Abstract

Links between species and the effect they have on ecosystem function is becoming increasingly recognised. Examples of such links include the complex, often multi-staged process of animal mediated seed dispersal, seed-caching by mammals and the tripartite relationship that occurs between many fungus-eating (mycophageous) mammal species, mycorrhizal fungi and woody plants. In this thesis, the role a small omnivorous marsupial, *Bettongia penicillata ogilbyi* (woylie), plays in ecosystem function, using its interaction with *Santalum spicatum* (Western Australian sandalwood) as a model, was examined.

The study was conducted in a semi-arid open wandoo (*Eucalyptus wandoo*) woodland, in Western Australia. Dryandra Woodland, (32°48'S, 116°54'E) 160 km southeast of Perth, is one of the largest and most diverse remnants supporting over 800 native plant species and 24 mammal species, seven of which are threatened. Dryandra Woodland experiences a Mediterranean climate with warm to hot, dry summers and mild, wet winters.

Bettongia penicillata Grey 1837 is a small, nocturnal marsupial within the family Potoroidae. Since European settlement, the distribution and numbers of woylies have decreased dramatically. Factors attributed to this decline include habitat loss, the introduction of feral predators such as the cat (*Felis catus*), the European red fox (*Vulpes vulpes*) and competing herbivores. From its original distribution across the south-western third of the continent, only three remnant natural populations remain in the south-west of Western Australia at Perup Nature Reserve, Tutanning Nature Reserve and Dryandra Woodland representing a reduction in range of approximately 97%.

Santalum spicatum (R. Br) DC (Western Australian sandalwood), family Santalaceae, is a small, hemiparasitic tree which has virtually disappeared from the 300-600 mm rainfall zone due to widespread clearing of natural woodland and excessive unregulated harvesting. Furthermore, it has been suggested that seed dispersal is limited in areas where woylies have become extinct.

Four broad objectives were addressed in this thesis:

1. To determine the population and dietary requirements of woylies in Dryandra Woodland

2. To determine the impact woylies have on the regeneration of sandalwood, by measuring recruitment of sandalwood in an area where woylies are present compared with an area where they are absent

3. To examine, in detail, seed dispersal and seed caching behaviour in the woylie by radiolabelling seeds with scandium 46. This will include the types of seeds cached, how caches are located, whether secondary or tertiary caching occurs, seed preference, germination rate from caches and seedling predation

4. To develop a rationale for woylie conservation and reintroduction based on an understanding on the woylie's role in ecosystem function and species' co-existence.

Two experimental sites, with both woylies and sandalwood, and a control site, with sandalwood but no woylies were used in this study. Site A is outside the main block of Dryandra Woodland, 1.65 km from the main entrance to the Woodland. This site lies parallel to the main Wandering-Narrogin road and across from farmland situated next to woodland. The sandalwood was planted in the 1950s and has numerous mature sandalwood trees, saplings and seedlings. Site B is within the woodland, 3.2 km from

the Dryandra village. Site B was planted in the late 1970s to early 1980s and has very few new sandalwood recruits and the few seedlings that did occur were found on soil mounds along the fenced area parallel to and across from a dirt road. The control site, with sandalwood but no woylies was at the Wickepen Water Reserve, 40 km south-east of Dryandra Woodland. This site had many mature sandalwood trees but very little recruitment growing away from the parent crown.

Trapping sessions, lasting four nights per experimental site, were commenced in May 2002 and repeated at regular intervals until December 2005 giving a total of 1300 trap nights at site A and 1400 at site b. The 2005 trapping sessions were carried out four times a year to cover the seasons for dietary analysis for *Bettongia penicillata ogilbyi* (woylie) and *Trichosurus vulpecula* (brushtail possum). Because the number of trapped and re-trapped woylies were so low, the Jolly-Seber method, used to estimate possum numbers, could not be used to estimate woylie populations at either site. Instead, the minimum number of animals known to be alive (KTBA) was calculated by counting the number of times an individual woylie was trapped over at least three sessions. Findings from this study indicate that woylie numbers are declining in Dryandra. Site A had a very low number of woylies KTBA with only two females trapped once in June 2002 and one individual male, trapped intermittently until February 2004. Subsequent trapping failed to catch woylies until December 2005. Because of the low number of woylie captures at this site no population data could be analysed. The number of woylies KTBA at site B was consistently higher than site A, although still low. A total of 11 individual females and 17 individual males were trapped between May 2002 and December 2005 giving a sex ratio of 1:1.5 females to males.

