Mutualistic seed dispersal by the Cape spiny mouse (*Acomys subspinosus*)

John Claude Midgley
Supervisor: Prof J.J. Midgley

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Contents:

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Abstract:

Acomys subspinosus, a nocturnal, endemic murid species of the SW Cape, has recently been shown to scatter-hoard seeds (Midgley *et al*, 2002). The extent of the seed dispersal relationship was explored by releasing four species of seeds that were labeled with brightly coloured, 10cm long strings in the evening and relocating them the following morning. After recording dispersal distance, fate and location, a linear regression was performed on the fate and location of the four types of seeds. The dimensions of holes were recorded after seeds that I had buried were excavated by *A.subspinosus*. The experiments show that *A.subspinosus* effects a more uniform distribution of seeds throughout the environment by dispersing them a mean distance of 1.9m and allows plants to colonize new habitats. Significant differences were obtained from the comparison between seed removal rates during the day vs. night. Most seeds (822/1200) were eaten. The maximum depth that *A.subspinosus* is prepared to dig down to was calculated at around 1.7cm.

Introduction:

A variety of nut-producing plants have mutualistic seed-dispersal interactions with animals that scatter hoard their nuts in soil (Van der Wall, 2001). Some may have originated as early as the Paleocene (60mya). Many nut producing species are found in mesic forests or semiarid regions with prolonged dry season eg: *Juglans, Carya, Quercus, Fagus, Castanae, Castanopsis, Lithopsis, Corylus, Aesculus* and *Prunus*. Most of these genera appear to have evolved from ancestors with wind-dispersed diaspores (Van der Wall, 2001). Food-caching rodents and jays appear to have played an important role in shaping Holocene plant communities in the deciduous forests of North America, Europe and Asia. These animals effected nut dispersal over a range of distances from meters to kilometers, maintaining populations of nut-bearing plants on a local scale, transporting nuts to new environments and causing gene flow across a patchy landscape (Van der Wall, 2001).

The South-western Cape region is noted for containing some of the most floristically diverse vegetation on Earth in a relatively small area. Diversity in fynbos extends to all aspects of plant life histories and strategies including diverse mutualisms between plants and insects (Johnson, 1992). Seed dispersal by ants (myrmecochory) was, until recently, seen as the only true biotic dispersal mechanism of any importance in fynbos (Slingsby and Bond, 1981; Johnson, 1992). Mutualistic relationships between plants in at least 25 families (Bond and Slingsby, 1983) and indigenous ant species have developed to the point that ants are supplied with a reward (a nutritious elaiosome) in exchange for transporting the seed back to the ant nest. Post dispersal seed predation is one of the factors with the highest impact on the reproductive success of a species (Johnson, 1992). Bond and Breytenbach (1985) argued that rodent seed predation in fynbos can be a major limiting factor for the successful expansion of plant communities. It is important to understand the impact of the fauna of the region, which contains denser rodent populations than similar shrublands in Australia and California (Fox *et al*, 1985), on fynbos plant populations and apply this knowledge to biodiversity conservation management strategies. Some 36 fynbos shrub species rely, to some extent, on mutualistic relationships with small mammals for pollination (Rebelo and Breytenbach, 1987) and seed dispersal (Wiens *et al*, 1983). Unfortunately, it is difficult to calculate the proportion of seeds that are merely consumed compared to the proportion that are genuinely dispersed to favorable sites or new habitats.

The term "scatter hoarding" refers to when animals bury one or a few items in many widely dispersed sites (Jensen, 1985) so that food is stored for future seasons. Scatter hoarding rodents benefit from seed dispersal mutualisms as nuts are nutritious food sources, often with high levels of lipids or proteins and a caloric value of 10-100 times greater than wind dispersed seeds (Van der Wall, 2001). Survival without another source of food would presumably be longer and by the same principle, more individuals can be produced by a population of scatter hoarders if nut bearing plants dominate their habitat.

The reproductive strategy of the nut bearing plant is focused on maximizing quality, instead of the number of propagules produced. Big seeds are more valuable or "expensive" than small seeds as they represent a greater investment from the parent plant. There are several ways in which a mutualism with a scatter-hoarding rodent is advantageous to a nut-bearing plant species:

1. Nuts transported away from source plants (Van der Wall, 2001).
2. Scattering nuts provides more uniform distribution of nuts over/in the environment than nuts that simply fall under the parent tree, where it has been shown that density dependent mortality is high (Janzen, 1971; Sork, 1983b).
3. Dispersal distances range up to about 100m for squirrels, chipmunks, mice and other rodents (Stapanian and Smith, 1978; Sork, 1984; Tamaru *et al*, 1999). Nearest neighbor distances between caches are usually several meters (Stapanian and Smith, 1978; Jensen, 1985).

4. Burial ensures good rooting (Griffin, 1971; Sork, 1983a). As seed size increases, the probability of burial by physical processes rapidly diminishes (Chambers *et al*, 1991).
5. Animals often cache nuts in habitats and microhabitats that favor establishment. The greater the value of the nut to the hoarder, the more likely it is that a rodent or corvid will move the nut out of the environment where it was harvested.
6. Scatter hoarding removes nuts from other sources of nut predation.

Jacobs (1992) hypothesized that the time it takes for a forager to handle a nut influences its likelihood of caching that nut. The most energy efficient behavior for animals would therefore be to cache items that take longer to eat, while feeding on smaller items that require less handling time. This would also optimize the mutualism, as only the most "expensive" seeds would get buried. The optimal spacing of caches is thought to be a compromise between minimizing energy and time investment by the animal in transporting items to distant sites while maximizing the number of stored items that the cacher can eventually retrieve (Stapanian and Smith, 1978, 1984; Clarkson *et al*, 1986; Tamaru *et al*, 1999).

Relocation of hidden seeds is due to the hoarders extensive spatial memory of cache sites although, for rodents, olfaction may also be important (Thompson and Thompson, 1980; Van der Wall, 1998, 2000). For this reason most cached nuts are eventually recovered by vertebrates as was the case in the study by Tamaru *et al* (1999), where animals use stored nuts as sustenance during the autumn and winter. 12% of nuts were untouched and healthy in Thompson and Thompson (1980) experimentally buried chestnut population. Similar results were obtained for a smaller sample of rodent caches (Thompson and Thompson, 1980). However, these values cannot be used uncritically to determine nut survival rate, because many of the nuts that animals remove from caches are relocated elsewhere (Van der Wall, 2000), so overall nut survival rate may be higher.

The Cape Spiny mouse (*Acomys subspinosus*) is a known scatter hoarder (Vlok, 1995), but has only recently attracted some attention amongst ecologists (Fleming and Nicolson, 2002; Midgley *et al*, 2002) as a scatter-hoarder of Cape *Proteaceae* nuts. The aim of this project is to explore the dynamics of seed dispersal by *A.subspinosus* in order to gain deeper insight into the reproductive ecology of nut-bearing fynbos species. Assessment of *A.subspinosus* as a scatter-hoarder will allow prediction of mutualistic nut-fruited species population growth and migration rates. These factors are important in changing climate scenarios, because the furture distribution of *A.subspinosus* could possibly be predicted according to bioclimatic modelling methods. Areas where scatter hoarding can occur could potentially limit plant species distributions if they rely on *A.subspinosus* for successful seedling recruitment.

It is difficult to quantify the fate of seeds in a natural community, because retrieval of seeds is nearly impossible unless they were labeled prior to their release. Van der Wall (1993) used radioactively labeled seeds in his investigation to allow relocation of seed caches with a gamma radiation counter. A more practical method of relocating seeds was developed by Midgley *et al* (2002). A 10 – 15cm length of lightweight, brightly coloured, waterproof string, known as dacron, is glued to the seed coat before its release. Caches can occur in the walls of shallow rodent burrows (Jensen, 1985) and this is the only factor limiting the percentage of seeds that could potentially be recovered.

After performing pilot studies with dacron labeled string at De Hoop Nature Reserve, Jonaskop and Bainskloof, evidence of scatter-hoarding was found at a site on the Sir Lowrys Pass (34° 09'S, 18° 55'E), 50km from Cape Town.

The main aims of this project were:

1) Assess the effectiveness of *A.subspinosus* as a scatter hoarder in terms of removal distance, burial rate and burial depth.

2) Identify a possible preference towards one of the four nut species used in the project.

3) Expand the field data on the mutualistic dispersal of nut-fruited plant species by rodents.

The overall aim was to explain the evolution of the rodent dispersal guild. Differences in dispersal distances and dispersal microsites, as well as of the distribution patterns of the different dispersers were identified as the main factors required for this purpose by Midgley *et al* (2002).

Materials and Methods:

Study sites:

Jonaskop

Protea magnifica dominated the vegetation on rocky outcrops, situated about 50m apart. *Protea lorifolia* and grasses dominated the vegetation in the plains between rocky outcrops. The rocky outcrops provided many positions of shelter for rodents under rocks and plants, while the plains offered considerably less shelter or no shelter at all from avian predators. To determine the extent of rodent seed predation vs. burial, 300 seeds (100 *P.lorifolia* and 200 *P.magnifica*) were released on the 13th and relocated on the 21st of July 2002.

Bainskloof

Protea repens and *Leucadendron sessile* dominated the site on the Bainskloof pass. Patches of grass and bare ground occurred between stands of larger shrubs. Soil surface was firm and covered with small pebbles. To determine the extent of rodent seed predation vs. burial, 200 sunflower seeds were released on the 28th of July and relocated on the 4th of August 2002.

Sir Lowrys pass

Protea repens and Leucadendron sessile dominated site near N2 highway. Intermittent grassy patches as well as some dense *Cliffortia* stands occurred throughout the site. The study site was on a slope with a slight angle and few rocky outcrops were present. Soil surface was sandy and slightly clayey, but not rocky. Experiments at Sir Lowrys pass were performed on the 6th and 7th (200 sunflower and 200 *Leucadendron sessile* seeds), 10th and 11th (100 sunflower, 100 *Leucadendron sessile* and *100 Wildenowia incurvata* seeds) of August. Seeds were released to determine the extent of rodent seed predation vs. burial rates.

Experimental design:

Seed burial experiments

To determine burial microsites, it was imperative to relocate seeds after dispersal and burial. To achieve this, seeds were marked with fluorescent orange and yellow dacron. Dacron is a light weight string that is conventionally used in flyfishing. The dacron was attached to the seeds with superglue. A small portion of the seed surface was roughened with fine sandpaper as the superglue didn't bond quite as well with smooth surfaces. "Caches" are clusters with the same number of seeds in them. Seeds were placed "under" bushes or left in the "open" as in Leaver and Daly's (2000) cache experiments. A cache was said to be "under" a bush if it were directly under the canopy of a shrub of height >25cm. This definition allows the vegetation dominated by *Protea repens* and *Leucadendron sessile* to be distinguished from the grass dominated vegetation, because the grass reaches a maximum vertical height of around 25cm. Therefore, the final category of vegetation was under bushes with maximum height <25cm. Seeds left in the "open" had no shelter around them, thus exposing any rodent to airborne avian predators for the time spent at the cache. Caches were also placed further than 25cm from any plant material, so that no permanent plant cover could be exploited as shelter by rodents visiting the cache. Distances of seed relocation and vegetation heights were measured with a 5m measuring tape. Cache locations were marked by tying strips of plastic to the vegetation close to the cache. These locations were also recorded on a hand-drawn map of the site.

