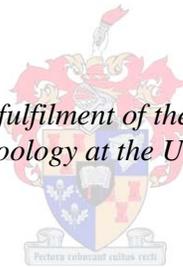


Scatter-hoarding in *Acomys subspinosus*: the Roles of Seed Traits, Seasonality and Cache Retrieval

by
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Declaration

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly stated below), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

The initial idea for this project, to investigate rodent seed dispersal behaviour, was conceived by Bruce Anderson and seen as an in-depth inquiry into earlier findings on rodent dispersal behaviour (Midgley *et al.* 2002). The methods in Chapter 1 and 2 on seed dispersal were based on suggestions by Bruce Anderson. The faecal analysis in Chapter 2 was the initial idea of Jeremy Midgley, however I designed and executed the study.

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Abstract

With growing concerns about current environmental issues, such as climate change, that affect ecosystems around the world, understanding ecosystem function is becoming increasingly important. In this study, I investigate the plant – seed disperser mutualism between an endemic scatter-hoarding mouse *Acomys subspinosus* and its Proteaceae plant counterpart *Leucadendron sessile* in the biodiversity hotspot of the Cape Floristic Region, South Africa.

The main objective of this thesis is to investigate the seed selection and caching behaviour of *A. subspinosus*. First, I determined the seed selection strategy for dispersal and burial by *A. subspinosus*. *Acomys subspinosus* may exert stabilizing selection pressure onto *L. sessile* seeds by dispersing and burying medium seeds with medium hull-thicknesses. Small seeds were eaten *in situ* and large seeds left at depots. I concluded that the buried *L. sessile* seeds may have a competitive advantage when it comes to seedling establishment in a post-fire environment, since seeds dispersed by rodents in the fynbos, such as *L. sessile*, are much larger in size and therefore have more stored nutrients and rapid growth capabilities than seeds dispersed by other vectors. Secondly, I documented rodent dispersal behaviour over a full year's time. *Acomys subspinosus* dispersal behaviour changed significantly over the seasons, which I attributed to a change in food availability as the year progressed. *Acomys subspinosus* buried seeds in autumn after mass seed drop but began to recover caches and consume seeds during winter and spring. The rodent switched to an insectivorous diet in spring. I propose that the *A. subspinosus* – *L. sessile* relationship is mutualistic during the year, but the relationship does shift in the favour of the rodent during winter and spring. Lastly, I address the scatter-hoarding behaviour of *A. subspinosus* and cache recovery ability of its assumed closest food competitor *Rhodomys pumilio*. I found that cache size has a profound influence on pilferage rates of *L. sessile* seeds. *Acomys subspinosus* scatter-hoarded the majority of seeds singly in the field and *R. pumilio* had difficulties finding those single-cached seeds in dry substrate under controlled experimental conditions, serving as evidence that scatter-hoarding is an effective method of pilferage mitigation by *A. subspinosus* during the dry summer months.

Relatively little was known about this plant – disperser mutualism and how it functions before this thesis were conducted. I have provided insights into the influence of rodent disperser behaviour on seed morphology development, seed fate and seed persistence in the field and suspect that the plant –disperser relationship may have a larger influence on ecosystem dynamics than previously anticipated. Further research on this system is of importance, especially with today's emerging environmental instability and human interference that threaten the robustness of highly interconnected ecosystems like the fynbos.

Opsomming

Met die huidige omgewingskwessies, soos die klimaatsverandering, wat ekosisteme wêreldwyd affekteer, word die begrip van ekosisteenfunksionering toenemend belangrik. In hierdie studie ondersoek ek die dier – saadverspreidingsmutualisme tussen die endemiese verstrooiings-storing muis *Acomys subspinosus* en sy Proteaceae plant eweknie *Leucadendron sessile* in die biodiversiteit ,hotspot’ van die Kaapse Floristiese Ryk, Suid-Afrika.

Die hoof doelwit van die tesis is om die saadseleksie en storingsgedrag van *A. subspinosus* te ondersoek. Eerstens het ek die saadseleksie strategie vir die verspreiding en begraving deur *A. subspinosus* bepaal. *Acomys subspinosus* het direksionele druk uitgeoefen op *L. sessile* sade deur mediumgrootte sade met medium saadhuiddiktes te versprei en te begrawe. Klein sade was *in situ* geëet en groot sade was gelaat by afgesette plekke. Ek het die gevolgtrekking gemaak dat die *L. sessile* sade wat begrawe is ‘n kompeterende voordeel mag hê wanneer dit kom by die vestiging van saailinge in ‘n afgebrande omgewing, aangesien sade wat in die fynbos deur knaagdier versprei word, soos *L. sessile*, baie groter is en dus meer gestoorde voedingstowwe en spoedige groeivermoëns het, as sade wat deur ander vektore versprei word. Tweedens het ek die knaagdier verspreidingsgedrag oor die tydperk van ‘n jaar gedokumenteer. *Acomys subspinosus* se verspreidingsgedrag het beduidend verander deur die verloop van die jaar, wat ek toegeskryf het aan die verandering in voedselbesikbaarheid soos wat die jaar gevorder het. *Acomys subspinosus* het sade begrawe in die herfs na grootskaalse vrylating en val van die sade, maar het gestoorde sade begin terug kry en sade begin eet gedurende die winter en lente. Die knaagdier het na ‘n insekvtende dieët omgeskakel in die lente. Ek stel voor dat die *A. subspinosus* – *L. sessile* verhouding nie die hele jaar mutualisties is nie, maar eerder antagonisties, in die knaagdier se guns, gedurende die winter en lente. In die laaste hoofstuk spreek ek die verstrooiings-storingsgedrag van *A. subspinosus* en storingsverkrygingvermoë van sy naaste voedselmededinger en deponeringsdief *Rhabdomys pumilio* aan. Ek het gevind dat die storingsgrootte ‘n beduidende invloed het op die koers van diefstal van *L. sessile* sade. *Acomys subspinosus* het die meerderheid van die sade gestoor in die veld en *R. pumilio* het die enkelgestoorde sade in droë substraat onder gekontroleerde eksperimentele kondisies moeiliker gevind. Dit is ondersteunende bewyse dat verstrooiings-storingsgedrag ‘n effektiewe metode is om diefstal te verminder in die droë somer in die fynbos.

Relatief min was bekend oor hierdie dier – saad verspreidingsmutualisme en hoe dit funksioneer voordat die studie uitgevoer was. Ek het insig verskaf oor die invloed van knaagdier verspreidingsgedrag op saadmorfologie ontwikkeling, die lot van sade en die tydperk wat dit begrawe is in die veld. Ek vermoed dat die mutualisme ‘n hoeksteenproses is in die fynbos en die invloed daarvan op ekosisteemdinamika mag dalk groter wees as wat voorheen verwag was. Verdere navorsing oor hierdie sisteem is belangrik, veral met vandag se opkomende omgewingsonstabiliteit en menslike inmenging wat die robuustheid van hoogs verbonde-netwerk ekosisteme soos die fynbos bedreig.

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Chapter 1

Introduction

Scientists continuously discover new flora and fauna despite the planet's rapid loss of species (e.g. Louis *et al.* 2006). New interactions between plant and animal subjects, even with species that have long been known, are still being described and make it apparent that ecosystems are extremely complex (Morin 2007). Mutualistic interactions in particular, form complex, inter-dependant networks that have been described as the architecture of biodiversity (Bascopte & Jordano 2007). The fact that discoveries of new mutualistic interactions, even of simple relationships between a plant and animal entity, are slow to be investigated and described (Bascopte & Jordano 2007) creates a fundamental problem: mutualisms are dependant on environmental predictability and stability. Ecosystems are quickly degrading due to climate change which can alter both environmental predictability and stability (Six 2009). This in turn can create negative effects on mutualisms that are reliant on consistent environmental conditions. Therefore, in order to understand ecological systems and prevent the collapse of such systems, mutualisms must be described and their robustness established (Bronstein 2009, Six 2009).

Bronstein (1994) established that the conditional outcome of mutualisms depends on the inter-dependency of the plant and animal entities. In other words, the less obligate the mutualism, the more external influences or “ecological variability” can be tolerated by the relationship without a catastrophic loss of fitness benefits by any of the affiliated entities. For example some ant species in a mutualistic interaction with nectar-rich plants provide defence against herbivores as a return for consuming nectar (Bronstein 1994). However, this mutualism is facultative and the plant can survive initial herbivory attacks without the presence of the ant while the ant is not dependant on nectar-based foods. This is a mutualism that could survive a high degree of environmental variability even if it may change the functionality of either or both entities.

One mutualism that has been discovered recently is a plant – seed disperser relationship between an endemic rodent scatter-hoarder and a Proteaceae plant in the fynbos of South Africa (Midgley *et al.* 2002). The fynbos is increasingly threatened by environmental instability, through increased frequency of fires (human and natural ignition), higher temperatures and

fluctuations in rainfall patterns caused by climate change (Wilson *et al.* 2010). To understand the consequences of this increased environmental variability on the Proteaceae – scatter-hoarder mutualism, the inter-specific reliance between the two subjects needs to be established (Theimer 2005). Additionally, it is of importance to determine how identified plant and rodent dispersers in the fynbos act within their respective mutualism, as a change in the subjects' roles may change the mutualisms in space and time. These changes can have a significant impact on ecosystem composition and function (Bascope & Jordano 2007).

Seed Dispersal Mutualisms

The interaction between seed-bearing plants and scatter-hoarding rodents originated in the Palaeocene, approximately 60 million years ago and the success of this mutualistic interaction was thought to be strengthened through co-evolution between the species involved (Vander Wall 2001). While debates continue over exactly how this interaction began, the most plausible theory lies in the innovation of “masting”, an intermittent production of large amounts of seeds by certain plant species (Kelly 1994). It is proposed that plants began to produce large seed crops at intermittent intervals to satiate predators. Aptly called the “predator satiation hypothesis” it stipulates that if plants release large quantities of seeds into the environment, seed predators will become satiated allowing some seeds to escape these predators (Kelly 1994, Kelly & Stork 2002). Rodents are thought to have adapted to these conditions and began to hoard excess seeds to prepare for food scarce times which often follow a masting event (Vander Wall 1990, Vander Wall 2002). Rodent dispersed plant species continued to undergo co-evolutionary development, evolving specific traits that further encourage rodents to disperse the plants' seeds (Vander Wall 2001). These traits include large, nutritious, nut-like seeds, that do not have wings or elaiosomes (a lipid-rich, fleshy structure attached to the seed that is attractive to ants). Seeds are usually high in protein and lipids making them attractive for rodent dispersers to cache, and have thick hulls or secondary chemicals such as tannins that increase the handling costs and encourage rodents to hoard seeds rather than consume them immediately (Vander Wall 2010).

Rodents, in turn, have also developed caching strategies to minimize pilfering and maximize seeds available for consumption. The two strategies used by seed hoarding animals are larder- and scatter-hoarding. Larder-hoarding is characterized by having a few large seed storage

sites deep underground that are often situated close to the burrow of the hoarder so that they can be actively defended (Vander Wall 1990, Vander Wall *et al.* 2005). Plants often do not benefit from this hoarding strategy as seeds may be too far below the surface to germinate and if germination is successful, the intra-specific competition between seedlings for space and nutrients is likely to be very high (Vander Wall 1990). Scatter-hoarding on the other hand, does not involve active defence by the hoarder. Instead, few seeds are dispersed in shallow caches at large distances from the food source or the cacher's burrow. This low seed cache density strategy can markedly decrease pilferage rates because the small caches are difficult to find and the low rewards discourage random searching behaviour by pilferers (Vander Wall 2003, Jansen *et al.* 2004, Moore *et al.* 2007). Scatter-hoarding benefits the plant's reproductive strategy as a large number of caches are spaced at great distances which increases the probability that the rodent neglects to retrieve some caches (Theimer 2001, Vander Wall 2001). Plants have evolved to produce seeds that do not emit strong odours which apparently decrease the chance of a cache being found through olfaction by a seed predator (Vander Wall 2010).

Nut-bearing plant – disperser animal interactions have evolved into highly sophisticated mutualisms that rely on the plant to provide food resources for the disperser and the disperser to move seeds away from the parent tree to favourable micro sites for potential germination. Disperser behaviour and mutualisms vary among ecosystems, however parameters of scatter-hoarding behaviour generally remain the same: rodents select specific types of seeds for caching (e.g. Theimer 2003, Zang & Zang 2008), seed size is correlated with dispersal distance (Xiao *et al.* 2005, Moore *et al.* 2007) and burial depths are an optimal trade-off between pilferage protection and cache retrieval ability (Vander Wall 1993b).

Seed selection for scatter-hoarding has been studied in numerous rodent populations with the general consensus being that larger seeds, that have greater nutritional value, are dispersed and cached for food-scarce times (e.g. Wang & Chen 2009) while small seeds are eaten at the seed source (e.g. Reichman & Fay 1983, Theimer 2003). Small seeds are not worth the energetic expenditure of caching and are easier to handle, hence they are eaten as “fast food” *in situ* (Woodrey 1990). Seed value is important for seed selection by hoarders, especially for long term scatter-hoarders (Jansen & Forget 2001). Additional to seed size (usually associated with

nutritional value), seed value can depend on hull-thickness and tannin content that can extend a seed's shelf-life. Thick hulls are hard to crack and herewith cached for a later time (Reichman & Fay 1983) but thick hulls also provide an improved barrier against fungi and bacteria attacks that may degrade the seed while in the soil. Similarly, tannins may deter insects from infesting the seed (Jansen & Forget 2001). Therefore, seed value, not only in terms of nutrition but also shelf life, may play an important role in seed selection. Despite being able to draw general conclusions about seed selection in rodent dispersers, the morphology and behaviour of rodents varies across ecosystems, which is reflected in the species' seed choices for caching. For example, Theimer (2003) found that White-tailed rats (*Uromys caudimaculata*) preferentially disperse medium-sized seeds and consume small and large seeds *in situ*. Similarly, Brewer (2001) found that the Spiny pocket mouse (*Chaetodipus spinatus*) preferentially disperses small seeds. According to Munoz & Bonal (2008) this is most likely due to the seed size – body ratio where seed size places an upper limit constraint on seed transport of smaller sized rodents. The authors found that a seed weighing in excess of 7% of the rodent's body mass saw a significant decrease in removal numbers. Additionally, gape width and masseter muscle size may be a constraint when it comes to rodent seed selection based on size; a large seed may be too wide in diameter for the gape widths of a small rodent or the hull too thick to crack open or gnaw through (Tamura & Hayashi 2008, Zang & Zang 2008). Therefore anatomical features of dispersers such as body mass and jaw size/strength may place another important constraint on seed selection for caching and consumption in rodents.

Once seed selection has commenced, rodents have to find optimal caching sites to bury the seed(s). It has been found that rodents cache large seeds farther from the seed source than small seeds (Xiao *et al.* 2005, Moore *et al.* 2007). Because large seeds represent a more valuable source of nutrition, these are often cached at larger distances to minimize density-dependent cache robbery (Vander Wall 2003, Jansen *et al.* 2004). However, the energetic trade-off between dispersal distance and transport costs is a factor that influences dispersal distance (Munoz & Bonal 2008). Seeds should be dispersed as far as possible from the source without incurring negative energy costs i.e. the energy expenditure to move the seed cannot be higher than the nutritional value of the seed (Munoz & Bonal 2008). Additionally, cache size should be large enough to be energetically viable to retrieve it, without being too large to be easily discovered by

a pilferer through olfactory means (Geluso 2005). Scatter-hoarders, as the name implies, often hoard no more than 1-5 seeds per cache, depending on the size of the seeds (Vander Wall 1990). Scatter-hoarded caches of large-sized seeds often contain fewer seeds than caches with small seeds, so that overall seed mass in caches is similar (Vander Wall 2003). Scatter-hoarded caches are still very small in size compared to larders (Vander Wall *et al.* 2005). Burial depth is a third parameter which the disperser has to take into consideration. The cache needs to be situated deep enough to avoid olfactory detection by pilferers while still being detectable by the disperser through olfactory means for later recovery (Vander Wall 1993b, Vander Wall 2000). Therefore several parameters during seed caching are important to minimize pilferage rates while the hoarder should still be able to relocate its caches.

Relocating caches is the second behavioural phase and often vital for survival of the hoarder, especially specialist scatter-hoarding species. Behavioural recovery efforts can be grouped into three categories: memory, olfaction and exploratory digging (trial-and-error). Cachers will most likely recover seeds with the former two efforts while pilferers use the exploratory digging strategy in conjunction with weak olfactory cues or in the absence of olfactory cues (Vander Wall 1991). Cachers attempt to rely on memory as much as possible; however memory is fallible especially after some time has lapsed since the cache was dug. Hence cachers as well as pilferers often rely on their olfactory acuity to retrieve caches (Vander Wall 2000, Vander Wall & Jenkins 2003). Retrieving caches successfully, either as a cacher or as a pilferer relies upon two main factors: the rodent's olfactory acuity and environmental conditions (Johnson & Jorgensen 1981). The environmental condition of soil moisture is critical during cache re-location (Vander Wall 1998, Vander Wall 2000, Vander Wall 2002), since moisture determines how many organic molecules leak from the seed coat into the soil as the seed imbibes (Vander Wall 2003). This determines how much molecular vapour will escape the soil into the air. The drier the soil, the less molecular vapour escapes into the air, demanding increased olfactory acuity from the rodents in order to detect the seeds (Vander Wall 2003). Variability in the olfactory acuity of rodents has been demonstrated numerous times. It seems that granivorous scatter-hoarders from arid environments, where seeds are cached in very dry substrates, have a higher olfactory acuity than pilferers or granivorous dispersers from temperate climates (Johnson & Jorgensen 1981, Vander Wall *et al.* 2003). Therefore, dry soil conditions

may have implications on cache recovery success of scatter-hoarders that may affect the overall rodent-seed interaction (Vander Wall 1998).

