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Seed predation and potential dispersal of *Ceratocaryum argenteum* (Restionaceae) nuts by the striped mouse (*Rhabdomys pumilio*)

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ABSTRACT

This study aimed to better understand the role of rodents as seed predators and dispersers in the fynbos biome at De Hoop Nature Reserve, South Africa in May and June, 2013. Based on previous studies I hypothesised that the large, nut-like seeds of *Ceratocaryum argenteum* (Restionaceae) are scatter-hoarded by rodents and that rodent seed choices and seed fates are affected by seed size and hull thickness. Field trials using three seed types showed that smaller seeds with a high reward and low processing cost were consumed significantly ($p < 0.01$) more than large, thick hulled seeds. Application of wire tags to facilitate discovery of relocated seeds had no significant influence on seed choice ($p > 0.05$), but further research should be conducted to determine if spooling of *C. argenteum* seeds influences rodent seed choice. Smaller seeds with a high-reward and low processing cost showed a significantly greater percentage of usage ($p < 0.01$) where seed stations were encountered and exploited. *Rhabdomys pumilio* was confirmed as being the most common murid at the study site, however, it seems unlikely that it scatter-hoards *C. argenteum* seeds, as no consumption or burial of seeds was observed. However, *R. pumilio* did show an interest in *C. argenteum* seeds and attempted to consume some seeds or carried seeds over distances not significantly different ($p > 0.05$) from the observed distances between nearest neighbour *C. argenteum* plants before discarding them on the soil. Additionally, the maximum distance *R. pumilio* moved *C. argenteum* seeds was commensurate with the maximum distance between nearest neighbour *C. argenteum* stands, so the end fate of the seeds remains unknown. Further research should account for seasonal variability in scatter-hoarding behaviour.

INTRODUCTION

Seed predation can take the form of pre-dispersal or post-dispersal predation. A great variety of animals are known to be post-dispersal seed consumers, including insects, molluscs, mammals (especially small rodents) and birds (Hulme, 1998). Rodent granivory is known to have a considerable impact on seed populations and is suggested to have greater potential influence on seed dynamics in most temperate ecosystems than other seed predators (Hulme, 1998). Patterns of post-dispersal seed predation show great variation in relation to habitat, plant species, seed density and the depth of seed burial (Hulme, 1998). Rates of predation by rodents are variable across a spatial scale from habitats to within a single microhabitat (Hulme, 1997). The abundance of small mammals tends to be positively associated with vegetative cover (Hulme, 1997) meaning seed predation is spatially heterogeneous and less seeds are removed in open areas. Seed predators' preferences for certain species' seeds are related to many factors, including characteristics of the seed (seed size/shape, nutritional content, toxicity), seed predator (body size, susceptibility to toxins, olfactory ability, hunger) and of the environment (food abundance, soil characteristics, vegetation cover) (Hulme, 1998). Seed size is a critical factor determining the degree to which seed predation is density-dependent. This is most evident in seed predation by rodents on small seeds, whereas removal of relatively large seeds is rarely density-dependent (Hulme, 1998). Certain seeds may be preferentially chosen for concentrations of minerals and/or amino acids to compensate for dietary deficiencies or concentrations of soluble carbohydrates in semi-arid or arid ecosystems (Hulme & Benkman, 2000). Other factors that affect seed choice include the distribution and detectability of the seeds, the risk of predation and the abundance of predators (Hulme & Benkman, 2000). Importantly, rodents are known to disperse seeds, as well as consume them.

Scatter-hoarding animals can play a major role in the fate of seeds, both as consumers and dispersers (Jansen et al, 2004). Accumulated evidence has shown that scatter-hoarding has a positive net contribution to seedling recruitment in large-seeded plant species (Vander Wall, 1990). Many plant species that depend on scatter-hoarding animals for the dispersal of their seeds release their seeds in masting events. These plants generally produce large seeds that are far greater in size than those produced by species that rely on wind or fruit-

eating animals (Vander Wall, 2001). Masting and large seed size have been suggested as adaptations by scatter-hoarded plants to increase seed dispersal and reduce seed predation (Jansen et al, 2004). Masting is the synchronous production of large seed crops at intermittent intervals to satiate predators. The “predator satiation hypothesis” (Kelly, 1994) is a functional explanation for masting events and proposes that mast seeding is an evolutionary response to severe seed predation. It stipulates that if plants release large seed crops into the environment creating a glut of seeds, seed predators will become satiated allowing some seeds to escape predation (Kelly, 1994). In response to masting, rodents are thought to have adapted by hoarding seeds that could not be consumed for times when food is scarce, which often follow masting events (Vander Wall, 1990). Scatter-hoarded plants continued to co-evolutionary develop, evolving specific seed traits to encourage seed dispersal, such as larger seeds (Vander Wall, 2001). Large seed size has been explained as an adaptation to the seed-choice preferences of scatter-hoarding animals for large, more nutritious seeds (Vander Wall, 2001; Jansen et al, 2004).

Seed traits and Seed predation

Scatter-hoarders selection for specific seed traits has been well documented, with a general consensus that larger seeds with a greater nutritional value are dispersed and cached for when food resources are scarce, while small seeds are eaten *in situ* (Vander Wall, 1990; Brewer, 2001; Theimer, 2003). The ‘value’ of a food item can be measured in many different ways, such as, caloric value, nutritional content or on its shell-life, all of which may play an important role in seed selection (Leaver, 2004). Although general conclusion can be drawn, seed selection by rodents varies across ecosystems (e.g. Theimer, 2003; Brewer 2001), which is likely due to seed size-body ratios, where seed size places an upper limit constraint on smaller sized rodents (Munoz & Bonal, 2008).

Foraging theory predicts that after an animal has encountered an item of food when foraging it will invest more effort in searching over the same area (Leaver, 2004). Models of cache spacing predict that pilferers will intensify their search if a highly valuable food is encountered (Hulme & Benkman, 2000; Leaver, 2004). Due to this, scatter-hoarders compensate by caching more valuable foods at lower densities, decreasing the risk of pilferage. However, caching seeds at lower densities requires more travel time and effort,

meaning there is an energetic trade-off between dispersal distance and transport costs that influences dispersal distance (Moore et al, 2007; Munoz & Bonal, 2008). Another important factor for scatter-hoarders is the depth at which seeds are buried (Rusch, 2011). Seeds must be buried deep enough to avoid pilferers discovering the caches by smell, but not too deep that the scatter-hoarder cannot relocate its own caches (Rusch, 2011). Rodent's olfactory ability and environmental conditions are two of the most important factors in determining whether caches can be successfully located (Rusch et al, 2012).

Seed choice is particularly important during masting events, where rodents are presented with a large variability in seed sizes and attempt to remove and bury as many seeds as possible in a short amount of time. Rodents are more likely to first consume small and thin hulled seeds as an easy and accessible energy source due to lower handling times required for consumption (Forget, 1993). This allows rodents proportionally more time to disperse and bury larger, more valuable seeds (Rusch et al, 2012). In the fynbos, Rusch et al (2012) found *Acomys subspinosus*, the Cape spiny mouse, ate small or thin-hulled seeds *in situ*, preferentially dispersed and buried seeds with average size and hull thickness and frequently left large or thick-hulled seeds at depot sites. It has also been found that rodents cache larger seeds further away from seed source than smaller seeds (Moore et al, 2007).

Scatter-hoarding

Food-hoarding animals are known to either larder- or scatter-hoard seeds (Vander Wall, 1990). Larder-hoarding occurs when animals cache many seeds in one or a few sites that are often situated in or near their nest burrow deep underground so that they can be actively defended (Vander Wall, 1990; Vander Wall, 2001). Larders are generally poor sites for the emergence and establishment of seedlings, as seeds may be too deep underground to germinate and successful germination would likely result in very high levels of intra-specific seedling competition (Vander Wall, 1990). Where there are high rates of cache pilferage, aggressive defence of larders is thought to be essential for most larder-hoarders (Vander Wall, 2003). Scatter-hoarding of seeds is suggested to provide protection against major loss to pilferers by increasing the area pilferers must search (Leaver, 2004). Scatter-hoarders are required to memorise a larger number of caches and this cost is shown by the

relative increase in hippocampal size in scatter-hoarders compared to larder-hoarders (Leaver, 2004).