Removal of untagged seeds:

To determine the rodents preference towards one of the nut species, untagged seeds (Sunflower, *Leucadendron sessile, Wildenowia incurvata* and *Ceratocaryum virgata*) were released in groups of 25 seeds per species, resulting in caches of 100 seeds. Each experiment consisted of 10 caches, half were released under bushes and the other half released in the open to test whether rodents preferred to forage in sheltered locations. A total sample size of 1000 seeds was thus achieved per experiment. The number of seeds of each species that had been removed was recorded between 17:30 and 18:00 for the day experiments and between 08:00 and 09:00 for the night experiments to determine at what time of the day most seeds are removed. Caches were refilled upon inspection and locations were recorded on a hand-drawn map of the study site.

Two days and two nights of seed removal experiments were performed between 08:00 on the 20th of August and 09:00, 22 August at Sir Lowrys pass. Weather conditions during this time were warm and windy during the day with clear, cold nights.

Retrieval of experimentally buried seeds

Sunflower, *L.sessile, C.virgata* and *W.incurvata* seeds were experimentally buried 5cm apart in grids of 5 by 4 rows at shallow depths (1cm vs. just below surface). The purpose of the experiment was to establish whether *A.subspinosus* could detect seeds that had been previously scatter hoarded and what its behavior would be towards this type of seed. Four seed types were included to test whether *A.subspinosus* has a particular preference toward seed of a local (*L.sessile*) vs. alien (*sunflower*) species. Caches were positioned in the open or under bushes in order to assess the degree of shelter under which rodents would forage for seeds.

In the first experiment, on the night of the 15th of August, 10 caches were prepared, each containing 10 sunflower and 10 *L.sessile* seeds. Half the seeds were buried just below the surface, while the other half were buried at 1cm below surface level. Holes were prepared by pressing a dowel marked at 1cm from the end into the ground until the mark was at the soil surface level. A seed was then placed in each hole and pushed down with the dowel until the seed reached the end of the hole.

In the second experiment, during 20 August and 22 August, 10 caches were prepared, each containing 5 sunflower, 5 *L.sessile*, 5 *W.incurvata* and 5 *C.virgata* seeds. The seeds were buried at 5cm below the surface in order to assess the maximum effort that the rodents were prepared to invest in order to obtain a seed. Caches were prepared in the open or under bushes as in the first experiment. The *depths* and *widths* of holes created by rodents were measured with a stainless steel ruler accurate to 0.5mm. The number of each seed type that a rodent had attempted to excavate was recorded. Cache locations were recorded on a hand-drawn map of the site.

Rodent trapping experiments

The first night of trapping was the 15th of August 2002, when 19 Sherman live traps were baited with sunflower seeds and placed in two transects, 10m apart. Traps were emptied at 08:00 the following morning and species abundance was recorded with the location of the traps. These experiments were conducted to determine the densities of different species of rodents in the environment. Specimens were photographed before being released.

The remaining trapping experiments were performed between the 20th and 22nd of August when 11 traps were baited with sunflower seeds and set in a grassy clearing surrounded by mostly *P.repens*. The clearing was within 50m of the seed burial, recovery and removal experiments.

Choice of study site:

At Jonaskop, situated in the Riviersonderend mountains (33° 55'S, 19°31'E) near Worcester, two species of seeds were released (*Protea magnifica* and *Protea lorifolia*) in order to test whether rodents preferred bigger or smaller seeds. The presence of *A.subspinosus* was recorded at Jonaskop by rodent census studies (Fleming and Nicolson, 2002) and it was hypothesized that seed burial would be demonstrated at the site. Furthermore, it was thought that burial rates would be higher in rocky areas than in open plains, because *A.subspinosus* has shown preference towards rocky outcrops (Bond *et al*, 1979).

Bainskloof:

As a pilot study, 200 sunflower seeds were released in order to test for the presence of scatter-hoarding rodents (*A.subspinosus*). Further species comparisons would be possible if the presence of *A.subspinosus* was confirmed by the burial of some sunflower seeds. A week after the seeds had been released only two seeds had been buried and the experiment was terminated.

At this point it was decided that a site adjacent to Sir Lowrys Pass should be the focus of this experiment as *A.subspinosus* has been shown to scatter-hoard seeds of Cape *Proteaceae* species (Midgley *et al*, 2002).

Variables:

To further explore the dynamics of seed dispersal by *A.subspinosus*, the following variables were recorded (statistical analysis was performed with “Statistica v5.5” and “Genstat v6.0”)

1. Location: The removal rates of seeds from sites offering shelter (under bush) were compared with removal rates from completely exposed sites (open). The final location (in situ, in a crack between rocks, in the open or under a bush) of seeds was also recorded.
2. Seed type: The seeds of four species (Sunflower, *Leucadendron sessile, Wildenowia incurvata* and *Ceratocaryum virgata*) were released in order to test whether *A.subspinosus* displayed preference towards a particular seed type.
3. Fate: Relocated seeds were classified as either ignored, eaten or buried.
4. Distance from cache: This variable was recorded to ascertain the spatial component of seed dispersal by scatter-hoarding rodents.
5. Distance from nearest adult plant stem: This variable was recorded for all buried seeds.

6. Burial depth: This variable would provide insight into the effort that *A.subspinosus* will invest in seed burial. Depths of holes created by *A.subspinosus* during retrieval of buried seeds would provide insight into the effort that the mice will invest on relocating seeds

A regression analysis (binomial distribution) in Genstat v6.0 was used to calculate the statistical probability of a category compared to a reference level category. The category is chosen automatically by Genstat according to alphabetical order and results are presented as the likelyhood of the other categories relative to it (Antilog of estimate). The strength of statistical significance for each result lies in the t pr. value. A low value, indicates a more significance result.

Results:

De Hoop Nature Reserve:

In the late afternoon, 80 sunflower seeds were released into a stand of *Leucadendron tinctum* situated on a slope with rocky outcrops for one night, no seeds were buried.

Seed recovery rates:

At least two thirds of seeds were recovered after release at any site. Of the recovered seeds, the highest burial rate was at Sir Lowrys Pass (Table 1)

Table 1. Recovery and burial rates for all seeds at each site:

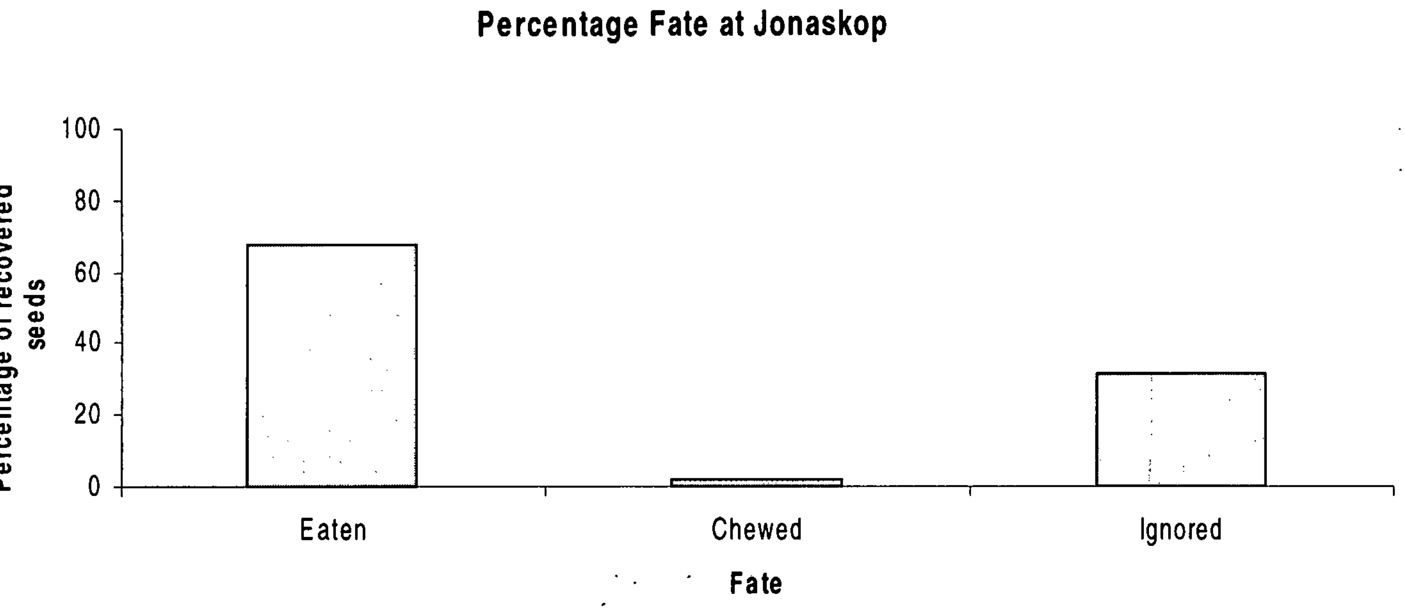
Site	Recovered (%)	Buried (%)
Jonaskop	77.67	0
Bainskloof	100	1
Sir Lowrys Pass	66%	7.3

Table 2: Percentage of seeds recovered from each site ("****" indicates that the seed type was not released at that site).