Scatter-hoarding and its Influence on Nut-bearing Plants

The plant's reproductive cycle is influenced by scatter-hoarders as they select what type of seeds are dispersed and buried (e.g. Theimer 2001), the type of (favourable) micro site seeds are cached in and the depth the seeds are cached at (Jansen *et al.* 2004). In addition, rodents control seed retrieval from caches which influences seed bank volume and ultimately determines how many seeds remain in the seed bank for germination (Hulme 1998). Scatter-hoarding behaviour may therefore influence the evolution of seed morphology (Vander Wall 2001) as well as play a role in determining plant community structure.

The selection of large and thick-hulled seeds for dispersal by rodents has distinct advantages for the plant. Large seeds will have the greatest chance of survival to germination if buried, because of their large nutrient storage volume (Vander Wall 1990). Thicker hulls also decrease susceptibility to infestation by small seed predators such as insects and pathogens which increases the seeds' shelf life (Janzen & Forget 2004). This increases the chances of survival in the ground, especially if the time period from seed set to germination is long (Howe & Richter 1982). It has been shown that the larger the seeds, the farther from the parent tree the seeds are dispersed (Xiao *et al.* 2005). Therefore, seeds with the highest chance of germination are moved the farthest from the parent tree. This decreases competition for suitable micro sites and nutrients between the parent tree as well as same-generation offspring (Vander Wall 2002, Vander Wall 2003, Jansen *et al.* 2004). Lastly, while selection for specific seed traits and dispersal distances are of importance for the survival and germination of rodent dispersed seeds, the burial depth is also of importance for successful germination (Vander Wall 1993b). It is especially important that the seeds are not buried too deep, which would decrease the chance of germinating seedlings to reach the soil surface. With scatter-hoarders caching seeds at shallow depths, plants have evolved to germinate successfully from the burial depth windows established by the hoarder (Vander Wall 1993b).

For the plant-animal disperser mutualism to function, it is important for rodents not to be able to re-locate all the seed caches. This can occur in a variety of ways. Firstly, scatter-hoarders can forget their caching location. Chances of this scenario occurring are heightened by the increased dispersal distances from the source tree and increased distances between individual cache sites (Vander Wall 1993a). An increased time lapse between cache establishment and recovery also increases the chance of seed germination (Briggs & Vander Wall 2004). Secondly, if a rodent is killed or forced to re-locate, the caches are left behind. In Mediterranean-like climates, such as in California USA and the fynbos, South Africa, fire can often play a major role in post-fire seed fate (Bond 1984). Hot, rapid fires may kill a large portion of the rodent population, leaving a large number of seeds to germinate in the post-fire environment. Slow and cool fires may force rodents to temporarily re-locate and leave enough time for the abandoned caches to germinate after fire (Bond 1984).

Soil aridity may be another factor influencing germination success rates in rodent dispersed plants. The ability to re-locate caches for scatter-hoarders and pilferers is most difficult when the soil is dry, which increases the chance of a seed cache remaining undetected. However, the evolution of increased olfactory acuity in granivorous scatter-hoarders from arid environments increases their chance of re-locating caches (Johnson & Jorgensen 1981, Vander Wall *et al.* 2003). Despite the possibility of dispersers relocating most of their caches, studies show that in a variety of plant-rodent disperser mutualisms, seedling establishment is substantial following mast seeding years (Forget 1993, Theimer 2001, Vander Wall 2003). This is confirmation that there are benefits to plants that have dispersal relationships with scatter-hoarders.

The Plant (Leucadendron sessile) – Animal (Acomys subspinosus) Interaction

To better understand seed disperser behaviour and its effects on the plant reproductive system in the fynbos, I investigated a fynbos-endemic plant species, *Leucadendron sessile*, and *Acomys subspinosus*, a scatter-hoarding mouse known to disperse the *L. sessile* seeds. *Acomys subspinosus*, a fynbos endemic (Breytenbach 1982) and is known as the Cape spiny mouse due to its spiny black hair running along the spine from the shoulder blades to the tail (Figure 1.1b). The mouse is easily identifiable with a rusty brown colour covering the flanks of the body, with

exception of the spinal region, and a white snout, breast, ventral side and paws (Figure 1.1a). The tail is approximately the length of the body with short, black hair covering the tip (Chimimba & Bennett 2005). *Acomys subspinosus* is a relatively small rodent at 20g body weight and is nocturnal. It is found throughout the southwestern Cape from Citrusdal, Cederberg Mountains in the northwest to Knysna in the southeast of the region (Figure 1.3). The mouse is generally found on rocky mountain slopes and nests in rock cracks and crevices (Chimimba & Bennett 2005). Its diet consists mainly of seeds (Vlok 1995, Midgley *et al.* 2002, Chimimba & Bennett 2005) but observations of nectar feeding have occurred (e.g. Fleming & Nicolson 2001).



Figure 1.1. *Acomys subspinosus* identification. (a) Dorsal view of *A. subspinosus*; rusty brown colour on flanks visible as well as the white snout and breast (photo: N. Neall). (b) Ventral view of *A. subspinosus*; the black spiny spinal hair is clearly visible as well as the white ventral side and paws (photo: author).

Midgley *et al.* (2002) observed that *A. subspinosus* scatter-hoards nuts from *Leucadendron sessile* (Proteaceae). *Leucadendron sessile* forms part of the southwestern sun conebush family and can be identified during flowering season by their bright yellow inflorescences (Figure 1.2a, Rebelo 2001). *Leucadendron sessile* is a shrub that reaches up to 1.5 meters in height with a single main stem. Flowering season is at the end of winter (July to August) after which fruit formation takes four months. The floral bracts develop into a cone and the fruit is a nut (Figure 1.2c, 4.2 mm long, 0.48g weight) which is dropped from the bush annually in summer (November - December) (Manning 2007). The fynbos is a fire-prone system

and *L. sessile* plants burn completely during fire; only the buried seeds remain, which germinate in the post-fire environment.



Figure 1.2. *Leucadendron sessile* identification (a) *L. sessile* in flower (photo: R. Saunders) (b) mature female *L. sessile* with young cone (photo: K. MacDonald) (c) *L. sessile* cone with nuts (photo: SANBI)

Leucadendron sessile is a regional endemic and its distribution is limited to granite clay soils on mountain slopes near the coast where winter precipitation is high (Rebelo 2001). *Leucadendron sessile* stands are found in two distinct patches: along the Witsenberg, Elands Kloof (Sir Lowry's Pass) and Hottentots-Holland Mountains (including Kogel Bay) and an inland patch that stretches from Jonkershoek near Stellenbosch to Kogelberg (Figure 1.3, Rebelo 2001). *Leucadendron sessile* is listed as Near Threatened by the SANBI interim red list (2007) due to increasing destruction of habitat for agriculture and forestry.



Figure 1.3. Distribution map. *Leucadendron sessile* (Proteaceae) stands occur mainly along the southwestern coast of the Western Cape province of South Africa, and fall well within the *Acomys subspinosus* distribution range.

One further study species, the striped field mouse (*Rhabdomys pumilio*) was utilized in experiments for Chapter 4. Description and distribution information for *R. pumilio* can be found in Chapter 4 under Materials and Methods.

Specific Thesis Objectives

The main objective of this thesis is to gain insights into the seed dispersal and cache retrieval behaviour of *A. subspinosus* by investigating seed selection, seasonal changes in caching behaviour and cache retrieval abilities. Seed selection and dispersal behaviour is covered under Chapters 2 and 3 while cache retrieval behaviours are covered in Chapter 4. Below I outline the main questions that are addressed in the chapters to follow. Due to the chapters being prepared for publication, duplication of information between the introduction and the chapters are inevitable.

Chapter 2: *Rodent Dispersers Select for Specific Seed Traits* – Rodent seed dispersal is a relatively new discovery in the fynbos, as myrmecochory was thought to be the only intentional, animal-mediated seed dispersal system for over 150 years. Despite this recent discovery, very little is known about rodent seed dispersal in the fynbos. It is known that *A. subspinosus* scatter-

hoards *L. sessile* seeds (Midgley *et al.* 2002, Midgley & Anderson 2005), that *A. subspinosus* has relatively large home ranges and that it can distinguish between healthy and parasitized seeds for consumption (van den Heuvel 2007). In Chapter 2 I attempted to gain insight into *A. subspinosus* seed dispersal behaviour by investigating seed selection for caching and consumption. The specific goals were as follows: (1) to determine if *A. subspinosus* selectively caches and consumes seeds with particular morphological characteristics, namely seed size and hull thickness; (2) to test whether the caloric value of seeds have an influence on the seed selection process of *A. subspinosus*.

Chapter 3: *Food Availability Affects Hoarding Behaviour of a Rodent Seed Disperser* – Literature on the effect of food availability on the behaviour of scatter-hoarders is relatively weak. *Acomys subspinosus* only receives a small window of time each year where a glut of fresh seeds enters the environment during the annual seed drop in summer. I anticipated that seed availability would therefore fluctuate greatly during the year, which may lead to changes in dispersal and consumptive behaviour in *A. subspinosus*. Hence, the goals for this chapter were: (1) to determine at what time of the year most rodent dispersed/consumed seeds were to be found on the soil surface; (2) to determine whether rodent dispersal and consumptive behaviour changed as the temporal gap to the seed drop event increased and seed availability changed; (3) to determine if *A. subspinosus*' diet changed over the year

Chapter 4: *Rodent Seed Competitors Drive the Evolution of Scatter-hoarding: Why Rodents don't put their Seeds in One Larder* – Scatter-hoarding rodents cache seeds for food scarce times. Therefore, when food resources dwindle the cached seeds are recovered for consumption. Scatter-hoarding is a technique used by rodents to minimize pilferage rates, as small-sized caches are hard to detect via olfactory means (Geluso 2005) but the hoarder itself is still able to retrieve the caches either through memory or an acute sense of smell. *Acomys subspinosus* scatter-hoards its seeds in low numbers, presumably for pilferage mitigation, so I determined whether *Rhodomys pumilio*, the main seed predator and potential pilferer at the field site, could retrieve small, scatter-hoarded caches. In order to establish whether scatter-hoarding is an effective anti-pilfering strategy in *A. subspinosus* I (1) determined relative cache recovery success of *A. subspinosus* and *R. pumilio* by manipulating cache size; (2) determined cache

recovery success in dry and wet substrate as increasing soil moisture increases olfactory detection ability of seeds (Johnson & Jorgenson 1981)

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Chapter 2

Rodent Dispersers Select for Specific Seed Traits in the Fynbos

The seed characteristics selected for scatter-hoarding by rodents can have a large impact on seed morphology development, seedling establishment and ultimately on plant community structure. Using the Proteaceae plant *Leucadendron sessile*, it was recently discovered that rodents are seed dispersers in the fynbos biome of South Africa. However, little is known about the characteristics of rodent-dispersed seeds and the selective influence rodents have on seed morphology in this biome. I investigated the caching behaviour of a known rodent disperser (*Acomys subspinosus*) and whether seed selection for caching or consumption is influenced by morphological seed traits such as size, hull thickness and caloric value. *Acomys subspinosus* tended to disperse and bury, rather than consume, medium sized unmodified *L. sessile* seeds with medium hull thickness. These seed trait parameters were most often encountered naturally in the field. In contrast, small or thin hulled seeds were preferentially eaten *in situ* and were seldom buried. Large seeds or seeds with thick hulls were often left untouched at depots. Caloric value had a negligible influence on seed selection behaviour. My results suggest that *A. subspinosus* may impose stabilizing selective pressures on seed size and hull thickness traits. Furthermore, seeds selected for burial by rodents are large in comparison to other seeds in the fynbos which may give rodent-dispersed seeds a competitive advantage for surviving veld fires and during subsequent seedling establishment.

Introduction

The propensity for organisms to converge upon similar traits when they are under similar selective pressures is one of the hallmarks of natural selection. Perhaps some of the most striking and well documented examples of this are pollination guilds, where groups of unrelated plants share a set of recognizable floral traits (Van der Pijl 1961). Also known as pollination syndromes, these traits are thought to be an adaptive response of the plant to a particular pollinator (Van der Pijl 1961). Similarly, the seeds of different plants may converge upon a similar set of character traits when they are dispersed by the same vector, creating a seed

dispersal syndrome (Forget & Milleron 1991). The fynbos biome of South Africa, for example, has two well established seed dispersal syndromes associated with it: myrmecochory and serotiny. Myrmecochorous seeds have a fleshy elaiosome attached to the seed hull that serves as a food source for ants. They consume the elaiosome while the rest of the still viable seed is dispersed underground where it is safe from granivores and fires (Christian & Stanton 2004). In contrast, serotinous seeds usually possess wings for wind dispersal and are only released from fire-resistant cones after burning (Midgley *et al.* 2002). Recently, a third group of large bodied fynbos seeds without wings or elaiosomes and a thick hull were identified as being most likely rodent-dispersed (Midgley *et al.* 2002, Midgley and Anderson 2005). These seeds are often released by plants *en masse* during the summer months. *En masse* seed release is a typical trade mark of rodent-dispersed plants as this satiates the rodents and encourages caching (Vander Wall 1990, Forget 1993).

Seed selection behaviour for dispersal in rodents is largely tied to energetic and temporal efficiency; rodents cache seeds which are large and/or thick hulled or seeds which are high in caloric value by nutritional standards (Vander Wall 1990, Brewer 2001). Small and thin-hulled seeds are generally eaten on site, as they have too little nutritional content to warrant the energy expenditure of dispersal and burial (Reichman & Fay 1983, Jacobs 1992, Theimer 2003, Jansen *et al.* 2004). Especially during *en masse* seed release when rodents attempt to disperse and bury as many seeds as possible, small and thin hulled seeds are used as “fast food,” since handling time for ingestion, e.g. cracking hulls, is minimal (Woodrey 1990, Vander Wall 1990, Forget 1993). Ingesting small or thin-hulled seeds allows the rodent to spend more time dispersing and burying larger, viable seeds for food scarce times and less time dealing with seeds for immediate ingestion.

Despite rodent seed dispersal being widespread, the morphology and behaviour of rodents varies across ecosystems, therefore the selection pressures of rodents imposed on seed traits are variable. Some rats and field mice in Australia and China, select medium-sized seeds for dispersal and caching (Theimer 2003, Zang & Zang 2008) while squirrels and chipmunks in the USA select large seeds (Vander Wall 2003). Nutrient and tannin content may also play a role in influencing seed choice (Wang & Chen 2009). As specific seed selection behaviour by rodents

varies between ecosystems and dispersers, my aim was to identify what specific seed traits are associated with rodent dispersal in the fynbos.

In the fynbos of South Africa, Midgley *et al.* (2002) confirmed that at least one species of Proteaceae with large and thick-hulled seeds (*Leucadendron sessile*) is dispersed by the Cape spiny mouse *Acomys subspinosus*. Later Midgley and Anderson (2005) showed that several other, similar looking seeds from the *Leucadendron* genus may also be dispersed by rodents and that the *Willdenowia incurvata* (Restionaceae) seeds found in sand plain fynbos are dispersed by the hairy-footed gerbil (*Gerbillurus paeba*). I used the rodent *A. subspinosus* to determine if it selectively consumes and caches seeds of certain morphological traits thus potentially exerting selective pressures on characteristics that enhance dispersal and burial. To determine which specific seed traits are selected for, I manipulated seed size and hull thickness, (two traits which may affect rodent handling efficiency) to determine if rodent dispersal versus consumption behaviour changed with respective seed manipulations. I also tested the influence of caloric value of seeds on seed selection by *A. subspinosus*. I expected that rodents would preferentially disperse and cache large, thick-hulled seeds and consume small thin-hulled seeds *in situ*. I also anticipated that higher caloric values in seeds will increase selective behaviour towards dispersal and burial.

Materials and Methods

Study Site and Species

The study was conducted in April 2010 on the slopes of Sir Lowry's Pass in Somerset West, Western Cape, South Africa (400m elevation). The field site consists of mostly undisturbed fynbos with a single, overgrown road transecting the site. The fynbos last burnt five years prior to my study being conducted. Fynbos goes through an irregular burn cycle which introduces a succession of dominant plant stages as the temporal gap to the burn event increases. At the time of the study, the site was predominantly a *Leucadendron* (suncone bushes), *Protea* and Restionaceae community, confirming the maturity of the stand. *Leucadendron sessile* is a single-stemmed shrubby bush growing up to 2.0 meters tall, which flowers in late winter (July – August) and drops its seeds annually. Peak seed drop is in late November/beginning of

December (early summer). At the Sir Lowry's Pass field site, mass seed drop windows of rodent-dispersed/consumed plant species during the summer months ensures a large seed bank until March to April (see Chapter 3 results). I conducted the experiment in the narrow one-month window of April to ensure that *A. subspinosus* were satiated from the full seed bank, encouraging the caching of experimental seeds. The duration of one month for this study had to be sufficient as *A. subspinosus* markedly decreases scatter-hoarding activity by May (early winter) (see Chapter 3).

Previously published (Midgely *et al.* 2002) and unpublished studies recorded three rodents and one Macroscelid at the research site: *Acomys subspinosus*, *Rhabdomys pumilio*, *Micaelamys namaquensis*, and *Elephantulus edwardii*. *Micaelamys namaquensis* is a nocturnal omnivore and only an opportunist granivore while *E. edwardii* is a nocturnal insectivore not known to consume seeds (Chimimba & Bennett 2005). Neither of the two species is known to disperse or bury seeds. *Rhabdomys pumilio* is a diurnal omnivore and opportunist seed predator that does not cache seeds and dominates the diurnal population census (Appendix 1). The nocturnal *A. subspinosus* is the only scatter-hoarding rodent at the site and dominated nocturnal population samples. *Acomys subspinosus* is a small (< 22 grams) rodent with clear identifiable markings; it has beige – grey coloured fur with a white ventral side and paws, and dark coarse hair running along the spine. Tail length is approximate to body length. *Acomys subspinosus* is a specialized granivore but specifics on diet and seed handling behaviour are not well known. It is known that *A. subspinosus* is capable of distinguishing between parasitized and healthy seeds (Van den Heuvel 2007) and that *A. subspinosus* switches to an insectivorous diet during spring when the seed bank is depleted and annual seed drop of *L. sessile* has not yet occurred (see Chapter 3).