Secondary seed dispersal, by frugivores and granivores, is recognised as an important process in the life cycle of some plants (Vander Wall and Longland, 2004). There are often multiple steps in seed dispersal that can involve two or more dispersal vectors (Vander Wall et al, 2005). An example of diplochory is found in warm temperate and tropical regions where dung beetles are common. Dung beetles are known to transport and bury herbivore dung at depths that favour seed germination and seedling emergence (Vander Wall and Longland, 2004; Vander Wall et al, 2005). The scatter-hoarding of seeds has several potential advantages to scatter-hoarded plants that increase the probability of seeds surviving, germinating and establishing if seed caches are not recovered (Jansen & Forget, 2001). Howe and Smallwood (1982) described three categories of potential benefits: (1) the risk of mortality due to pathogens and pests associated with the parent plant decreases and most importantly, the risk of distance- and density-dependent mortality by seed predators is reduced (Jansen & Forget, 2001); (2) The transportation and scattering of seeds away from both the parent plant and siblings, decreases the risk of intraspecific competition; and (3) directed dispersal via a non-random process to specific sites that infer a much higher probability of seedling establishment (Vander Wall, 1990).

Examples of scatter-hoarding exist world-wide, including Japanese horse chestnut (*Aesculus turbinata*) by large Japanese field mouse (*Apodemus speciosus*) in Asia, *Beilschmedia bancroftii* by white-tailed rat (*Uromys caudimaculatus*) in Australia, black walnut (*Juglans nigra*) by fox squirrels (*Sciurus niger*) in North America, Brazilnut (*Bertholletia excels*) by red-rumped Agouti (*Dasyprocta leporine*) in South America (see Jansen et al, 2004), and *L. sessile* by Cape spiny mouse (*A. subspinosus*) in South Africa (Midgley et al, 2002). The role of scatter-hoarding animals in plant dispersal has been well documented in temperate regions (Vander Wall, 1990) while more recently studies have focused on neotropical and paleotropical rainforests (Forget & Vander Wall, 2001).

Seed predation and Scatter-hoarding in the CFR

In the fynbos biome of the Cape Floristic Region, South Africa there are two predominant dispersal mechanisms: myrmecochory, dispersal by ants which are attracted by the nutritious elaiosome on the seeds, and serotiny, where seeds are stored in the canopy and dispersed post-fire (Midgley & Anderson, 2005). These were previously believed to have evolved as anti-rodent predation characteristics. In the fynbos, the effects of seed predation by rodents and ants have been well documented, while the relative role of birds has been poorly studied (Pierce & Cowling, 1991; see Midgley & Anderson, 2005).

Serotinous plants have small seeds that are only released after fire events when rodents are scarce, due to a lack of cover and resources (Bond, 1984). Germination of serotinous seeds takes place in the first winter after fire (Midgley & Anderson, 2005). Bond (1984) recorded higher levels of seedling regeneration in the Proteaceae after autumn fires compared to summer fires. Summer fires leave a far greater time period available for seed predation before seeds can germinate, meaning plants would favour autumn fires over summer fires. Myrmecochory is argued to have evolved as a result of granivory by small mammals (Midgley & Anderson, 2005). Bond & Breytenbach (1985) found no difference in seed removal rates of myrmecochorous seeds when exposed to only ants or small mammals (who can also detect the nutritious elaiosomes of myrmecochorous seeds). They argued that the major benefit of myrmecochory in Proteaceae is the escape from mammal seed predators and that all seeds taken by ants were protected against granivory.

However, more recently, scatter-hoarding has been identified as an important process and potentially widespread mechanism for seed dispersal in the fynbos biome (Midgley et al, 2002; Midgley & Anderson, 2005; Rusch, 2011). Midgley et al (2002) found the endemic *A. subspinosus* to scatter-hoard seeds of *Leucadendron sessile* in the south-west, Cape mountains. Midgley & Anderson (2005) later extended the scatter-hoarding phenomenon geographically (the Namaqualand and Overberg regions of the Cape Floristic Region) and to a new plant (*Willdenowia incurvata*: Restionaceae) and animal (*Gerbillurus paeba*: Cricetidae) family. Furthermore, rodent burial is similar in depth to that of ant burial and post-fire regeneration rates are commensurate with serotinous species (Midgley et al, 2002). These findings bring into question whether serotiny and myrmecochory are in fact

anti-rodent predation adaptations. In the fynbos, there are many nut-fruited species whose seeds are neither serotinous, as they are not released post-fire, nor myrmecochorous, as they lack an elaiosome and are too large for ants to disperse (Midgley & Anderson, 2005). Midgley & Anderson (2005) estimated that there are 60-100 plant species within the Proteaceae and Restionaceae families alone that are primarily dispersed by rodents. Rusch (2011) found that at least 32 nut-fruited species, 23 within the genus *Leucadendron* (Proteaceae) and nine with the Restionaceae family, correspond to the general set of seed characteristics favoured by *A. subspinosus*, a known fynbos scatter-hoarder. Up to this point, all *in situ* evidence has been circumstantial for cases of scatter-hoarding in fynbos.

Aims and predictions

This study aimed to assess the role of rodents as seed predators and dispersers in the fynbos biome of South Africa, at a site where the only confirmed rodent scatter-hoarder (*A. subspinosus*) is absent. I hypothesised that the most ubiquitous rodent seed predator in the fynbos, the striped mouse *Rhabdomys pumilio*, would not only be the most frequent seed predator, but possibly a seed disperser, owing to its overlapping distribution with the nut-fruited *Ceratocaryum argenteum*, which has been predicted to be rodent dispersed (Midgley et al, 2002) and the lack of a recognised scatter-hoarder. To confirm the species responsible for seed predation and dispersal *in situ*, camera trapping was used. Furthermore, this study aimed to determine rodent seed choices when presented with seeds of different value: *C. argenteum* - large, thick hulled seeds with high energy rewards and potentially long shelf life but high processing costs; sunflower seeds - small seeds with a thin hull offering a high energy reward with low processing costs and shelf life; and *L. sessile* – medium-sized and hulled seeds with an intermediate shelf life and cost-benefit ratio. It was expected that: sunflower seeds would be chosen first and always eaten; *L. sessile* seeds would be mostly eaten and some dispersed; and *C. argenteum* seeds would be occasionally eaten and mostly dispersed. Lastly, different seed relocation methodologies were tested to determine if the fate of rodent-dispersed seeds differed depending on the relocation technique.

METHODS

Study site and species

The study was conducted during the winter months (May and June 2013) at De Hoop Nature Reserve, South Africa (-34.399907°, 20.554238°). This reserve is approximately 340 km² in area and has a Mediterranean-type climate. Summers are generally warm, while winters are mild, with an annual rainfall of approximately 380 mm. De Hoop forms part of the Cape Floristic Region, which is dominated by the fynbos biome and has exceptionally high plant diversity (11 420 plants species) with high levels of endemism (8 900 plant species) (Manning et al, 2012). The reserve has the largest conserved area of lowland fynbos, with the study site being located in Albertinia Sand Fynbos (Mucina and Rutherford, 2006). This vegetation is found on acid, coarse textured, sandy soils and is adapted to frequent fires (every 5-25 years) and drought. The dominant growth forms are the Restionaceae and Ericaceae families and the *Protea* and *Leucadendron* genera.

The study site supports numerous stands of *C. argenteum*, a dioecous plant restio that grows approximately 2-3 m tall. These rhizomatous plants typically occur in groups of 5-15 individuals and have indehiscent fruits with a hard, woody ovary wall. Nuts are approximately 10-12 mm long and 8-9.5 mm in diameter and lack an elaiosome (eMonocot Team Literature, 2013). Flowering occurs in late winter and seeds are dropped *en masse* annually. The mastig period extends from November to January with the peak seed drop period in early summer (J.J. Midgley, pers. comm.). No *L. sessile* or sunflower plants occur naturally at this site.

Eighty-six mammal species are found in the reserve, with small mammal abundances being well documented over the last 10 years (G. Bronner, pers. comm.). *R. pumilio* is the numerically dominant rodent species at the field site (Fig 1). Other less common small mammals include the pygmy mouse (*Mus minutoides*) and vlei rat (*Otomys irroratus*). *R. pumilio* is a diurnal, opportunistic omnivore that weighs approximately 40 g (Skinner & Chimimba, 2005). It is a well-known seed predator, but is not known to bury seeds (Rusch, 2011) *R. pumilio* is widespread throughout the Cape Floristic Region and is often the most abundant rodent (Rusch, 2011). It tends to forage primarily under bushes where food

densities are higher and predation risk is relatively low (Skinner & Chimimba, 2005). *R. pumilio* has a general diet consisting mostly of vegetative matter, seeds and insects and accesses these food resources opportunistically (Rusch, 2011). Given that *R. pumilio* is ubiquitous, a major seed predator and has an overlapping range with *C. argenteum*, it seems plausible that a masting event could induce burial due to satiation (Kelly, 1994).