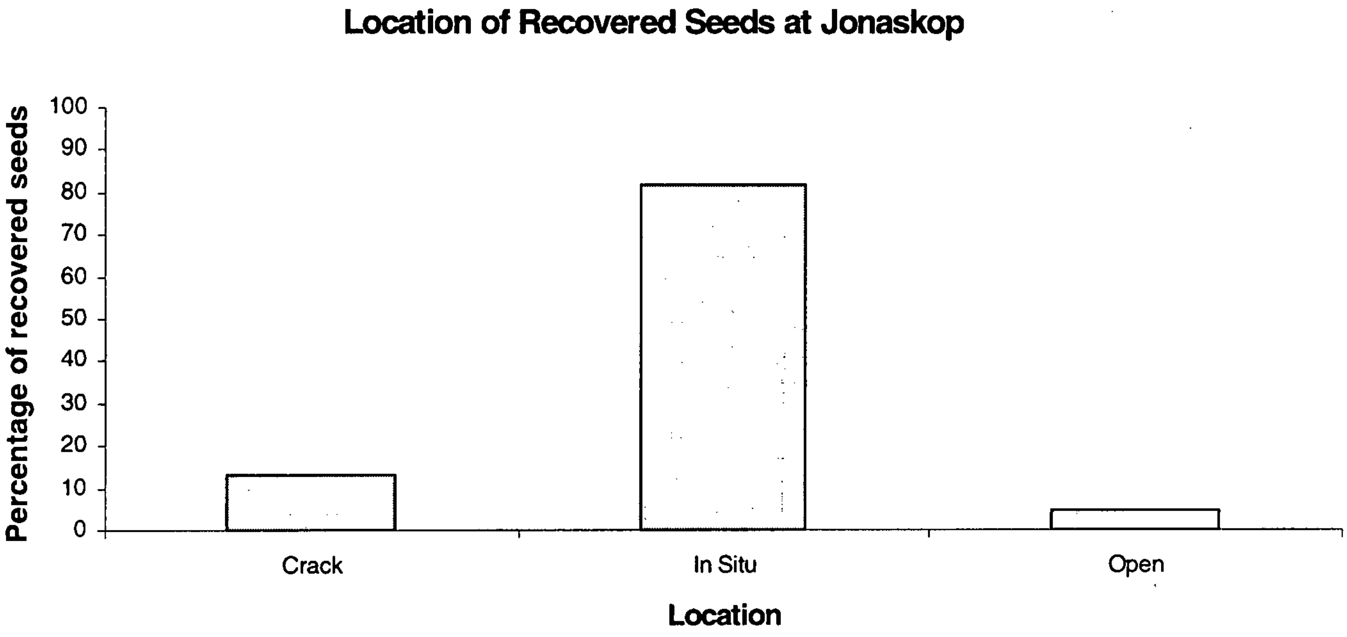
			Seed Type (%)			
	Nuts	Pappus	Sunflowers	*L.sessile*	*W.incurvata*	Unknown
Site						
Jonaskop	70	93	***	***	***	***
Bainskloof	***	***	100	***	***	***
Sir Lowrys Pass	***	***	51.3	31	31	61.6

Jonaskop:

Two species of seeds (300 seeds in total) were released into 2 contrasting environments that occurred adjacent to each other. The rocky outcrops were inhabited by mice (pers. obs.) at least during the day, while at least one mouse burrow was discovered in the plains. Evidence for seed burial was not found upon inspection of the site one week after the seeds were released (Table 1). Nearly 70% of seeds were eaten (Graph 1) and the most common location of recovered seeds (Graph 2) was in situ (80%). It is impossible to say which species of rodent was the main seed predator at this site, but the method of opening the nuts found at Jonaskop was very similar to that of *A.subspinosus*. Seed coats of seeds eaten by *A.subspinosus* are characterized by the presence of a neatly chewed hole on one hemisphere of the seed (J.Midgley, pers. obs.)



Graph 1: Percentage Fate at Jonaskop.



Graph 2: Location of Recovered Seeds at Jonaskop.

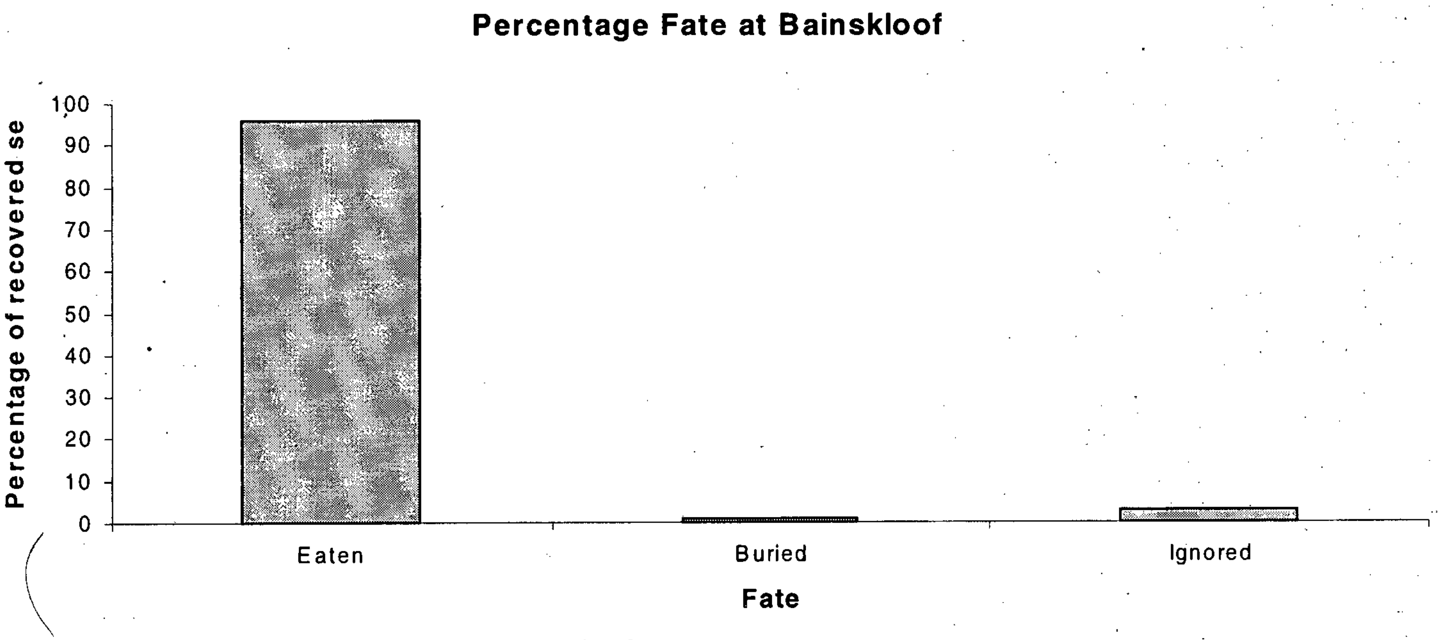
Table 3: Probability of fate for seed types at Jonaskop: (Nut and Eaten as reference level)

Type	Fate	t pr.	Antilog of Estimate
Pappus	eaten	0.79	2.07
Pappus	ignored	0.80`	0.48
Nut	eaten	0.00	1.00

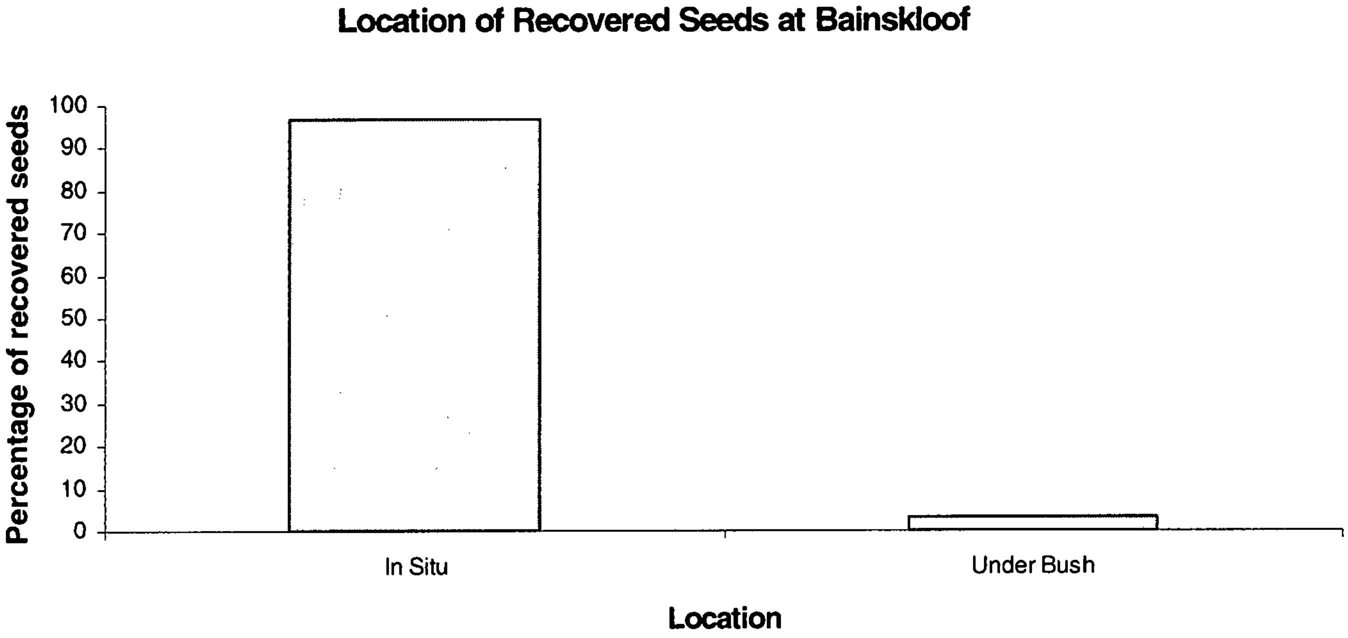
The probability of the seed predator eating a pappus seed was twice that of it eating a nut (Table 3). It is also evident that a pappus seed has half the chance of being ignored than a nut has of being. These results have no statistical significance, therefore they are only indications of the rodents preference.

Bainskloof:

Only sunflower seeds were released at Bainskloof as the initial evidence for seed burial was required before species comparisons could commence. Most seeds were eaten (Graph 3) in situ (Graph 4) when seeds were retrieved a week after release.



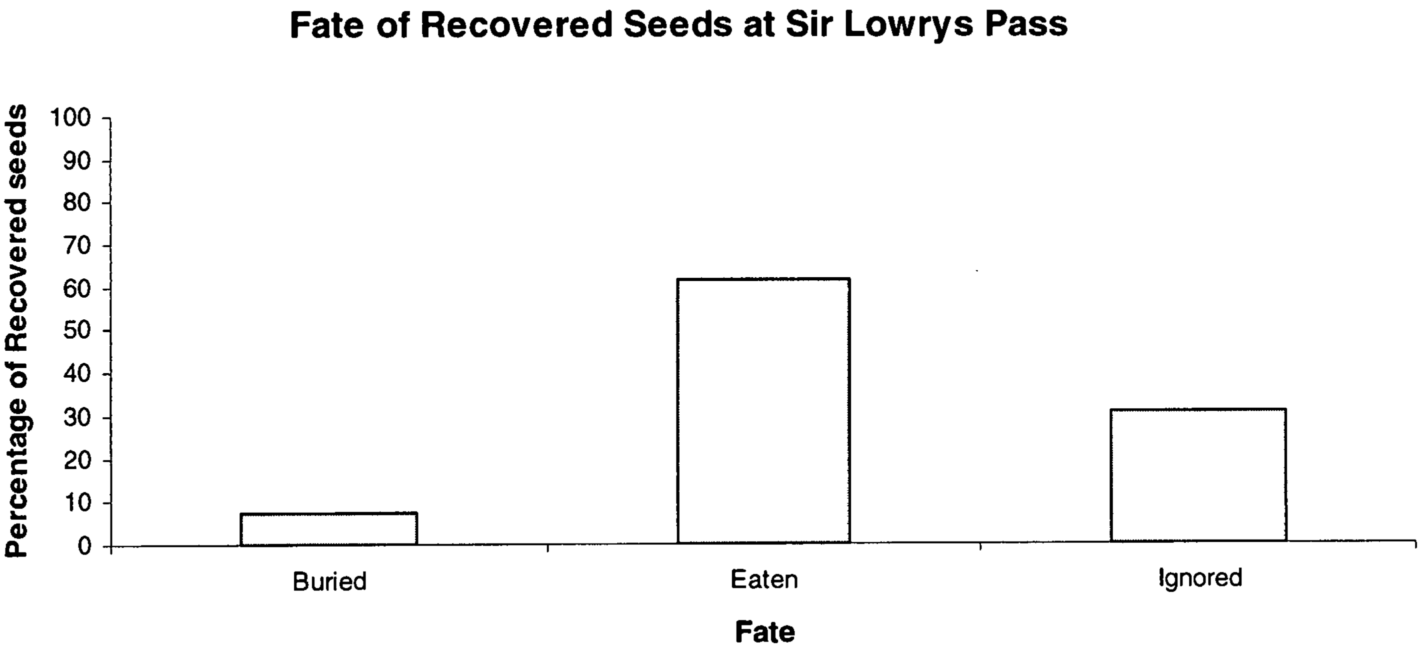
Graph 3: Percentage Fate at Bainskloof.



