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Factors influencing the olfactory capabilities of foraging rodents

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By

Amy E. Seaman

Dr. Stephen B. Vander Wall/Thesis advisor

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AMY SEAMAN

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Stephen B. Vander Wall, Ph.D., Advisor

Matthew L. Forister, Ph.D., Committee Member

Gary J. Blomquist, Ph.D., Graduate School Representative

Marsha H. Read, Ph. D., Dean, Graduate School

December, 2012

ABSTRACT

Granivorous rodents living within rich communities of scatter-hoarding species partially rely on olfaction as a foraging strategy to locate buried seeds. While abiotic factors are well known to influence the olfactory capabilities of foraging rodents, we know less about how seed olfactory cues may change over time, how attributes of seed resources themselves affect olfactory cues, or exactly what type of odors are being detected. Such questions are worth investigating, as olfactory cues may influence resource partitioning in arid ecosystems through their importance to a rodent's foraging strategy. I investigate three hypotheses in the first chapter, 1) the olfactory signal of seeds buried in dry soil diminishes over time, 2) the presence of a seed's hardened seed coat acts to diminish olfactory cues, and 3) the olfactory signal of buried seeds increases following rain events. Data collected using artificial foraging grids in Little Valley, NV, and laboratory foraging trials, suggest that a seed's soil residence time does not alone significantly affect rodents' ability to detect cached seeds, and in fact, localized disturbances associated with seed burial may slightly increase cache detection. Alternatively, a seed's hardened, durable shell does appear to play an important role in reducing detection by naïve foragers. Further, my experimental evidence showed that rodents were more likely to retrieve their own caches, than pilfer, in moist soil conditions. In chapter two I investigated the hypotheses that 1) Jeffrey pine seeds contain volatile terpenoids (VTs) that contribute to seed odor, 2) VTs are used as cues when rodents forage for buried seeds, and 3) rodents can detect individual macronutrients. I used similar investigative techniques as those in chapter 1, with the addition of solid phase micro-extraction (SPME) paired with gas chromatography/mass spectrometry

(GC/MS) headspace analysis. Jeffrey pine seeds were found to contain 16 compounds, most of which were released from whole, wetted seeds. The majority of compounds appeared to emanate from the seed coat rather than the nutrient-containing seed embryo. One of these, (-)ß-pinene, was found to illicit digging behavior in the lab, while another, (-)R-Limonene, had no such effect. When isolated macronutrients were presented to chipmunks, lipids and proteins were found most successfully, while carbohydrates were not. Cumulatively these data suggest that the chemosensory information available within seed resource may be as important in making foraging decisions as they are in social and predator awareness contexts. As effective density dependent predators, rodents pressure plant resources to balance selective costs of dispersal with loss to seed predators, pathogens, and fungi. The high preference of rodents for Jeffrey pine seeds and the presence of a variety of terpenoid compounds within them suggest that these balancing mechanisms have already been at work shaping plant-animal interactions in an animal-dispersed pine.

DEDICATION

For the natural world and all with whom I share it.

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CHAPTER 1: Factors influencing the olfactory capabilities of foraging rodents

INTRODUCTION

Granivorous rodents in semi-arid ecosystems rely on olfaction to locate and evaluate seed resources that have been buried abiotically or scatter-hoarded (cached) by other animals, when detection using visual cues or spatial memory is restricted (Vander Wall 1998, 2003a). Although cache detection is essential for food hoarders in their management of scattered caches and/or successful preparation of winter larders, rodent species vary in their olfactory capabilities, that are further modified by abiotic factors including relative humidity, soil moisture, soil type, cache depth, number and species of seeds (Johnson & Jorgensen 1981, Vander Wall 1998, Vander Wall 2003b, Taraborelli et al. 2009). Differences between rodent olfactory abilities and the influence of abiotic conditions are amplified when resource levels are depressed by drought or natural fluctuation, as is often the case in arid environments (Johnson & Jorgensen 1981). Furthermore, deserts and semi-arid locales represent extremes in temporal and spatial food availability, areas where olfaction is inherently less effective due to extremely low moisture (Downs & Vander Wall 2009). In this respect, factors influencing the olfactory capabilities of foraging rodents are significant because many are reliant on locating seeds, a limited ephemeral resource, to obtain metabolic water and nutrients while surviving periods of food scarcity under a tight water budget (Frank 1988, Hulbert & MacMillen 1988).

Rodent granivores are the most diverse and abundant group of mammals in arid environments, and their presence in a community frequently overlaps with seed-hoarding species from diverse taxa. Many granivorous species, in general, act as facultative seed

consumers, with the ability to track and exploit seed species as they ripen progressively throughout the growing season. This behavior may cause intense inter and intra-specific competition for shared resources. Differences in cache depth, cache size, and cache microsites may mitigate some affects of competition between species, but also act to create a dynamic soil seed bank when many individuals are scatter-hoarding (Vander Wall & Jenkins 2003). Although individual scatter-hoarding animals retrieve their own caches more frequently than not, pilferage between sympatric species can be high. Cache removal rates by naïve foragers in one arid habitat were measured in the field to range between 2-30% a day, enough to decimate long-term rodent and corvid seed stores (Vander Wall and Jenkins 2003). These high pilfering rates suggest that competition for below ground resources may be as important in shaping resource dynamics as competition for above ground resources (Vander Wall 1998).

In addition to being seed predators, scatter-hoarding rodents and corvids can act as effective plant dispersal agents, scatter-caching seeds into microsites that promote plant regeneration (Vander Wall et al. 2005, Briggs et al. 2009). For many plants, especially moderately large-seeded pines such as Jeffrey pine (*Pinus jeffreyi*), this dispersal service is one of the dominant processes influencing population spatial demographics (Nathan & Muller-Landau 2000). At least 20 of the remaining108 pine species worldwide have also developed a rodent facilitated dispersal modality from predominantly wind-dispersed ancestors over evolutionary time; a trend that is particularly strong in semi-arid ecosystems (Vander Wall et al. 2006). Where a network of species participating in this pine seed dispersal mutualism exist, animals place selective pressure on plants to develop mechanisms capable of balancing pre-dispersal

mortality and propagule consumption with attracting dispersers and providing adequate nutrients to developing seedlings (Jorgensen & Chesser 2000).

Pines existing under co-evolutionary pressure from scatter-hoarders have responded with changes in cone morphology and seed size. The resulting seeds are not only more accessible to dispersers, but also have a higher net energy reward and attractiveness (Thayer & Vander Wall 2005). Seed resources additionally contain amino acids, proteins, other organic acids, sugars, lipids, and ions that contribute to a bouquet of odors foraging rodents perceive (Vander Wall 1998). Inter and intraspecific differences in the ability to use chemosensations (tastes and smells) in the environment to locate seeds could impact resource partitioning within a community, and contribute to competitive exclusion via unequal resource discovery and pilfering. As mentioned before, in communities rich with seed hoarding species, pilfering is widespread. As seed dispersers, the unequal effectiveness of each pilferer as a seed disperser may therefore affect subsequent plant recruitment (Thayer and Vander Wall 2005).

Behaviors that lead to seed dispersal, and the sheer abundance of granivorous rodents, enables dispersers to exert a strong influence on seedling recruitment, reseeding operations, plant establishment, succession, and the colonization of new habitats (Johnson & Jorgensen 1981, Leaver & Daly 2001,Vander Wall et al. 2005). This includes impacting population genetic structure at local and landscape levels (Jorgensen 2001, Levin et al. 2003). Consequently, an animal's foraging strategy contributes to an individual's overall fitness, the redistribution of local plant resources and resource partitioning within a given scatter-hoarding community (Johnson & Jorgensen 1981, Smallwood & Peters 1986). A forager's olfactory abilities are an important aspect of their

overall foraging strategy, and in this way indirectly contribute to plant demographics, granivore survival, and the evolutionary relationship between dispersers and their resources.