Seed Preparation

To test if *A. subspinosus* seed selection differed according to seed size and hull thickness, I used a range of three different hull thicknesses and three different seed sizes. I determined, by analyzing 100 randomly chosen *Leucadendron sessile* seeds, that mean seed size was 4.2 mm/0.48g but that seeds could be up to 50% smaller and lighter than the mean and 25% larger.

Hull thickness also varied between 1.2 mm to 2.5 mm with a mean of 2 mm. This baseline data were used to create standardized dimensions of the manipulated seeds.

Table 1. Seed Dimensions of *L. sessile* seeds. Stated are seed ranges, mean seed dimensions and mean hull thickness.

	Smallest Seeds	Largest Seeds	Mean Seeds
Seed Mass	0.23g	0.98g	0.4 ± 0.48g
Seed Dimensions	2.7 mm length	7.9mm length	0.3 ± 4.2 mm length
	1.8 mm breadth	5.5 mm breadth	0.2±3.2mm breadth
Hull Thickness	1.2 mm	2.5 mm	0.1 ± 2.0 mm

Since it is impossible to determine hull thickness or flesh mass without damaging the seeds I was unable to use natural variation in seed traits to determine their effects on rodent behaviour. Instead I used standardized *L. sessile* seeds of mean size and weight, and perforated plumber's putty (to allow seed scent to pass) to manipulate hull thickness. Plumber's putty dries odourless with the same hardness and texture as natural seed hulls, hence, the presence of putty on seeds should not alter the behaviour of rodents towards the treated seeds. I used three hull-thickness treatments based on my baseline data: hull-thickness 50% thinner than mean *L. sessile* hull-thickness (1mm), mean hull-thickness (2mm) and hulls 25% thicker than the mean (2.5mm). *Leucadendron sessile* seed hulls were shaved off with sand paper by 1mm for the thin seed hull treatment, unmodified *L. sessile* seeds were used as the mean hull thickness treatment (control) and *L. sessile* seeds with a 1mm perforated putty layer represented the thick seed hull treatment. I prepared 300 seeds, 100 for each treatment, and all seeds were fitted with a 30cm fluorescent yellow tracking string which was attached onto the hull with a 2mm by 2mm piece of plumber's putty. Stripes, coding for different seed modifications, were marked on the tracking strings to identify seeds which had been consumed. Since plumber's putty was used on all seeds, regardless of the treatment used, the presence of the putty on seeds was controlled for. Tracking strings were used in previous studies (e.g. Midgley *et al.* 2002) and were not believed to have had an influence on seed selection by rodents.

Macadamia nuts (*Macadamia integrifolia*) were used to simulate different seed sizes by shaving down de-hulled, consumer grade nuts to the recorded smallest, mean and largest dimensions of naturally occurring *L. sessile* seeds. I fitted all nuts with a standard 2 mm (mean natural *L. sessile* hull thickness) artificial hull of perforated plumber's putty. I used macadamia nuts as they are easy to shave down and herewith ensure consistent seed size manipulations. The manipulated seed dimensions were based on the range of dimensions found in the *L. sessile* seed samples. Small seed samples (based on the smallest *L. sessile* seeds found) were generated by shaving macadamia nuts down to 2.0 mm/0.25g. The control seeds were nuts shaved to the size of a *L. sessile* seed with mean dimensions (4.2 mm/0.48g) and large seeds were generated by increasing the seed size to 9.0 mm and up to 1.0g in weight. Again, I prepared 100 seeds for each treatment and embedded a 30cm fluorescent tracking string into all artificial hulls. Strings were marked with stripes according to the manipulation. The use of the artificial plumber's putty hull on all seeds controlled for the putty's presence.

Experimental Design

To determine seed selection behaviour in *A. subspinosus*, I placed seeds with varying hull thickness and size into the field overnight and recorded seed fates the following morning. I used the overgrown road transecting the study site and marked five transects running north, perpendicular to the road. I distanced transect lines 20 m to 25 m from each other, since *A. subspinosus* seldom disperses seeds more than 10 m (personal observation). Each transect line contained two seed depot sites (5 transects x 2 depot sites) with the first depot in the transect line 5 m from the road and the second depot 20 m from the first depot. Depot sites were selected based on cover, slope and soil abundance. I chose depot sites under mature *L. sessile* bushes, with at least 50% cover in a 3 m² area surrounding the depot. Slope never exceeded 15° and only sites with at least 5 cm soil depth in a surrounding 5 m² area were chosen.

Seed Size Trial

I placed ten macadamia seeds from each size class category (small seeds, "normal seeds" and large seeds) making a total of 30 seeds per depot. Seeds were mixed and randomly dispersed over 1 m² under the respective depot site's *L. sessile* bush. This procedure was repeated for all ten depot sites for a total of 300 seeds deposited. Deposition took place shortly before sunset and

recovery took place shortly after sunrise the next morning to avoid as many diurnal rodent foragers as possible. Seed recovery was conducted by first checking a depot for non-dispersed/eaten seeds and then I completed a full search in a 10 m radius around the depot site by walking in a spiral fashion away from the depot, recovering any dispersed, buried or eaten seeds. Seeds were presumed eaten when I found the hull cracked open and the seed inside missing. Usually the tracking string was still attached to part of the seed hull which allowed me to identify the type of seed that was ingested based on the stripe coding on the string. If the seed was within 1 m of the depot site, intact and not buried, it was considered “not touched”. Seeds that were never found were noted as “unknown fate”.

Hull Thickness Trial

Forty eight hours after the seed size trial with macadamia nuts, I returned to the field site and repeated the experiment described above with *L. sessile* seeds. I placed ten seeds of each hull thickness (thin, normal and thick) at each depot for 30 seeds per depot and 300 seeds for the trial. Since *L. sessile* seeds were used two days after the macadamia nuts (seed size trial), the two seed species were never mixed upon presentation at depots.

Caloric Comparison

To determine whether *A. subspinosus* selected for calorie-rich seed species, I determined the caloric values of *L. sessile* and macadamia nuts and compared seed fates in the field of the two species. I prepared 20 *L. sessile* seeds (hulls removed) and three macadamia nuts by grinding them into a fine powder and drying them in an oven at 70° C for 48 hours. The powders were then stamped into small tablets (+/- 3.0g) and placed into an SDC313® bomb calorimeter. I repeated testing for each of the two samples three times to receive a mean caloric value for each seed species.

Additionally, I compared the removal and ingestion rates of the unmodified *L. sessile* seeds and the standardized macadamia nut (same size and artificial hull thickness as a mean *L. sessile* seed) from the data collected during the seed dispersal experiments. I inferred whether caloric value of macadamia and *L. sessile* seeds may have had an influence on seed choice.

Statistical Analysis

Data sets were tested for homogeneity and normality, to satisfy the requirements to perform ANOVA's with a Bonferroni post-hoc test to test for differences in recovery percentages of each seed treatment within each seed fate group (not dispersed, dispersed and eaten). All percentage values were arc-sin root transformed. All analyses were conducted with SPSS 18.0 (SPSS 2010). I used a chi-square test to test for caloric differences between the two seed types and a t-test was used to examine the proportions of each seed fate between the two different seed types for the caloric comparison.

Results

Seed Dispersal

Unmodified “normal” *L. sessile* were preferentially removed and buried by *A. subspinosus* (Fig. 2.1, ANOVA $F_{10,13} = 16.94$, $p < 0.001$). Thin-hulled *L. sessile* seeds had significantly higher *in situ* ingestion rates than normal and thick-hulled *L. sessile* seeds ($F_{10,13} = 13.34$, $p < 0.001$). Thick-hulled seeds were most often left at the depot site compared to normal and thin-hulled seeds ($F_{10,13} = 3.0$, $p < 0.051$).

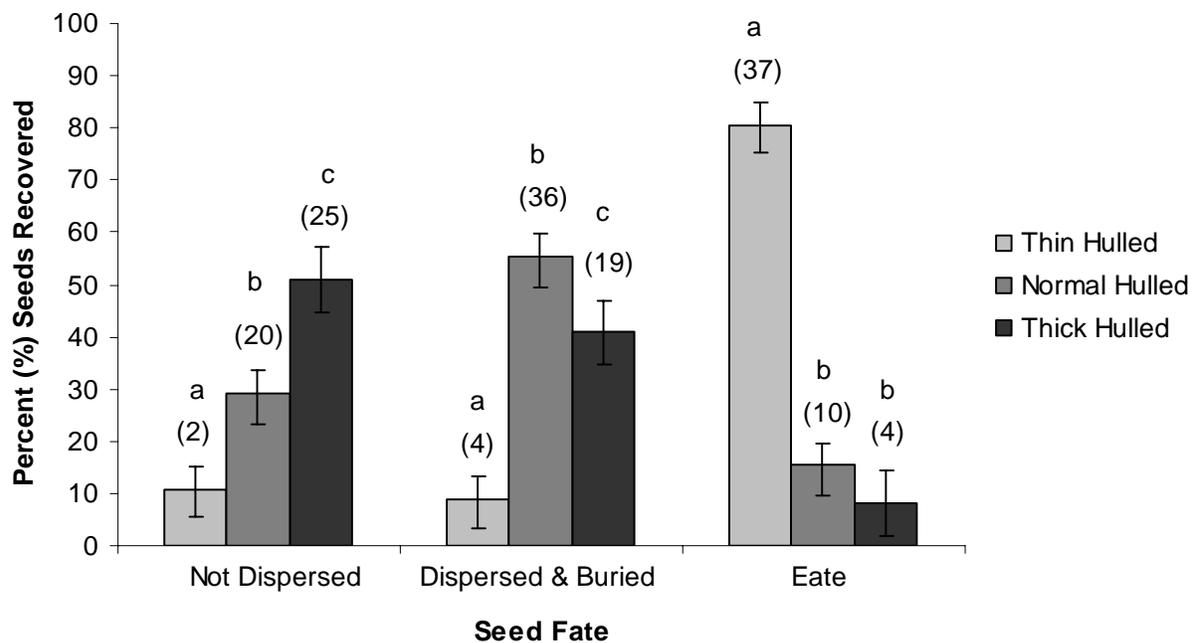


Figure 2.1. Fates of *L. sessile* seeds with three different types of hull thicknesses after a 12 hour exposure to *A. subspinosus* in the field. Percentage seeds recovered that share the same letter are not significant ($P > 0.05$), numbers in brackets indicate number of seeds recovered

Similar to the hull thickness trial, macadamia nuts that were modified to resemble normal-sized/hulled *L. sessile* seeds, had significantly higher dispersal and burial rates than small and large seeds (Fig. 2.2, ANOVA, $F_{10,13} = 8.93$, $p = 0.01$). Small seeds had significantly higher *in situ* ingestion rates than normal and large seeds ($F_{10,13} = 5.55$, $p = 0.01$) and large seeds were left at depot sites significantly more often than small or normal sized seeds ($F_{10,13} = 3.2$, $p = 0.053$).

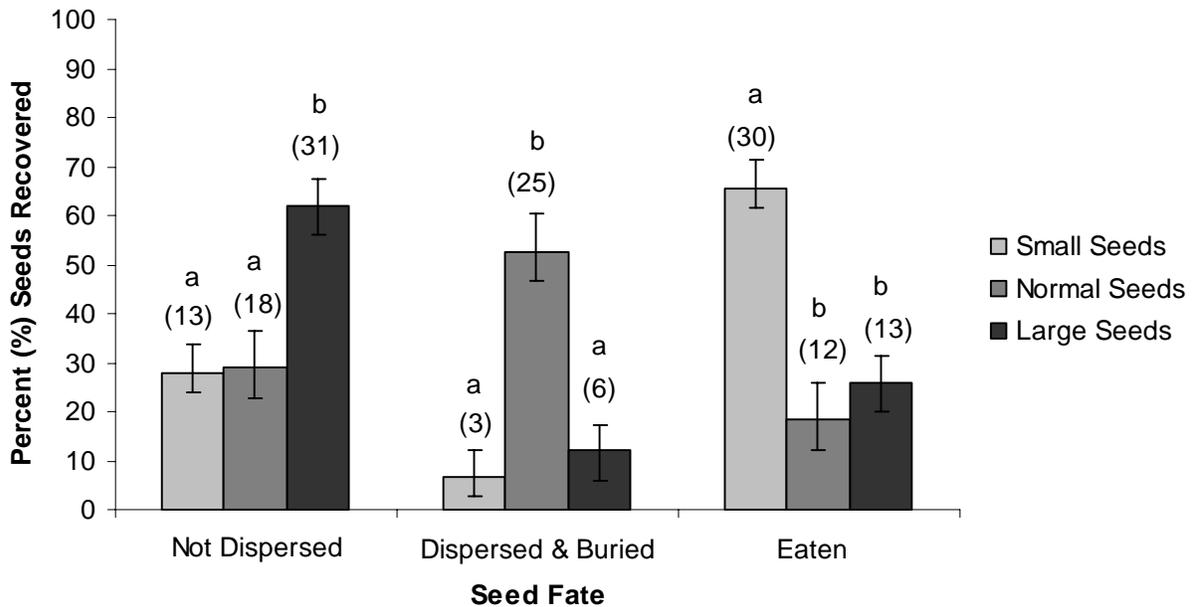


Figure 2.2. The fates of macadamia nuts modified into three different sizes after exposure to *Acomys subspinosus* for 12 hours in the field. Percentage seeds recovered that share the same letter are not significantly different ($p > 0.05$), numbers in brackets indicate the number of seeds recovered

Caloric Comparison

Macadamia and *L. sessile* seed samples were analyzed for caloric content to determine differences in caloric value. Macadamia seeds had a mean value of 33.45 kJ/g and *L. sessile* 26.5kJ/g, rendering macadamia nuts significantly higher in caloric value ($X^2 = 3.659$, $df = 5$, $p = 0.044$).

I compared seed fates between “normal sized” macadamia nuts with artificial hulls made of plumber’s putty at the same thickness as natural *L. sessile* hulls and unmodified “normal sized” *L. sessile* seeds with natural hulls and small patches of plumber’s putty to attach tracking strings. I found that differences within fates of the two seed types were not significant neither for non-dispersed, dispersed or eaten seeds respectively (Fig. 2.3, $t_{37}= 2.46$, $p= 0.9$, $t_{61}= 1.71$, $p= 0.12$, $t_{22}= 3.03$, $p= 0.6$)

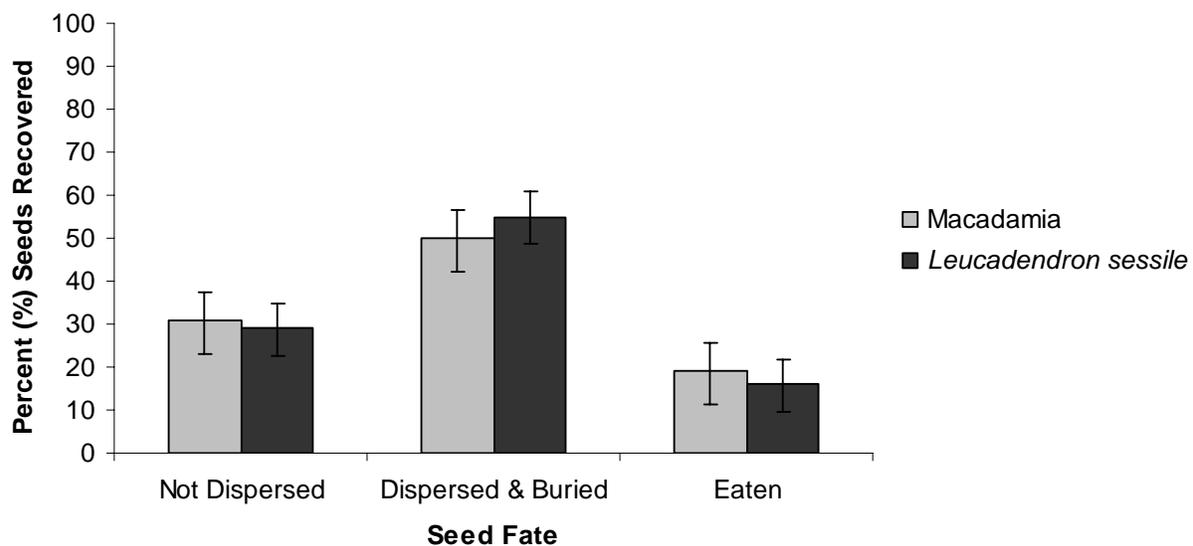


Figure 2.3. Comparison within seed fate groups (not dispersed, dispersed and eaten) of unmodified *Leucadendron sessile* seeds and artificially hulled macadamia nuts of the same size/hull thickness. Seed fate for the two seed species did not differ significantly.