The trapping effort in the spring of 2003 resulted in an estimated population of brushtail possums ranging from 17 at site A to 70 at site B thus giving a density of approximately 1.4 and 5.8 brushtail possums ha^{-1}, respectively. The trapping effort in spring 2005 resulted in an estimated population of brushtail possums of 25 at site A and 35 at site B, thus giving a density of 2.1 and 2.9 brushtail possums ha^{-1}, respectively. The trap success per 100 trap nights for brushtail possums was significantly (p < 0.0001) higher than that of woylies at both experimental sites during 2003 and 2005. Total trap success was compared between sites for woylies and brushtail possums. There was a significant ($\chi^2_5 = 16.41$, p < 0.01) difference in the trap success between the sites for woylies, and a highly significant ($\chi^2_5 = 42.04$, p <0.001) difference for brushtail possums for the 2003 trapping. The trap success for woylies, per 100 trap nights, was not significantly ($\chi^2_2 = 4.1358$, p >0.100) different between seasons for site B in 2005. Similarly, the trap success for brushtail possums, per 100 trap nights, was not significantly ($\chi^2_2 = 2.8565$, p >0.200) different. However, brushtail possums, for all seasons, had a significantly ($\chi^2_1 = 103.9$, p < 0.0001) higher trap success compared with woylies for site B. Site A was not analysed due to the lack of woylies at this site from 2004 onwards. Trapping at the control site failed to catch woylies, and only one possum was caught during the trapping effort.

Fungal spores occurred in 100% (n = 16) of woylie scats for winter, spring and summer. During the three seasons analysed, woylies consumed an equal balance of spore types from hypogeal (n = 7) and epigeal (n = 7) fungi. Overall, woylies ate eight spore types in winter and 12 spore types in spring. Fungi appeared to be important in the diet of brushtail possums during autumn and summer as a higher percentage of brushtail possums had fungal spores in their scats during these seasons compared with spring

($\chi_1^2 = 13.94$, p < 0.001) and winter ($\chi_1^2 = 8.65$, p < 0.005). The percentage of brushtail possums that consumed fungi during winter and spring did not ($\chi_1^2 = 1.128$, 0.250 > p < 0.500) differ, nor did the percentage of brushtail possums that ate fungi in autumn and summer ($\chi_1^2 = 0.00046$, 0.925 > p < 0.99).

The major spore type consumed throughout the year by both the brushtail possum and the woylie was *Mesophellia*. However, the amount of *Mesophellia* consumed by the brushtail possum differed between seasons (F = 83.472, df =1, p <0.001) as it was only dominant in the diet during the hotter months (i.e. summer and autumn). *Austrogautieria* and *Mesophellia* were the only spore types present in both brushtail possum and woylie scat samples in summer. In contrast to *Mesophellia*, spores of *Gastrotylopilus* dominated the brushtail possum and woylie scat samples during the cooler seasons (winter and spring) and were absent in summer and autumn. They occurred in significantly higher densities in winter than spring (F= 10.390, df = 1 p = 0.002) in brushtail possum scats. Overall, woylie scats contained a higher density of spores for all spore types than in brushtail possum scats. The total spore densities per gram of scat for woylies were $348.4 \times 10^4 \pm$ SE 277.1×10^4 for winter, $170.7 \times 10^4 \pm$ SE 110.3×10^4 for spring and $1353.4 \times 10^4 \pm$ SE 450.6×10^4 for summer.