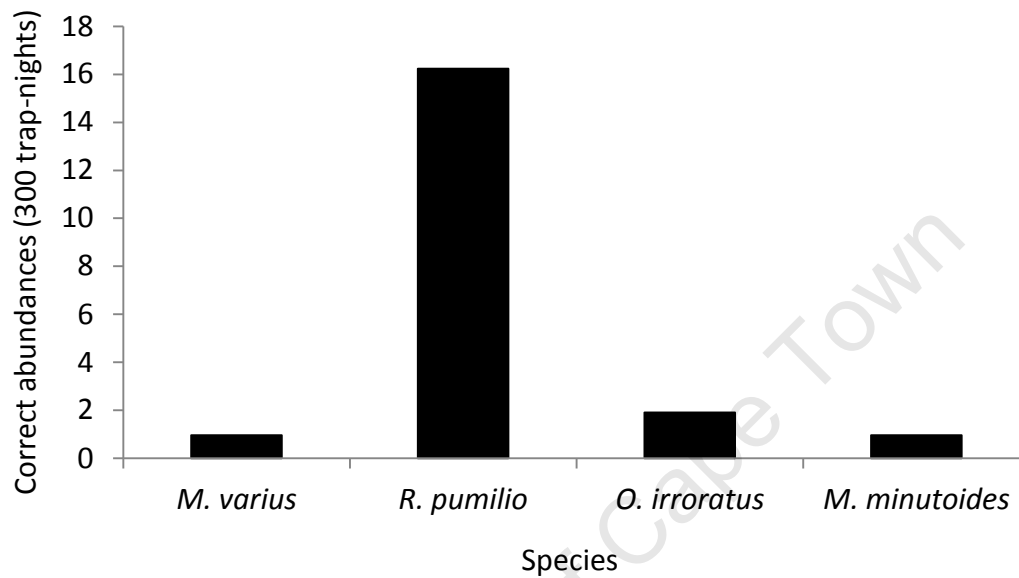


Figure 1. Small mammal abundances corrected per 300 trap-nights at a young Albertinia Sand Fynbos site at De Hoop Nature Reserve, South Africa (February 2013).

Seed removal

To determine if seeds of *C. argenteum* were removed by animals, 12 seeds (six *C. argenteum* and six *L. sessile* a scatter-hoarded plant with a documented mutualism with *A. subspinosus*) were assembled at six seed stations, spaced 15 m apart on three separate transects, two of which were in close proximity to adult *C. argenteum* stands. At certain sites rodents were also presented with a choice between *C. argenteum*, *L. sessile* and commercial sunflower seeds.

At each seed station, seeds were presented cafeteria-style on the ground within 50 cm of the plant cover. Three seeds of each type were left unmarked as controls. To determine the fate of the relocated seeds, three seed marking-relocation techniques were used for *C. argenteum* and *L. sessile* seeds. Firstly, three seeds of each species were tagged by either:

gluing 150 mm lengths of brightly coloured, fluorescent thread with quick drying glue (Midgley et al, 2002) or inserting 200 mm long (0.2 mm thick) lengths of wire threaded through each seed (Yi et al, 2008) with a 20 mm square of bright insulation tape attached to aid relocating removed seeds. Another three seeds of each seed type were spooled by gluing the end of threads of reverse-wound cotton spools to seeds, thus allowing for the rodent's movement with the seed to be tracked by following the direction of the thread from the buried spools.

Seed stations were checked in the early morning and late evening. The numbers of seeds remaining at the seeds station (or within a 50 cm radius of it) were recorded, as were the seed fates (uneaten; eaten as evidenced by husk remnants; buried or removed). In an attempt to relocate removed seeds searches were conducted by walking in spirally outwards from the seed stations for up to 5 m. The distances and fates (eaten/buried uneaten/discarded on the surface) of recovered seeds from the seed station were then recorded. For spooled seeds, each separate linear movement and angle relative to the position of the last movement was recorded. If seeds were relocated, they were classified as: (1) removed where seeds had been left on the surface further than 50cm from the seed station, (2) consumed where seeds had clearly de-hulled and consumed with the tracking device (e.g. wire) still attached and lastly (3) buried, if the seed had been buried in the ground.

Nearest neighbour correlations

The nearest-neighbour distances between *C. argenteum* individuals and between distinct stands of *C. argenteum* were measured (to the nearest cm) to assess how these distances compared to those between relocated seeds and seed stations.

Camera trapping

Camera trapping has been used in many seed dispersal studies, as it allows indirect monitoring of seeds (e.g. Miura et al, 1997, Beck & Terborgh, 2002, Seufert et al, 2009). The advantages of camera trapping include the reduced disturbance to animals, the ability to monitor nocturnal and cryptic species and the ability to monitor sites regardless of weather conditions (Seufert et al, 2009).

To determine potential seed dispersers and predators, 19 automatic, motion-sensing Ltl Acorn camera traps (6210M) were set-up on steel rods at each of 19 seed stations for two consecutive days and nights. Each time the motion detector of a camera was activated it took three consecutive 5MP photographs, immediately followed by a high-resolution 60 sec video recording and automatically recorded the date and time. Owing to the difficulty of distinguishing among individual animals of the same species, it was not possible to discriminate between independent visits by different animals and repeated visits by the same individual. A visit was defined as each time an animal entered or re-entered the frame of the photograph/video. The observed behaviour of the animal with regard to the seeds provided was recorded as either: (1) ignored, when no interest was given to the seeds; (2) explored, when seeds were picked up for more than 5 seconds, but not consumed; (3) handled and consumed, when seeds were visibly de-hulled and consumed or a substantive attempt to do so was made; and (4) handled and removed, when seeds were removed from the seed station and field of view of the camera. For each visit, the duration was defined as the difference between the times of the last photograph/video and the first photograph/video of the visit.

Statistical analyses

All statistical analyses were conducted using STATISTICA Version 11 (StatSoft, Inc., 2012). Kolmogorov-Smirnoff d-tests showed that the raw seed removal variables did not conform to a normal distribution. As these variables were expressed as proportions, arc-sin transformations were performed in an attempt to induce normality, but without success. Consequently all dependent seed variables were subjected to non-parametric analyses. Kruskal-Wallis ANOVA by ranks was used for one-way analysis of variance, with post-hoc multiple comparisons tests to determine further differences among independent (grouping) variables. Mann-Whitney U tests were used for comparing two independent samples' measurements of non-parametric data. Two sample T-tests comparing independent means were used to individually compare the mean distances of spooled *C. argenteum* seed movements by *R. pumilio* with the mean distances between nearest neighbour *C. argenteum* individuals, *L. sessile* seed movements and nearest neighbour *C. argenteum* stands, as all data sets were normally distributed.

Furthermore, encounter versus exploitation analyses were conducted. An encounter was described as the first use (removal or consumption) of a seed from a station and all subsequent removals were described as exploitation, regardless of seed type or treatment. For the encounter versus exploitation data the percentage of seeds removed and eaten were combined and called percentage seeds used.

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RESULTS

Camera trapping

A total of 3 h 29 min of animal activity at seed stations was recorded by camera trapping. Three different rodent species visited the seed stations, with single visits from a dung beetle species and a bird species respectively were recorded (Table 1). Three of the *M. minutoides* visits and one *O. irroratus* visit were recorded at night. All other visits were recorded during the day. *R. pumilio* was the only animal observed interacting with the seeds and was the dominant visitor, accounting for 97.5% of all visits, with 43% of these visits observed as seed removal events (Table 1). All other species ignored the seeds at seed stations. Six percent of the *R. pumilio* visits were observed as seed consumption events, while seed handling was observed 18% of the time (Table 1). No seeds were observed being buried. On nine occasions, two *R. pumilio* individuals were observed foraging together simultaneously.

Table 1. Number of visits to seed stations by each animal species captured by motion-sensing cameras and the assigned behaviour observed at seed stations at De Hoop Nature Reserve, South Africa.