Currently, our knowledge about what influences the olfactory cues foraging rodents perceive pertains to environmental factors such as precipitation related abiotic conditions and physical cache characteristics. Controlled field and laboratory studies have tested soil moisture, seed water content, relative humidity, cache depth, and cache size for their impact on seed detection via olfaction. It is well known that increases in relative humidity or soil moisture lead to an increase in seed detection rates by naïve foraging rodents (Johnson & Jorgensen 1981, Vander Wall 1998, 2000, Downs & Vander Wall 2009), while detection is reduced as cache depth increases and as the number of seeds in a cache decreases (Vander Wall 1998). Much less is known about temporal changes of caches characteristics, or what attributes of the seed resource itself affect olfaction.

Most dry seeds are highly hygroscopic, readily taking up water from their surroundings (Vander Wall 1998). When a fresh seed is buried in dry soil it loses moisture to the surrounding environment because of its greater water content. Moisture exchanged between the seed and soil is coupled to the escape of volatile compounds from seeds into the surrounding soil and atmosphere (Simon & Raja Harun 1972, Vander Wall 1998, 2003a). However, after a period of time the seed and soil should come into moisture equilibrium, reducing the amount and concentration of volatile compounds released through the seed coat (Vander Wall 2003b). Time since burial should contribute to diminish a seed's olfactory signal because volatiles compounds are present in a finite

amount within plant tissues (Dudareva et al. 2006). The time frame over which this process may occur is unknown, however in many cache removal studies, rates of detection rapidly decline within the first few days (Vander Wall 2008).

For plant species with life cycles requiring a period of dormancy prior to germination, the presence of a hardened seed coat allows for seeds' persistence in harsh environments (Rolston 1978, Barnett 1998, Moïse et al. 2005). The presence of increasingly durable seed coats is typical for animal dispersed plants (Stiles in Abrahamson 1989, Thayer & Vander Wall 2005, Vander Wall 2010), and although the seed coat is necessary for protecting developing embryos, it may also form a barrier limiting the seed's ability to exchange moisture, and therefore volatile compounds, with its surroundings. In this way the seed coat may modify the odor a seed emits (Simon & Raja Harun 1972, Barnett 1998). Additionally seed coat characteristics may influence foraging decisions of rodents by increasing handling costs, such that rodents are encouraged to cache rather than consume seeds when they are discovered (Vander Wall 2010). Under this premise, seeds lacking seed coats altogether would rapidly exchange moisture with the soil environment, driving volatiles rapidly into the environment before seeds quickly reach moisture equilibrium. Initially rapid cache detection by foraging rodents would decrease quickly, concomitant with the diminishment of seed olfactory cues.

A decrease in seed odor (as indicated by diminishing harvesting rates) over time may indicate important physical interactions between a seed and its soil environment that can be considered when asking why certain seeds are being removed from, or are allowed to persist in, the soil's seed-bank. Exactly how rodents use olfaction, what odors they are

detecting, and on what time frame olfactory cues influence foraging decisions are still unclear (Vander Wall 2003b). Furthermore it is unclear how seed characteristics themselves may influence olfactory cues. To begin answering some of these questions, I investigated, under field and laboratory conditions, the following. 1. Is there an effect of a seed's soil residence time on the removal rate of seed caches? 2. Does the presence of a hardened seed coat affect the removal rate of cached Jeffrey pine seeds? 3. Does the occurrence of a rain event influence the rate of pilferage on a population of caches?

In the selected study system, Little Valley, NV, both sciurid and murid rodent species are dispersal agents of the locally abundant Jeffrey pine (*Pinus jeffreyi)*. Yellow-pine chipmunks (*Tamias amoenus*), lodgepole chipmunks (*T. speciosus*), golden-mantled ground squirrel (*Spermophilus lateralis*), Douglas squirrel (*Tamiasciuris douglasii*), California ground squirrel (*Spermophilus beecheyi*), and deer mouse (*Peromyscus maniculatus*) are all found at the study site, and many of them have been shown to use a combination of olfaction, random digging, and spatial memory to locate buried seeds (Thayer & Vander Wall 2005, Vander Wall et al. 2009). Corvid dispersers such as Clark's nutcracker (*Nucifraga columbiana*) and Stellar's jay (*Cyanocitta stelleri*) also cache seeds within Little Valley, but lack the ability to use olfaction (Vander Wall 1982). Yellow-pine chipmunks are particularly efficient dispersers of Jeffrey pine seeds; systematically caching seeds in non-random locations, positively affecting pine seedlings, and also avoiding areas of dense litter where fitness is reduced (Briggs et al. 2009).

METHODS

Study Site

Field experiments were conducted at the University of Nevada's Whittell Forest and Wildlife Area in Little Valley, Washoe County, Nevada, during the summer and fall of 2010 and 2011. The fairly high elevation valley floor sits at ~2000 m and is ~12.8 km in length, running north south on the eastern flank of the Sierra Nevada. Jeffery pine (*Pinus jeffreyi*) is the dominant tree species intermixed with lodgepole pine (*Pinus contorta*). The understory is dominated by antelope bitterbrush (*Purshia tridentata*), green-leaf manzanita (*Arctostaphylos patula*), tobacco brush (*Ceanothus velutinus*) and Sierra bush chinquapin (*Castanopsis sempervirens*). Decomposed granitic soils, scattered boulders and plant litter form the caching substrate. Climatic conditions are semi-arid, with an average annual precipitation of 87.5 cm falling mainly in the winter as snow.

Foraging Grids

I established foraging grids at six open pine sites within Little Valley. Each of the six sites was dominated by Jeffrey pine and an open understory of manzanita and bitterbrush shrubs. I avoided placing grids in areas containing large amounts of pine litter. Each 10 x10 foraging grid covered ~2500 m^2, and contained 100 cache sites. I spaced cache locations 5 to 8 m apart along parallel grid lines. I placed small pieces of flagging approximately every 5 m along gridlines, always between adjacent cache sites. Each cache contained two seeds buried ~1 cm deep using forceps to avoid transferring human odor. I selected this depth to allow for comparisons with previous removal studies

(Vander Wall 1998), and to stay within the 5-25 mm depth typical of Jeffrey pine caches available for pilfering by scatter-hoarding species in the area (e.g., Briggs et al. 2009).

Experiment 1: *Soil Residence Time*

I used three sites (sites 1, 2, and 3) to investigate how a seed's soil residence time (the number of days a seed spends buried in the soil) affects the rate at which foraging rodents find seeds using olfactory cues. I weighed Jeffrey pine seeds collected in 2009 and selected for those within 2 SD of the mean mass (160.2 $\pm$ 54.2 mg) (Vander Wall 2008), a parameter assumed to influence odor. I then marked half of the weighed seeds red and half black using a Sharpie® to facilitate identification, and radio-labeled both with scandium-46 (Sc^{46}), a gamma-emitting radionuclide with a half-life of 87.5 days. Sc^{46} is not known to have a detectable odor or taste (Parmenter unpub. data), and does not appear to affect removal rates (Vander Wall 2008).

I prepared 300 seeds of two treatment types: 1) soil residence time 10 days, hereafter aged seeds, and 2) soil residence time 0 days, hereafter referred to as fresh seeds. I buried aged seeds under ~10 mm of dry soil within a 0.6 m x 1.5 m rodent proof exclosure, made of 6mm wire-mesh, for 10 days before trial initiation. I removed aged seeds from the exclosures and cached the aged and fresh seed treatments early on the day I initiated trials. I randomly assigned 50 aged seed treatments and 50 fresh seed treatments to cache to locations within each of the three grids and I repeated the experiment on two occasions; 17 July 2010 (Experiment 1a) and 11 September 2010 (Experiment 1b).