At site A the spatial distribution of adult sandalwood trees, adult to juvenile, adult to seedling and juvenile to juvenile were aggregated (p < 0.05), whilst seedling to seedling distribution was highly aggregated (p < 0.005). The density of adults was approximately 24 trees ha^{-1}, there were approximately 107 saplings ha^{-1} and approximately 128 seedlings ha^{-1}. The furthest sandalwood seedling found growing away from an adult was 91 m. At site B the distribution of mature adult trees, adults to saplings and saplings to

saplings were all highly aggregated (p < 0.005). Adults occurred at an approximate density of 81 trees ha^{-1}, and saplings at 38 trees ha^{-1}. At the control site, mature trees were highly aggregated (p < 0.005) with a density of 15.7 trees ha^{-1}. There were very few seedlings and saplings at the site, all of which were clumped under or around the crown of the parent tree.

Log-linear analysis indicated a significant two-way interaction between the presence of woylies and the distance the offspring were found in relation to adult trees ($\chi_2^2 = 288.4$, p < 0.0001) and between the presence of woylies and the age of the offspring (saplings and seedlings) ($\chi_2^2 = 34$, p < 0.0001). Thus, in Dryandra, where woylies were present, there were higher numbers of both seedlings and saplings growing more than 1 m from adult trees, compared with Wickepin Water Reserve, where woylies were not present. Conversely, in the absence of woylies, more seedlings and saplings grew less than 1 m from adult trees. For the three year survival rates of seedlings surveyed for the three sites, log-linear analysis indicated a significant two-way interaction between site and seedling survival ($\chi_3^2 = 218$, p < 0.0001) and between seedling survival and under/away from parent crown ($\chi_3^2 = 107$, p < 0.0001). Site A experienced significantly higher mortality of seedlings growing away from the crown after three years compared with site B, whilst the control site suffered significant seedling mortality under the crown.

There was a highly significant (one-way ANOVA, $F_{(2,99)}= 125.58$, p < 0.0001) difference between Dryandra Woodland and Wickepin Water Reserve in the mean number of whole seeds found under adult sandalwood trees. The mean number of whole seeds under the parent crown at site A was 0.97 ± 0.16, range 1 to 2 (n = 34). At site B the mean was 0.76 ± 1.67, range 1 to 9 (n = 34), while at the control site the mean was 59.97 ± 11.19, range 10 to 330 (n = 34). *A posteriori* analysis indicated that the mean

number of whole seeds under the parent crown at Wickepin Water Reserve, where woylies were extinct, was significantly greater than those at Dryandra Woodland where woylies were still present (p < 0.0001). Log-linear analysis indicated a significant two-way interaction between the presence of woylies and the distance the offspring were found in relation to adult trees ($\chi^2_2 = 288.4$, p < 0.0001) and between the presence of woylies and the age of the offspring (saplings and seedlings) ($\chi^2_2 = 34$, p < 0.0001).

To enable large numbers of seeds to be tracked effectively over a period of time, two experiments were udertaken using a labelling technique with the isotope scandium-46 (Sc^{46}). Scandium-46 is a moderate beta and a high level gamma-emitting radionuclide with a half life of 83.6 days and a maximum photon energy of 1.12 Mega electron volts (MeV) 100%. It is the high gamma emissions that allow seeds labelled with Sc^{46} to be tracked using a Geiger Muller (GM) counter to determine their fate. Scandium-46 is absorbed by the seed hull and each seed typically received ~1 microcurie (μCi) [37 kilobecquerel (kBq)] of activity, sufficiently strong to allow detection of buried seeds from about 30 cm with a GM counter. The seeds labelled for the May 2005 experiment were sandalwood, *S. acuminatum* (quandong), *Acacia acuminata* (jam) and *Gastrolobium microcarpum* whilst in the February 2006 experiment only sandalwood seeds were used.