Discussion

Seed Dispersal

Acomys subspinosus clearly displayed preferences in terms of which seeds were dispersed and buried or consumed. It seems that seeds with intermediate, “normal” size and hull thickness dimensions (corresponding with values of natural *L. sessile* seeds) dimensions were preferentially dispersed and buried. Small or thin-hulled seeds were most often eaten *in situ* whereas large or thick-hulled seeds were frequently left at the depot sites. Thus *A. subspinosus* may be exerting stabilizing selective pressure on hull thickness and seed size whereby seeds

outside of the “normal” size and hull thickness range are unlikely to be dispersed and buried by *A. subspinosus*. I specifically targeted the nocturnal scatter-hoarding *A. subspinosus* in these field experiments by placing seeds into the field overnight. The only other nocturnal rodent present at the site that may have consumed seeds was the omnivore *Micaelamys namaquensis* that is not known to scatter-hoard seeds. I’m confident that all seeds which were found to be scatter-hoarded were done so by *A. subspinosus*. It is possible that *M. namaquensis* was responsible for part of the seed consumption recorded in these experiments however based on its low relative abundance at the field site (Appendix 1), this percentage is minimal. The use of plumber’s putty as artificial seed hulls also did not appear to alter seed choice in rodents. In the seed size experiment with macadamia nuts, the use of plumber’s putty was controlled for, as all seeds were fitted with a 2 mm thick artificial hull. The amount of putty used in the hull thickness experiment with *L. sessile* seeds was variable, however putty was used on all seeds. Additionally, I showed that “normal sized” *L. sessile* seeds (i.e. unmodified *L. sessile* seeds with exception of a 2 mm by 2 mm plumber’s putty patch to attached the tracking string to the seed) and “normal sized” macadamia nuts (macadamia nuts shaved down to unmodified *L. sessile* seed specifications and with an artificial hull) were consumed, cached and left at depots at similar rates (see caloric analysis results). This confirms that the plumber’s putty as an artificial seed hull had no effect on seed choice in rodents as seeds without (*L. sessile*) and with artificial hulls (macadamia nuts) were selected for their respective seed fates at similar proportions.

Other studies (e.g. Vander Wall 2003) found that large seeds, which have a higher nutritional value than smaller seeds, should present the most energetically viable option for dispersal and burial in rodents (Pulliam 1974, Kerley & Erasmus 1991). I hypothesized that *A. subspinosus* should preferentially disperse and bury large seeds. However my results highlight the fact that handling, transport and burial costs as well as the body size of *A. subspinosus* have to be taken into consideration when looking at trait-specific seed selection. Large seeds require increased handling and transport time thereby increasing exposure risk to predators (Lima 1985, Jacobs 1992). Additionally, transport and handling itself is an energetic cost which has to be taken into account. Large seeds, while higher in nutritional value, also require more energy to transport. It is therefore important that the trade-off between the energetic value of the seed is not negative in relation to the energy spent transporting it (Stapanian & Smith 1978, Tamura *et al.*

1999 Moore *et al.* 2007). Given the relatively small body size of *A. subspinosus* (+/- 20g) compared to other fynbos rodents such as *Micaelamys namaquensis* (+/- 60g), a larger seed can rapidly become a negative energy cost to *A. subspinosus* during transport and handling. This makes it energetically more economical to transport smaller sized seeds, in this case the “normal” sized *L. sessile* seeds. Jansen *et al.* (2002), Theimer (2003) and Munoz & Bonal (2008) all suggest a transport threshold where seeds above a certain percentage of the rodent’s body weight are too heavy to carry, constraining or even preventing long-distance transport for these seeds. The transport threshold seems to vary dependant on the ecosystem, rodent body size and rodent species. Munoz & Bonal (2008) found that Algerian mice (*Mus spretus*) and wood mice (*Apodemus sylvaticus*) would transport Holm oak (*Quercus ilex*) acorns weighing up to 60% of their body weight. In contrast, Jansen *et al.* (2002) found that scatter-hoarding rodents moved seeds weighing up to 7% of their body weight. For example, in Brewer’s (2001) study, seeds weighed in excess of 25% of rodent body mass which apparently restricted their physical ability to transport the seeds. Hence seed removal rates dropped substantially because long distance transport was constrained (Munoz & Bonal 2008). At an average of 0.48 g per unmodified *L. sessile* seed (2.5% of body mass), *A. subspinosus* displayed a trade-off between a nutritious seed without being too heavy, where as the larger seeds weighed up to 1 g or 5% of *A. subspinosus* body mass. Therefore, I suggest that the transport threshold of seeds for *A. subspinosus* may lie around the 2.5% weight margin.

Tamura and Hayashi (2008) also identified the issue of hull thickness and corresponding seed size as a factor in seed selection in mice. Seeds with thicker and/or harder hulls are often larger in size, making it increasingly difficult for smaller mice, like *A. subspinosus*, that have comparatively smaller masseter muscles and herewith lower jaw pressure (Zang & Zang 2008), to gnaw into or crack these hulls. This increases the time and energy spent in trying to get to a seed thereby increasing the time exposed to predators and may require such large energy expenditure that attempting to extract these seeds from their hulls is not energetically viable. This may explain why my thick-hulled seeds were often left at depots by *A. subspinosus*.

Similarly, the size of the seed also plays a role in being able to crack the hull. In order to get to the seed, *A. subspinosus* needs to be able to take the seed into its mouth and bite down

onto the seed to crack the hull. However if the seed is larger than the rodents jaw gape, cracking the hull will either be impossible, or if the rodent elects to gnaw a hole into the hull, may be so time and energy consuming that it will not be energetically worth it (Tamura & Hayashi 2008). This provides a second possible explanation, as to why large seeds, that were almost double the diameter (mean 9.0 mm) compared to an unmodified *L. sessile* seed (mean 4.8 mm), were often left at depots by *A. subspinosus*.

Caloric Comparison

Previous studies have shown that caloric/nutritional value of seeds influence seed choice in rodents (Jansen *et al.* 2004, Xiao *et al.* 2005, Wang & Chen 2009). Having hypothesized that a seed higher in caloric value would be selected for dispersal and burial more often than a seed with the same dimensions and hull thickness but lower in caloric value, I found that *A. subspinosus* did not seem to favour seeds based on caloric value. As both macadamia nut (at 33.45kJ/g) and *L. sessile* seeds (at 26.5kJ/g) are very high in energy, selection for either seed may be irrelevant to *A. subspinosus*. Similarly, while the macadamia nut is statistically significantly higher in energy than *L. sessile*, the difference in energy content may not have been large enough for *A. subspinosus* to detect, hence it dispersed and buried both seed types at the same rate. Alternatively, *A. subspinosus* may not be able to distinguish calorific differences in seeds at all. This would have to be tested in a future study.

Ecological Significance

It appears that *A. subspinosus* may be exerting stabilizing selective pressures for medium hull thickness/sized seeds because these traits enhance caching. Plants benefit from caching because it protects the seeds from the biotic environment (e.g. seed predators) as well as the abiotic environment (e.g. fires) (Vander Wall 1990). Fires are thought to play a particularly important role in the fynbos since many seeds require fire to germinate (Rebelo 2001). Plants usually have adaptations for their seeds to survive fires, such as exhibiting specific seed traits to encourage underground caching by animals. Rodents tend to bury much larger seeds than other fire-adapted plants dispersed by other mechanisms such as wind or ants (Bond *et al.* 1999). It is possible that large seeds may be advantageous if they enable germination from deep within the soils and therefore survive particularly hot fires. Rodents bury their relatively large seeds to a

maximum of 4 cm (Midgley *et al.* 2002). However, seeds which are a quarter of the size of rodent-dispersed seeds, and presumably use a dispersal vector other than rodents (*Leucospermum* genus), are capable of germinating from the same depth (Bond *et al.* 1999). Despite similar germination depth ranges, large rodent-dispersed seeds may have an advantage over smaller seeds because their extra resources provide them with consistent and rapid growth rates after fire. This may allow them to survive the harsh, post-fire conditions and out-compete seedlings establishing from smaller seed bodies.

Additionally, rodent-dispersed seeds have thick hulls which may increase their resistance to heat as well as insect, fungi or bacterial attacks (Jansen & Forget 2001). Holmes & Newton (2004) determined that Proteaceae seeds, some of which are thought to be rodent-dispersed, display long-term persistence in the soil (half-life exceeding 2 years). This may add time to the shelf-life of rodent-dispersed seeds and increases the chance of germination not only by increasing the chances of survival during veld fire, but also by increased chances of survival in the soil during the pre-fire period.

These results are the first effort to characterize rodent dispersal syndrome in the fynbos by using foraging behaviour to build a putative profile of rodent-dispersed seeds in the Western Cape region. My results suggest that seeds from Cape plants that do not have wings or elaiosomes, are in the 0.5g weight range (up to 2.5% of *A. subspinosus* body mass) with hulls up to 2.0 mm thick and seed dimensions of 2.0 to 5.0 mm in diameter are likely to be dispersed by *A. subspinosus*. Within the genus *Leucadendron* (Proteaceae) there are at least 23 species that correspond to this general set of seed traits and within the Restionaceae family there are a further nine species (Appendix 2). This profile can herewith be used to preliminarily identify potential rodent-dispersed plant species in the fynbos that can then be confirmed through research experiments similar to the one conducted in Midgley *et al.* (2002) and this study. The method may be applied to other Mediterranean-like ecosystems where rodents play a role in seed dispersal. This study suggests that rodents may have a larger influence on plant populations in the fynbos than previously thought. Although it is well known that rodents influence Cape plant communities through herbivory (Christian 2001), my study suggests that caching behaviour may also have an important effect on plant community structure.

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Appendix 1. Relative abundances of three rodent species and one Macroscelidid found at the field site. One rodent was diurnal (*Rhabdomys pumilio*) and one a nocturnal insectivore (*Elephantulus edwardii*), therefore only *Micaelamys namaquensis* may have had an influence on my seed experiments which were conducted overnight. Hence I calculated relative abundance for nocturnal and diurnal animals (RA based on activity) to obtain a clearer picture of the number of rodents that are active based on time of day. RA was calculated as number of captures per six trapping sessions and re-captures were not included.

Species	Relative Abundance (RA)	Active	Diet	RA based on activity
<i>Acomys subspinosus</i>	0.412	Nocturnal	Specialized granivore Scatter-hoarder	0.60 (nocturnal)
<i>Micaelamys namaquensis</i>	0.118	Nocturnal	Omnivore	0.16 (nocturnal)
<i>Elephantulus edwardii</i>	0.176	Nocturnal	Insectivore	0.24 (nocturnal)
<i>Rhabdomys pumilio</i>	0.294	Diurnal	Omnivore	1.0 (diurnal)

Appendix 2. Plant species identified as potentially rodent-dispersed based on the seed trait parameters of: nut, wingless, no elaiosome, thick hull and annual mass seed drop

Group	Plant Species	Seed Characteristics	Seed Drop
Proteacea			
<i>Leucadendron</i>	<i>L. brunioides</i>	Nut, covered in hairs	Dec - Jan

<i>L. sessile</i>	Nut, hairless, 4mm diameter, 0.48g	Nov – Dec
<i>L. stellare</i>	Nut, covered in hairs	Jan – Feb
<i>L. thymifolium</i>	Nut, covered in hairs	Jan – Feb
<i>L. concavum</i>	Nut, hairless, large: 9mm diameter	Nov
<i>L. dubium</i>	Nut, hairless, large: 8mm diameter	Nov
<i>L. remotum</i>	Nut, hairless, 4mm diameter	Nov
<i>L. bonum</i>	Nut, hairless, 4mm diameter	Jan
<i>L. pubescens</i>	Nut, hairless, 4mm diameter	Oct
<i>L. arcuatum</i>	Nut, hairless, 4mm diameter	Jan
<i>L. ericifolium</i>	Nut, short-haired	Nov
<i>L. verticillatum</i>	Nutlet, short-haired, diameter: 2-3mm	Feb
<i>L. corymbosum</i>	Nutlet, short-haired, diameter: 2-3mm	Jan – Feb
<i>L. laxum</i>	Nutlet, short-haired, diameter: 2-3mm	March
<i>L. barkerae</i>	Nut, hairless, large: 5mm diameter	Dec
<i>L. tinctum</i>	Nut, hairless, large: 4-6mm diameter	Nov
<i>L. cordatum</i>	Nut, hairless, large: 4-6mm diameter	Nov
<i>L. gydoense</i>	Nut, hairless, large: 4-6mm diameter	Feb
<i>L. daphnoides</i>	Nut, hairless, large: 4mm diameter	Oct - Dec

<i>L. sheilae</i>	Nut, hairless, large: 5mm diameter	Nov
<i>L. loranthifolium</i>	Nut, hairless, large: 4mm diameter	Nov
<i>L. chamelaea</i>	Nut, hairless	Jan
<i>L. elimense</i>	Nut, hairless	Feb

Restionaceae

<i>Ceratocaryum argentum</i>	Nut, 10mm diameter, round and woody	?
<i>Wildenowia incurvata</i>	Nut, 8mm diameter, woody	?
<i>Elegia filacea</i>	Triangular nutlet, 3mm diameter	?
<i>Staberoha banksii</i>	Nutlet, 3mm diameter	?
<i>Nevillea obtusissima</i>	Nutlet, 2-chambered, 2mm diameter	?
<i>Anthocortus crinalis</i>	Nutlet, soft-walled, 4mm long	?
<i>Hydrophilus rattrayi</i>	Nutlet, 3mm long	?
<i>Calopsis paniculata</i>	Nutlet	?
<i>Calopsis viminea</i>	Nutlet	?

Chapter 3

Food Availability Affects Hoarding Behaviour of a Rodent Seed Disperser

Scatter-hoarding rodents may play a pivotal role as seed dispersers in plant communities that contain mass seed-drop species. Dispersal patterns, however, may vary between food rich and food poor months. I set out to investigate whether a scatter-hoarder, *Acomys subspinosus*, in an interaction with an annual mass seed drop plant species, *Leucadendron sessile*, changed its dispersal behaviour over the course of one year based on food availability. I established the *en masse* seed drop windows and estimated seed mass of potentially rodent-dispersed and/or consumed plant species, monitored seed dispersal behaviour of wild *A. subspinosus* throughout one year and conducted regular faecal analyses to determine the mice's diet. Potentially rodent-dispersed and consumed seed species, identified through seed characteristics, showed a tendency to drop from the plants from early to late summer (November to March) with peak seed drop in December. Rodent seed dispersal and consumptive behaviour was highly variable and ranged from scatter-hoarding experimental seeds in late summer/early autumn to high seed ingestion rates in winter and spring. Faecal analyses supported the inferences made from experimental seed fates and showed a dietary switch by *A. subspinosus* from seeds to insects in spring, which was reversed in early summer. The results suggest that *A. subspinosus* seed dispersal and consumptive behaviour is influenced by seed availability and thus the temporal outcome of the relationship between disperser and plant may vary over the seasons.

Introduction

Plant-animal interactions form the basis of a complex and dynamic ecological network that determines the overall functionality of ecosystems (Darwin 1859, Strauss & Irwin 2004). These interactions can be crudely categorized into positive (mutualism), neutral (commensalisms) or negative (parasitism) interactions (Bronstein 1994). Mutualisms are of special interest in ecology as they represent conditional relationships between two entities that range from loosely associative to obligatory (Bronstein 1994, Theimer 2005). However, the

relationship is not “friendly” but highly competitive and both entities act selfishly by struggling to incur fewer costs while obtaining more benefits from the interaction (Anderson & Midgley 2007, Bronstein 2009). For example, nectar-producing plants spend a large portion of their photosynthate on nectar production. The plant then provides the pollinator with the smallest possible portion of nectar that ensures maximum visitation, unless the pollinator finds a more rewarding plant entity (Bronstein 1994). Like pollinator relationships, mutualistic interactions between a seed disperser and the seed-bearing plant display similar cost-benefit struggles (Hulme & Benkman 2002).

Rodents can affect seed banks as seed predators, seed dispersers/cachers or both. Rodents disperse and cache seeds that have been dropped *en masse* by a plant in a short window of time. *En masse* seed drop allows for rodents to become satiated quickly and encourages caching behaviour (Vander Wall 1990, Forget 1993, Vander Wall 2002). Caching then becomes beneficial to the plant as the seeds are stored safely underground, away from seed predators and environmental factors such as fire. This facilitates an increase in seed bank volume and ultimately seedling establishment (Forget 1993, Theimer 2001). However, caches are often recovered by the scatter-hoarding rodent once food resources decline. This makes the outcome of the plant-disperser interaction variable depending on the rodent species as well as the availability of microhabitat for seed germination (Maron & Simms 1997, Orrock *et al.* 2006), available seeds (with respect to seed size) (Hulme 1998) and size of the canopy seed bank (Borchert *et al.* 2003). For example, Maron & Simms (1997) found that rodents acted as seed predators at critical microhabitat sites ideal for seed germination hence rodents had a magnified negative influence on the seed bank. Borchert *et al.* (2003) however, found that a significant amount of serotinous seeds released after a fire were dispersed and buried by rodents, increasing seed bank volume. While rodent seed predation is more prominent in ecosystems than dispersal and caching (e.g. Cabin *et al.* 2000), the documented incidences of seed caching may affect the seed bank positively and increase seed bank persistence (Crawley 1992, Hulme 1998).

Highly dynamic seed banks, such as those affected by rodent dispersal, are characterized by either an annual mass seed drop event or a masting event (Forget 1993, Vander Wall 2002). This causes the seed banks to go through cycles of replenishment and depletion. Seed banks are

replenished shortly after mass seed drop when rodents begin to scatter-hoard seeds. At this point in time, the plant – disperser interaction is completely mutualistic (Forget 1993). After mass seed drop and the caching period, the seed bank volume will decline steadily as cachers recover their seeds and pilferers find caches (Hulme 1998). The plant-disperser relationship may display a negative fate for the plant during the cache recovery period as previously cached seeds are now recovered and consumed. Therefore, seed bank volume as a food source for granivorous rodents may fluctuate greatly between seed drop or masting events (Hulme & Benkman 2002), causing a shift in the plant – disperser interaction over time that favours the rodent during non-mast months.