Species	Seeds Ignored	Seeds Explored	Seeds Handled & Consumed	Seeds Handled & Removed	Total number of Visits
<i>R. pumilio</i>	128	72	23	169	392
<i>M. minutoides</i>	5	0	0	0	5
<i>O. irroratus</i>	3	0	0	0	3
Dung beetle spp.	1	0	0	0	1
Bird spp.	1	0	0	0	1

Table 2. Seed fates after *R. pumilio* visits captured by motion-sensing cameras at De Hoop Nature Reserve, South Africa.

Species	Explored	Handled & Consumed	Handled & Removed
<i>C. argenteum</i>	60	0	60
<i>L. sessile</i>	11	23	111

In total, 120 events involving *C. argenteum* were recorded, with half of these being explored and half being removed (Table 2). Most *L. sessile* seeds were observed to be removed, while 23 consumption events and 11 exploratory events were recorded (Table 2).

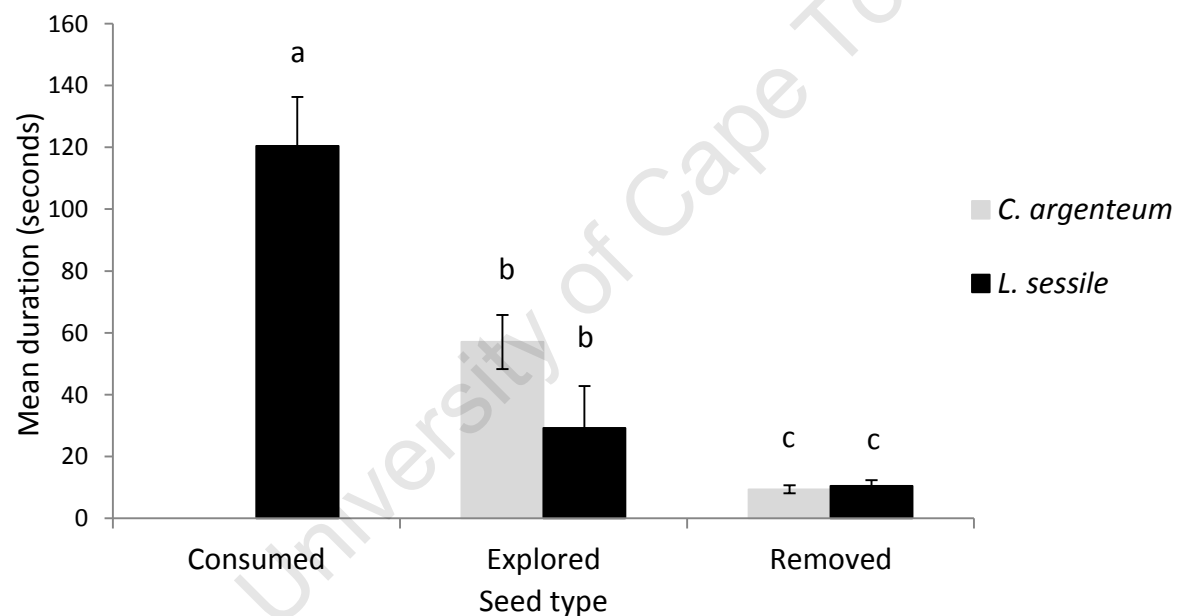


Figure 2. The mean durations (seconds with standard errors) of observed *R. pumilio* behaviours when *C. argenteum* and *L. sessile* seeds were encountered at all seed stations.

No *C. argenteum* seeds were observed being consumed, although there were many cases of *R. pumilio* attempting to eat them, indicated by the exploratory behaviour (Fig 2.). The mean duration for *R. pumilio* to consume a *L. sessile* seed was approximately 120 seconds (Fig 2). The mean exploration and removal time of *C. argenteum* seeds was not significantly different from that of *L. sessile* seeds ($p > 0.05$) (Fig 2).

Seed removal

No significant difference in seed fate was found between sites where *C. argenteum* stands were present or absent ($p>0.05$), therefore data for these sites were combined for further analyses.

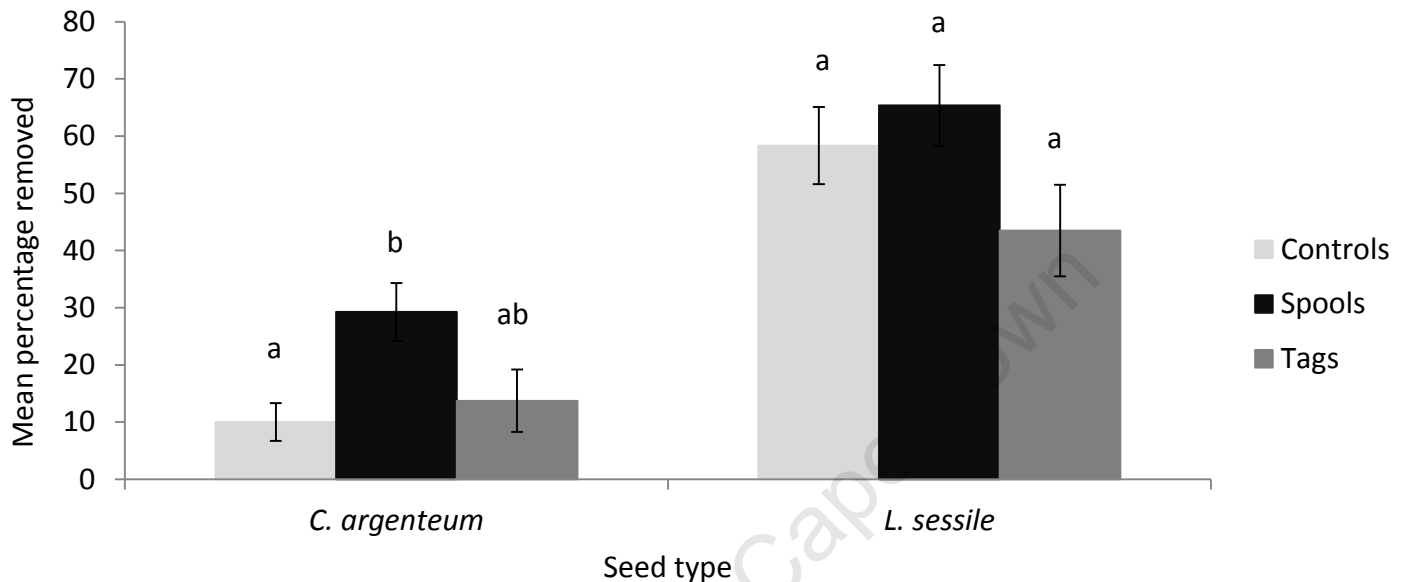


Figure 3. The mean percentage (with standard errors) of *C. argenteum* and *L. sessile* seeds removed for three different treatments of the seeds by *R. pumilio* at all seed stations.

No sunflower seeds were removed for any seed station. There were no significant differences in mean removal percentages for the control, spooled and tagged *L. sessile* seeds ($p>0.05$) (Fig 3). For the *C. argenteum* seeds, there was no significant difference in the mean removal percentages of control and tagged seeds or between spooled and tagged seeds, but significantly more spooled than control seeds were removed ($H_{2,133}=12.70$, $p<0.01$) (Fig 3).

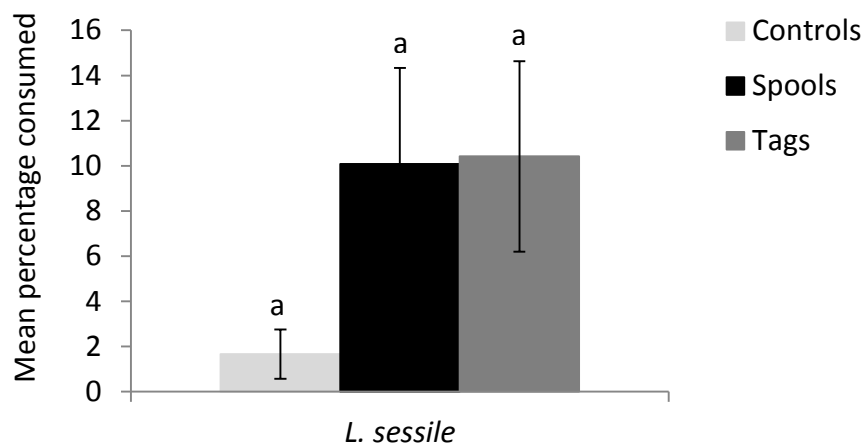


Figure 4. The mean percentage (with standard errors) of *L. sessile* seeds consumed by *R. pumilio* for the three different seed treatments at all seed stations.