I checked caches with a portable Geiger counter (Eberline ASP-1 meter and SPA-3 probe; ThermoFisher Scientific, Santa Fe, New Mexico, USA) every 24 hours for the first three days and then on days 5, 7, and 10. I surveyed grids for signs of cache detection around noon to avoid disturbing rodent foraging activities. When a radio signal was absent the site was searched for physical disturbances caused by digging. If dig marks were apparent, I searched the soil for seeds using forceps. If seeds were found in these locations, I re-cached them and they remained within the sample. I considered a cache detected when rodents: 1**)** had removed entire seeds (one or both), 2) had exposed seeds, or 3) had eaten seeds (shells nearby). After day 10 the remaining seeds were removed from the grids to verify presence/absence and to prevent rodents from learning to forage in those locations.

I determined minimum daily seed and soil moisture content (% by mass) to monitor changes during both trials. 21 caches of each treatment (labeled with a Sharpie®, soaked in distilled water and dried for 48 hrs as methodological controls) were buried ~10 mm deep in a separate 0.6 m x 1.5 m wire-mesh exclosure. I made 21 aged caches in the exclosure 10 days before trial initiation, and an additional 21 fresh caches on the day trials were initiated. Each day that I monitored grids for removal I dug up three caches of each treatment. I placed seeds from individual caches immediately into whirl-pack bags, sealed them tightly, and placed them in a cooler for transport back to the lab. I then weighed samples, oven-dried them at 80 ºC for 48 hours, and re-weighed them obtaining percent water content. I quantified soil moisture on each foraging grid by collecting 5 ~20 g soil samples at random pre-determined locations within each foraging grid. I took soil from the same depth as seed caches and sealed samples in whirl-pack bags. In the lab

I sifted soil samples through a 2.33 mm sieve to remove small pebbles, and then weighed, oven-dried them at 80 ºC for 48 hours, and re-weighed them in the same manner as the seeds. Although seed samples were not taken from within foraging grids, they were taken ~100 m from site 1 and in a similar habitat and microsite. Rain gauges were placed in the center of each foraging grid to record chance rain events. I placed 5 ml of mineral oil into rain gauges so that small or localized rain events could still be recorded.

Experiment 2: Effects of seed coat

To consider how a seed's shell effects the rate of cache detection by foraging rodents I cached seeds of two treatments types at three sites (sites 4, 5, and 6), 1) shelled Jeffrey pine seeds, and 2) whole Jeffrey pine seeds. I shelled seeds using forceps and wearing nitrile gloves. I cached seeds on 9 September 2010 and monitored them with a Geiger counter on days 1, 2, 3, 5, 7, 10. Cache detection, soil water content, and seed water content were all determined in the same manner as experiment 1.

Experiment 2a: Effects of the seed coat in laboratory foraging trials

The effects of a seed's shell on rodent foraging success was tested inside using 8 yellow-pine chipmunks that were captured in Little Valley, NV, during October 2010. Following capture, and while trials were conducted, I housed rodents at the Fleischmann Agriculture building in separate 48 x 27 x 20 cm plastic cages and provisioned them with quart-sized glass jars and cotton bedding for nesting, Sani-chips® on the cage floor, black-oil sunflower seeds, Hekklah® rodent pellets, and ad libitim access to water. Lighting in the room operated on a 12 hour light-dark cycle. I cared for animals

according to University of Nevada, Reno's, Institutional Animal Care and Use Committee protocol, and they were consistent with guidelines set by the American Society of Mammalogists (Gannon & Sikes 2007).

Experiments were conducted within a 2.4 x 3.6 m indoor arena located in the basement of the Fleischman Agriculture building. The arena floor contained 48 equal sized holes, spaced 26.5 cm apart in a 6 x 8 array. I placed cups made of PVC tubing (52-mm diameter x 110 mm deep) into each whole, flush with the plywood flooring and completely filled them with clean dry sand. When wet sand was used, I applied 3 ml of distilled water directly to top of the sand in each cup. A CCD-VXS Sony Hi-8 Video Camcorder was mounted on the ceiling to record each individual's foraging trial during experiments. Following an experiment, I watched the videos to note the order in which cups were visited and the action taken during each visit. I recorded actions as checking (the rodents nose directly over a cup), successful dig (digging and exposing contents when a treatment is present), or unsuccessful dig (digging when no treatment is present, or digging when a treatment is present and failing to find seeds). I also recorded the time of subsequent visits to cups containing treatments, and the action of all subsequent visits. I watched individual videotapes for a period of 1 hour after the chipmunk began to forage, however, if less than half of the cups had been visited following the completion of an hour, I continued to watch the tape for another half-hour. If all treatments had been dug up before the hour was over, the trial was considered complete.

Two experimental trials were run to determine if the complete removal of Jeffrey pine seed shells affects digging success. Trials were run between 25 February and 3 March 2011, using dry sand and again between 7 March and 10 March 2011, using wet

sand, to see if removal trends are similar under both conditions. For both trials, I used two treatments Jeffrey pine seeds; shelled (seeds with the shell completely removed), and whole seeds. I shelled seeds using forceps and wearing nitrile gloves to avoid transferring human odor. During trials I buried 1 seed of each treatment type into 6 individual, randomly selected cups, so that individuals had a 14.3% likelihood of finding a seed of either treatment type by chance.

Between 31 January 2012 and 1 February 2012, I ran one non-choice trial burying half-shells of Jeffrey pine seeds separately in 16 cups to see if rodents would dig for shells alone. In the trials, subjects had a 33.3% likelihood of finding a treatment by chance. Another two trials, one with dry sand and one with wet sand, were then run comparing the removal of Jeffrey pine shells to the removal of shells containing half of a Jeffrey pine seed. These later two trials took place between the 14 through 17 February and 18 through 22 February. In these trials 8 of each treat was present, giving chipmunks a 20.0% chance of finding a cache at random.

Experiment 3: Interactions between soil residence time and disturbance

I determined if a seed's soil residence time interacts with the age and presence of a soil disturbance (caused by digging) to affect the rate of cache detection by making 20 caches of 5 treatment types at 2 sites (sites 4 & 6) on 20 August 2011. I again weighed seeds and marked them with Sharpies® but did not radio-label seeds. I marked exact cache locations by mapping their relationship to nearby unmoved natural objects and very detailed site maps. The five treatments were based on a semi-factorial design: 1) Seeds buried for 10 days prior to the trial on the foraging grid under small 10 x 10 x 10 cm wire

mesh cages (hereafter aged seeds with old dig marks), 2) sham caches dug 10 days prior to the trial to create soil disturbances on the foraging grid and covered with wire mesh baskets (sham caches with old dig marks), 3) seeds aged in rodent proof exclosures and cached on the grid the day the trial was initiated (aged seeds with new dig marks), 4) sham caches (i.e fresh disturbances) dug the day the trial was initiated (sham caches with new dig marks), and 5) fresh seeds with new disturbances (fresh seeds). Sham treatments contained no seeds to test for direct affects of small-scale soil disturbance on the rate of cache detection. The latter three treatments were placed directly into grids the day the trial was initiated, however all cache sites were covered with wire-mesh baskets and weighted with rocks, to control for animals potentially learning sites while caches were aging. Baskets were removed from the grids when the trial began. Removal was monitored beginning 21 August and again on days 2, 3, 5, 7, 10, however I did not quantify seed and soil moisture. This trail was repeated in September. Rain gauges were present on grids.