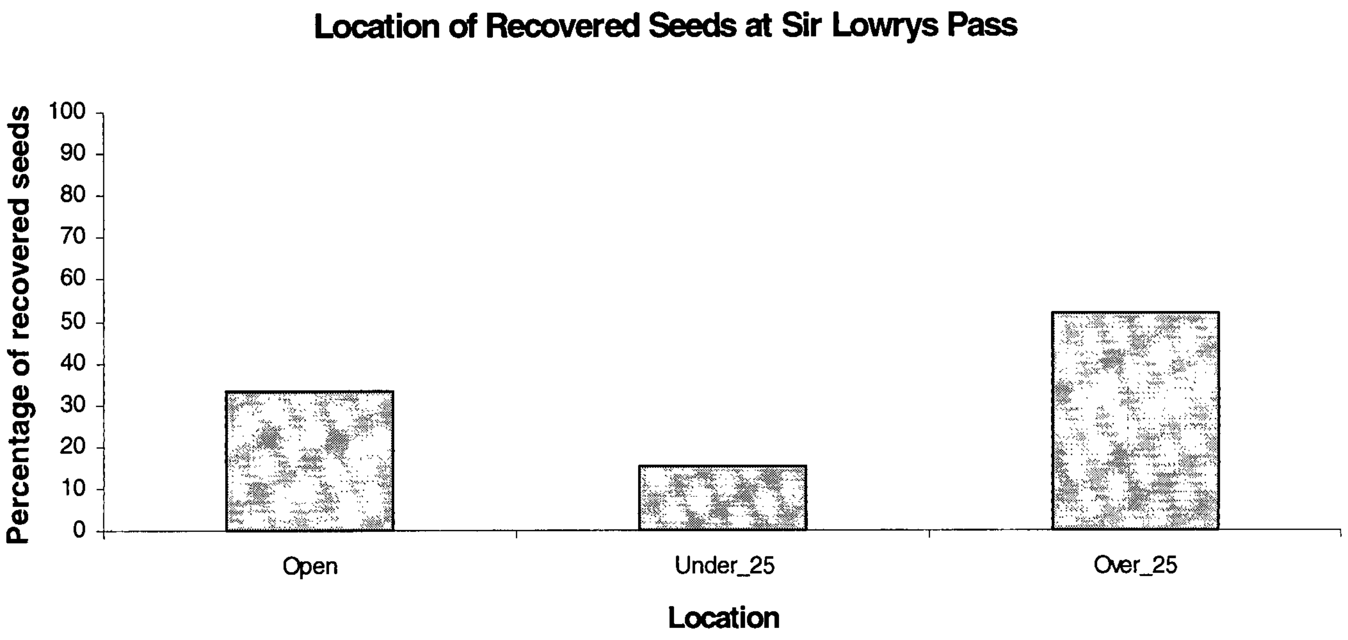
Graph 4: Location of Recovered Seeds at Bainskloof.

Sir Lowrys Pass:

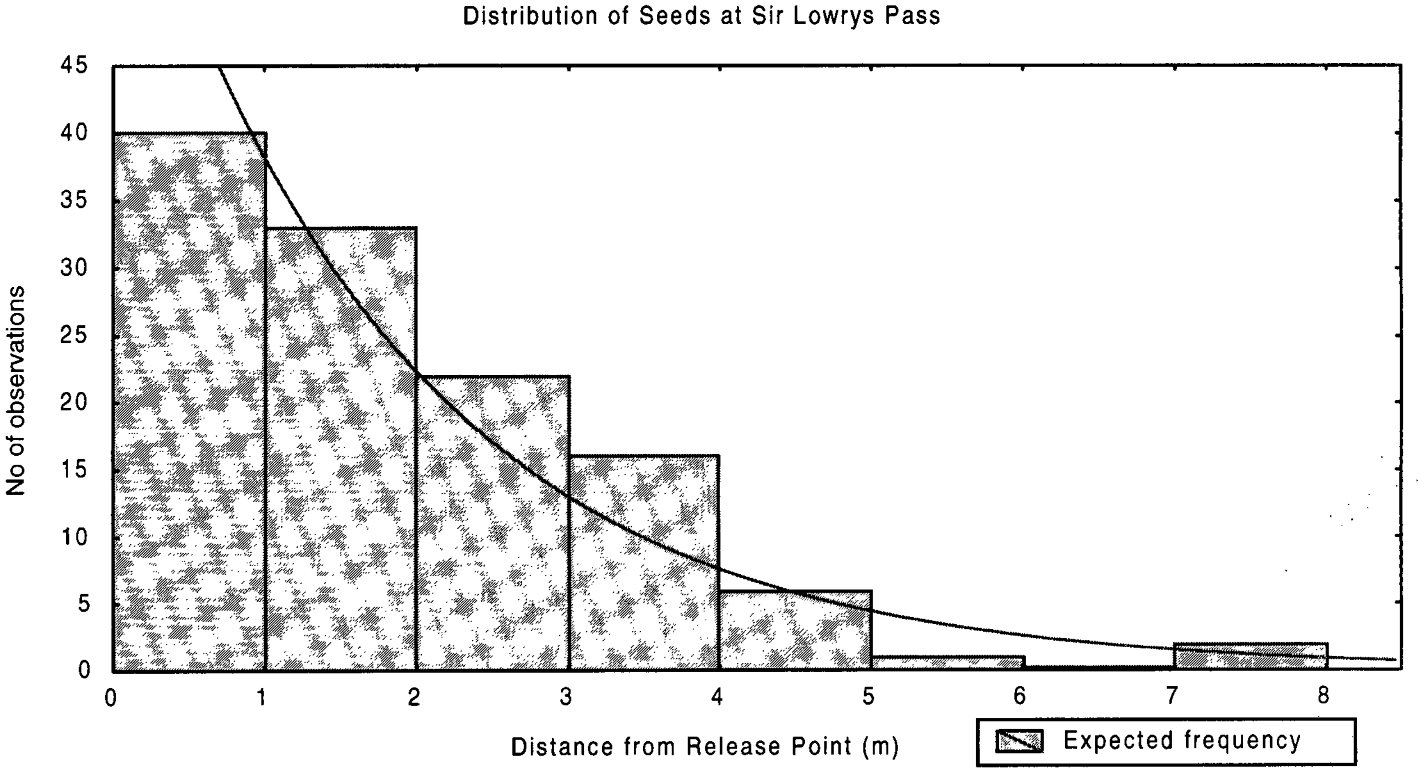
Evidence for burial was found (Graph 5), but rates were lower (7.3%) than those found by Midgley *et al* (2002) at the same site (47%) during summer.



Graph 5: Fate of Recovered Seeds at Sir Lowrys Pass.



Graph 6: Location of Recovered seeds at Sir Lowrys Pass.



Graph 7:Distribution of Seeds at Sir Lowrys/h Pass .

Seed Removal:

The statistical probability that a particular seed type will be removed by a rodent was calculated relative to the reference level selected by Genstat, which was the removal probability of *C.virgata* (Table 4). It was found that sunflower seeds were the most likely seed type to be removed.

Table 4: Probability of seed removal at Sir Lowrys Pass (*C.virgata* as reference level):

Type	t pr.	Antilog of Estimate
Sunflower	0.236	66728.00
L.sessile	<0.001	278.50
C.virgata	<0.001	1.00
W.incurvata	<0.001	0.29

Regression analyses were performed to test the correlation between seeds that were located in the open and those under bushes (Table 5), seed removal rates during the day vs. during the night (Table 6), seed type vs. fate (Table 7)

Table 5: Results of a linear regression between seed type and their location. The relative likelihood (antilog of estimate) and the probability (t pr.) of each category are based on a comparison with *C.virgata* and "Open" as the reference level).

Type	Location	t pr.	Antilog of Estimate
L.sessile	under	0.64	104.90
Sunflower	under	0.95	0.43
W.incurvata	under	0.57	0.85
C.virgata	under	<0.001	1.00

Statistical evidence for the removal of Sunflower seeds was obscured as all seeds were removed from most sites. This result makes accurate estimation of mean, SD and subsequently the probability and antilog of the estimate impossible.

Table 6: Results of a linear regression between daytime removals and nighttime removals for all seed types at Sir Lowrys Pass (*C.virgata* and daytime as reference level).

Type	t pr.	Antilog of Estimate
Sunflower	<0.001	189.90
L.sessile	<0.001	36.50
C.virgata	<0.001	1.00
W.incurvata	<0.001	0.29

Sunflower and *L.sessile* seeds were more likely to be removed during the night than *C.virgata* and *W.incurvata* seeds (p<0.001 significance level).

Table 7: Probability of seed removal at Sir Lowrys Pass: (*L.sessile* and Buried as reference level)

Type	Fate	t pr.	Antilog of Estimate
Sunflower	Eaten	0.98	1.16
Sunflower	Ignored	0.95	1.16
W.incurvata	Ignored	0.94	0.73

The results are not statistically significant, but the trend in the data suggests sunflowers were more likely to be eaten than *L.sessiles* are to be buried.

Rodent Trapping Experiments:

A.subspinosus was the dominant species captured at the site (Table 8). In total, 30 traps were set and 11 individuals were caught during two nights and one day. All *A.subspinosus* specimens were trapped at night.