In the fynbos of South Africa, rodent seed dispersal and caching has only recently been discovered (Midgley *et al.* 2002). Little is known about how rodent dispersal and consumption behaviour is influenced by fluctuating food availability or how rodent caching and seed consumption affects the seed bank, a food source for rodents. However, it is known that native seed dispersing ants may act as keystone species in the fynbos as they transport a variety of seed species to sites with favourable microclimates, resulting in a species rich and dynamic seed bank (Christian 2001). It has been shown that seed-dispersing ants greatly increase seed bank volume and persistence by burying seeds deep enough to minimize rodent predation (Christian & Stanton 2004) a primary factor of decreasing seed bank volume in the fynbos (Pierce & Cowling 1991). Unlike the positive role played by ants on fynbos seed banks, rodents have thus far been studied purely as seed predators (Pierce & Cowling 1991). It is unknown whether the seed caching behaviour of rodent species, most notably *Acomys subspinosus* (Midgely *et al.* 2002, Midgley & Anderson 2005), has a positive impact on the plant –disperser relationship, similar to ants, or whether rodents turn into seed predators as the temporal gap to the seed drop event increases.

I determined the seasonal seed burial and consumptive behaviour of the scatter-hoarding rodent *Acomys subspinosus* in the fynbos of South Africa based on food availability in the environment. I predicted that the varied availability of food throughout the year would affect rodent dispersal and consumptive behaviour towards food resources and affect rodent diet. I anticipated that rodents would disperse and bury seeds when food is plentiful (i.e. after mass seed drop) but that rodent behaviour towards seeds would change to a consumptive pattern later

in the year. I determined the seed mass (in counts) and timing of seed drop of suspected rodent dispersed/consumed seed species at my field site to estimate seed volume during mass seed drop. This created a temporal reference point that could be used to compare rodent behaviour based on food availability over the year. To determine whether *A. subspinosus* seed selection behaviour for caching and consumption changed over the seasons based on seed volume, I placed traceable *Leucadendron sessile* seeds into the field, exposing them to the scatter-hoarding rodent. Additionally, I conducted a full-year faecal analysis for an in-depth look at *A. subspinosus* diet. A comparative faecal analysis with the generalist feeder and possible cache pilferer *R. pumilio* was also completed. I anticipated that the faecal analysis for *A. subspinosus* would reflect the changes in seed selection trends found through the seasonal seed fate results and that *R. pumilio* is an omnivorous seed predator and anticipated cache pilferer.

Materials and Methods

Study Site and Species

Field work was carried out on the western-facing slopes of Sir Lowry's Pass, Somerset West, Western Cape, South Africa between April 2010 and April 2011. The field site consists of fynbos which was last disturbed by fire approximately five years ago. The site consists mainly of *Leucadendron* (suncone bushes), *Protea* and Restionaceae plant species. *Leucadendron sessile*, the plant species used in this study, is a single-stemmed shrubby bush growing up to 2.0 m tall, which flowers in late winter (July – August) and drops its seeds annually. Peak seed drop is late November to beginning of December (early summer). *Leucadendron sessile* seeds possess numerous seed characteristics that conform to the rodent seed dispersal syndrome. These characteristics include large hairless nut, absence of an elaiosome or wings, thick seed hull with a relatively short, annual mass seed drop event. *Leucadendron sessile* seeds are documented to be dispersed by the rodent *A. subspinosus* in the fynbos (Midgley *et al.* 2002, Midgley & Anderson 2005). I found three rodent species and one Macroscelidea species were present at the site, including *Acomys subspinosus*, *Rhabdomys pumilio*, *Micaelamys namaquensis*, and *Elephantulus edwardii* respectively (see Chapter 2, Appendix 1) *Acomys subspinosus* the main rodent subject in this study is a small (< 22g), nocturnal, beige-coloured rodent with distinguishing markings including a strip of dark fur running down the length of the spine, a

white ventral side and white paws. The species is known to be a granivorous scatter-hoarder. *Rhabdomys pumilio*, also known as the Striped field mouse, is a diurnal omnivore (< 30g) with dark brown fur and three distinctive white and black stripes running along the dorsal side of the body.

Seed Availability Estimation

As a crude estimation of when seeds will be most and least abundant for rodents, I estimated when large and non-serotinous seeds are released at the field site, by identifying all fynbos plants with the following characteristics: nut-like seeds that do not have wings and are dropped annually. I included seeds with elaiosomes attached (indicates myrmecochory) in this seed availability estimation as rodents predate on ant-dispersed seeds which can aid in satiating the mice (Bond & Breytenbach 1985, Christian & Stanton 2004). I randomly chose eight sites at the field site on Sir Lowry's Pass and used 5 m² quadrants to identify potentially rodent dispersed plant species. Once identified, the plants' flowers or spiklets were counted. From the counted flowers I estimated seed production for each plant in the respective quadrant based on field guides and the literature (Haaksma & Linder 2000, Rebelo 2001, Manning 2007). I divided total seed drop numbers from each plant species from all quadrants by five to estimate the number of seeds per plant species per 1 m². I then used field guides (Haaksma & Linder 2000, Rebelo 2001, Manning 2007) to estimate seed drop windows for each species. If seed drop windows extended over more than one month, I assumed that maximum seed drop occurred mid-way through the seed drop window (normal distribution). I used a modified quartile formula equation to calculate a normally distributed seed drop per month, based on the total number of seeds dropped during the number of months that the respective plant's species seed drop window lasted. I did this by modifying the equation $(1/4(n-1) + 1)$ (upper quartile) and $(3/4(n-1) + 1)$ (lower quartile) into "tertiles" $(1/3(n-1) + 1)$ and $(2/3(n-1) + 1)$ respectively for 3 month windows, upper and lower quartile for 4 month windows etc. where n= the total number of seeds dropped over the whole seed drop window length.

Seasonal Seed Dispersal

To determine if rodent dispersal behaviour changed seasonally, I placed seeds out each season and determined seed fate. The field site is bisected by an overgrown road that was used as

a reference point for seed depots. I laid five transect lines perpendicular to the overgrown road, each line 20 m apart with two seed depots per transect line, for a total of ten depot sites. Depots were spaced at least 5 m from the road and 20 m from each other. All depot sites were on bare patches of ground which had aerial protection from a *L. sessile* plant. Seeds were scattered randomly in a 1 m² area underneath the respective plant. I followed the fate of seeds by attaching pieces of 30 cm long yellow fly fishing backing line to seeds with plumber's putty, which dries odourless and is similar in texture and density than seed hulls. This allowed us to easily spot seeds in the field. Seeds were recovered by checking the depot site first before beginning to search for moved seeds by walking in a spiral fashion up to 10 m away from the depot site. Seeds were categorized as either not dispersed (left at depot site), dispersed and buried or consumed. Consumed seeds were identified when the seed hull with tracking string attached had been cracked. I placed 300 *L. sessile* seeds into the field at the ten depot sites with 30 seeds per depot. Seeds were deposited at sunset and retrieved seeds 8-10 hours later at sunrise before diurnal seed predators became active. I repeated this procedure 48 hours later with another 300 seeds. This procedure was carried out every three months for one year (four trials in total) with each trial completed in the middle of each season (mid-April, mid-July, mid-October and mid-January). For more detailed information on depot site selections and procedures refer to Chapter 2.

Faecal Sampling

To determine rodent diet over the course of one year, I trapped rodents at regular intervals and collected and analysed their faecal pellets. I trapped *A. subspinosus* and *R. pumilio* with PVC Sherman live traps (each trapping session is mentioned as a "trial" from hereon) every six to eight weeks starting in August 2010 and ending in June 2011. During cold winter months wood shavings were provided as bedding in the traps. I used the depot sites from the seed dispersal trials as trapping locations, placing seven traps per depot for a total of 70 traps per trial. Each trial consisted of two consecutive nights of trapping. I set traps one hour before sunset and collected them the next morning, one hour after sunrise, to ensure that both the nocturnal *A. subspinosus* as well as the diurnal *R. pumilio* was captured. Rodents were released immediately upon identification and the faecal matter collected from the trap. I collected four faecal pellets per trap. Faecal matter was placed into separate airtight bags and then transferred to vials containing 70% ethanol for preservation. Prior to dissection, faecal samples were soaked for 48

hours in a saturated bicarbonate solution to stain any seed fragments in the samples a reddish colour (Dacar & Gianonni 2001). Faecal pellets were then dissected under a dissection microscope and percentage of different food particles (seed fragments, insect parts, plant matter; leaf matter or roots) were recorded, based on overall faecal sample volume. I grouped faecal collecting dates into four seasonal categories (autumn, winter, spring and summer) to allow data pairing with seed dispersal results.

Statistical Analysis

For seasonal seed fate data, I tested for differences in seed fate by converting data of seed fates into percentages of total seeds found, arcsin square root transformed the percentages and conducted ANOVA's for each seed fate over the four seasons in PASW Statistics 18 (SPSS Inc.). Tests for homogeneity and normality were satisfactory to perform the ANOVA analysis. For the faecal analysis, percentage data were arcsin square root transformed, tests for homogeneity and normality performed to satisfy the required parameters to use a MANOVA with Bonferroni post-hoc tests for the analysis in PASW Statistics 18 (SPSS Inc.).

Results

Seed Availability Estimation

The annual seed drop windows of plants identified as possibly rodent-dispersed/consumed show a trend towards seed drop from late spring to mid-summer (Fig.3.1) with a mean seed drop window of 3.7 months. Maximum seed drop, in terms of number of seeds released, occurs in December with an estimated 1100 seeds dropped.

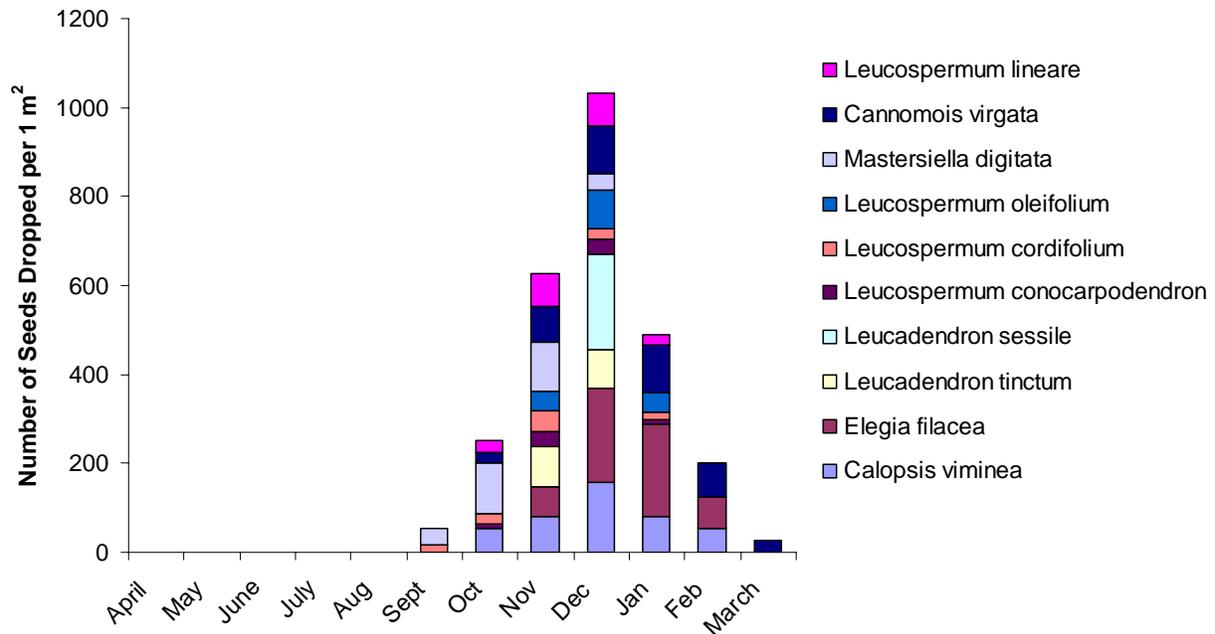


Figure 3.1. Estimated seed quantities released during the respective seed drop windows of rodent-dispersed plant species (including plants that produce seeds with elaiosomes) at the Sir Lowry's Pass field site.

Seasonal Seed Dispersal

Seed dispersal patterns by *A. subspinosus* changed over the course of a one year period (Fig. 3.2). Significantly more seed burial occurred in autumn than in any other season (ANOVA $F_{11,8} = 9.99$, $p = 0.012$). In contrast, a significant majority of seeds were consumed in winter ($F_{11,8} = 4.46$, $p = 0.04$) compared to moderate consumption in autumn and spring. Significantly lower consumption and burial rates, compared to the other three seasons, were recorded in summer ($F_{11,8} = 6.10$, $p = 0.029$) with most seeds left at depots untouched.

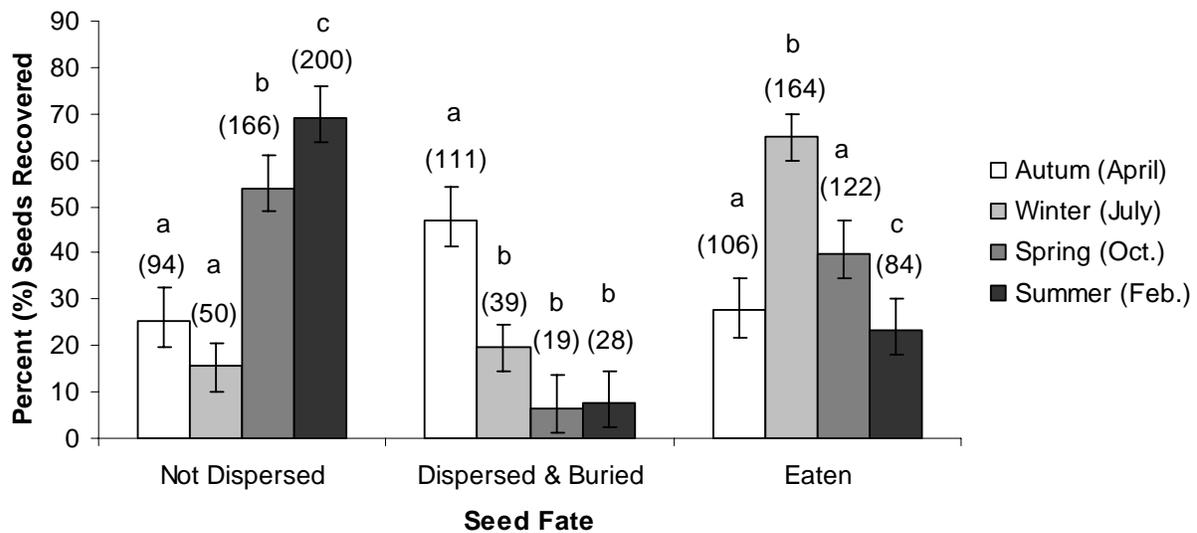


Figure 3.2. Seed fate after exposure to *A. subspinosus* for 12 hours in the field in each season. Experimental seed fate is reciprocal to natural seed fate: e.g.: if consumption of experimental seeds increases, fewer seeds were found in the natural environment. Percent seeds recovered that share the same letter are not significantly different ($p > 0.05$), numbers in brackets indicate number of seeds recovered during that respective season

Faecal Sampling

I found significant quantities of seed fragments, vegetation remains and insect material in *A. subspinosus* faeces. Vegetation remained a consistent component of the diet throughout the year ($F_{10,13} = 0.712$, $p = 0.57$) while seed fragment and insect material quantities fluctuated significantly. I found a significantly higher percentage of insect matter in samples from the winter ($n = 17$) and spring ($n = 13$) months compared to summer ($n = 21$) and autumn ($n = 21$) (Fig. 3.3. $F_{10,13} = 10.97$, $p = 0.002$) with seed fragments completely absent. An abrupt change from insect-based to seed-based diet was recorded in late spring with seed fragments representing a major proportion of faecal volume in summer and autumn ($F_{10,13} = 24.56$, $p > 0.001$) compared to winter and spring. In comparison, *R. pumillio*, a generalist feeder, had a consistent diet throughout the year with a significant majority of vegetative matter in faecal samples ($F_{10,13} = 16.72$, $p > 0.001$) in winter ($n = 16$), spring ($n = 14$), summer ($n = 21$) and autumn ($n = 25$). Seed fragments and insect parts were found to be low in volume throughout the year and never varied significantly throughout the year ($F_{10,13} = 0.82$, $p = 0.49$ and $F_{10,13} = 1.21$, $p = 0.09$)

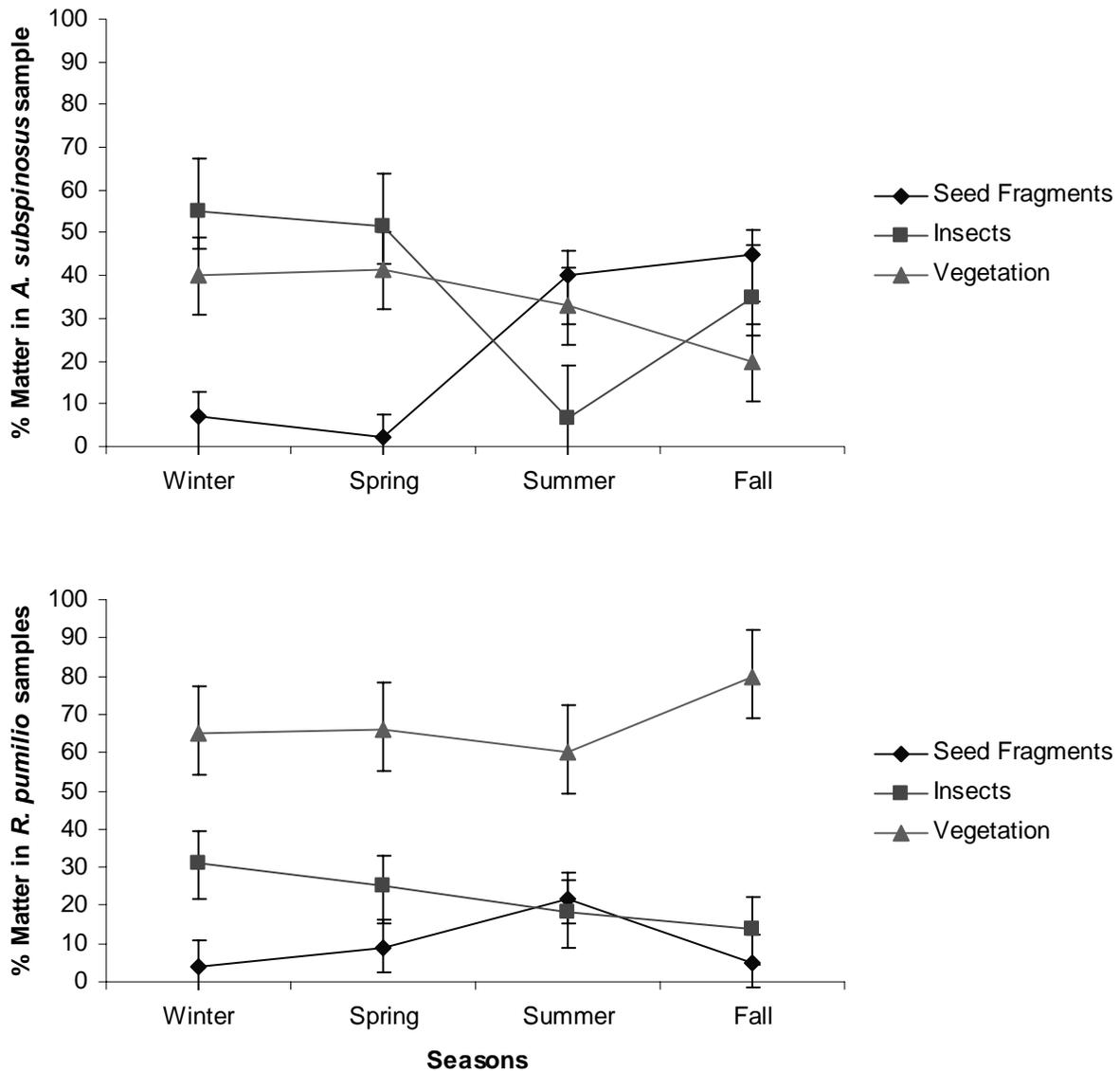


Figure 3.3. Food matter found in faecal samples of *Acomys subspinosus* (top) and *Rhabdomys pumilio* (bottom) throughout the year. *Rhabdomys pumilio* shows indications of a generalist feeder with pilfering tendencies while *A. subspinosus* is a seasonal specialized feeder.