Experiment 4 Cache recovery vs. pilferage under moist conditions

Under dry conditions, rodents show a preference for the recovery of their own caches versus the pilferage of conspecific caches. Following a rain event on 12 October 2011, I set out three sets of 150 Jeffrey pine seeds in three open Jeffrey pine sites (sites 1, 2 and 4) in Little Valley. I set out another 150 Jeffrey pine seeds at site 3 on 13 October, and another 150 at site 5 on 22 October. I radio-labeled seeds with Iron-59 (FE^{59}), a gamma-emitting radionuclide that has a half-life of 44.4 days. I soaked seeds in an FE^{59} and distilled water solution until each had adsorbed ~37 GBq of radioactivity and they

were allowed to dry for 48 hours. I piled seeds in a small soil depression at each site and marked them with colorful pin-flags to attract rodents. I allowed 24 hours for seeds to be removed before surveying for caches. I surveyed around the source in concentric 5 m circles, until a 40 m radius was surveyed around seed sources. When caches were found, I temporarily marked them with pin-flags. After locating as many caches as possible, the number of seeds, depth of cache at the top of seeds, and cache microsite were recorded. Microsite was determined as mineral soil, light litter, or heavy litter. For each cache that a rodent made, I made another cache, identical in seed number, depth, and microsite, ~30 cm away using forceps. Pin-flags were removed and all cache sites were surveyed 24 hours later, and again every other day until 3 November 2011 when a snowstorm prevented access to Little Valley. When a cache appeared to be missing, I surveyed for secondary caches within a 3 m radius of the original site. When I found secondary caches, I continued to monitor them. As in experiment 1, a cache was considered removed when rodents, 1**)** had removed entire seeds, 2) had exposed seeds, or 3) had eaten the seeds.

Rodent Abundance

The abundance and species of small mammals were determined by trapping for five consecutive days, between 9 October and 13 October, at three sites (sites 1, 3 and one site not used for caching grids) in 2010 and between 8 October and 11 October at three sites (sites 1, 3 and 4) in 2011. 40 Sherman live traps were set in a 4 x 10 array with ~10 m spacing. Traps were covered with pine needles for shade, baited with black-oil sunflower seed and checked in the early morning and early evening. Captured rodents

were identified to species, sexed, weighed (in grams), checked for reproductive status, ear-tagged, and released.

Data Analysis

I used multi-sample survival analysis in Program R (*package-survival*) to analyze removal within foraging grids and to determine the mean number of days caches survived. A Weibull distribution and interval censoring was used due to sampling on non-consecutive days 1, 2, 3, 5, 7, 10. Survivorship was analyzed using the last day a cache was present and first day a cache was absent as parameters. Multiple comparisons of mean survival were run to decipher treatment effects. Log-transformed daily number remaining data were used to fit decay lines comparing the percent removal per day between treatment types. Paired T-tests were used to compare the retrieval of animal prepared caches to artificial paired caches in experiment 4 and to compare seed and soil moisture. Chi-square goodness of fit analyses were used to compare laboratory digging success rates to random success. One-way ANOVAs were used with arcsine-transformed data to compare digging success, digging likelihood and the likelihood of failure between treatments in indoor foraging trials. Digging success was defined as the number of seeds dug/ number of cups dug in. Digging likelihood was the number of seeds dug in/ the number of cups containing seeds that were visited, and the likelihood of failure was the number of seeds missed / number of cups containing seeds that were visited.

RESULTS

Experiment 1: Soil Residence Time— Treatment removal rates did not differ between sites ($\chi 2 = 4.07$, df = 2, $P = 0.130$), so data presented are pooled. Cache removal

was low in general; 42 of 150 fresh caches were removed (3.2% per day), and 36 of 150 aged caches were removed (2.7% per day)(Figure 1). The slightly higher removal rate of fresh seeds resulted in a lower mean number of days (52.3) estimated for cache survival compared to 69.4 days for aged caches, however this difference was not significant ($\chi 2$= 0.72, df = 1, P = 0.390). The same trend was seen when the experiment was repeated about two months later, although removal rates almost doubled. Data were again pooled because removal did not differ between sites ($\chi 2$= 3.09, df = 2, P = 0.210); a total of 79 of 150 fresh caches (7.2% per day) and 71 of 150 aged caches were removed (6.2% per day)(Figure 2). Again, although fresh seeds were removed more quickly, there were no differences in survivorship between treatments ($\chi 2$= 0.23, df = 1, P = 0.630) (Figure 2). Mean survival was estimated to be 34.4 days for fresh caches and 38.7 days for aged caches, values about half of those estimated in the first run of the experiment (Table 1).

During the two trials of experiment 1, soil moisture content ranged from 0.22 - 0.39% and from 0.28 - 0.37% percent, respectively. Seed moisture ranged between 1.97 and 4.7% in the first trial and between 3.06 and 3.12% in the second trial. There were no differences between treatments' seed water content in the July trial (t = 1.7367, df = 9.9, P = 0.110), or during the September trial (t = .7841, df = 8.6, P =0.450) (Table 2).

Experiment 2: Effects of the seed coat—Animals removed 98 of 120 shelled caches (15.5% per day) and 63 of 120 whole caches (7.2 % per day) when data were pooled between sites (Figure 3). The doubling in removal rate of seeds lacking shells was reflected in significant differences in treatment survival at two of the three sites (Table 3), and when data were pooled ($\chi 2$= 23.98, df = 1, $P < 0.001$). The difference in the mean

number of days each cache type was estimated to survive was, however, not large. Shelled caches survived a mean of 8.24 days, and whole caches survived 11.55 days (Table 3). Soil moisture ranged between 0.39 and 0.54% and although both cache types water content fluctuated slightly, there were no differences between treatments ($t = 2.16$, $df = 1$, $P = 0.062$) (Table 4).

Experiment 2a : Effects of the seed coat in laboratory foraging trials—When both dry and wet sand were used to compare chipmunks' success at finding whole and shelled seeds, 12 cups contained treatments so that each chipmunk had a 14.3% probability of finding seeds of each treatment type, present in 6 of 42 cups, at random. Chipmunks found whole Jeffrey pine seeds at a rate of 11.6 ± 4.7% and found shelled seeds at 11.7 ± 6.4% (Figure 4) when dry sand was used. This slight difference between the success rates of both treatments was not significant, nor did chipmunks dig at either treatment more often than random (Table 5). When using wet sand, chipmunks found whole Jeffrey pine seeds marginally less than seeds lacking shells (18.0 ± 4.2% vs 19.5 ± 6.0%), a difference that was not significant (Figure 4). Chipmunks again did not find treatments more successfully than random under wet conditions (Table 5). There were no differences between a chipmunks' likelihood of digging up whole (60.6 ± 24.7%), or shelled (59.4 ± 23.3%) treatments under dry conditions, or under wet conditions (83.3 ± 17.8% vs. 78.1 ± 17.2% for whole and shelled seeds, respectively) (Figure 5). Likelihood of failure also did not differ during either trial (Figure 6).

When both dry and wet sand were used to test chipmunks' ability to locate Jeffrey pine seed shells, chipmunks had a 20.0% likelihood of finding a cache by chance. Chipmunks were 26.9 ± 9.3% successful at finding Jeffrey pine shells under dry

conditions and 58.5 ± 19.1% successful under wet conditions (Figure 7). Under wet conditions, chipmunks dug in cups containing seeds and shells significantly more often than random ($\chi2$= 85.62, df = 6, $P < 0.001$ and $\chi2$= 59.09, df = 6, $P < 0.001$, respectively). Chipmunks had a high likelihood of digging at both treatments (Figure 8) and a low likelihood of failure (Figure 9). In the non-choice experiment, 16 cups had Jeffrey pine shells in them so that individuals had a 33.3% chance of finding a cache by chance. Chipmunks located shell treatments slightly more often than chance, 48.6 ± 25.4, and were 63.8 ± 37.6% likely to dig when cups containing shells were encountered. They failed to dig when visiting cups containing shells 36.2 ± 37.6% of the time (Figure 10).