Table 8: Rates of rodent capture at Sir Lowrys Pass:

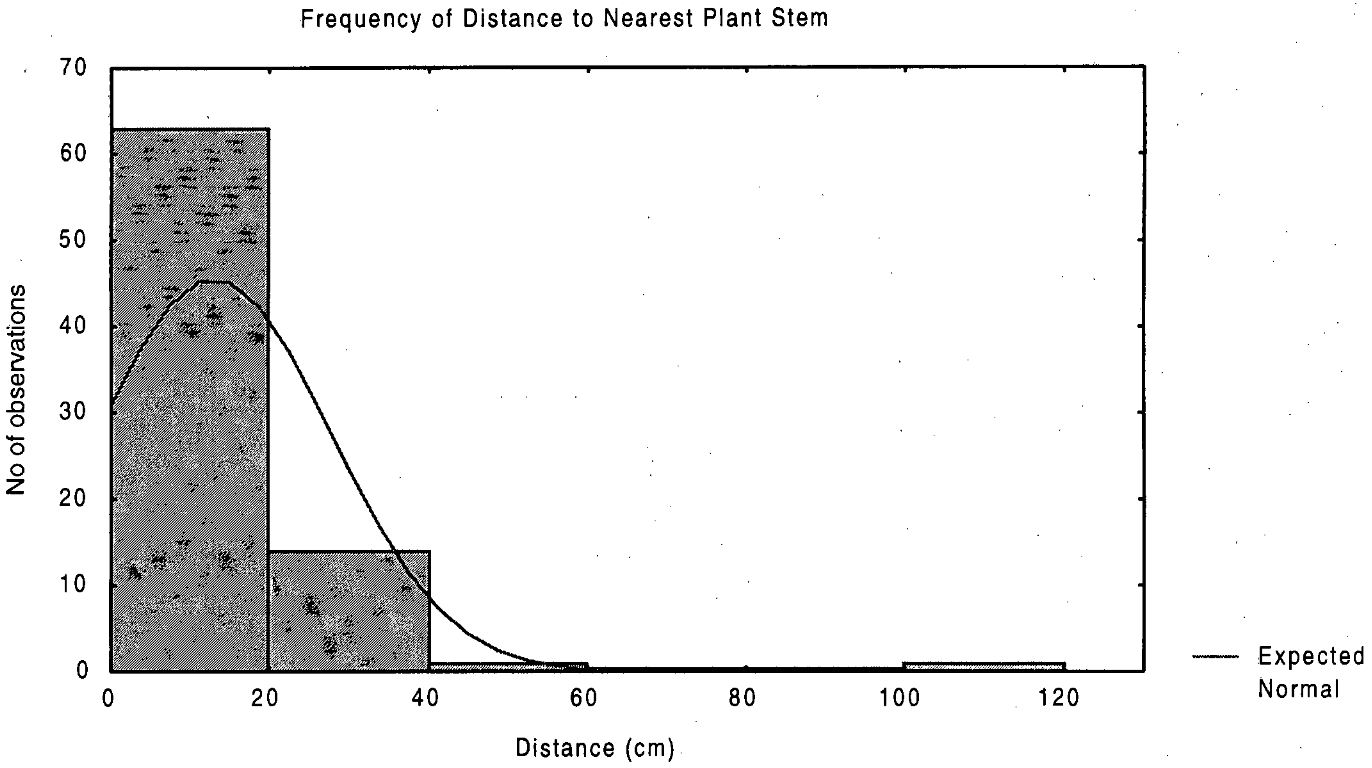
Species	No. of Individuals
Acomys subspinosus	7
Rhabdomys pumilio	2
Elephantulus edwardii	1
Vlei Rat	1

Experimentally Buried seeds:

80% of buried seeds were within 20cm of an adult plant stem (Graph 8). The average depth of burial was 1.68 cm (Table 11). On average, seeds were buried closer to adult plants than to another buried seed (Table 9). *A.subspinosus* displayed a preference towards burying *L.sessile* seeds (Table 10)

Table 9: Average distances (m) that a buried seed was from an adult plant stem and another marked seed:

	Mean	Maximum	Standard Deviation
Nearest plant stem	0.13	1.13	0.15
Nearest marked seed	0.74	3.30	0.95



Graph 8: Frequency of Distance to Nearest Plant Stem

Table 10: Burial rates for seed types at Sir Lowrys Pass:

Seed Type	% of Buried Seed
Leucodendron	65.0
Sunflower	30.5
W.incurvata	4.5

Table 11: Mean, minimum, maximum and standard deviation of the 31 seeds buried by *A.subspinosus*

	N	Mean	Minimum	Maximum	Std.Dev.
Burial Depth	31	1.68	1.00	2.00	0.48

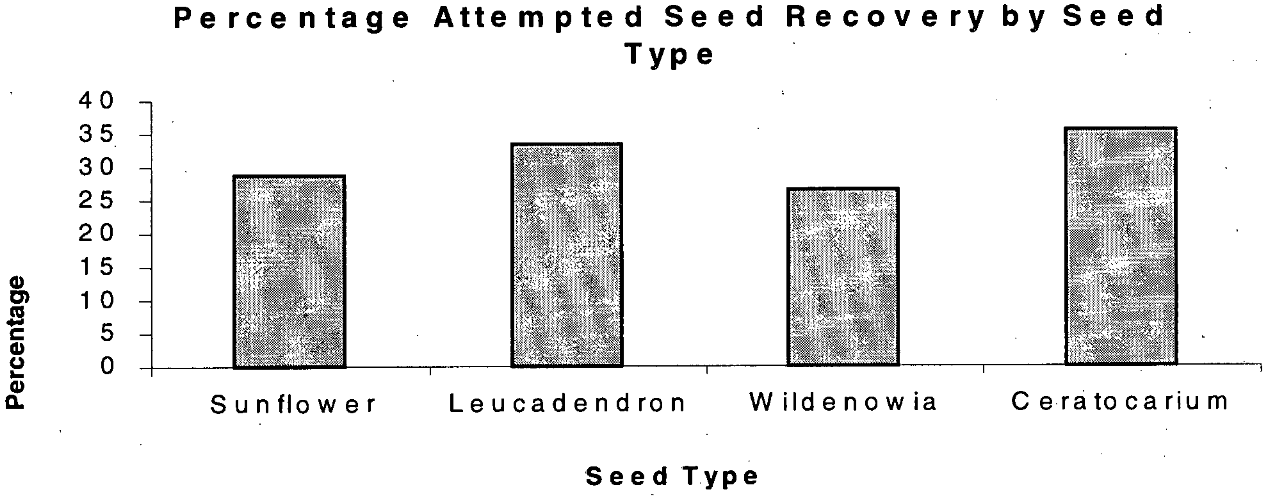
Seed Retrieval:

A nocturnal rodent, probably *A.subspinosus*, located and excavated some of the experimentally buried seeds (Graphs 12 and 13). Most seeds buried in open sites were retrieved on the first night (Graph 10) while most seeds buried under bushes were retrieved on the second night (Graph 11) following burial. All seeds buried at 1cm below the surface and just below the surface were removed by a rodent. The rodent could only locate 65% of seeds buried at 5cm below the surface. None of these seeds were completely excavated by a rodent and no preference towards attempted retrieval of any species was visible in the data (Graph 9). Holes that were created by *A.subspinosus* to excavate seeds that were buried at 1cm below the surface were shown to be statistically smaller than holes created to excavate seeds buried at 5cm (Table 12). The hole volumes were shown to be normally distributed (Graph 14) and Box and Whisker plots of hole volume (Graph 15) and hole depth (Graph 16) clearly display the difference in energy investment by the excavator.

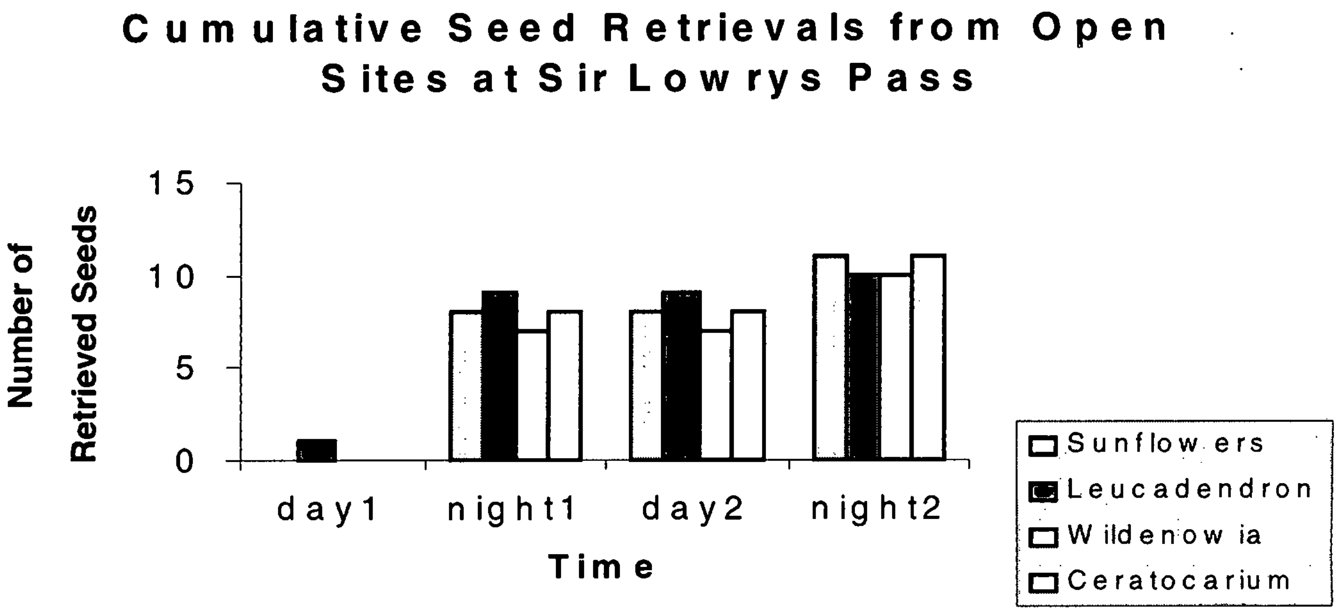
Table12: Results from the T-test for Dependent Samples performed on the hole volumes from 1cm and 5cm trials at Sir Lowrys Pass
Values are significant at $p < 0.05$

	Mean	Std.Dv.	N	Diff.	Diff.	t	df	p
5cm	3.46	1.28						
1cm	2.72	0.88	38	0.73	0.42	10.59	37	0.000

