatistics*, 2nd edn, Freeman and Company, New York.

SONIA, L. & HESLEHURST, M. R., 1978. Germination characteristics of some *Banksia* species. *Australian Journal of Ecology* 3: 179–186.

TAYLOR, A. & HOPPER, S., 1988. *The Banksia Atlas*. Australian Flora and Fauna Series No. 8, Canberra Publishing and Printing Company, Canberra, pp. 216–217.

THIELE, K. R., 1993. A Cladistic Analysis of *Banksia* L. f. Ph. D. Thesis. School of Botany, The University of Melbourne.

THIELE, K. R. & LADIGES, P. Y., 1994. The *Banksia integrifolia* L. f. species complex (Proteaceae). *Australian Systematic Botany* 7: 393–408.

TRAN, V. N. & CAVANAGH, A. K., 1984. Structural aspects of dormancy. In *Seed Physiology, Germination and Reserve Metabolization*, Vol. 2, D. R. Murray, ed., Academic Press, Australia, pp. 1–40.

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

WHELAN, R. J. & 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.

WHIFFIN, T. & LADIGES, P. Y., 1992. Patterns of variation and relationships in the *Eucalyptus alpina–E. baxteri* complex (Myrtaceae) based on leaf volatile oils. *Australian Systematic Botany* 5: 695–709.

WITKOWSKI, E. T. F., LAMONT, B. B. & CONNELL, S. J., 1991. Seed bank dynamics of three co-occurring banksias in south coastal Western Australia: the role of plant age, cockatoos, senescence and interfire establishment. *Australian Journal of Botany* 39: 385–397.

WRIGLEY, J. W., 1989. 'Banksias, Waratahs and Grevilleas and all other plants in the Australia Proteaceae family.' Collins Publishers, Australia.

ZAMMIT, C. & HOOD, C. W., 1986. Impact of flower and seed predators on seed-set in two *Banksia* shrubs. *Australian Journal of Ecology* 11: 187–193.

ZAMMIT, C. & WESTOBY, M., 1988. Pre-dispersal seed losses, and the survival of seeds and seedlings of two serotinous *Banksia* shrubs in burnt and unburnt heath. *Journal of Ecology* 76: 200–214.