Discussion

Acomys subspinosus seed dispersal and consumptive behaviour towards *L. sessile* seeds was affected by seasonal food availability. *Acomys subspinosus* displayed mostly scatter-hoarding behaviour in autumn but began to consume increasing volumes of experimental seeds in the winter and spring months. Seeds were an important dietary component during summer and

autumn when they were presumably plentiful as mass seed drop occurs during those months. However, fewer seeds were available during winter and spring and may have accounted for a dietary switch from seeds to predominantly insects.

Seed Availability Estimation

The seed drop window for potentially rodent dispersed seeds at the field site on Sir Lowry's Pass is relatively small. The seed availability estimation showed a peak in December, in the middle of the overall seed drop window period for the site. Much of the extension of the seed drop window was due to the addition of myrmecochorous plants that have a much longer seed drop window (mean window length of 4.4 months) than the elaiosome-less *Leucadendrons* (mean seed drop window of 2 months). I added the myrmecochorous plant species as rodents often prey on seeds containing elaiosomes (Bond & Breytenbach 1985, Christian & Stanton 2004). By including seeds containing elaiosomes that may be consumed by *A. subspinosus*, I obtained a more complete picture of the number of seeds in the environment that may be ingested or dispersed. Although very crude, the estimation provides an important overview of seed mass numbers at the field site. It is unlikely that the addition of seed mass data into these figures will significantly change the overall trend, as all of the plants disperse their seeds over a similar time window.

Seasonal Seed Dispersal

In mid-summer, during the occurrence of the mass seed drop event by the Proteaceae and Restionaceae species, *A. subspinosus* dispersed or ate a limited quantity of experimental seeds. This indicates that such a high abundance of seeds lying on the soil surface that the chances of the experimental seeds being consumed or dispersed was small. Caching of experimental seeds commenced in autumn (end March to April) suggesting that there were enough seeds in the environment to satiate the rodents but not enough for them to ignore a potential food source on the soil surface (Forget 1993). Experimental seed ingestion increased significantly during the winter months, suggesting that seeds available in the natural environment were decreasing in volume. Consumption of the majority of experimental seeds also suggests that rodents were no longer satiated enough for caching behaviour to be displayed. It is likely that at that stage of the season, rodents were recovering seed caches that were originally cached in autumn. This was

confirmed by the low but significant amounts of seed fragments that were found in early-winter faecal samples. I found that these low volumes of seed fragments were consistent across faecal samples of numerous individuals, indicating that cache recovery did indeed take place. These seed fragments could not have originated from freshly dropped seeds, as no species of seed-bearing plants, regardless whether serotinous, myrmechocorous or rodent-dispersed at the field site, has a seed drop window during the months of May to August.

I expected that rodents would continue to ingest experimental seeds at high rates into spring, however experimental seed ingestion dropped significantly and a significantly higher volume of seeds were left at depots compared to winter. The higher number of seeds left at the depots in spring and lower ingestion rates cannot be attributed to lower *Acomys subspinosus* numbers, as the population numbers were similar during winter and spring (see Appendix 1). *Micaelamys namaquensis* could not have been responsible for the high seed ingestion in winter as population numbers were low compared to *A. subspinosus*. Additionally, *Micaelamys namaquensis* numbers remained constant over the two seasons, suggesting that high seed ingestion rates would have had to be recorded for both winter and spring if *Micaelamys namaquensis* was responsible for a significant portion of the ingested seeds recorded in winter.

The changes in behaviour towards the experimental seeds by *A. subspinosus* suggest that rodents found another food source that led to a decreased interest in experimental seeds as available food. Through a comprehensive, full-year faecal analysis I detected a dietary change from seeds to insects in spring. This change in diet explains the decrease in experimental seed ingestion in spring, as mice were exploiting a high-energy, high-protein food source that left them relatively satiated. Similar findings were reported by Harris (1986) with Kangaroo (*Microdipodops pallidus*) and Deer mice (*Peromyscus maniculatus*). Arthropods seem to play a larger role in the diet of scatter-hoarding rodents than previously thought (Harris 1986). Compared to *R. pumilio*, *A. subspinosus* displayed a highly specialized diet that centred around two essential food components, seeds and insects, with vegetative matter as a constant supplement. The predictions on *R. pumilio* displaying a general diet with signs of being a cache pilferer were confirmed by the results. I found a significant amount of vegetative matter as part of the diet of *R. pumilio* throughout the year. Seed fragments and insect volume were fluctuating

with seasons, suggesting that these food sources were opportunistic. The presence of seed fragments in faecal samples of *R. pumilio* during the winter and spring, points to the possibility of *R. pumilio* being a cache pilferer since this rodent is not known to cache seeds which it could retrieve.

The dietary switch I observed by *A. subspinosus* may help to establish what happened to *L. sessile* seeds as a food source from autumn to late winter. It is possible that some seeds were left in the seed bank after consistent and frequent cache recoveries in winter that may have prompted the diet switch. Based on the *R. pumilio* faecal analysis, this explanation may be supported. Seed fragment quantity did increase in *R. pumilio* faecal pellets from winter to spring, however the increase was marginal (3.6%) and does not suggest that a substantial amount of seeds were still available in the seed bank. This explanation would support the prediction that the seed bank depletes to low levels by spring due to cache recoveries and pilfering by *A. subspinosus* and *R. pumilio* respectively. This would mean that seeds are a highly fluctuating food source for *A. subspinosus* through the year, and may force the rodent to switch to insects by spring.

Alternatively, the *A. subspinosus* diet switch may be explained by its reproductive process or simply by an opportunistic diet switch. Insects would be increasingly abundant in numbers and diversity in spring with rising daytime temperatures and after a rainy winter. Fleming & Nicolson (2002) suggested that *A. subspinosus* is an opportunistic breeder. If *A. subspinosus* is indeed an opportunistic breeder, then young should be born in late spring when mass seed drop occurs in the Proteaceae plants. However, if the insect influx is high enough in early spring, *A. subspinosus* may switch to an insectivorous diet and match their breeding cycle to increasing insect densities since insects provide a higher food reward for breeding rodents (Harris 1986). However, the rodents used in Fleming & Nicolson's (2002) observations, were primarily nectar feeding, whereas *A. subspinosus* is a seed eater and may have a fixed breeding cycle, as in the case of *Acomys spinosissimus* that bears young in late spring/early summer (Fleming & Nicolson 2002). If *A. subspinosus* has a fixed breeding cycle occurring in late spring, then the dietary switch to insects in early spring may be a) due to decreased seed availability or b) simply be opportunistic since insect abundance increases in spring and the energy trade-off

between catching high-energy insects and retrieving comparatively lower-energy seeds may favour an insectivorous diet.

Temporal Fluctuations in the Outcome of the Plant – Rodent Relationship

The plant – scatter-hoarder relationship I described showed signs of temporal variations in its outcome. Soon after seed release, the cost to plants (seed consumption by the scatter-hoarder) were relatively low as *A. subspinosus* was apparently satiated from the seed drop and only a minimal amount of seeds were consumed while large amounts of seeds were cached. However, as the temporal gap to the seed drop event increased, surface seeds were no longer available and rodents began to consume seeds that had been cached earlier. Consumption of previously cached seeds can be so high that *A. subspinosus* may run out of stored seeds by spring. Although the *L. sessile* – *A. subspinosus* relationship is most likely mutualistic overall, it shows tendencies of predation by the rodent during the winter and spring months when seeds are consumed and not cached. The consumptive patterns of *A. subspinosus* did not only reflect on the availability of surface food during summer and autumn but may also reflect on the availability of cached food later in the year. Consumptive patterns suggest that seed bank volume is low throughout the year with the exception of late summer and autumn. Since the fynbos is a fire prone system where plants rely on fire for regeneration, I predict that the timing of fires may have significant effects on seedling recruitment and therefore the outcome of the plant – scatter-hoarder relationship. Fires in late summer or autumn would lead to high seedling recruitment and would be highly beneficial to plant regeneration as scatter-hoarders have cached most seeds by that time. However, fires during any of the other seasons, particularly late spring, may be near-detrimental to the plant species' local population as scatter-hoarders would have recovered most of their caches, leaving the seed bank depleted at the time of fire. Given the important ecological role scatter-hoarding rodents, such as *A. subspinosus*, may play in the fynbos, understanding rodent behaviour based on food availability and rodent influence on seed bank dynamics is crucial to the understanding of ecosystem dynamics. The interaction between fynbos rodent dispersers and plants seems to grow increasingly complex the more it is investigated, demonstrating that for successful management of a delicate, fire-prone system like the fynbos with a large number of endemic plant and animal species, knowledge of the system becomes very important.

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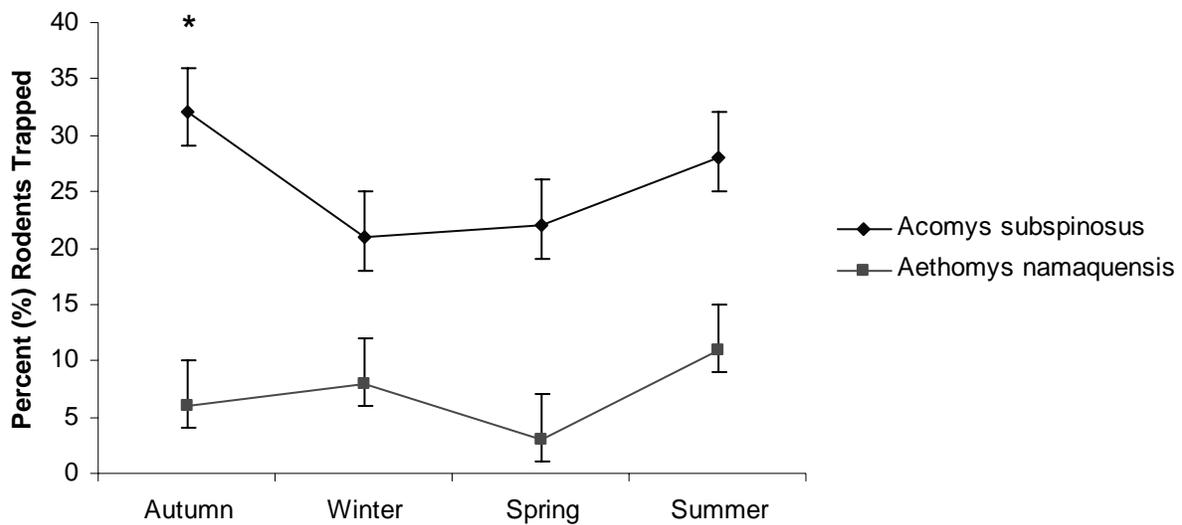
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Appendix 1. Population census survey over a one year period at the Sir Lowry's Pass field site. This population census was completed to be able to compare temporal, nocturnal rodent population fluctuations with seed fate in the field (see discussion). RA was based on number of captures per six trapping nights each season, with re-captures within a season not re-counted. As trapping sessions were temporally independent, I analyzed *A. subspinosus* RA with a negative binomial test for over-dispersion of data before performing a Kruskal-Wallis test over the four seasons. *Acomys subspinosus* numbers dropped significantly from autumn to winter ($H_6 = 8.52$, $p = 0.014$). *Micaelamys namaquensis* population numbers remained relatively constant over the year but always significantly lower than *A. subspinosus*. Whiskers denote SD, star denotes significant difference within species population at $p > 0.05$



Chapter 4

Competing Seed Consumers Drive the Evolution of Scatter-hoarding: Why Rodents don't put all their Seeds in One Larder

Competition for food resources amongst animal seed dispersers and pilferers has driven the dispersers to increasingly innovative seed caching methods. I determined cache sizes in the field as well as seed cache recovery ability of an endemic scatter-hoarder in the South African fynbos, *Acomys subspinosus*, and compared these results to its seed competitor, the omnivorous seed predator *Rhabdomys pumilio*. I found that up to 76% of *A. subspinosus* caches in the field contained one seed and that *A. subspinosus* was able to find caches of all sizes equally well under wet and dry soil conditions. In comparison, *R. pumilio* was able to find caches of all sizes in wet soil conditions but recovery success of small caches (single-seed) was poor in dry soil conditions. This suggests that scatter-hoarding is an effective anti-pilfering strategy, at least in dry conditions where cache pilferers have difficulty locating small caches through olfaction. I also suggest that high pilfering rates may have been a driving factor behind the evolution of scatter-hoarding in *A. subspinosus*.

Introduction

Many plants, in a variety of ecosystems, rely on animals to disperse their seeds (Vander Wall 1990). These plant – disperser relationships can often be viewed as part of a keystone process that frequently affects ecological dynamics far beyond the initial interaction (Briggs *et al.* 2009). Briggs *et al.* (2009) found that directed seed dispersal by chipmunks is part of a keystone process that places pine seeds under ideal conditions for seedling establishment. Seed caching can have important positive effects on seed persistence and seedling germination (Vander Wall 1990, Theimer 2001, Briggs *et al.* 2009). Seed hoarding does present numerous advantages for the dispersed plants: seeds are moved away from the parent site which decreases intra-specific competition upon germination, the probability of established pathogens being present at new sites are lower, scattering seeds decreases the chance of density-dependent seed/seedling mortality, and it contributes towards (re)colonization of the plant community

(Vander Wall 1990, Hulme 2002). Finally, burying seeds underground provides them with protection from seed predators and the elements.

The behaviour of hoarding seeds evolved when animals began to stock seeds during plentiful times for subsequent food-scarce times (Vander Wall 1990). Two main types of hoarding are recognized: larder hoarding, where seeds are amassed in large numbers in a few underground nests or deep caches and actively defended (Vander Wall 1990, Daly *et al.* 1992, Vander Wall *et al.* 2005) and scatter-hoarding, where seeds are usually cached a considerable distance from the source, either singly or in low numbers (Vander Wall 1990). Larder hoarding may be beneficial from an energetic view point because food is stored at few sites in large quantities often close to the burrow (Vander Wall *et al.* 2005). This limits the energetic expenditure needed to retrieve food once the seeds are hoarded. However, if a single larder is pilfered, the hoarder stands to lose a considerable portion of its food resource. However, some larder hoarding rodents use scatter-hoarding as an additional seed caching method, to prevent catastrophic loss of food supply should the larder-hoarded cache(s) be raided (Daly *et al.* 1992). Larder hoarding does present numerous problems to plants as caches are often too deep underground to allow for germination and intra-specific competition upon seedling establishment is so high that few seedlings survive (Vander Wall 1990).

In contrast, scatter-hoarded caches are not guarded by the disperser. Here, cache pilfering is mitigated by having small seed caches spaced at considerable distance and buried at micro sites which decrease the chance of olfactory detection by seed predators (Clarke & Kramer 1994, Vander Wall 1998, Tamura *et al.* 1999). However, the disadvantage of scatter-hoarding is that seeds are usually moved large distances from the seed source or burrow which increases energetic expenditure of the caching rodent to recover seeds (Vander Wall 1990). Despite that, seed dispersing animals often rely solely on scatter-hoarding, demonstrating that it is the more successful seed hoarding strategy, compared to larder hoarding, in most environments (Vander Wall 1990, Vander Wall 2002, Vander Wall *et al.* 2005). Studies have shown that scatter-hoarding may be of benefit to plants as the small caches (one to five seeds) remain undiscovered significantly longer than large seed caches (Geluso 2005). Scatter-hoarding is also most likely to result in seedling establishment due to shallow burial of seeds, low seed numbers in caches and

low cache density in a given area, which minimizes intra-specific competition between seedlings for space, light and nutrients (Hollander & Vander Wall 2004).