There were no significant differences in the mean consumption percentages of the control, spooled and tagged *L. sessile* seeds ($H_{2,120}=4.77, p>0.05$) (Fig 4). No *C. argenteum* seeds were consumed. Mean percentage of sunflower seeds consumed is shown in Figure 5.

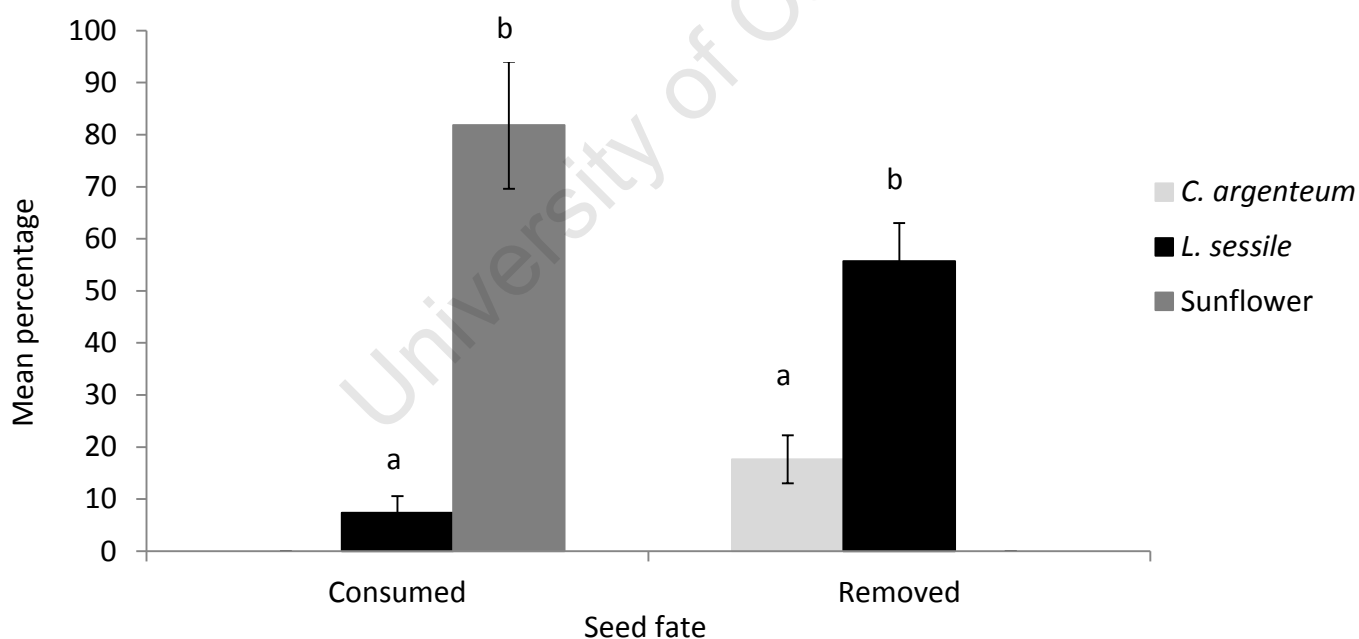
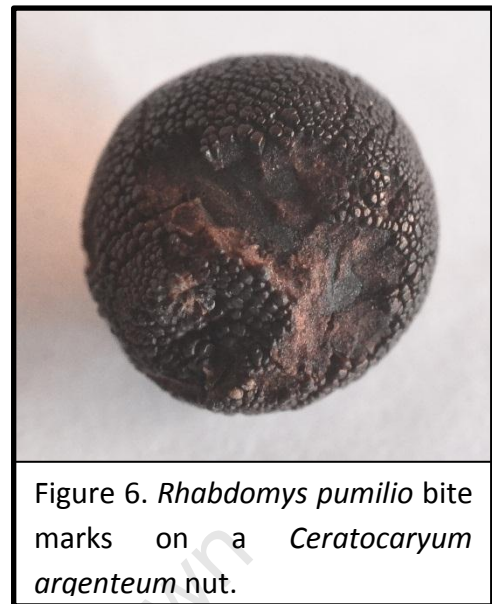


Figure 5. The mean percentage (with standard errors) of control and marked *C. argenteum* and *L. sessile* seeds and control commercial sunflower seeds consumed and removed by *R. pumilio* at all seed stations.

No seeds were observed to have been buried at seed stations. Although no *C. argenteum* seeds were consumed *in situ*, bite marks were regularly found on the hull of the seeds (Fig 6). There was a significantly greater mean percentage of sunflower seeds consumed *in situ* than *L. sessile* seeds ($H_{2,264}=89.87$, $p<0.01$) (Fig 5). Significantly more seeds of *L. sessile* were removed ($p<0.01$), but no sunflower seeds being removed from any seed station (Fig 5).



Encounter vs. Exploitation

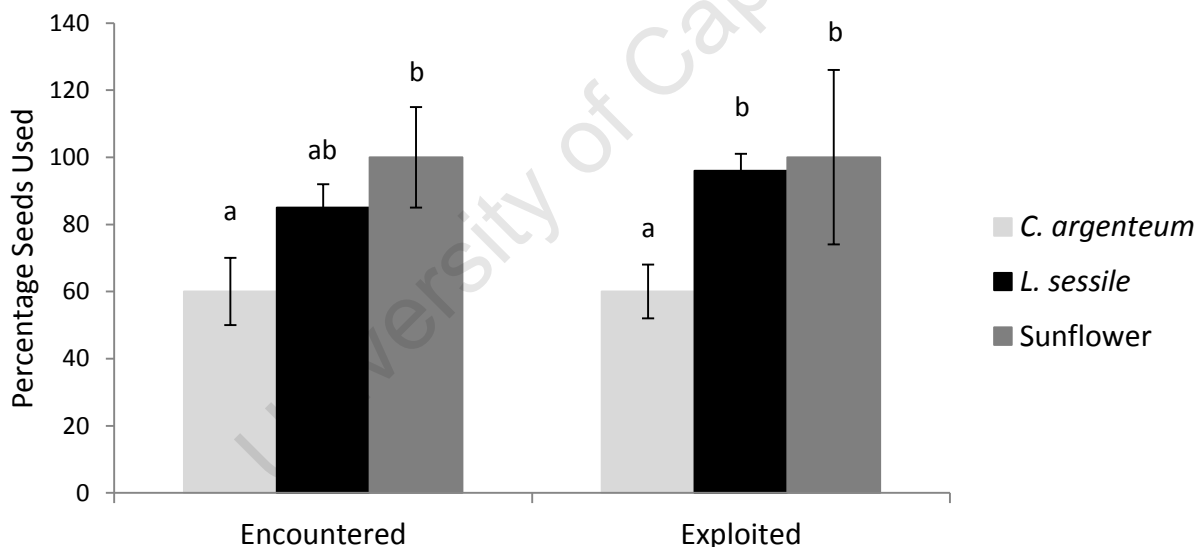


Figure 7. The mean percentage (with standard errors) of seed of each type used (removed & consumed) when a seed station was first encountered or subsequently exploited by *R. pumilio* at all seed stations.

A significant difference was found in the encounters percentages per seed type ($H_{2,45}=10.45$, $p<0.01$) (Fig 7). First encounter percentages of commercial sunflower seeds were significantly higher than for *C. argenteum* seeds, but *L. sessile* seed encounters did not significantly differ from the other seed types (Fig 7). Once a seed station was encountered,

the exploitation of commercial sunflower seeds and *L. sessile* seeds was not significantly different, but both of these seed types were exploited significantly more than *C. argenteum* seeds ($H_{2, 84}=37.55$, $p<0.01$). For both encounters and exploitation, *C. argenteum* seeds showed lower levels than seed usage than the *L. sessile* or the commercial sunflower seeds.

A significant difference was found between encounters and exploitation for *L. sessile* seeds ($p<0.05$), but no significant difference ($p>0.05$) was found between encounters and exploitation for *C. argenteum* or sunflower seeds.