Experiment 3: *Interactions between soil residence time and disturbance*—While testing for interactions between cached seeds' soil residence time and physical disturbances caused by seed burial, animals removed 21 of 40 fresh caches (2.7% per day), 11 of 40 aged caches with new dig marks (1.1% per day), 11 of 40 sham caches with new dig marks (1.1% per day), 17 of 40 aged caches with old dig marks (2% per day), and 8 of 40 sham caches with old dig marks (.75% per day) (Figure 11). The interaction between cache type (fresh, aged, or sham) and cache age (old or new) significantly affected cache survivorship ($\chi2$ = 11.77, df = 5, P = 0.038) (Table 6) and so separate analyses were conducted on the effects of burial age and treatment type. Differences between treatment type survival (aged caches, fresh caches and sham caches) were significant ($\chi2$ = 8.98, df = 2, P = 0.011), however differences between age since burial alone, were not ($\chi2$ = 0.23, df = 1, P = 0.630) (Table 7). During a second trial, initiated about 1 month later, the removal pattern was similar although aged caches

associated with old dig marks were removed less quickly. Animals removed 5 of 20 fresh caches (2.2% per day), 5 of 20 aged caches associated with new dig marks (2.2% per day), 2 of 20 sham caches associated with new dig marks (0.7% per day), 2 of 20 aged caches associated with old dig marks (0.7% per day), and 1 of 20 sham caches associated with old dig marks (0.28% per day) (Figure 12). The interaction between cache age and disturbance age did not have a significant effect on cache removal although there were sometimes large differences in the mean predicted survivorship (Table 6 & 7).

Experiment 4 Cache recovery vs. pilferage under moist conditions—Animals removed 105 of the initial 150 seeds from site 1 and made 18 caches containing 2-35 Jeffrey pine seeds. Caches ranged from 1 to 43 mm deep and the deepest was also the largest cache. The shallowest cache contained 4 seeds. Survivorship between caches made by animals and the artificial paired caches I made was significantly different ($\chi 2 = 5.58$, df = 1, $P = 0.009$). Animal caches were estimated to survive for a mean of 4.03 days after being removed at a rate of 18.6% per day, and paired caches were estimated to survive for 14.92 days after being removed at 8.0% per day (Table 8, Site 1). A total of 73% of animal caches and 60% of paired caches were retrieved (Figure 13, Site 1).

At the second site 24 seeds were removed by animals and scattered into 14 caches containing between 1 and 3 seeds. Cache depths ranged from 3 to 20 mm. 27 of 33 animal caches were removed at 28.8% per day and 25 paired caches were removed at 24.6% per day (Figure 13, Site 2). Animal caches were estimated to survive for a mean of 4.16 days and paired caches were predicted to survive for 18.63 days, a difference that was again significant ($\chi 2 = 5.09$, df = 1, $P = 0.024$) (Table 8, Site 2).

77 seeds were removed at site 3 and animals made 34 caches containing 1-5 seeds. Cache depths ranged from 1 to 23 mm. Animal caches were again removed at higher rate than paired caches, 8.3% per day versus 5.4% per day. A large difference in mean survival was estimated between cache types, 14.13 days for animal caches and 50.13, although the difference between cache types survivorship was not significant ($\chi 2$= 1.1, df = 1, P = 0.290) (Table 8, Figure 13, Site 4).

Rodent Abundance—Trapping between 9 October and 13 October 2010 at three sites yielded 87 yellow-pine chipmunks, 15 deer mice, 15 long-eared chipmunks and 5 golden-mantled ground squirrels. There was an average of 29.0 ± 14.7, 5.0 ± 2.1, 5.0 ± 9.2, and 1.7 ± 2.1 animals per species per site, respectively. In 2011, between 8 October and 11 October we trapped 56 yellow-pine chipmunks, 14 deer mice, 3 long-eared chipmunks, 8 golden-mantled ground squirrels, and 1 jumping mouse (*Zapus princeps*). This latter species has not been caught during previous trapping events at any of these sites. Numbers were overall lower in 2011 and averaged 18.7 ± 8.4 yellow-pine chipmunks per site, 4.7 ± .6 deer mice, 1 long-eared chipmunk and 2.7 ± 1.5 golden-mantled ground squirrels per site.

DISCUSSION

I hypothesized, under the assumption that seeds contain volatile compounds in a finite amount, that a seed's soil residence time would affect rodents' ability to detect caches in dry soil. Lower rates of removal for caches containing aged versus fresh seeds were predicted as experimental evidence for the presence of this effect. Although at most sites the removal of fresh seeds occurred at a slightly higher rate than aged seeds (Figure 1 & 2), the trend was not statistically evident in either iteration of the experiment. Grids

surveyed in the summer (July) had lower overall removal rates than trials in the fall (September), although the minor effects of seed age on cache removal were stronger under summer conditions (Table 1). At one of the three sites used during both trials, cache removal rates were consistently higher for caches containing aged seeds. No differences were found between treatment seed moisture, and soil moisture remained below 0.39% (Table 2), indicating that the study sites were experiencing conditions typical of the study area's dry soil regime (<0.50% soil water content) (Vander Wall 1998). Removal rates of naïve foragers are typically low under these conditions, 0.33 ± 0.61 % per day (Vander Wall 1998), and the overall low removal rate of caches recorded here, 2.7 – 7.2%, were typical of the foragers in the study area.

Given that dozens of animals had access to each foraging site and given that dry conditions were sustained between July and September 2010, removal patterns suggests a seed's soil residence time does not act to influence the olfactory signal of buried seeds. Furthermore, the removal pattern was atypical, contrasting removal patterns on artificial foraging grids that show initially high, but exponentially declining rates of removal during the first few days following seed burial (Vander Wall 2008). It was this typical pattern that suggested time as a mechanism functioning to reduce the amount of volatiles a seed releases as it reaches moisture equilibrium with the soil. The low removal rates I found, especially early in the season, may reflect a lack of natural animal caches in the soil, and therefore a lack of animals actively searching for caches. Annual seed resources were not locally abundant during either trial, so background rates of scatter-caching and cache management may have been low. Consequently animals may have been focused on harvesting resources such as arthropods and vegetation.

Since the soil residence time of a cache alone did not seem to affect its likelihood of removal, we tested the prediction that a seed's soil residence time would interact with the physical soil disturbance caused by seed burial to affect detection. Fresh seeds associated with new dig marks were expected to be the most apparent caches, followed by aged seeds with new dig marks, aged seeds with old dig marks, sham caches with new dig marks, and sham caches with old dig marks. The pattern found was close to the prediction, and all cache types experienced some "removal". Fresh seeds were removed most quickly, followed by aged seeds associated with old dig marks, aged seeds associated with new dig marks, sham caches associated with new dig marks, and sham caches associated with old dig marks (Figure 11). The fact that any sham caches were removed supports the idea that digging disturbances at cache sites likely influence exploratory digging by animals. The removal rate of aged seeds with old dig marks was higher than expected, but not significantly lower than fresh caches. When repeated a second time, the results followed the predicted pattern more closely, and aged seeds with old dig marks were removed less quickly (Figure 12).

Only in the first iteration of the experiment were there significant differences between cache types' survival (Table 6). Differences were due to a significant interaction between the age of burial and treatment type, however when effects were isolated, the age of the disturbance associated with burial did not significantly contribute to statistical differences. Cache type, fresh, aged, or sham, was more important in affecting removal rates, and fresh seeds were removed consistently faster than aged seeds or sham caches (Table 7). This indicates that olfactory cues, due to a seed's presence, do increase successful cache removal, and that seed age may have a minor influence on reducing a

seed's olfactory cues. Removal rates of aged seeds associated with fresh dig marks suggest that small-scale disturbances created as rodents manage caches may create visual cues that counteract losses in seed odor over time, allowing caches to remain apparent to foraging rodents.