Scatter-hoarded seeds are retrieved later on either by the cacher itself through memory or olfaction or by a pilferer through exploratory digging or olfaction (Vander Wall 1990). Since memory fades as time passes and exploratory digging is energy and time consuming, rodents often rely on olfaction to retrieve caches (e.g. Briggs & Vander Wall 2004). It has been shown that a variety of factors contribute to the success of cache retrieval, most notably environmental conditions such as soil moisture and the rodent's olfactory acuity (Johnson & Jorgenson 1981, Vander Wall 2000, Vander Wall *et al.* 2003). The lower the moisture content of the soil, the more difficult it is for rodents to detect seed caches (Vander Wall 1998, Vander Wall 2000). However, some granivorous scatter-hoarders from arid environments display high olfactory acuity compared to rodents from temperate climates (Johnson & Jorgensen 1981, Vander Wall 1998, Vander Wall *et al.* 2003), suggesting that in order for the former to survive, an acute olfactory system is needed to re-locate caches in extremely dry substrates.

Recently, a plant- animal disperser interaction was discovered in the fynbos of South Africa where the Cape spiny mouse (*Acomys subspinosus*) scatter-hoards seeds of a suncone bush, *Leucadendron sessile* (Midgley *et al.* 2002). *Acomys subspinosus*' most common sympatric seed predator/potential pilferer is thought to be *Rhabdomys pumilio*, an omnivore known to consume but not bury seeds (J.J Midgley personal communication). Since scatter-hoarding may have evolved as a means to decrease pilfering rates without having to actively defend caches, I first established the sizes of the caches dug by *A. subspinosus* in the field. I then determined the ability of *A. subspinosus* to find caches of differing sizes using olfaction only and compared these to *R. pumilio*. Additionally, I was interested in whether *A. subspinosus* was able to detect seed caches more readily than *R. pumilio*, so I investigated the effect of soil moisture content on cache recovery success in both rodent species. I predicted that *R. pumilio* should find it difficult to recover seed caches in dry substrates and of similar size to natural *A. subspinosus* caches found in the field. However, I anticipated that caches larger in size than those made by *A. subspinosus* in the field may be much easier for the *R. pumilio* to find. In addition, an increase in soil moisture was expected to influence the cache recovery rates positively in both species.

Materials & Methods

Field Site Description

All field experiments and the trapping of experimental individuals commenced at a field site on the western-facing slopes of Sir Lowry's Pass near Somerset West, South Africa. The field site is a five year old fynbos stand that contains plant species from the genus *Leucadendron* as well as *Protea* and Restionaceae. Previous studies (Midgley *et al.* 2002), as well as my own population census, documented the following rodent species at the site: *Acomys subspinosus*, *Rhabdomys pumilio*, *Micaelamys namaquensis*, as well as the Macroscelided *Elephantulus edwardii* (see Appendix 1, Chapter 2). *Acomys subspinosus* is a small mouse (< 22g) and the only known nocturnal scatter-hoarder at the site while *R. pumilio* is a diurnal omnivore and suspected seed pilferer (see Chapter 3 results).

Cache Density Survey

As part of the seasonal seed dispersal experiments (see Chapter 3) I conducted a full-year cache size analysis between April 2010 and February 2011. I completed this by recording all experimental seeds that were buried by *A. subspinosus* in the field and noting cache size. I performed this analysis by dropping two times 300 seeds for a total of 600 seeds into the field every three months (four times over a one-year period). Seeds were tagged, for easy retrieval, with yellow fluorescent, 30cm long, fly-fishing backing line. The tracking strings were attached by plumber's putty that dries odourless and retains a similar texture and hardness to seed hulls. The seeds were placed at ten depot sites that were equally spaced at 20 m throughout the field site. I dropped 30 *L. sessile* seeds per depot site, for 300 seeds dropped per session (two sessions per season). Seeds were placed at sunset when the nocturnal *A. subspinosus* becomes active and retrieved the next morning at sunrise. Any experimental seeds that were dispersed and buried were noted along with cache size. The second session with another 300 seeds followed 48 hours later (for more detailed methods, see Chapter 2 and 3). The sole purpose of the cache size analysis, which was conducted at the same time as the dispersal experiments in Chapter 3, was to determine the cache size of the experimental seeds that were buried by *A. subspinosus* in the field.

Capture & Housing

Seven *Acomys subspinosus*, three females and four males and seven *Rhabdomys pumilio*, five males and two females, were captured in PVC Sherman live traps between April 11, 2011 and April 20, 2011 at the Sir Lowry's Pass field site. The mice were transported via car in 40 cm x 40 cm x 20 cm transport cages to Stellenbosch University, South Africa. The holding facility was a 7 m by 12 m shade-cloth greenhouse with a concrete floor and waterproof roof. Mice were housed individually in large glass terrariums (64L) on a natural light-dark cycle. Ambient temperatures fluctuated between 12° C (night time) and 20° C (daytime). Sand was used as substrate in the terrarium. Each terrarium contained a nesting box in the form of a 2 L glass jar filled with wood shavings, along with shelters in the form of bark and eggshell cartons. Water was provided *ad libitum*, with food given once per day in the morning unless mice were assigned a trial within 48 hours, at which point food was revoked. Food consisted of a mix of sunflower seeds, oatmeal, carrots, apples and lettuce.

Cache Recovery Experiment

To determine cache recovery ability of *A. subspinosus* and *R. pumilio*, I cached seeds in a sand arena and exposed rodents to the caches over a 24 hour period. All trials were conducted in the same greenhouse as the mice were kept. I used two 1.5 m by 2.1 m enclosures constructed of 30 cm high wooden walls with wire meshing on top to prevent escaping. The arenas were constructed on the concrete floor, underlain with waterproof tarps to prevent water seepage and filled with construction sand 10 cm deep.

For each trial, *Leucadendron sessile* seeds were randomly cached in one arena by hand. I placed seven single-seed caches, seven three-seed caches, seven five-seed caches and seven placebo caches (3 seed-sized stones) per trial. Seeds were cached at 2.5 cm depth with gloved hands to avoid scent deposition. Caching sites were smoothed to avoid leaving visual cues. Five *L. sessile* seeds were strewn on the sand surface to signal seed presence and encourage searching behaviour. Caches were marked by taping a piece of masking tape with the cache's seed number directly above the cache on the wire meshing. The respective mouse's nesting box was placed in the centre of the arena for shelter. After each trial the mouse was removed by trapping it with a Sherman live trap and returned to its terrarium. A visual survey of the arena was completed and

recovered caches were noted. Subsequently each cache was double checked for remaining seeds with a hand rake garden tool and seeds in non-recovered caches were retrieved. When necessary, samples of arena soil and intact seeds were taken, to measure moisture content. The whole arena was then raked to flatten the surface and new seeds were cached for the next trial.

Seven *A. subspinosus* and seven *R. pumilio* were trialed in dry sand (30% humidity on sunny or overcast days). The trials were then repeated with the same mice in wet sand (80% humidity or higher on rainy days only). Each mouse spent 24 hours in the arena per trial. Food was removed from the respective mouse 48 hours prior to a trial. Fresh lettuce and water was available *ad libitum* up to the start of the trial. Water and food, with exception of the cached seeds, was withheld in the arena. Mice were placed in the arena between 8:00 and 10:00. Arena sand was moistened for wet trials with 3.0 litres of distilled water to reach a benchmark moisture content of 0.4 g water per gram sand. Seeds for wet trials were kept in paper bags to allow moisture absorption from the air. Seeds for dry trials were kept in sealed plastic bags after being exposed to 30% humidity in a humidity chamber for four days.

Statistical Analysis

I calculated cache recovery as the percentage of caches recovered by each rodent and arcsin root transformed the percentage data. A general linear model multivariate analysis with a Bonferroni test was used to compare *A. subspinosus* versus *R. pumilio* cache recovery ability in dry and wet sand. I also compared cache recovery ability within species between wet and dry sand using pair-wise t-tests. Analyses were completed in PASW Statistics 18 (SPSS Inc.).

Results

Cache Density Survey

Of the seed caches I recovered in the field (n= 186, Fig. 4.1), 76% were buried by *Acomys subspinosus* in single-seed caches (141 caches), while two-seed caches made up 20% (38 caches), three-seed caches 3% (five caches) and four-seed caches 1% (two caches) of the total cache count.

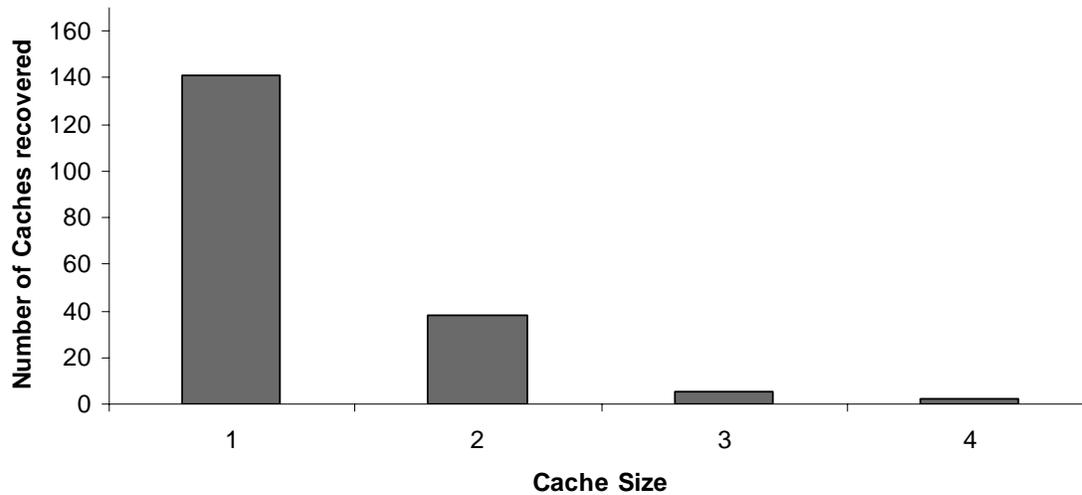


Figure 4.1. Number of caches and their respective size (number of seeds) dug by the scatter-hoarding mouse *Acomys subspinosus* in the natural environment.

Cache Recovery Experiments

Seed cache recovery in dry sand was significantly higher in *A. subspinosus* than in *R. pumilio* for single-seed caches (Fig. 4.2, $F = 13.2$, $df = 13$, $p \leq 0.001$) but not for three- or five-seed caches ($p = 0.339$, $p = 0.644$) respectively. Cache recovery in wet sand (0.45 g water/g sand \pm 0.28g water) was not significantly higher in *A. subspinosus* compared to *R. pumilio* for one-seed caches (Fig. 4.3, $F = 2.13$, $df = 13$, $p = 0.116$), three-seed caches ($p = 0.82$) or five-seed caches ($p = 0.93$). Placebo caches were not recovered by either mouse species in the dry or wet trial series.

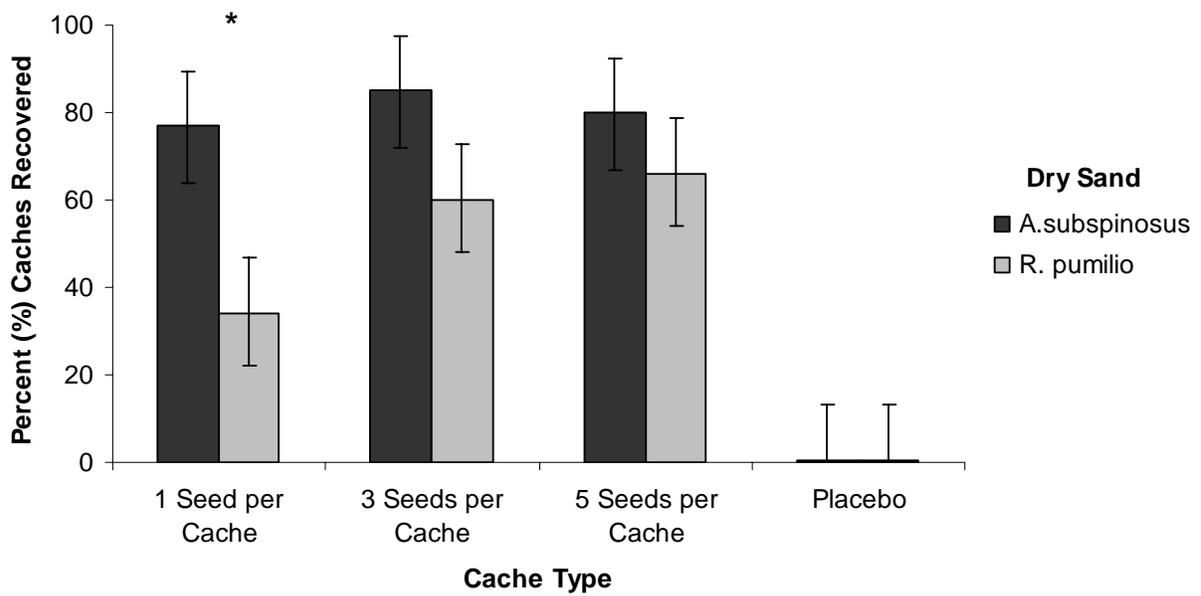


Figure 4.2. Comparison of seed cache recovery ability between *Acomys subspinosus* and *Rhabdomys pumilio* in dry substrate (0.02 g water/g sand). *Acomys subspinosus* recovered a significantly higher number of one-seeded caches than *R. pumilio* * denotes significant difference at $p < 0.01$, whiskers denote SE

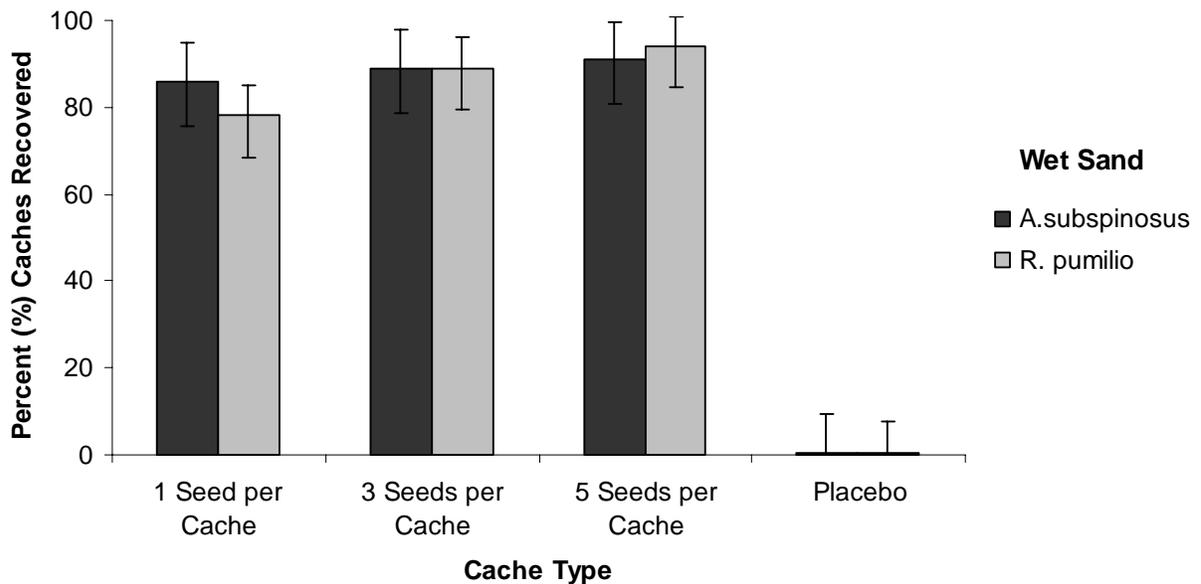


Figure 4.3. Seed cache recovery ability of *Acomys subspinosus* and *Rhabdomys pumilio* in wet substrate (0.42g water/ g sand). Both mice species recovered caches at similar rates. Whiskers denote SE

Within-species comparison showed that *A. subspinosus* did not recover a significantly higher amount of caches in either wet or dry soil. In addition, the number of seeds per cache, whether one, three or five seeds did not affect the recovery rate of *A. subspinosus* (Fig. 4.4, $t = 0.9$, $df = 6$, $p = 0.741$, $t = 0.3$, $df = 6$, $p = 0.85$, $t = 1.4$, $df = 6$, $p = 0.096$). On the other hand, *R. pumilio* recovered a significantly smaller number of one-seed caches in dry conditions compared to one-seed caches in wet soil (Fig. 4.5, $t = 4.6$, $df = 7$, $p = 0.003$) but did not have significantly higher cache recovery success in three- and five-seed caches in wet soil compared to dry soil ($t = 0.16$, $df = 6$, $p = 0.151$, $t = 0.17$, $df = 6$, $p = 0.149$)