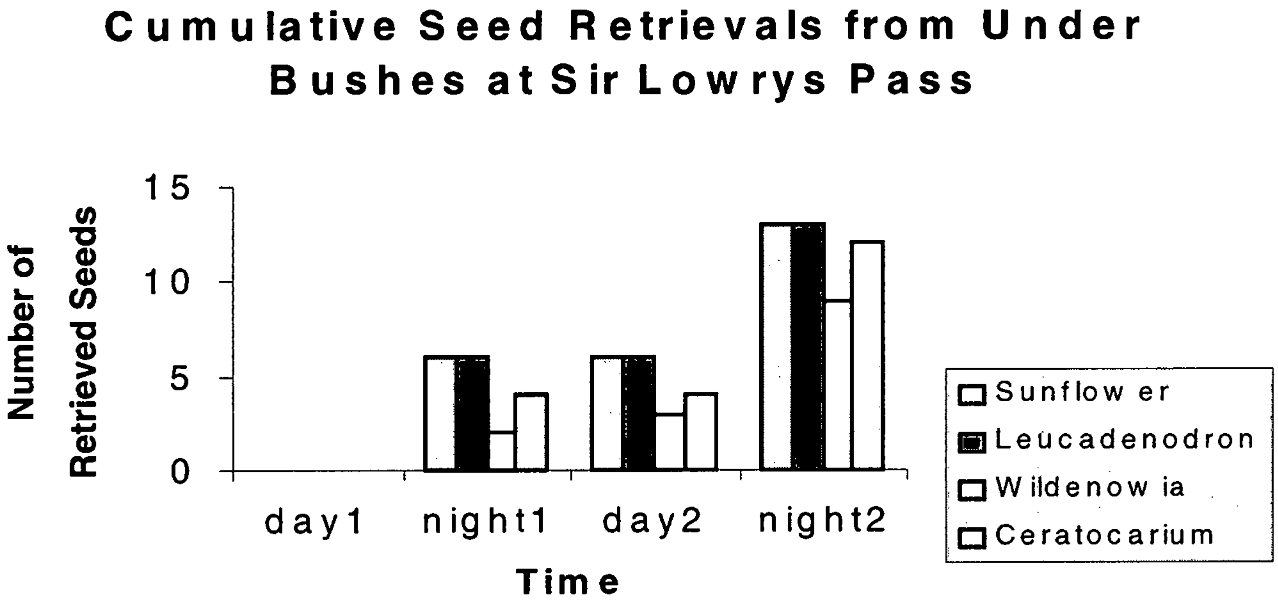
This test shows that the samples are not dependent (p<0.05).



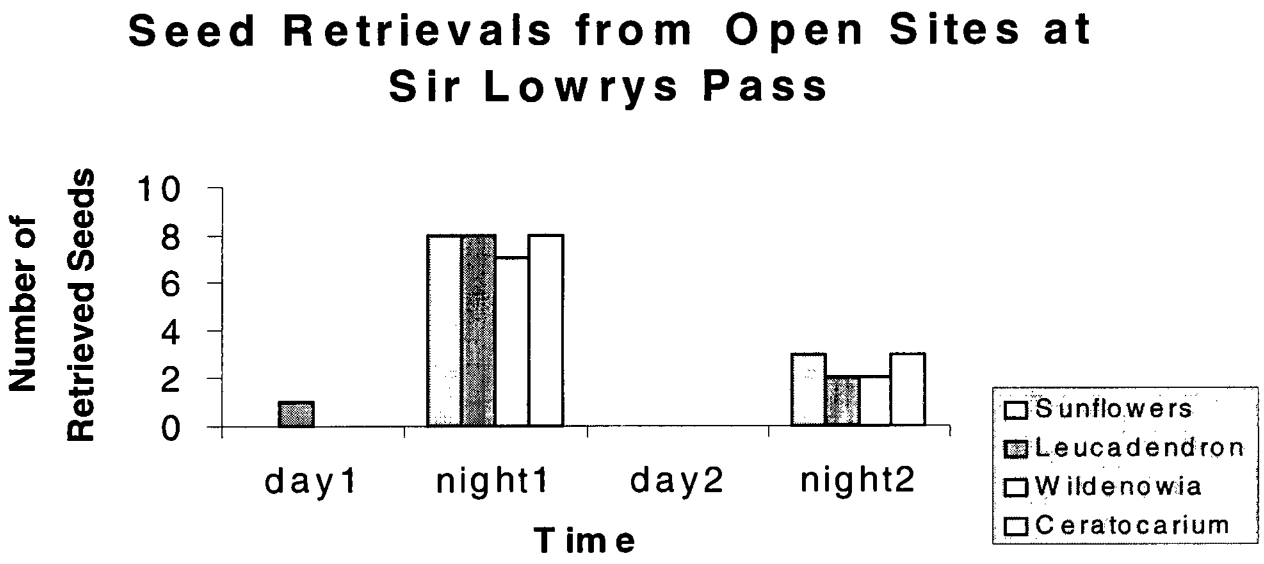
Graph 9: Percentage attempted seed recovery by seed type at Sir Lowrys Pass.



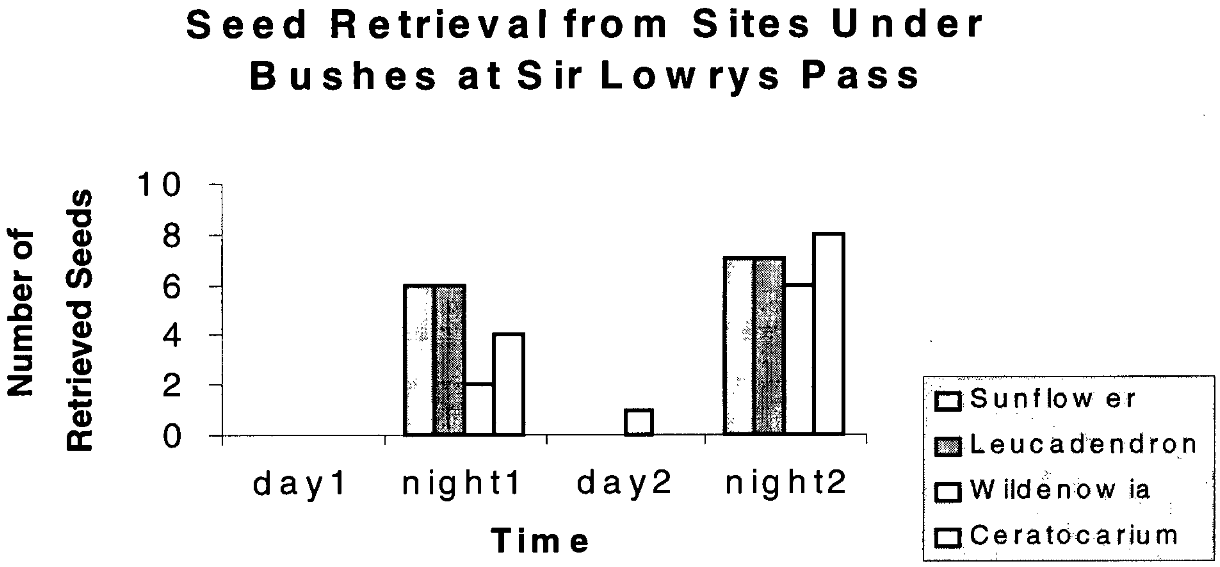
Graph 10: Cumulative seed retrieval from open sites at Sir Lowrys Pass.



Graph 11: Cumulative seed retrievals from under bushes at Sir Lowrys Pass.

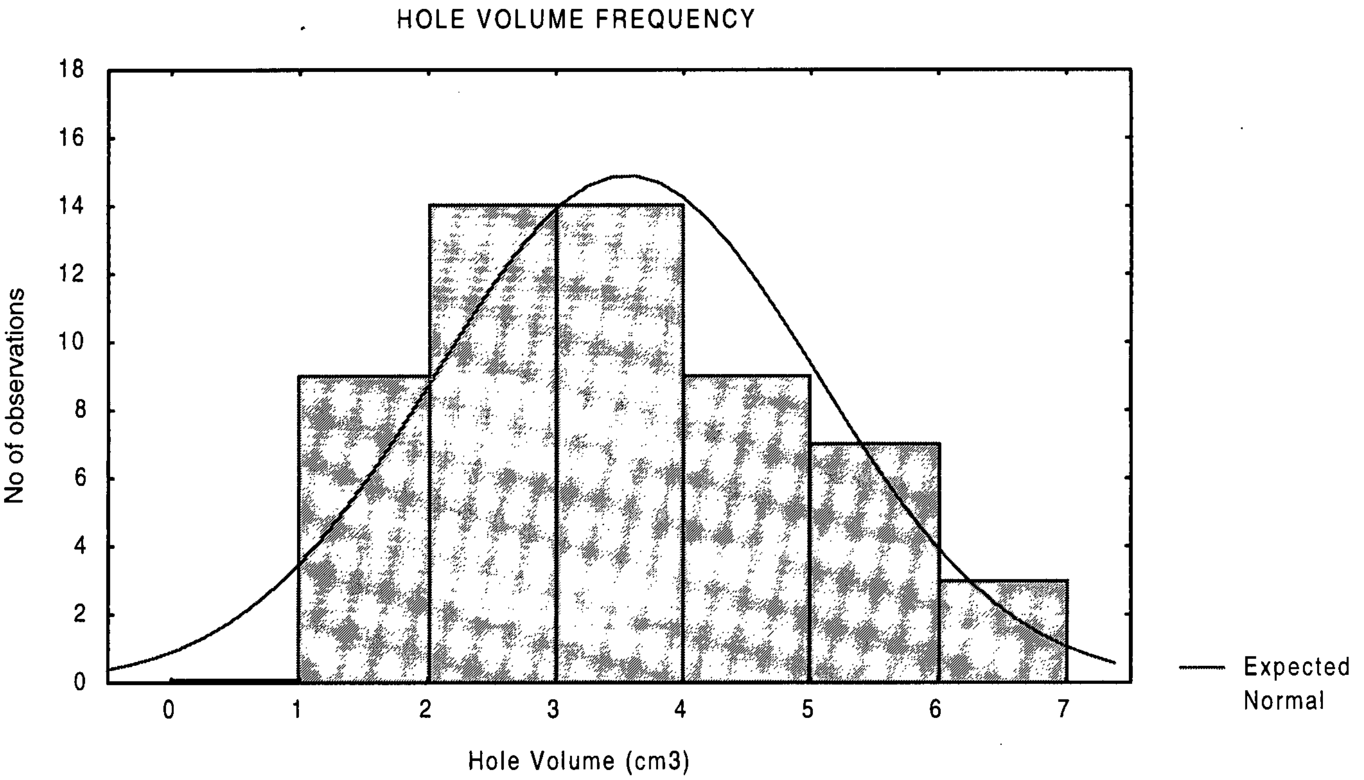


Graph 12: Seed retrievals from open sites at Sir Lowrys Pass.