In the May 2005 experiment, woylies ate or cached all the sandalwood seeds before any interaction with either *S. acuminatum* (quandong), *Acacia acuminata* (jam) or *Gastrolobium microcarpum* seeds ocurred. In the first night all the sandalwood seeds had been removed with 26 cached and 24 seeds eaten *in situ* under or within 1 m from the source tree. The woylies took three nights to remove all the quandong. On the third night the rate of removal increased which resulted in 6 cached seeds and 39 seeds were

consumed *in situ*. The next type of seed to be removed was the *Acacia acuminata* which were all consumed by one woylie. No *Acacia* seed caches were found. On the fifth night of the experiment the majority of *Gastrolobium* seeds were observed to be eaten *in situ* by one woylie. Two caches of *Gastrolobium* seeds were located, one cache with 22 seeds and one with 15 seeds buried within 8 m of each other.

In the February 2006 experiment, of the 500 seeds deployed under the source tree over four nights, 211 (42.2%) were eaten *in situ*, 185 (37%) were cached and 104 (20.8%) had an unknown fate. Individual seeds were buried between 1 and 6 cm deep (mean 3.15 ± SE 0.11 cm). By November 2006, 185 primary, 120 secondary, 52 tertiary, 35 quaternary and 17 quintic caches had been located. Of the 185 seeds initially buried, 40 (21.6%) seeds had been dug-up and eaten *in situ*, 5 (2.7%) were left undisturbed in the caches, 20 (10.8%) were removed from the area, their fate unknown, and the remaining 120 (65%) seeds were re-cached into secondary caches. Of the 120 secondary caches, 38 (32%) seeds were dug up and eaten *in situ*, 12 (10%) were left undisturbed, 18 (15 %) were removed from the area, their fate unknown, and 52 (43%) had been re-cached into tertiary caches. Subsequently, seven (13.5%) of the seeds were consumed from these caches, 10 (19.2%) were left undisturbed and 35 (67%) seeds were re-cached to quaternary caches. From the quaternary caches, 12 (34.2%) seeds were dug-up and eaten *in situ*, 6 (17.1%) caches were left undisturbed and 17 seeds were dug-up and re-cached for a fifth time. Of these quintic caches, 4 were dug-up and eaten, 5 (29.4%).

The most common site for primary through to quintic caches was *A. acuminata*. The next most common area for primary to quaternary caches was out in the open not near any particular vegetation type. The exception was the location of quintic caches which had a higher percentage of caches under fallen logs (24% n = 4) compared with out in

the open (18% n = 3). There were a relatively high number of seeds cached within 30 cm of a sandalwood tree in all types of caches. The fate of 17 individual seeds that were cached and re-cached in five different caches was mapped, however, it remains unknown whether the same woylie that made the primary cache went on to move the seeds five times or whether it was several different woylies moving the seeds around.

The fate of cached seeds was monitored for germination rates *in situ*. All the seeds were eventually consumed from the May 2005 experiment before any germination could take place. For the 2006 experiment, overall germination rates were low with only 6 (15.8%) of seeds from the 38 undisturbed caches germinating. Of these, two from the secondary caches germinated out in the open and did not survive. The single seed from an undisturbed tertiary cache germinated beneath a fallen log as did one of the seeds that germinated and survived from the undisturbed quintic cache while another successfully germinated beneath a *G. microcarpum* bush.

Olfaction appeared to be the principle method used by woylies to locate buried seeds, a process which they are very efficient at. There was a significant ($\chi_1^2 = 6.5$, $0.025 > p < 0.01$) difference in the number of artificially cached seeds dug-up by woylies compared with disturbance of the control 'caches'. Of the 80 caches, 65 (81.2%) were located by woylies over three nights. During the same time period, 39 (48.7%) of the control 'caches' showed signs of being disturbed, thus suggesting that woylies were, at least in part, responding to the smell of disturbed soil as well as the actual seed. Furthermore, there was also a highly significant ($\chi_1^2 = 7.5$, $0.01 > p < 0.005$) difference between the number of seeds located by woylies under leaf litter compared to the control. All of the 20 seeds under the leaf litter were located within two nights

compared with only six (30%) of the control 'caches' in the leaf litter showing signs of disturbance.