***C. argenteum* and *L. sessile* seed movements and nearest-neighbours**

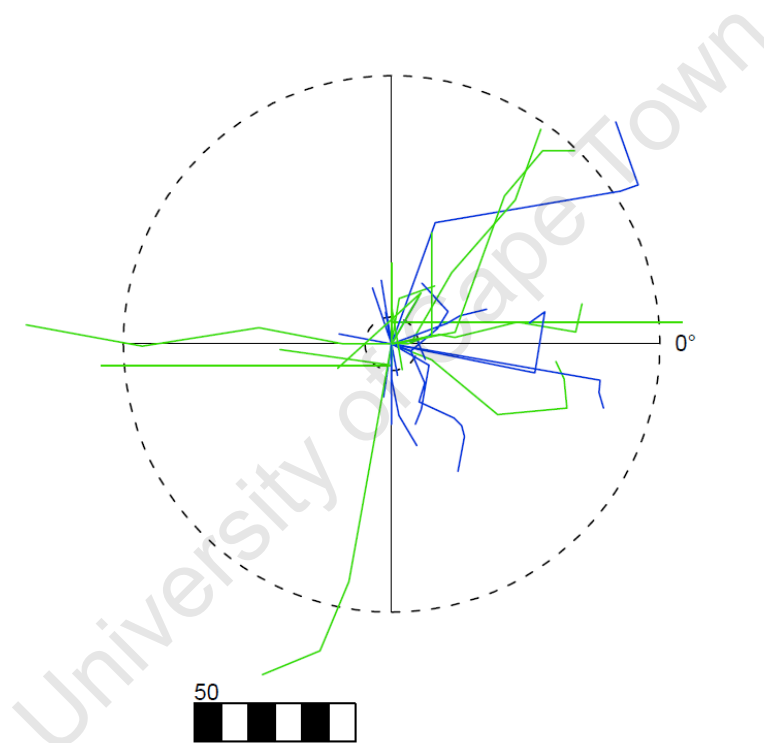


Figure 8. Relative movement paths of spooled *C. argenteum* seeds that were removed from all seed stations by *R. pumilio* at De Hoop Nature Reserve ($n=37$). The radius of the inner and outer circle is 50 and 500 cm, respectively. (Red lines = seed consumed; blue lines = seeds discarded on soil surface; green lines = seed fate unknown).

A total of 86% of the recovered spooled *C. argenteum* seeds were removed within 50 to 500 cm of the seed station, while 5 (14%) of the seeds were removed in excess of 500 cm (Fig 8) with a maximum of 830 cm. The median distance of spooled *C. argenteum* seed removals

was between 50 and 100 cm, with the greatest frequency of seeds being dropped closer to the seed station and slowly decreasing with an increase in distance away from the seed station. A total of 65% of *C. argenteum* seeds were discarded and 35% were not recovered. No *C. argenteum* seeds were buried or consumed.

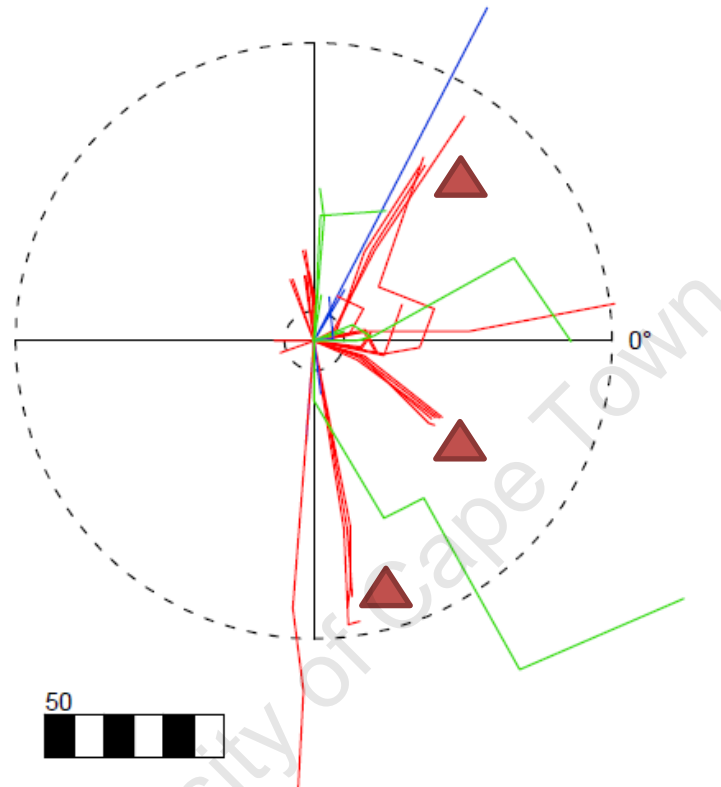


Figure 9. Relative movement paths of spooled *L. sessile* seeds that were removed from all seed stations by *R. pumilio* at De Hoop Nature Reserve (n=54). The radius of the inner and outer circle is 50 and 500 cm, respectively. (Red lines = seed consumed; blue lines = seeds discarded on soil surface; green lines = seed fate unknown; red triangles = favoured feeding sites).

A total of 93% of the recovered spooled *L. sessile* seeds were removed within 50 to 500 cm of the seed station, while 4 (7%) of the seeds were removed in excess of 500 cm (Fig 9) with a maximum of 750 cm. The median distance of spooled *L. sessile* seed removals was between 50 and 100 cm, with the greatest frequency of seeds being dropped closer to the seed station and slowly decreasing with an increase in distance away from the seed station. A total of 74% of seeds were found to be consumed, 15% were not recovered and 11% were

discarded. No *L. sessile* seeds were found to be buried. A number of sites where *L. sessile* seeds were consumed were frequently re-used, indicated by adjacent red triangles (Fig 9).

Two sample T-tests comparing normally distributed, independent means were used to individually compare the mean distances of spooled *C. argenteum* seed movement by *R. pumilio* with the mean distances between nearest neighbour *C. argenteum* individuals, *L. sessile* seed movements and nearest neighbour *C. argenteum* stands (Fig 10).

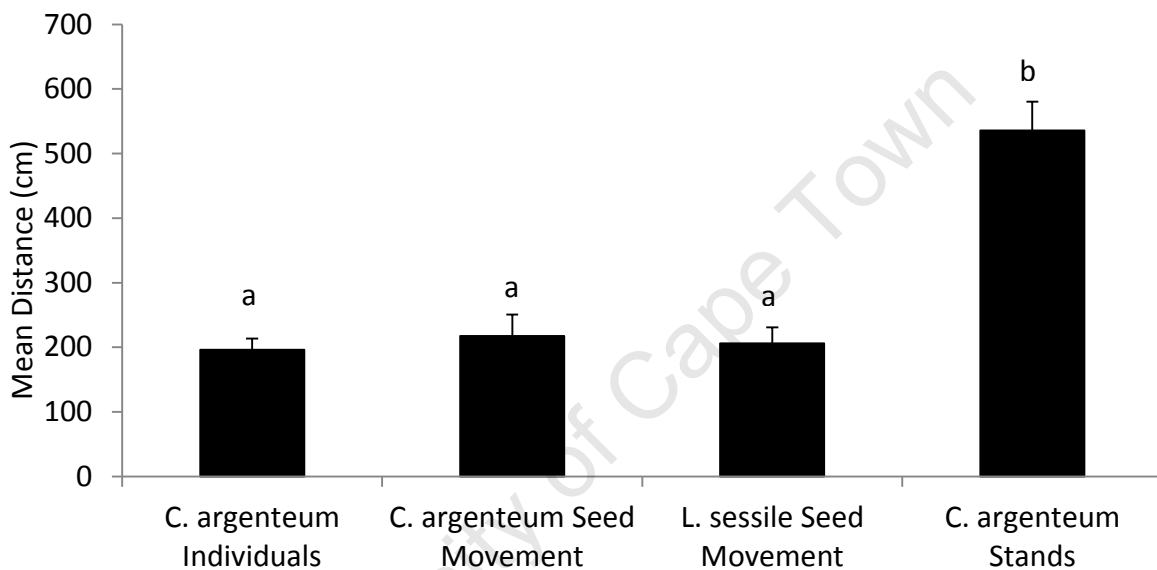


Figure 10. The mean distance (cm with standard errors) between nearest neighbour *C. argenteum* plants, spooled *C. argenteum* and *L. sessile* seed movements by *R. pumilio* at all seed stations and nearest neighbour *C. argenteum* stands.

Figure 10 indicates that there was no significant difference between the distances of nearest neighbours of *C. argenteum* individuals and the distances *R. pumilio* move the *C. argenteum* seeds ($p > 0.05$). The mean distance between *C. argenteum* stands was significantly greater than the mean removal distances of *C. argenteum* seeds by *R. pumilio* ($p < 0.05$) (Fig 10). The maximum distance recorded between nearest neighbour *C. argenteum* stands was 860 cm.