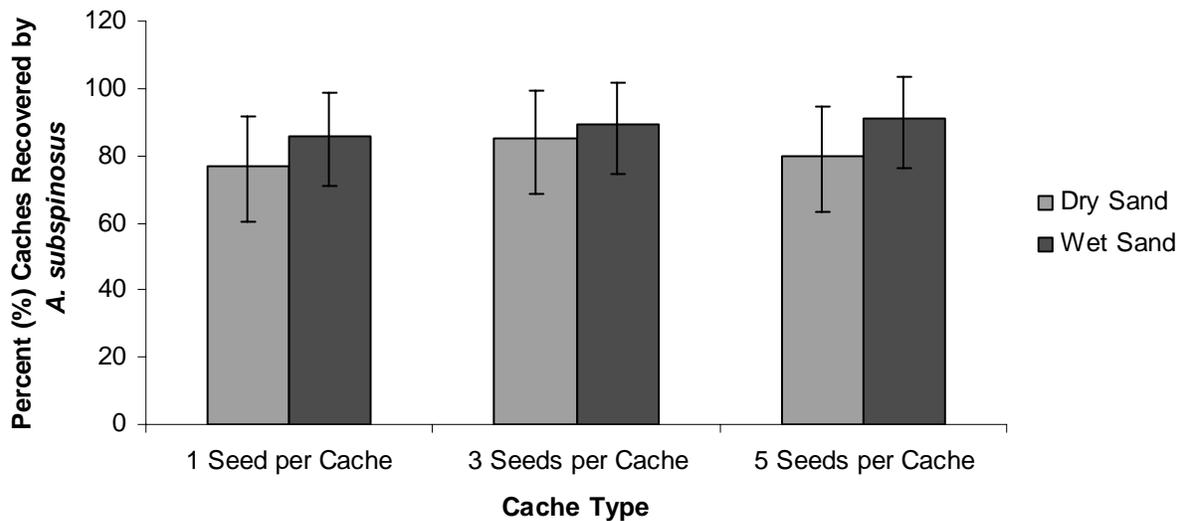


Figure 4.4. Percent of seed caches, with variable seed numbers, in both wet and dry sand recovered by *Acomys subspinosus*. Whiskers denote SE

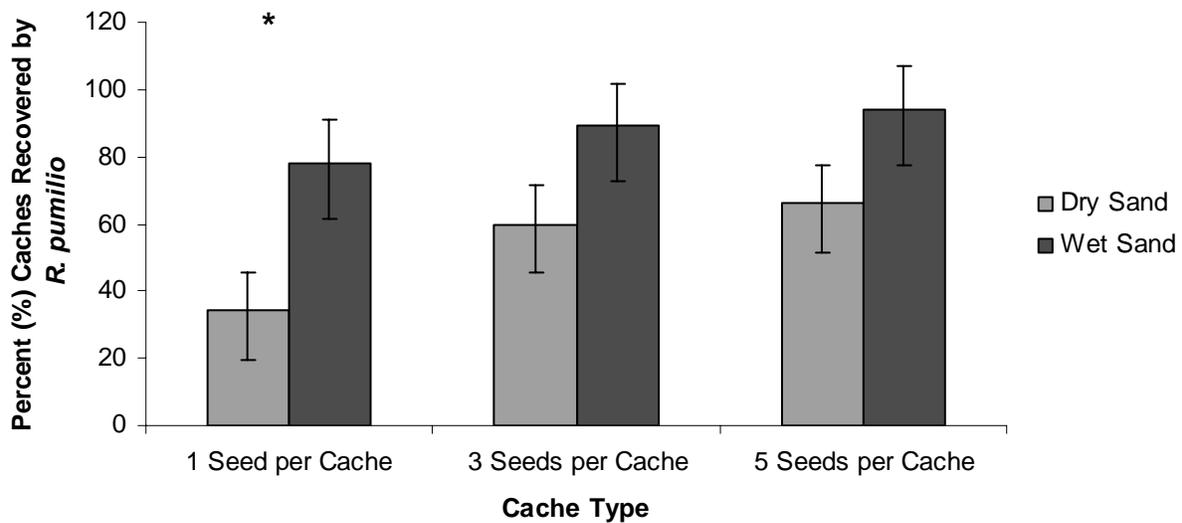


Figure 4.5. Percent of seed caches, with variable seed numbers, in both wet and dry sand recovered by *Rhabdomys pumilio*. * denotes significant difference at $p < 0.01$, whiskers denote SE

Dry sand trials were conducted at 30% humidity with mean sand moisture at 0.02g water/g sand (SE \pm 0.03g). Sand moisture did not significantly decrease or increase over the 24 hour trial period. *Leucadendron sessile* seeds kept at same humidity were significantly higher in moisture content compared to sand at 0.11g water/g seed (SE \pm 0.08g) ($t = 3.1$, $df = 10$, $p = 0.004$). Wet sand trials were conducted at 80% humidity or higher with mean sand moisture at 0.42g water/g sand (SE \pm 0.11g). Sand moisture did decrease significantly over the 24 hour trial periods ($t = 2.6$, $df = 11$, $p = 0.02$), however, at lowest moisture content, wet sand still had a water content six times greater than that of dry sand. *Leucadendron sessile* seeds kept at 80% humidity or greater had a mean water content of 0.51g water/g seed (SE \pm 0.06g) which was not significantly higher than wet sand moisture levels ($t = 6.2$, $df = 11$, $p = 0.07$).

Discussion

Acomys subspinosus buried the majority (76%) of its seeds singly, confirming similar single-seed cache rates (64%) observed by Midgley *et al.* (2002). I found that 97% of all caches contained two or fewer seeds, confirming *A. subspinosus*' scatter-hoarding behaviour. *Acomys subspinosus* was able to recover the vast majority (78%- 95%) of seed caches in the laboratory

experiment, regardless of cache size or soil moisture. Bond & Breytenbach (1985) reported similar results, where *A. subspinosus* showed a high tendency towards seed cache recovery (91%) of elaiosome-containing seeds regardless of cache depth (I did not test ant-dispersed seeds, and the authors do show that the elaiosome seems to act as a strong, additional scent cue for the rodents which increases cache recovery rates). *Rhabdomys pumilio* recovered significantly fewer single-seed caches in dry sand (34%) than *A. subspinosus* (77%) but had moderate cache recovery success with larger (three and five seeds) cache sizes (60% and 66% respectively). However, under wet soil conditions cache recovery of *R. pumilio* was no different from *A. subspinosus* for all three cache sizes. This increase in cache recovery success by rodents in wet soil has been recorded in other studies (Johnson & Jorgenson 1981, Vander Wall 1998, 2000, Geluso 2005) and is related to the increased organic molecule leaching from seed coats as they absorb moisture. These organic molecules rise from the soil surface as organic vapours which make seed cache detection easier (Vander Wall *et al.* 2003). The increase in soil moisture during my wet sand experiments increased the ability of *R. pumilio* to find low-density seed caches via olfaction.

With *R. pumilio*'s poor cache recovery rate of small-sized caches under dry conditions, scatter-hoarding becomes an effective way for *A. subspinosus* to protect its seed resources. The use of scatter-hoarding as a strategy to minimize cache pilfering has been reported by Vander Wall *et al.* (2005), where small-sized caches survived significantly longer in the presence of chipmunk pilferers than did larders. Similarly, Geluso (2005) found that pilferage of seeds by Ord's Kangaroo rat (*Dipodomys ordii*) in dry substrates is weak for small-sized caches. Both authors attributed the low success rates of small-sized cache retrievals to olfactory acuity in their respective pilferer subjects. Therefore, by scatter-hoarding the "right" number of seeds i.e. one to two seeds per cache, *A. subspinosus* not only mitigates inter-specific cache pilfering by keeping seeds out of an olfactory detection range suitable to pilferers, but manages to recover the majority of the cached seeds itself via olfaction. Scatter-hoarding must be a persistently successful method for acquiring and storing food in *A. subspinosus* since Preston & Jacobs (2001) found that scatter-hoarders changed their caching strategy to larder-hoarding when the scatter-hoarded caches were pilfered at high rates. Given that I found 97% of *A. subspinosus* caches containing two seeds or fewer, and never recorded a larder throughout the study, I can

conclude that scatter-hoarding is working effectively for *A. subspinosus* against pilferage, at least under dry soil conditions. Since scatter-hoarding is used as a means to decrease inter-specific competition for seeds as a food resource in other semi-arid and arid ecosystems (e.g. Vander Wall 1993), I suspect that competition for food between seed predators and seed consumers such as *R. pumilio* and *A. subspinosus* respectively, may have been the factor behind the evolution of scatter-hoarding in *A. subspinosus*.

I found that *A. subspinosus* had a significantly higher recovery rate of low density seed caches in dry sand than *R. pumilio*. This has previously been documented with rodent scatter-hoarders from arid environments (Johnson & Jorgenson 1981, Vander Wall 1995, Vander Wall *et al.* 2003) and suggests that the olfactory acuity of these scatter-hoarding granivores is very high irrespective of soil moisture (Johnson & Jorgensen 1981, Vander Wall 1995, Vander Wall 1998). Vander Wall *et al.* (2003) attributed several selective pressures to the development of more acute olfactory systems in granivorous scatter-hoarders from arid environments. Similar to most other desert rodent species, *Acomys subspinosus* are nocturnal which should automatically increase olfactory reliance for foraging since visual cues cannot be utilized. Additionally, humidity usually increases during the night which would increase olfactory signals from buried seeds (Vander Wall 1993). Lastly, rodents with a granivorous diet such as *A. subspinosus*, may be more specialized at detecting seeds than omnivores such as *R. pumilio* (Vander Wall *et al.* 2003). These selective pressures may explain the high cache recovery success of *A. subspinosus* and its ability to find low-density seed caches in dry sand. *Rhabdomys pumilio* on the other hand, is a diurnal, omnivorous rodent and opportunistic pilferer found in a variety of arid to mixed temperate climates, and is not exposed to the selective pressures set out by Vander Wall *et al.* (2003) for a highly developed olfactory system.

Unlike other semi-arid to arid environments in which olfactory research has been conducted (e.g. Beck & Vander Wall 2010), the South African fynbos experiences annual winter rains. My results suggest that *A. subspinosus* may lose its cache protection advantage by scatter-hoarding once the Cape winter rains set in and soil moisture increases to levels where small caches are more readily detected by potential pilferers such as *R. pumilio*. This demonstrates that weak cache detection ability in pilferers can significantly increase the effectiveness of a

scatter-hoarding strategy, at least in dry substrates. This is especially the case if the cacher is able to recover its own caches under any substrate conditions in case memory fails.

The fynbos is host to a variety of granivorous and omnivorous rodents that compete for limited food resources in an arid and fire-prone environment. Scatter-hoarding may have evolved as an effective food storage method with low pilferage rates for small rodents such as *A. subspinosus* that do not possess the body size to defend larders effectively. The high cache detection ability of *A. subspinosus*, compared to *R. pumilio*, can be attributed to a set of environmental and behavioural factors that have facilitated the development of an olfactory system that allows for high cache recovery success. Scatter-hoarding would not be a successful cache protection strategy if the scatter-hoarder could not “out-sniff” the pilferer to re-locate its caches if its memory of the location fails. My experiments have contributed to a greater understanding of scatter-hoarding and cache retrieval in the fynbos; however the influence of cache establishment and subsequent removal on the plant community is unknown. It is therefore important for future research to attempt to link the scatter-hoarding and cache recovery behaviours of rodents with the consequences for plant community dynamics, such as seed bank persistence and seedling establishment.

Acknowledgements

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Chapter 5

General Conclusion

In this thesis I set out to investigate the seed dispersal and cache recovery behaviour of the endemic, scatter-hoarding mouse *Acomys subspinosus*. Despite being a floristic hotspot, relatively little is known about the plant-animal interactions operating in the ecosystem. This is reflected by the discovery of rodent seed dispersal in 2002 (Midgley *et al.* 2002). For over 150 years it was not known for certain whether rodents were a dispersal vector in the fynbos. However, it was predicted that rodents may play an important role as seed dispersers in the ecological processes of the fynbos and this thesis was completed to address the gap in basic knowledge on seed dispersal and cache recovery behaviour of *A. subspinosus* as well as the rodent's influence on plant reproductive strategy.

In chapter 2 I investigated the basics of seed selection by *A. subspinosus*. Existing literature suggested that rodent-dispersed seeds are generally nut-like, do not possess wings or elaiosomes and have thick, hard hulls. The goal of this chapter was to determine what specific seed characteristics (seed size and hull thickness) the rodent selects for consumption or caching. Seed selection by *A. subspinosus* showed a potentially stabilizing selection pressure towards seeds with hull thickness/size of mean *L. sessile* dimensions that are found naturally in the field. Small/thin-hulled seeds were consumed in situ, as expected based on the literature (e.g. Theimer 2001) and large/thick-hulled seeds were left at depots. Large seeds were presumed too heavy to transport based on a transport threshold rule (Munoz & Bonal 2008), or too large for *A. subspinosus*' gape width to crack the seed hull (Tamura & Hayashi 2008). This suggests that seeds larger than *L. sessile* with rodent-dispersed seed characteristics, such as *Ceratocaryum argenteum* (group Restionaceae), may be dispersed by a larger rodent. I confirmed *A. subspinosus*' specific seed selection process and projected that rodent seed disperses may have a significant influence on seed morphology development in the fynbos.

Based on my chapter 2 findings, I suggested that seeds with the following parameters may be dispersed by *A. subspinosus*: 0.5g to 1.4g weight range (up to 2.5% of *A. subspinosus* body mass) with hulls up to 2.0 mm thick and seed dimensions of 2.0 to 5.0 mm in diameter. I

identified 32 possible Proteaceae and Restionaceae species that conform to the above parameters and are within *A. subspinosus* habitat. In order to understand how rodent seed selection may influence seed morphology, it is of importance to investigate whether those 32 plant species are indeed rodent-dispersed and if *A. subspinosus* selects for the same seed characteristics and dimensions as with *L. sessile* seeds. Seeds which are above the size or weight range of *A. subspinosus* may well be dispersed by a larger rodent such as the Hairy footed gerbil (*Gerbillurus paeba*) that is known to disperse *Willdenowia incurvata* seeds (Midgley & Anderson 2005). It is of importance to identify these potential rodent seed dispersers in the fynbos, in order to understand the magnitude of influence that rodent dispersal vectors may have on plant reproduction and community structure in the fynbos.

In chapter 3 I extended the experimental protocol from the previous chapter to cover a full year and determine whether seasonal changes in food availability altered the seed dispersal behaviour of *A. subspinosus*. I found that *A. subspinosus* changed its behaviour towards experimental seeds significantly over the course of one year. A decrease in natural seed availability during winter and spring meant that the burial rates of experimental seeds decreased and consumption rates increased. I found that the fate of experimental seeds was affected by what was happening in the field (e.g. if seed availability/seed bank volume was declining, consumption of experimental seeds increased). *Acomys subspinosus* seemed to rely primarily on cached seeds for food in winter; however, I recorded a dietary change in spring when the usually granivorous *A. subspinosus* switched to an insectivorous diet. This diet change was reflected in the decreased consumption of experimental seeds in the field compared to winter, suggesting that the rodents were more satiated due to the availability of insects. I concluded that the interaction between *A. subspinosus* and *L. sessile* was mutualistic overall, but that the relationship had a negative aspect to it during winter and spring when seed caches were consumed by *A. subspinosus* and little burial took place.

I predicted that seeds were most abundant in summer and early autumn and least abundant in late spring before the next seed drop event occurs. I observed a switch in diet from seeds to insects in spring, however I was not able to determine whether this dietary change was due to a lack of seeds in the seed bank or if the switch was opportunistic. Future research should

therefore focus on the seed bank dynamics of rodent dispersed seeds and how rodent behaviour influences seed bank volume. Knowing the seed bank biomass in autumn, after seeds have been cached by rodents, and in spring, before mass seed drop occurs, would allow one to estimate the influence rodents have on the seed bank over winter and determine the cause of the dietary switch in spring. In addition, knowing the seed bank volume over an entire year would significantly contribute to the understanding of rodent dispersal behaviour and the influence it has on seed bank persistence. Being able to predict seed bank persistence would be of great aid in determining the effect of fires on seedling establishment rates and plant regeneration capabilities at any given time of year.

In chapter 4 I confirmed that *A. subspinosus* is a scatter-hoarder, caching 97% of seeds singly or in pairs, and investigated *A. subspinosus*' as well as *R. pumilio*'s ability to recover seed caches of varying sizes in different soil moisture levels. Caches in wet substrate were recovered at high rates by both species, regardless of cache size, confirming previous findings in the literature that cache recovery is high under wet conditions regardless of the rodent species (e.g. Vander Wall 1998). Under dry substrate conditions *A. subspinosus* retrieved up to 90% of all seed caches regardless of cache size, while *Rhabdomys pumilio* performed poorly (36% recovery rate) when recovering small sized seed caches (one seed). *Acomys subspinosus*' scatter-hoarding behaviour then becomes an effective strategy to protect seeds from potential pilferers such as *R. pumilio*. I proposed that the scatter-hoarding behaviour of *A. subspinosus* evolved as a result of competition for food resources with other rodent species such as *R. pumilio*.

To my knowledge, this was the first cache recovery experiment conducted with fynbos endemic rodent species. While the experiment in chapter 4 answered the basic questions on the potential origins of scatter-hoarding behaviour in *A. subspinosus* and established seed cache detection abilities in both subject species, the environmental parameters used to determine cache recovery ability were narrow, as I only considered cache size and substrate moisture. A number of other factors may influence cache recovery success such as caching depth (Vander Wall 1993) and the presence of intra- and interspecific competitors (Preston & Jacobs 2001). Therefore, the high cache recovery success rate of *A. subspinosus* in a controlled environment may differ from the natural environment. A study in the field on *A. subspinosus* cache recovery rates may paint a

clearer picture on true recovery success and contribute to answering questions proposed in chapter 3 on *A. subspinosus* cache recovery rates during winter and its influence on seed bank volume.

This thesis has shed light on the mechanisms and behaviours of rodent seed dispersal in the fynbos. These experiments offer a glimpse of the highly dynamic and complex interaction between *L. sessile* and *A. subspinosus*. By establishing basic rodent dispersal and cache recovery behaviour, I have contributed towards the understanding of the mutualism from the rodent disperser's perspective. However, the effect that *A. subspinosus* behaviour has on *L. sessile* seed bank persistence, seedling establishment and plant (re)colonization/succession is largely unknown. Anywhere from 32 to 100 (Midgley & Anderson 2005) plant species, including endangered species such as *Widdringtonia cedarbergensis*, may be rodent-dispersed. Therefore, understanding the influence of the seed disperser on plant reproduction is important. In addition, the fynbos is struggling with present-day issues such as climate change, habitat fragmentation and frequent fires that may place considerable stress on the ecosystem.

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