Individual cache management strategies, such as the number and type of re-caching events, represent a type of resource processing that may intrinsically create heterogeneity in the soil seed bank; creating heterogeneity in the cues available for locating buried caches and preventing consumers' equal access to buried resources (Price and Mittler 2003). What is unique about this view of resource processing is that it can involve both competition and facilitation because ''upstream'' harvesters of primary resources may increase the availability of processed material to ''downstream'' consumers by increasing cache conspicuousness (Price & Mittler 2003). A heterogeneous template of buried resources arising through individual cache management strategies may be crucial in promoting the coexistence of multiple granivores in one community (Price and Joyner 1997). Unfortunately, the experimental design makes it impossible to know what individuals attempted to remove caches, or if the same individuals that located seeds also tended to locate sham caches. Vander Wall et al. (2009) showed that golden-mantled ground squirrels were not typically successful finding buried seeds using olfactory cues, unlike yellow-pine chipmunks and deer mice, that are well known to be effective olfactory-oriented foragers (Howard & Cole 1967). It is very likely most of the removal was due to the latter two foragers that were both very abundant in the study site. Additionally, yellow-pine chipmunks have demonstrated the ability to associate cache

markers with buried food items (Downs and Vander Wall 2009), indicating that subtle visual cues may help foragers locate caches.

It appears that the removal of Jeffrey pine seed coats greatly increases the ability of foraging rodents to locate seeds. At individual sites, and when data were pooled, rodents removed caches containing seeds lacking shells significantly more quickly than caches containing whole seeds (Figure 4). During this experiment, 2 caches of seeds lacking shells were successfully excavated, and partially consumed by ants. No seeds with intact shells experienced this fate. Again dry conditions were sustained; soil moisture ranged between 0.39-0.54%, and both seed treatments remained below 4.9% (Table 4). This pronounced removal pattern was also demonstrated in a separate field study (Chapter 2) investigating the interaction between the presence of a seed's shell and lipophilic seed compounds. Again, the removal of the seed coat proved a dominant factor in explaining increased removal rates. The foraging success of yellow-pine chipmunks for shelled and whole seeds in the laboratory were greater under wet conditions, although under both dry and wet conditions there were no differences between treatment removal rates (Figure 4) and success was never greater than random. Chipmunks were more likely to dig up seeds under wet conditions and more likely to fail to find seeds in dry conditions as expected (Figure 5 & 6). Because the success and likelihood of digging increased under wet conditions it appears that olfactory cues played a minor role in dictating when chipmunks decided to dig, however much of the success was likely due to exploratory digging. Moisture had a similar influence when I tested the ability of chipmunks to locate just the shells of Jeffrey pine seeds. Chipmunks' success was higher under wet conditions, but surprisingly there were never differences between chipmunks

success, likelihood, or failure of digging at cups containing shells or shells and seeds (Figure 7,8 & 9). During this experiment, under wet conditions, chipmunks did dig more frequently than random at both treatments. In a non-choice framework chipmunks did not dig for buried Jeffrey pine shells more often than random. The contrary results of these last two experiments seem to support the idea that rodents can detect the shells of buried seeds, but may only choose to dig when food rewards are expected.

Many animal-dispersed seeds are protected by a hardened seed coat (Stiles in Abrahamson 1989); its properties being molded by the need to balance access to legitimate dispersers with protection from non-dispersing seed consumers (Vander Wall 2010). One direct way the seed coat functions is to increase seed handling time. Increased handling time results in an increased likelihood that seeds will be cached rather than consumed when encountered. When shells are thin or lacking, seed quality can be assessed rapidly, and in study by Xianfeng et al. (2011), acorns lacking shells were consumed *in situ* significantly more than cached when encountered. In an earlier removal study of isolated seeds in tropical environments, the hardness/thickness of a shell fell out in a principle components analysis as a main determinant of seed removal (Blate et al. 1998).

Although a durable seed coat is necessary for plants that exhibit delayed germination or dormancy, it appears to also benefit plant fitness by acting as a barrier to the release of olfactory cues. In addition to reducing the passage of volatile organic compounds (VOCs), the seed coat may act as a diffusion barrier for odors associated with the seeds' nutritional components. The protein content of seeds often proves to be a driving factor determining seed preference, and lab studies with yellow-pine chipmunks

have demonstrated that these animals are able to locate pure protein and pure lipid, even under dry conditions (Chapter 2). A further possibility is that the shell itself contains anti-herbivory, anti-parasite, or anti-fungal compounds (Moïse et al. 2005, Dudareva et al. 2006), the absence of which increase the likelihood of cache excavation. Defensive compounds that may act to deter pre-dispersal mortality, such as terpenoids, are abundant in pines and other woody species (Dudareva et al. 2006).

Under wet field and laboratory conditions, rodents are more successful at locating cached seeds, whether because of increased olfactory cues related to volatile compounds, nutritional components of the seed, or other clues (Johnson & Jorgensen 1981, Vander Wall 1998, 2000, Downs & Vander Wall 2009). Under dry conditions they are less successful and rodents demonstrate a recovery advantage when relocating their own caches versus pilfering from others. In a study conducted during rain-free periods in 1997, Vander Wall et al. (2006) found that removal of animal caches was consistently 3.4 - 6.5 times as quickly as the removal of artificial paired caches. The recovery advantaged is expected because of enhanced spatial information about exact cache locations and a working preference for caching microsites. Using a similar methodology, but under wet conditions, I also found the mean survivorship of animal caches to be consistently fewer days than survivorship of paired caches. The difference was only significant at the first two sites (Figure 13), however at all three sites animal caches disappeared more quickly. In total 75.4% of animal made caches were removed, as were 64.6% of the paired caches (Table 8). Recover rates of animal caches were only slightly higher than values found under dry conditions by Vander Wall et al. (2006), and the large disparity recorded between removal of animal and paired caches was not nearly as dramatic under wet

conditions. It appears that animals' recovery advantage decreases following rain events, suggesting that rain may function to renew the olfactory cues released by seeds, at least until conditions dry out again.

In removal trials where only naïve foragers are present, it may be easy to underestimate the removal rates of rodent caches (Vander Wall 1993), however these rates do accurately reflect pilfering in the area. It is typically assumed that a cache will be excavated when it is detected, however, the presence of partially exposed seeds and dig marks directly over non-removed caches shows otherwise. In all trials there were dig marks above or one to the side of a few cache sites although the seeds had not been removed. Experiments by Vander Wall et al. (2009) showed that of 432 caches presented in field exclosures, 51 (11.8%) were dug directly over, but not removed by yellow-pine chipmunks. This suggests that many factors influence removal rates, whether it is simply not profitable to excavate caches at that time or there was a high risk of predation. In the indoor arena there were times when an animal dug, was disturbed for no apparent reason, and immediately stopped excavations to take refuge. This situation probably occurs regularly in the field when I am unable to detect it, and animals may have discovered caches that they did not attempt to retrieve, but rather chose to monitor subsequently. Thus, the removal rates found here may be lower than actual rates of cache detection by rodents using olfaction.

Factors that allow rodents to use olfaction when locating seeds all contribute to a foraging strategy that is essential to individual survival. Because I did not find support for the idea that a seed's soil residence time is a factor that acts to diminish the olfactory cue of buried seeds, it is still unclear what influences the typical rapid decrease in cache

removal rates over time. If the diminishment of seed odor is one driving mechanism, it may indicate important physical interactions between a seed and its soil environment, other than time since burial, that need to be considered when asking why certain seeds are being removed from or allowed to persist in the soil's seed-bank. The more quickly a seed becomes "invisible" to foraging rodents, the safer it is from predation, increasing the likelihood of germination. Interestingly, when the disturbance associate with burying seeds was factored into the effects of the seed's soil residence time, it appeared that removal rates were impacted by the age of the associated disturbance. This suggests that the rate and characteristics of an individual's cache management behavior may act as a form of resource processing; increasing resource heterogeneity in the soil seed bank and allowing for the coexistence of granivore communities (Price and Mittler 2003). Within such communities, it is also thought that a level of tolerance to pilferage has been the adaptive strategy, such that an animal's fitness will not be negatively influenced by pilferage of its own caches because that animal will itself pilfer from others (Vander Wall & Jenkins 2003). Where dozens of animals share overlapping home-ranges, for every cache an individual makes there are many times more in the ground belonging to other individuals. My research shows that storm events have the ability to increase the buried resources generally available to foraging rodents by increasing pilferer success relative to dry conditions. When temporal and abiotic conditions fluctuate so that years of extremely high cache abundance, caused by high resource availability during masting years, or many rain events occur, pilfering as an evolutionary strategy may become more profitable.