The predation rates of buried seeds and emerging seedlings was measured. After distributing 100 sandalwood seeds under a random sandalwood tree at each site, there was significantly ($\chi_1^2 = 12.25$, p < 0.0001) less seed disturbance at Site A on the first night compared to site B. Conversely, on the third night there was significantly ($\chi_1^2 = 10.96$, p < 0.0001) more seed disturbance at site A compared with site B. All of the 100 seeds had been eaten *in situ* or removed by woylies at the end of the third night at site B whilst at site A the 100 seeds were eaten *in situ* or removed by woylies by the end of the fourth night.

It is without doubt that woylies are prolific seed cachers and that their relationship with sandalwood is complex and mutual in nature. The woylies benefit by consuming some of the large nutritious seeds sandalwood produces. In-turn, sandalwood benefits by having a vector, the woylie, to disperse their seeds many of which are buried in areas conducive to germination, for example, under fallen logs and near sandalwood's host species. Woylies are able to very efficiently locate buried seeds by using olfaction and possibly visual clues of disturbed earth during random foraging for hypogeous fungi, although there was no evidence to suggest they use spatial memory to locate seeds.

To determine if woylies use emerging seedlings as markers, seedlings were grown in the greenhouse and transplanted in Dryandra. Of the 46 seedlings transplanted at site A, 14 (30%) were intact and growing after one month. A total of 23 seedlings (50%) were dug-up by woylies. Of these, 17 (37%) were discarded whilst the remainder of the endosperm was eaten *in situ* leaving the endocarp on the ground. In the remaining six (13%) seedlings the endocarps were removed from the area, presumably to be eaten

elsewhere rather than re-cached as they would have been split open during the germination process and, therefore, no longer suitable for storage. Similar numbers and fates were recorded at site B with 10 (43%) left intact, 22 (51%) of the seedlings had been dug-up by woylies and discarded whilst the remainder of the epicarp was eaten *in situ* leaving the endocarp on the ground. Three (7%) of the seedlings were dug-up by woylies but again the endocarps were removed from the area. Six (14%) seedlings at site B were also grazed and two (5%) seedlings died. There was no significant ($\chi^2_4 =$ 3.96, p < 0.25) difference between sites for the fate of seedlings. The majority of seeds that were retrieved by woylies from the seedlings were eaten *in situ* at both sites. There was no significant ($\chi^2_1 = 1.583$, 0.25 < p > 0.10) difference between sites for seeds eaten *in situ* or removed from the area. The fate of the seeds removed from the area was unknown.

It was concluded that woylies have both a mutualistic and antagonistic relationship with sandalwood and its propagules and as a result may strongly influence the recruitment and spatial distribution of sandalwood by reducing the number of seeds and seedlings available for dispersal and regeneration, respectively. Woylies are both pre and post-dispersal predators consuming seeds under the parent trees, seeds that have been dispersed away from the parent trees and buried and germinating seeds. Evidence has been provided to support the early anecdotal reports that woylies use emerging seedlings as 'markers' to locate buried seeds.

This is the first study of its type in Australia to demonstrate, unequivocally, the consequences of the loss of a key seed-dispersal vector, the woylie, on sandalwood recruitment and regeneration in Dryandra Woodland. Through seed-caching and seed-predation, woylies have been shown to substantially alter the fate and distribution of

sandalwood seeds and seedlings. The loss of woylies from 97% of their former range will undoubtedly have serious implications for sanalwood and possibly other plant species. In losing this seed-dispersing and seed-caching animal from our ecosystems we have lost a keystone species. The woylie sandalwood interaction seems to have shaped the morphology of the sandalwood seed and fruiting phenology by making the propagules attractive and rewarding to the woylie. In this way both the woylie and sandalwood benefit from this interaction. Such close interactions have a positive impact on the health and functioning of ecosystems and landscapes.