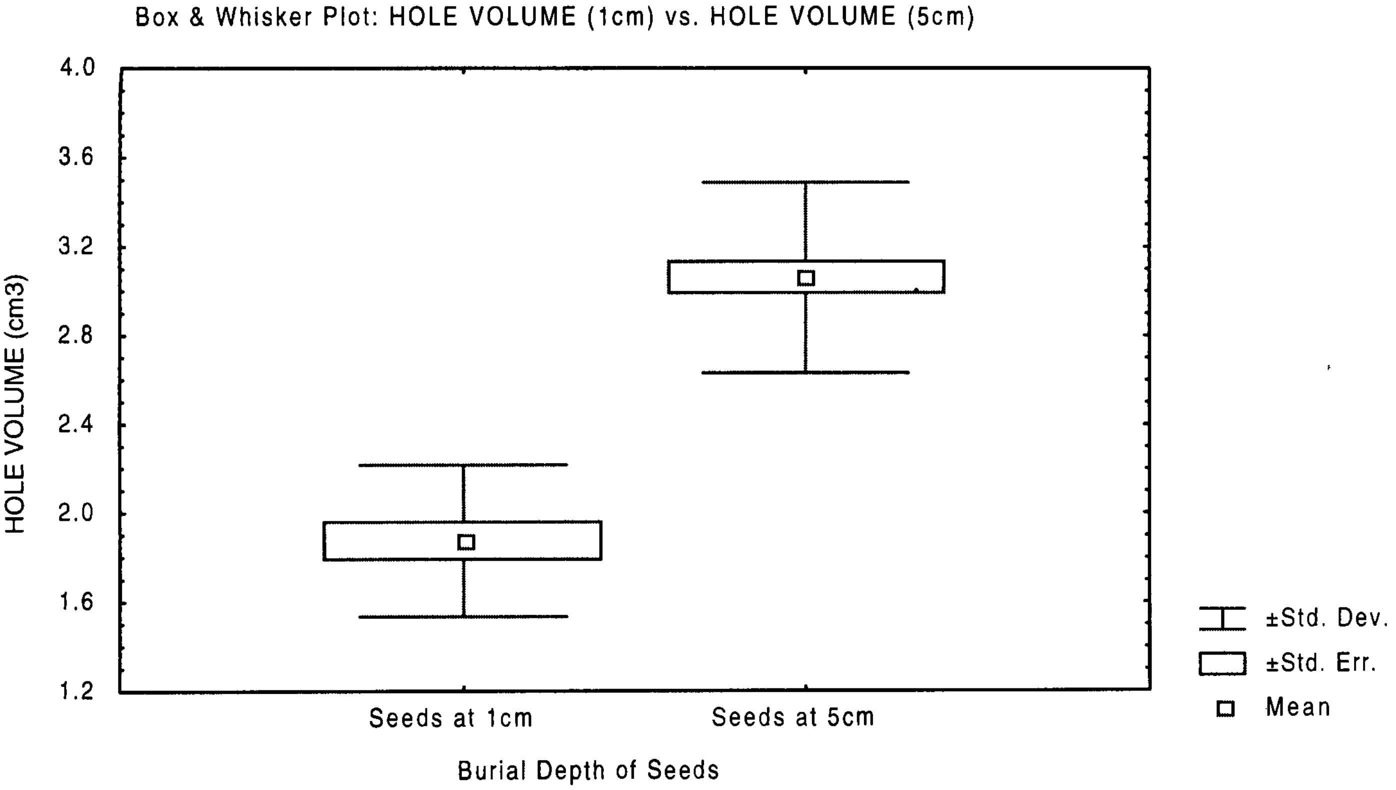


Graph 13: Seed retrieval from sites under bushes at Sir Lowrys Pass.

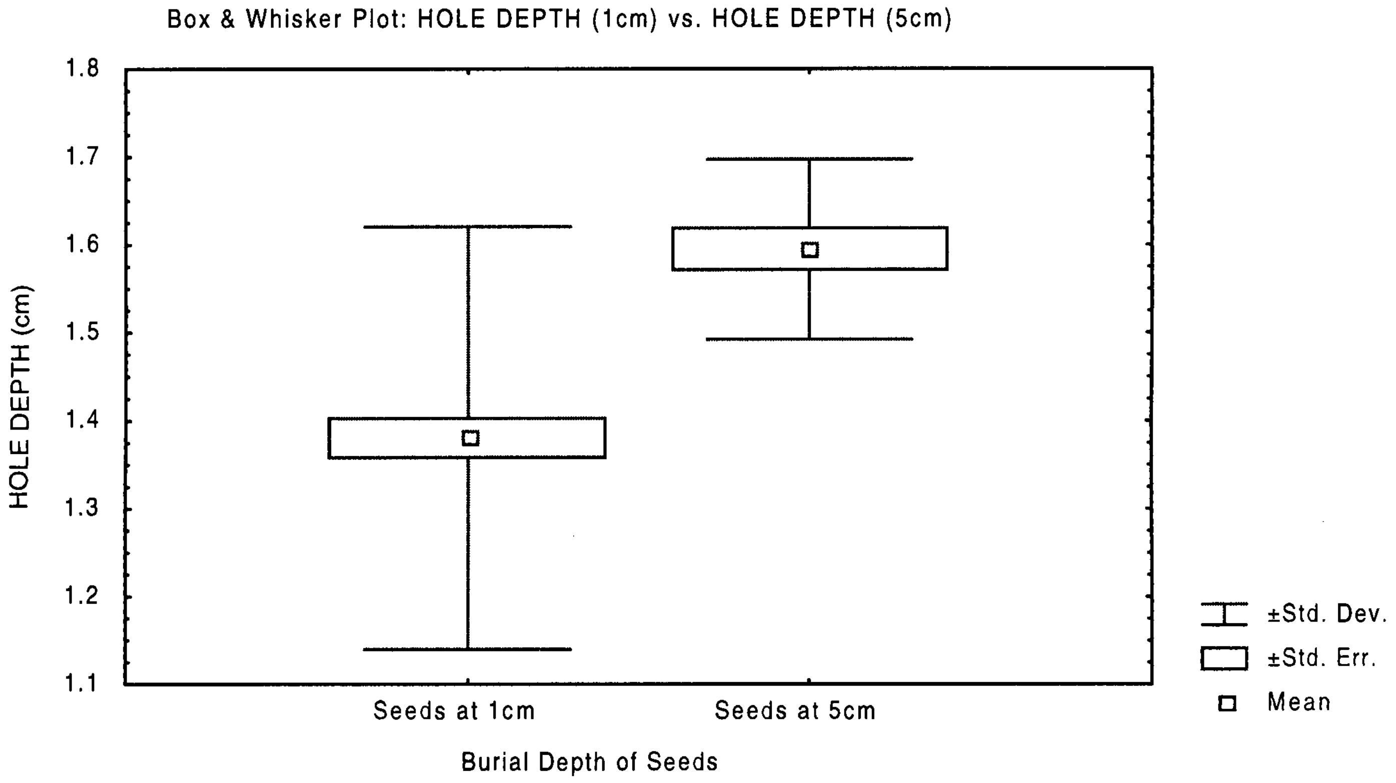
Hole Volume:



Graph 14: Hole Volume Frequency shows that most holes had volumes between 2 and 4 cm3. The frequency of hole volumes follows the expected normal distribution.



Graph 15: Box & Whisker Plot of Hole Volumes for Seeds Buried at 1cm vs. 5cm.



Graph 16: Box & Whisker Plot of Hole Depths for seeds buried at 1cm vs. 5cm.

Discussion:

Jonaskop:

A recent study at Jonaskop (Fleming and Nicolson, 2002) suggested that *A.subspinosus* feeds almost exclusively on pollen and presumably nectar during the winter months (while *P.humiflora* is in flower). Perhaps no other seeds are available for *A.subspinosus* to feed on during this season and the rodents behavior shifts from storing seeds to consuming them upon discovery. Fleming and Nicolson (2002) also suggest that *A.subspinosus* occurs in transient populations that seek flowering plants or plants setting seed and will breed opportunistically. Perhaps there was a stand of flowering *P.humiflora* some distance from the site I used at Jonaskop. If most *A.subspinosus* individuals were focussed on pollinating *P.humiflora*, few individuals would have been found at sites with *P.magnifica* and *P.lorifolia*. Either the absence of *A.subspinosus* or seasonality in hoarding behaviour could explain the absence of buried seeds.

This evidence suggests seasonality in the hoarding behavior of *A.subspinosus*. A different explanation for this discrepancy between the two experiments lies in the theory of transient populations, suggested by Fleming and Nicolson (2002). I would hesitate to suggest that most *A.subspinosus* individuals relocated to another site, thus causing lower burial rates. As the seed removal experiments showed, most seeds were removed during the night (Graphs10, 11, 12 & 13), strongly suggesting the presence of a healthy *A.subspinosus* population. I would suggest that the *A.subspinosus* population remains in one area, but alters its hoarding strategy during the winter.

Sir Lowrys Pass:

Location:

The most common location of relocated seeds was under bushes taller than 25cm ie: *P.repens* or *L.sessile* plants (Graph 6). Sunflower and *W.incurvata* seeds were more likely to be found at a site with vegetation taller than 25cm (Table 5). This suggests that rodents remove seeds from less sheltered locations to areas providing shelter from avian predators. Another reason for this phenomenon is that *A.subspinosus* burrows could be situated under tall bushes and that seed would be moved and eaten nearer to the rodents burrow. This would allow the individual to dash into its burrow if a predator is sensed. Both explanations are associated with shelter from predators. The nocturnal activity of *A.subspinosus* would lead one to believe that there is little threat to *A.subspinosus* from avian predators, perhaps there is no need for *A.subspinosus* to remove seeds to sheltered sites. Under bushes at the site, rodent pathways are abundant (pers. obs.), the dacron strings may have hooked onto surrounding vegetation separating the string from the seed while *A.subspinosus* was transporting them, resulting in many strings being relocated from under tall bushes. This could explain the dominance of unknown seed types being relocated (Table 2), as this category was assigned to strings found without an identifiable part of a seed attached to it.

The most common type of seed to be buried was *L.sessile* (Table 10) and this was also the most uncommon type of seed to be relocated (Table 2). Therefore, if the dacron marker was removed from the seed before burial, it is possible that more seeds were buried than are reflected in the data.

Distance:

The mean and maximum distance that seeds were moved from a cache was greater than at Bainskloof and Jonaskop (Graph 7), perhaps as an effect of greater *A.subspinosus* densities relative to other species. The negative exponential distribution shows that mice are likely to remain near to the seed cache. An effective method of tracking rodent movement in the wild is required. This would allow deeper interpretation of hoarding strategy.

Seed Preference:

Unmarked (stringless) seeds were released to calculate the preference toward species, if any existed. Table 4 compares the statistical preference of *A.subspinosus* towards sunflowers, *L.sessile*s and *W.incurvata*s with *C.virgata* as a reference level. It is evident that sunflowers and *L.sessile*s are preferred, while *W.incurvata* seeds are the least popular species. The *W.incurvata* seeds were the smallest and had the hardest seed coat of the four types. The reason for the low removal rates of *W.incurvata* could be that investment into opening the seed is more than the caloric value of the nut itself. Another reason could be that a high percentage of *W.incurvata* seeds are infertile and that *A.subspinosus* could detect this. Upon examination of ignored seeds by Midgley *et al* (2002), it was found that most were infertile (B. Anderson, pers. comm.). When the location of the seed is included in the regression analysis (Table 5), all statistical significance is lost. The trend in the data shows that *L.sessile*s are 104.9 times more likely to be removed from under a bush than a *C.virgata* is to be removed from an exposed clearing. A more significant result came from the regression between removals during the day and removals at night (Table 6) which clearly shows that sunflower seeds and *L.sessile*s are likely to be removed at night. Table 7 shows, with very little significance that sunflower seeds are more likely to be eaten, when compared with the probability that a *L.sessile* seed has to be buried. The low correlation in the regression analysis between these factors proves that this comparison is meaningless, because there are 3 potential fates (eaten, buried or ignored).