Plant adaptations that lead to the active animal relocation of seeds to favorable germination sites are thought to be under natural selection pressures (Briggs et al. 2009). However, plants must balance this need with the need to protect propagules from non-dispersing seed consumers (Vander Wall 2010). Although it is still unclear whether a seed's olfactory cues result from secondary compounds in the shell or seed, or whether they come from the nutritious embryo, it appears the increased thickness of seed coats associated with animal dispersed pines benefit the plant not only by allowing it to survive dormancy, but, by acting as a diffusion barrier to a seed's olfactory cues. Rodents may differentiate between odors on and in seeds, factors that could drive rodent selection on seed resources, having important implications for plant fitness. The degree to which olfactory cues mediate plant and animal interactions probably varies among ecological communities, however olfaction appears to have ability to impact the spatial heterogeneity of local plant communities and resource based competition between granivorous rodent assemblies in arid environments.

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Table 1. Mean number of days caches survived at three open Jeffrey pine sites during two trials comparing the removal of fresh and aged caches. Exp 1a started on 17 July and Exp 1b was started on 11 September 2010. The last day a cache was present and the first day a cache was absent were the only survival parameters. Chi-square survival analyses are shown for individual sites and for data pooled between sites. For all analyses df = 1. * indicates the only time that removal rates of aged caches were greater than fresh caches.

	Site 1	Site 2	Site 3	Fresh caches	Aged caches
a. July 2010					
Mean cache survival (Days)	60.7*	60.8	61.0	52.3	69.4
$\chi 2$	2.8	2.87	2.17	.72	
P value	0.094	0.090	0.140	0.390	
b. September 2010					
Mean cache survival (Days)	36.5*	36.6	36.6	34.4	38.7
$\chi 2$	.65	1.00	.44	.23	
P value	0.420	.320	.510	0.630	

Table 2. Percent (mean ± sd) of seed and soil moisture during Exp 1a (starting 17 July 2010) and Exp 1b (starting 11 September 2010) comparing the removal of fresh and aged caches. There were no differences in seed or soil water content during either trial.

	Soil			Fresh caches			Aged caches		
	Mean ± sd	Range	*n*	Mean ± sd	Range	*n*	Mean ± sd	Range	*n*
a. July 2010									
Water content (%)	0.30 ± 0.06	0.22- 0.39	35	3.2 ± 0.83	1.97 – 4.64	21	3.8 ± 0.51	3.20 – 4.79	21
b. September 2010									
Water content (%)	0.31 ± 0.03	0.28 - 0.37	30	4.4 ± 1.4	3.12 – 7.0	21	4.0 ± 0.66	3.06 – 4.91	21

Table 3. Mean number of days caches survived and comparisons between caches containing whole and shelled seeds (seeds lacking shells) for three open Jeffrey pine sites starting 9 September, 2010. Chi-square survival analyses are shown for individual sites. For all analyses df = 1. * indicates significant differences between treatment survivorship at two of three sites.

	Sites					
	4		5		6	
	Whole	Shelled	Whole	Shelled	Whole	Shelled
Number of caches removed / total number available	20/40	28/40	26/40	34/40	17/40	36/40
Mean cache survival (days)	12.7	9.31	9.88	7.55	11.95	8.23
$\chi 2$	3.33		5.78		21.54	
P value	0.068		0.016*		<0.001*	

Table 4. Percent (mean ± sd) of seed and soil moisture content starting 9 September 2010. Whole seeds contained a greater amount of water than shelled seeds (seeds lacking shells), and the difference was nearly significant (t = 2.16, df = 8.3, *P* = 0.062) (Figure 5).

	Soil (%)			Whole seeds (%)			Shelled seeds (%)		
	Mean ± sd	Range	*n*	Mean ± sd	Range	*n*	Mean ± sd	Range	*n*
Water content (%)	0.45 ± 0.06	0.39 - 0.54	35	3.9 ± 1.0	2.74 – 4.90	21	3.0 ± 0.45	2.69 – 3.82	21

Table 5. Foraging success (number of seeds found/number of cups dug in) of chipmunks during two laboratory trials comparing the removal of whole Jeffrey pine seeds to seeds with their shells removed. Chi-square analyses compare foraging success to random digging. For both trials df = 7.

	Dry sand		Wet sand	
	Whole seeds	Shelled seeds	Whole seeds	Shelled seeds
Percent success (mean ± sd)	11.6 ± 4.7%	11.7 ± 6.4%	18.0 ± 4.2%	19.5 ± 6.0%
$\chi 2$	4.06	7.10	10.73	6.35
P value	0.772	0.418	.151	.499

Table 6. Mean survival (days) estimated for treatments used to test for an interaction between cache type and age since cache burial. Exp 3a started on 21 August and Exp 3b was started on 20 September 2011. Chi-square survival analysis estimated significant differences in cache removal rates during the August trial ($\chi 2$ = 11.77, df = 5, P = 0.038), but not during the September trial ($\chi 2$ = 6.17, df = 5, P = 0.290).

	Treatment				
a. August					
Mean cache survival (days)	42.88	192.29	161.87	80.45	416.34
b. September					
Mean cache survival (days)	31.17	32.13	71.60	68.97	116.82

Table 7. Mean survival (days) estimated for each explanatory variable during two trials testing for an interaction between cache type and age since cache burial. Exp 3a started on 21 August and Exp 3b was started on 20 September 2011. Chi-square survival analysis estimated significant differences in cache type removal rates during the August trial ($\chi 2 = 8.98$, df = 5, $P = 0.011$), but not difference for the removal of caches based on the age of the digging disturbance ($\chi 2 = 0.23$, df = 5, $P = 0.630$. During the September trial there were not significant differences between either cache type ($\chi 2 = 4.15$, df = 5, $P = 0.130$) or age of digging disturbance ($\chi 2 = 3.26$, df = 5, $P = 0.071$).

	Explanatory variables				
	Age of digging disturbance		Cache type		
	New	Old	Fresh	Aged	Sham
a. August					
Mean cache survival (days)	113.67	139.38	45.57	119.78	233.94
b. Experiment 3b					
Mean cache survival (days)	45.64	92.90	31.17	50.55	93.66

Table 8. Mean survival (days) estimated for the removal of animal-made and artificial -paired caches in October 2011. For all sites df = 1. * indicates significantly lower rates of survival for animal-made caches at site 1 ($\chi 2 = 5.58$, $P = 0.018$), and site 2 ($\chi 2 = 5.09$, $P = 0.024$) using Chi-square survival analysis.

	Sites					
	1		2		4	
	Animal	Paired	Animal	Paired	Animal	Paired
Number of caches removed / total number available	14/18	8/18	27/33	25/33	7/14	6/14
Mean survival (days)	4.03*	14.92	4.16*	18.63	14.13	50.13

FIGURE LEGENDS

Figure 1: Rates of cache removal pooled between 3 open Jeffrey pine sites starting on 17 July 2010. Fresh caches were made that day and had never been in the soil previously. Aged caches were buried 10 days prior under exclosures and then relocated to the grids. For both treatments n =150. There were no differences in cache survival ($\chi 2$= 0.72, df=1, P = 0.390).