DISCUSSION

Seed choices

Removal and consumption of seeds between sites where *C. argenteum* stands were present or absent did not differ significantly, indicating that familiarity of rodents with the *C. argenteum* nuts did not affect seed usage patterns. Only *L. sessile* and sunflower seeds were consumed at seed stations, with far greater consumption rates for sunflower seeds (Fig 5). This supports a large body of literature, indicating that smaller seeds with no or thin-hulls are more likely to be consumed *in situ*, because of the almost immediate energy reward (Vander Wall, 1990; Forget, 1993; Brewer, 2001; Theimer, 2003). Larger, medium-hulled *L. sessile* seeds were most frequently removed (56%) from seed stations, whereas the smaller sunflower seeds were only consumed *in situ* (Fig 5). However, significantly fewer of the larger, thick-hulled *C. argenteum* seeds were removed from seed stations than *L. sessile* seeds. These results, with the absence of any consumption (despite frequent attempts involving gnawing of nuts) suggests that the high processing costs resulting from the large size and thick hull of *C. argenteum* seeds deter *R. pumilio* (Hulme, 1998).

Different methods of seed marking for relocation have previously been shown to influence seed choice by rodents (Wrobel & Zwolak, 2012). Marking seeds with spools or tags did not affect the magnitude of consumption or removal of *L. sessile* seeds compared to controls (Fig 3). However, spooled *C. argenteum* seeds were removed significantly more than control seeds, indicating that these preferentially chosen by *R. pumilio*. The reason for this is unclear, but it shows that the removal of marked seeds can be influenced not only by the method of marking, but also seed type. Given the short duration of this study, further research is required to establish whether the apparent preference by *R. pumilio* for spooled *C. argenteum* seeds is a consistent and widespread phenomenon. If so, future studies will need to control for this.

Both encounters and exploitation were significantly lower ($p < 0.01$) for the larger *C. argenteum* seeds, which is presumably due to their high processing costs associated with the thick seed hulls (Fig 7). The smaller and thinner-hulled *L. sessile* and sunflower seeds, with a higher reward:processing cost ratio, were generally the first seeds to be encountered by *R. pumilio* (Fig 7). This may be because a thick hull reduces permeability of seed coat and

thus seed detectability (Vander Wall, 1990). Exploitation for the high-reward *L. sessile* seeds was found to be significantly ($p < 0.01$) greater than encounters. This finding indicates that once *L. sessile* seeds are encountered by *R. pumilio*, it is likely to return and exploit all the seed resources available (Hulme & Benkman; Leaver, 2004). These findings are comparable with those found by Hulme & Hunt (1999) where smaller wych elm (*Ulmus glabra*) seeds were encountered and exploited at higher rates than the bigger ash (*Fraxinus excelsior*) seeds. A significant difference between encounter and exploitation of sunflower seeds was not tested as the sample size was too small. However, preliminary evidence does suggest that once sunflower seeds are encountered all available seeds will be exploited (Fig 7).

Seed movements and nearest-neighbours

The majority of spooled *C. argenteum* seeds (86%) were moved within 500 cm of the seed stations (Fig 8), with the median removal distance being observed at 50 to 100 cm away. These findings indicate that *R. pumilio* can move *C. argenteum* seeds over distances that are commensurate with the observed distribution of nearest-neighbours *C. argenteum* plants (Fig 10). Furthermore, the maximum distance *R. pumilio* was recorded to move *C. argenteum* seeds (830 cm) is comparable with the maximum distance recorded between nearest neighbour *C. argenteum* stands (860 cm).

Seed movement paths of spooled *L. sessile* seeds showed a great consistency in chosen paths than spooled *C. argenteum* seed movements. Many of the *L. sessile* seeds were taken away from the seed stations to distinct feeding sites under vegetation cover, which *R. pumilio* individuals favoured (Fig 9). Due to high rates of predation and a lack of burial of *L. sessile* seeds it is plausible that the range of *L. sessile* occurrence may be limited by high densities of *R. pumilio*. This would require empirical evidence from sites where *L. sessile* naturally occurs.

Is *Rhabdomys pumilio* a scatter-hoarder?

Rhabdomys pumilio was the only observed animal to interact with the seeds placed at seed stations (Table 1). It is by far the most abundant small mammal species at the field site and is a reputed seed predator (Fig 1). *C. argenteum* nuts, which may be the largest in the fynbos biome, have a hard, woody ovary wall indicating they can stay dormant for long periods of time. Furthermore, *C. argenteum* drops its seeds to the ground *en masse* from November to January, creating a glut of seeds. *C. argenteum* lacks an elaiosome and therefore it seems likely that the seeds are scatter-hoarded. However, no burial of *C. argenteum* seeds was recorded *in situ* at De Hoop Nature Reserve. Additionally, no *C. argenteum* seed was found to be consumed (Fig 5). Were this species a scatter-hoarder, one would expect that it would preferentially select *C. argenteum* nuts as their large size presumably would offer a large energetic reward, and their thick hulls would decrease vulnerability and detection by other seed predators, and thus prolong shelf life (Jansen & Forget, 2001). The contradictory results I obtained, together with the lack of any seed burial by *R. pumilio*, thus indicate that this species is probably not a scatter-hoarder per se.

It is clear that although *R. pumilio* did not consume or bury any *C. argenteum* seeds, it showed a strong interest in them, as indicated by the long distances that seeds were moved away from seed stations (Fig 8 and 10), as well as the frequent bite marks found on *C. argenteum* seeds (Fig 6). As the mean distances that *R. pumilio* carried these seeds were commensurate with distances between nearest neighbour *C. argenteum* individuals and the maximum distances were commensurate with the maximum distances between *C. argenteum* stands, this rodent may indeed be a secondary disperser (Fig 10). However, dispersal of the *C. argenteum* seed would presumably only be beneficial to the parent plants if the seeds are buried, because seeds left lying on the ground would be vulnerable to attack by a wide variety of seed beetles (such as bruchid boring beetles) (Linzey & Washok, 2000) and would likely be incinerated by wildfires that are important for fynbos survival and regeneration. Furthermore, *C. argenteum* seeds require hypogeal germination and therefore must be buried for germination (Linder & Caddick, 2001). Therefore, it seems that while *R. pumilio* could serve as an effective disperser of *C. argenteum* seeds, it does not fulfil the role expected of a scatter-hoarder. It could be that there is further dispersal vector that potentially buries the seeds, such as the dung beetle observed by camera trapping (e.g.

Vander Wall & Longland, 2004; Vander Wall et al, 2005). It thus appears that *R. pumilio* did not scatter-hoard these nut-like seeds during my study, as I predicted. However, this study was short in duration and limited to only two sites, and there may be seasonal differences in seed consumption and dispersal that further research might demonstrate. Alternatively, their large size and thick hull or perhaps anti-predation compounds in the pericarp may be a deterrent (Hulme, 1998) and previous predications (Midgley et al, 2002; Midgley & Anderson, 2005) that seeds of this species are scatter-hoarded by rodents may be incorrect. Further studies should investigate seasonal differences in seed predation and dispersal and be conducted during the peak *C. argenteum* mast seeding season (December) to allow for a definitive conclusion to be drawn.

Conclusion

This study has described a number of novel behaviours, observed via camera trapping, of the ubiquitous fynbos Murid *Rhabdomys pumilio*. *R. pumilio* did not consume any of the large, thick-hulled *Ceratocaryum argenteum* seeds *in situ*, while the high reward, low cost *Leucadendron sessile* and commercial sunflower seeds were readily consumed. Removal patterns were irregular, with a favouring for the removal of the smaller *L. sessile* over the larger *C. argenteum* seeds. It is suggested that as *R. pumilio* did not bury or successfully consume any *C. argenteum* seeds and discarded any of the seeds that it moved onto the ground, its removal behaviour does not conform to that expected of a scatter-hoarder and instead preferentially remove smaller seeds for immediate consumption instead of caching. It appears that using wire tags has no significant influence seed choices, while further research should be conducted to determine whether spooled seeds influence rodent seed choice. Smaller seeds with a high-reward and low processing cost showed a greater percentage of usage when seed stations were encountered and exploited. However, *R. pumilio* does show an interest in the seeds with the mean and maximum distances of seed movements commensurate with the observed distances between nearest-neighbour *C. argenteum* plants and stands respectively, therefore the end fate of the seeds remains unknown. Further research should account for seasonal variability in scatter-hoarding behaviour.