Buried Seeds:

For the 31 buried seeds out of 463 recovered seeds, the mean depth was 1.68 cm (Table 11). This result is similar to that of Midgley *et al* (2002) who found that 98% of relocated seeds were buried <2cm deep. While recovering seeds, I noticed that many of the buried seeds were located close to the base of established adult plants. I decided to record this variable as it impacts on the survival potential of the seedling. The data shows that *A.subspinosus* buried 80% of seeds within 20cm of an adult plant stem (Graph 8). This is unfortunate for seeds, as competition with an adult, if it was a resprouter, for water and nutrients may reduce a seedlings chance of successful establishment (Johnson, 1992). However, after a fire has removed non-sprouting adult plants, seeds should be well distributed throughout the environment. The soil near to their germination point should be enriched with nutrients from decaying root material and burnt plant material. If *A.subspinosus* avoids burying seeds at the base of resprouters then the location of the microsites that *A.subspinosus* provides a seed with during burial could provide seedlings with a competitive advantage over other seedlings.

Nearest Seed:

The fact that seeds are buried singly on most occasions means that they are likely to have little competition from each other. Greater distance between seeds means more successful dispersal has occurred. The average distance that *A.subspinosus* disperses hoarders like American crows which can disperse seeds on a range of kilometers (Cristol, 2001).

Seed Retrieval:

When seeds were experimentally buried in grids (20cmX20cm), the holes that rodents created during their search for the seeds were measured. The volume of the hole is equivalent to the volume of soil removed by the rodent which, in turn, is a measure of the effort that the rodent invested into its search. Very little preference towards a particular species was observed in the attempted recoveries of these buried seeds (Graph 9). The range of hole volumes was shown to follow the normal distribution (Graph 14). The first experiment consisted of seeds buried at 1cm and just below the soil surface. It is evident that the range of hole sizes created during the recovery of these seeds was larger than that of seeds in the second experiment, when seeds were only buried at 5cm below the surface.

The second experiment shows that the maximum depth that rodents were prepared to dig to was within 0.1cm of the maximum depth that rodents dug to in the first experiment (Graph 16). This data suggests that if a mouse doesn't find a seed within roughly the first 2cm of soil, it will abandon its efforts to locate the seed. The maximum total effort invested (Graph 15) in terms of the volume of soil removed seems to be around 3.6 cm^3. This average effort was far greater than when seeds were recovered from 1cm, yet very little maximum depth was gained from this effort. Perhaps the hole gets so large, relative to the rodent, that increasing the maximum depth requires too much energy to be invested and the rodent realizes that its search is too costly. An alternative explanation could be that *A.subspinosus* only buries seeds between 1.5 and 1.7 cm deep. If it doesn't find its seed within this depth, then it starts to dig around, instead of deeper down. The hole volume will increase, without increasing the maximum depth.

The extraordinary strength of the olfactory ability in *A.subspinosus* is displayed by this experiment. I would suggest that *A.subspinosus* relies mainly on this sense in relocation of scatter hoarded seeds rather than any sort of extensive spatial memory of its environment.

Conclusion:

Fleming and Nicolson (2002) showed that *A.subspinosus* juveniles were the most abundant during August (50% of population). I think that this could explain the low numbers of buried seeds at Sir Lowrys Pass. It seems that *A.subspinosus* is able to bury so many seeds during the summer and autumn that the population size can be doubled during the winter months. The large numbers of juveniles in the population would require a large amount of food to survive the winter, therefore hoarding strategy is altered and all individuals consume a greater percentage of seeds that they discover. Three out of seven trapped *A.subspinosus* individuals from Sir Lowrys Pass on the 15th of August were juveniles, strongly supporting this hypothesis.

A.subspinosus seems to be a better dispersal mechanism for plants than simply dropping seeds to the ground as seed dispersal over the environment is more uniform when *A.subspinosus* is present. The only potential negative effect of dispersal by *A.subspinosus* is that seeds can be placed in competition with established resprouting plants. I observed a large proportion of buried seeds were dispersed into the grassy clearings between *P.repens* and *L.sessile* stands. Colonization of new habitats is therefore aided by the action of *A.subspinosus*.

A preference towards sunflower seeds was evident from the data and is hypothesized to be due to the ease of opening the relatively thin coat of the seeds compared to *W.incurvata*, *C.virgata* and *L.sessile* seed coats. Thinner seed coats would mean that the handling time of sunflower seeds is shorter than *L.sessile.* This demonstrates the principle proposed by Jacobs (1992), that seeds with a longer handling time should be stored, while those with short handling times are eaten immediately.

The evolution of non-myrmecochorus nut-fruited species is likely to be due to mutualistic relationships with rodent dispersers. Although scatter hoarding was hardly demonstrated at any new sites, seasonality in the hoarding behavior of the disperser is suggested as the cause for the lack of dispersal. If the distribution of *A.subspinosus* is sensitive to climate, subtle climate changes may have already caused the species to be lost from sites with nut-fruited species. The experiments performed in this project should be repeated in other seasons to assess the true macroscopic range of *A.subspinosus* as a scatter hoarder.

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References:

Bond, W.J, Ferguson, M. and Forsyth, G. (1979). Small mammals and habitat structure along the altitudinal gradients in the southern Cape mountains. *S. Afr. J. Zool.* **15(1)**, 34-43.

Bond, W.J. and Slingsby, P. (1983).Seed dispersal by ants in shrublands of the Cape Province and its evolutionary implications. *S.A. J. Sci.* **79**, 231-233.

Bond W. J. and Breytenbach G. J. (1985). Ants, rodents and seed predation in Proteaceae. *S. A. J. Zool.* **20**, 150-154

Chambers, J.C, MacMahon, J.A. and Haefner, J.H. (1991). Seed entrapment in alpineecosystems: Effects of soil particle size and diaspore morphology. *Ecology*, **72**, 1668-1677.

Clarkson, K.S, Eden, S.F, Sutherland W.J. and Houston, A.I. (1986). Density dependence and magpie food hoarding. *J. Animal Ecol.*, **55**, 111-121.

Cristol, D.A. (2001). American crows cache less preferred walnuts. *Animal Behavior* **62**, 331-336.

Fleming, P.A. and Nicolson, S.W. (2002). Opportunistic breeding in the Cape spiny mouse *(Acomys subspinosus). African Zool.* **37**, 101-105.

Fleming, P.A. and Nicolson, S.W. (2002). How important is the relationship between *Protea humiflora* (Proteaceae) and its non-flying mammal pollinators? *Oecologia* **132**, 361-368.

Fox, B. J, Quinn R. D. and Breytenbach G. J. (1985). A comparison of small mammal successionfollowing fire in Australia, California and South Africa. *Proc. Ecol. Soc. Australia* **14**, 179-197.

Griffin, J.R. (1971). Oak regeneration in the upper Carmel Valley, California. *Ecology,* **52**, 862-868.

Jacobs, L.F. (1992) The effect of handling time on the decision to cache by grey squirrels. *Animal Behaviour,* **43**, 522-524.

Janzen, D. (1971). Seed predation by animals. *Ann. Rev. Ecol. Syst.* **2**, 465-492.

Jensen, T.S. (1985). Seed-seed predator interactions of European beech, *Fagus silvatica* and forest rodents, *Clethrionomys glareolus* and *Apodemus flavicollis. Oikos*, **44**, 149-156.

Johnson, S. D. (1992). Plant-animal Relationships. In *The Ecology of Fynbos Nutrients, Fire and Diversity.* (ed Cowling R M) Oxford University Press, Cape Town, Oxford, New York. 175-205.

Leaver, L.A. and Daly, M. (2001). Food caching and differential cache pilferage: a field study of coexistence of sympatric kangaroo rats and pocket mice. *Oecologia*, **128**, 577-584.

Midgley, J, Anderson, B, Bok, A. and Fleming P.A. (2002). Scatter hoarding of Cape Proteaceae seeds. *Evol. Ecol. Res.*

Rebelo, A. G. and Breytenbach, G. J. (1987). Mammal pollination in the Cape flora. In *A preliminary synthesis of pollination biology in the Cape flora.* (ed. Rebelo A G) *South African National Scientific Programmes Report* **141**, CSIR, Pretoria. 109-125.

Slingsby, P. and Bond, W. J. (1981). Ants – friends of the fynbos. Veld and Flora **67**, 39-45.

Sork, V.L. (1983a). Distribution of pignut hickory (*Carya glabra*) along a forest edge transect and factors affecting seed recruitment. *Bull. Torrey Bot. Club,* **110**, 494-506.

Sork V.L. (1983b). Mammalian seed dispersal of pignut hickory during the three fruiting seasons. *Ecology*, **64**, 1049-1056.

Sork, V.L. (1984). Examination of seed dispersal and survival in red oak, *Quercus rubra* (Fagaceae), using metal tagged acorns. *Ecology*, **65**, 1020-1022.

Stapanian, M.A. and Smith, C.C. (1978). A model for seed scatterhoarding: Coevolution of fox squirrels and black walnuts. *Ecology*, **59**, 884-896.

Tamura, N. and Shibasaki, E. (1996). Fate of walnut seeds, *Junglans ailanthifolia,* hoarded by Japanese squirrels, *Sciurus lis. J. Forest Res.* **1**, 219-222.

Thompson, D.C. and Thompson, P.S. (1980). Food habits and cacheing behavior of urban grey squirrels. *Canad. J. Zool.* **58**, 701-710.

Tiffney, B.H. (1986). Fruitand seed dispersal and the evolution of the Hamamelidae. *Ann. Missouri Bot. Gard.* **73**, 394-416.

Van der Wall, S.B. (1993). Seed water content and the vulnerability of buried seed to foraging rodents. *Amer. Midl. Naturalist*, **129**, 272-281.
Van der Wall, S.B. (1998). Foraging success of granivorous rodents: Effects of variation in seed and soil water on olfaction. *Ecology*, **79**, 233-241.

Van der Wall, S.B. (2000). The influence of environmental conditions on cache recovery and cache pilferage by yellow pine chipmunks (*Tamias amoenus*) and deer mice (*Peromyscuc maniculatus*). *Behav. Ecol.* **11**, 544-549.

Van der Wall, S.B. (2001). The evolutionary ecology of nut dispersal. *Bot. Review*, **67**, 74-117.

Vlok, J.H. (1995). Fynbos and rodents. *Veld & Flora* December, 105-107.

Wiens, D, Rourke J. P, Casper, B. B, Rickardt, E. A. Lapine, T. R, Peterson, C. J. and Channing A. (1983). Nonflying mammal pollination of southern African proteas: a non-coevolved system. *Annals of the Missouri Botanical Garden* **70**, 1-30.