REFERENCES

- Anderson, B. & Midgley, J.J. 2007. Density-dependent outcomes in a digestive mutualism between carnivorous *Roridula* plants and their associated hemipterans. *Oecologia*. 152: 115-120.
- Beck, H. & Terborgh, J. 2002. Groves versus isolates: how spatial aggregation of *Astrocaryum murumuru* palms affects seed removal. *Journal of Tropical Ecology*. 18: 275-288.
- Bond, W.J. 1984. Fire survival of Cape Proteaceae – influence of fire season and seed predators. *Vegetation*. 56: 65-74.
- Bond, W.J. & Breytenbach, G.J. 1985. Ants, rodents and seed predation in Proteaceae. *South African Journal of Zoology*. 20: 150-154.
- Brewer, S.W. 2001. Predation and dispersal of large and small seeds of a tropical palm. *Oikos*. 92: 245-255.
- eMonocot Team Literature *Restionaceae* <http://restionaceae.e-monocot.org/biblio> (accessed 9 October 2013).
- Forget, P-M. 1993. Post-dispersal predation and scatterhoarding of *Dipteryx panamensis* (Papilionaceae) seeds by rodents in Panama. *Oecologia*. 94: 255-261.
- Forget, P-M. & Vander Wall, S.B. 2001. Scatter-hoarding rodents and marsupials: convergent evolution on diverging continents. *Trends in Ecology and Evolution*. 16 (2): 65-67.
- Hulme, P.E. 1997. Post-dispersal seed predation and the establishment of vertebrate dispersed plants in Mediterranean scrublands. *Oecologia*. 111: 91-98.
- Hulme, P.E. 1998. Post-dispersal seed predation: consequences for plant demography and evolution. *Perspectives in Plant Ecology, Evolution and Sytematics*. 1 (1): 32-46.
- Hulme, P.E. & Hunt, M.K. 1999. Rodent post-dispersal seed predation in deciduous woodland: predator response to absolute and relative abundance of prey. *Journal of Animal Ecology*. 68: 417-428.

Hulme, P.E. & Benkman, C.W. 2000. Granivory. In: Herrera, C.M. & Pellmyr, O. (eds) Plant-Animal Interactions. Blackwell Science, Oxford.

Hoshizaki, K., Suzuki, W. & Nakashizuka, T. 1999. Evaluation of secondary dispersal in a large-seeded tree *Aesculus turbinata*: a test of directed dispersal. *Plant Ecology*. 144: 167-176.

Howe, H.F. & Smallwood, J. 1982. Ecology of seed dispersal. *Annu. Rev. Ecol. Syst.* 13: 201-228.

Jansen, P.A. & Forget, P-M. 2001. Scatterhoarding rodents and tree regeneration. In: Bongers et al (eds.), Dynamics and plant-animal interactions in a neotropical rainforest. Kluwer Academic Publishers. 275-288.

Jansen, P.A., Bongers, F. & Hemerik, L. 2004. Seed mass and mast seeding enhance dispersal by a neotropical scatter-hoarding rodent. *Ecological Monographs*. 74(4): 569-589.

Kelly, D. 1994. The evolutionary ecology of mast seeding. *Trends in Ecology and Evolution*. 9: 465-470.

Leaver, L.A. 2004. Effects of food value, predation risk, and pilferage on the caching decisions of *Dipodomys merriami*. *Behavioural Ecology*. 15 (5): 729-734.

Linder, H.P. & Caddick, L.R. 2001. Restionaceae seedlings: Morphology, anatomy and systematic implications. *Feddes Repertorium*. 112 (1-2): 59-80.

Linzey, A. & Washok, K.A. Seed removal by ants, birds and rodents in a woodland savannah in Zimbabwe. *South African Journal of Zoology*. 35: 295-299.

Manning, J.C., Goldblatt, P., Duncan, G.D. 2012. Plants of the Greater Cape Floristic Region: 1: The Core Cape flora (*Strelitzia* 29). Pretoria: South African National Botanical Institute.

Midgley, J.J., Anderson, B., Bok, A. & Fleming, T. 2002. Scatter-hoarding of Cape Proteaceae nuts by rodents. *Evolutionary Ecology Review*. 4: 623-626.

- Midgley, J.J. & Anderson, B. 2005. Scatterhoarding in Mediterranean Shrublands of the SW Cape, South Africa. In: Forget, P.M. (Ed.), Seed Fate: Predation, Dispersal and Seedling Establishment. Ciba Publishing. 197-204.
- Miura, S., Yasuda, M. & Ratnam, L.C. 1997. Who steals the fruit? Monitoring frugivory of mammals in a tropical rain-forest. *Malayasian Nature Journal*. 50: 183-193.
- Moore, J. McEuen, B., Swihart, R., Cotreras, T.H., Steele, M. 2007. Determinants of seed removal distance by scatter-hoarding rodents in deciduous forests. *Ecology*. 88: 2529-2540.
- Mucina, L. & Rutherford, M.C. 2006. The vegetation of South Africa, Lesotho and Swaziland. Strelitzia, 19. South African National Biodiversity Institute, Pretoria.
- Munoz, A. & Bonal, R. 2008. Are you strong enough to carry that seed? Seed size/body size ratios influence seed choices by rodents. *Animal Behaviour*. 76: 709-715.
- Pierce, S.M. & Cowling, R.M. 1991. Dynamics of soil-stored seed banks of six shrubs in fire-prone dune fynbos. *Journal of Ecology*. 79: 731-747.
- Peres, C.A., Schiesari, L.C. & Dias Leme, C.L. 1997. Vertebrate predation of Brazil-nuts (*Bertholletia excelsa*, Lecythidaceae), an agouti-dispersed Amazonian seed crop a test of escape hypothesis. *Journal of Tropical Ecology*. 13: 69-79.
- Rusch, U.D. 2011. Scatter-hoarding in *Acomys subspinosus*: the roles of seed traits, seasonality and cache retrieval. MSc Thesis, University of Stellenbosch.
- Rusch, U.D., Midgley, J.J. & Anderson, B. 2012. Rodent consumption and caching behaviour selects for specific seed traits. *South African Journal of Botany*. 84: 83-87.
- Seufert, V., Linden, B. & Fischer, F. 2009. Revealing secondary seed removers: results from camera trapping. *African Journal of Ecology*. 48: 914-922.
- Skinner, J.D. & Chimimba, C.T. 2005. The mammals of the Southern African Subregion. Cambridge University Press (1st ed).
- StatSoft, Inc. 2012. STATISTICA (data analysis software system), version 11. www.statsoft.com

- Theimer, T.C. 2001. Seed scatterhoarding by white-tailed rats: consequences for seedling recruitment by an Australian rain forest tree. *Journal of Tropical Ecology*. 17: 177-189.
- Theimer, T.C. 2003. Intraspecific variation in seed size affects scatterhoarding behaviour of an Australian rain-forest rodent. *Journal of Tropical Ecology*. 19: 95-98.
- Vander Wall, S.B. 1990. Food Hoarding in Animals. University of Chicago Press. 1st ed.
- Vander Wall, S.B. 2001. The evolutionary ecology of nut dispersal. *Botanical Review*. 67: 74-117.
- Vander Wall, S.B. & Jenkins, S.H. 2003. Reciprocal pilferage and the evolution of food-hoarding behaviour. *Behavioural Ecology*. 14 (5): 656-667.
- Vander Wall, S.B., & Longland, W. S. 2004. Diplochory: are two seed dispersers better than one? *Trends in Ecology and Evolution*. 19: 297-314.
- Vander Wall, S.B., Kuhn, K.M., & Beck, M.J. 2005. Seed removal, seed predation, and secondary dispersal. *Ecological Society of America*. 86 (3): 801-806.
- Vander Wall, S.B. 2010. How plants manipulate the scatter-hoarding behaviour of seed dispersing animals. *Philosophical Transactions of The Royal Society*. 365: 989-997.
- Willson, M.F. & Traveset, A. 2000. The ecology of seed dispersal. In *Seeds: the ecology of regeneration in plant communities*. (Fenner M ed.) 2nd ed. 85-110.
- Wrobel, A. & Zwolak, R. 2012. The choice of seed tracking method influenced fate of beech seeds dispersed by rodents. *Plant Ecology*. 214: 471-475.
- Yi, X., Xiao, Z. & Zhang, Z. 2008. Seed dispersal of Korean pine *Pinus koraiensis* by two different tags in northern temperate forest, northeast China. *Ecological Research*. 23: 379-384.