Figure 2: Rates of cache removal pooled between 3 open Jeffrey pine sites starting on 11 September 2010. Fresh caches were made that day and had never been in the soil previously. Aged caches were buried 10 days prior under exclosures and then relocated to the grids. For both treatments n =150. There were no treatment differences in cache survival ($\chi 2$= 0.23, df=1, P= 0.630).

Figure 3: Rates of cache removal pooled between 3 open Jeffrey pine sites starting on 2 September 2010. For both shelled and unshelled seeds n = 240 caches. * indicates significant differences in removal between treatment types; caches with seeds lacking shells were removed significantly faster at two of three of the sites ($\chi 2$= 23.98, df=1, P <0.001).

Figure 4: Foraging success (number of seeds found/number of cups dug in) during two laboratory trials testing for seed coat removal effects. Point estimates are mean

± sd. In both experiments $n = 8$. There were no differences in success during either trial.

Figure 5: Likelihood of digging (number of cups containing seeds dug in/ number of visits to cups containing seeds) during two laboratory trials testing for seed coat removal effects. Point estimates are mean ± sd. In both experiments $n = 8$.

Figure 6: Likelihood of failure (number of seeds missed/ number of visits to cups containing seeds) for cups containing treatments and empty cups during two laboratory trials testing for seed coat removal effects. Point estimates are mean ± sd. In both experiments $n = 8$.

Figure 7: Foraging success (Number of seeds found/number of cups dug in) during two laboratory trials testing chipmunks' ability to locate Jeffrey pine seed shells. Point estimates are mean ± sd. In both experiments $n = 7$. * Indicates that under wet conditions chipmunks found shells and seeds more frequently than random ($\chi 2 = 85.62$, df = 6, $P < 0.001$ and $\chi 2 = 59.09$, df = 6, $P < 0.001$).

Figure 8: Likelihood of digging (number of cups containing seeds dug in/ number of visits to cups containing seeds) during two laboratory trials testing chipmunks' ability to locate Jeffrey pine seed and Jeffrey pine shells. Point estimates are mean ± sd. In both experiments $n = 7$.

Figure 9: Likelihood of failure (number of seeds missed/ number of visits to cups containing seeds) for cups containing treatments and empty cups during two laboratory trials testing chipmunks' ability to locate Jeffrey pine seed shells. Point estimates are mean ± sd. In both experiments $n = 7$.

Figure 10: Foraging success (Number of treatments found/number of cups dug in), likelihood of digging (number of cups containing treatments dug in/ number of visits to cups containing treatments) and likelihood of failure (number of treatments missed/ number of visits to cups containing treatments) during a non-choice laboratory trial testing whether yellow-pine chipmunks detect and dig for Jeffrey pine shells. Points are mean ± sd. The success of chipmunks did not differ from random expectation.

Figure 11: Rates of cache removal pooled between 2 open Jeffrey pine sites starting on 21 Aug 2011. For all treatments n=40. Fresh caches were made on day 0 and had never been in the soil previously. Aged seeds were buried 10 days prior to day 0, the same day aged sham caches were prepared. The interaction between cache type and age since burial significantly affected cache survivorship estimated using chi-square survival analysis ($\chi 2 = 11.77$, df = 5, $P = 0.038$).

Figure 12: Removal rates from 1 open Jeffrey pine site in September 2011. For all treatments $n = 20$. Fresh caches were made on day 0 and had never been in the soil

previously. Aged caches were buried 10 days prior to day 0, the same day aged sham caches were prepared.

Figure 13: Rates of cache removal for 3 open Jeffrey pine sites in October 2011 following a rain event. For site a, *n*= 36, for site b *n*= 28 and for site c, *n*=66. At each site half of the caches were made by animals and half of the caches were identical ones we made ~30cm away from the original. Differences in survival between original and paired caches were only seen at site A ($\chi 2 = 6.75$, df = 1, $P = 0.0094$) indicated by *.

Fig. 1

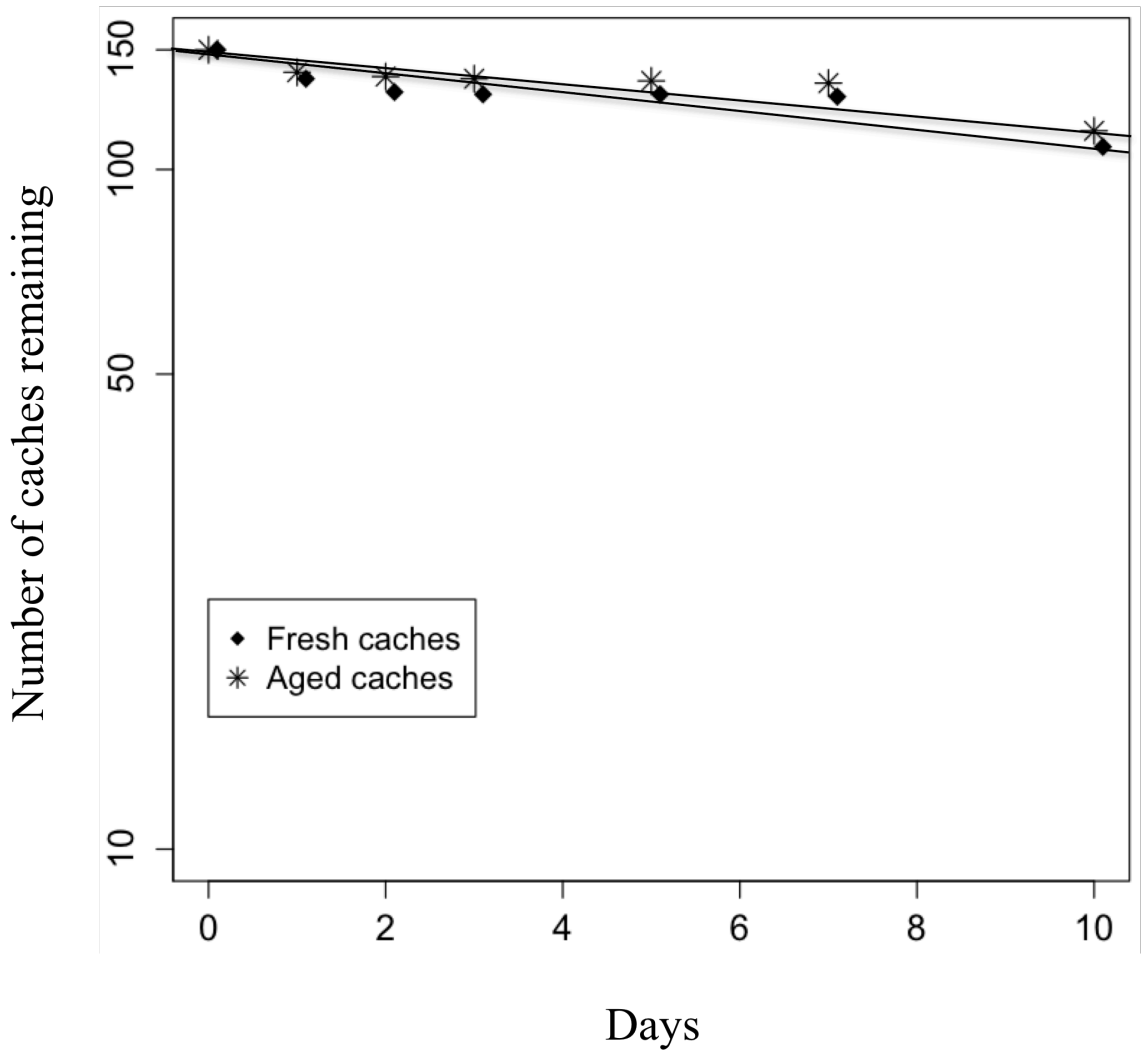

Number of caches remaining
150
100
50
10
0
2
4
6
8
10
Days
Fresh caches
Aged caches

Fig. 2

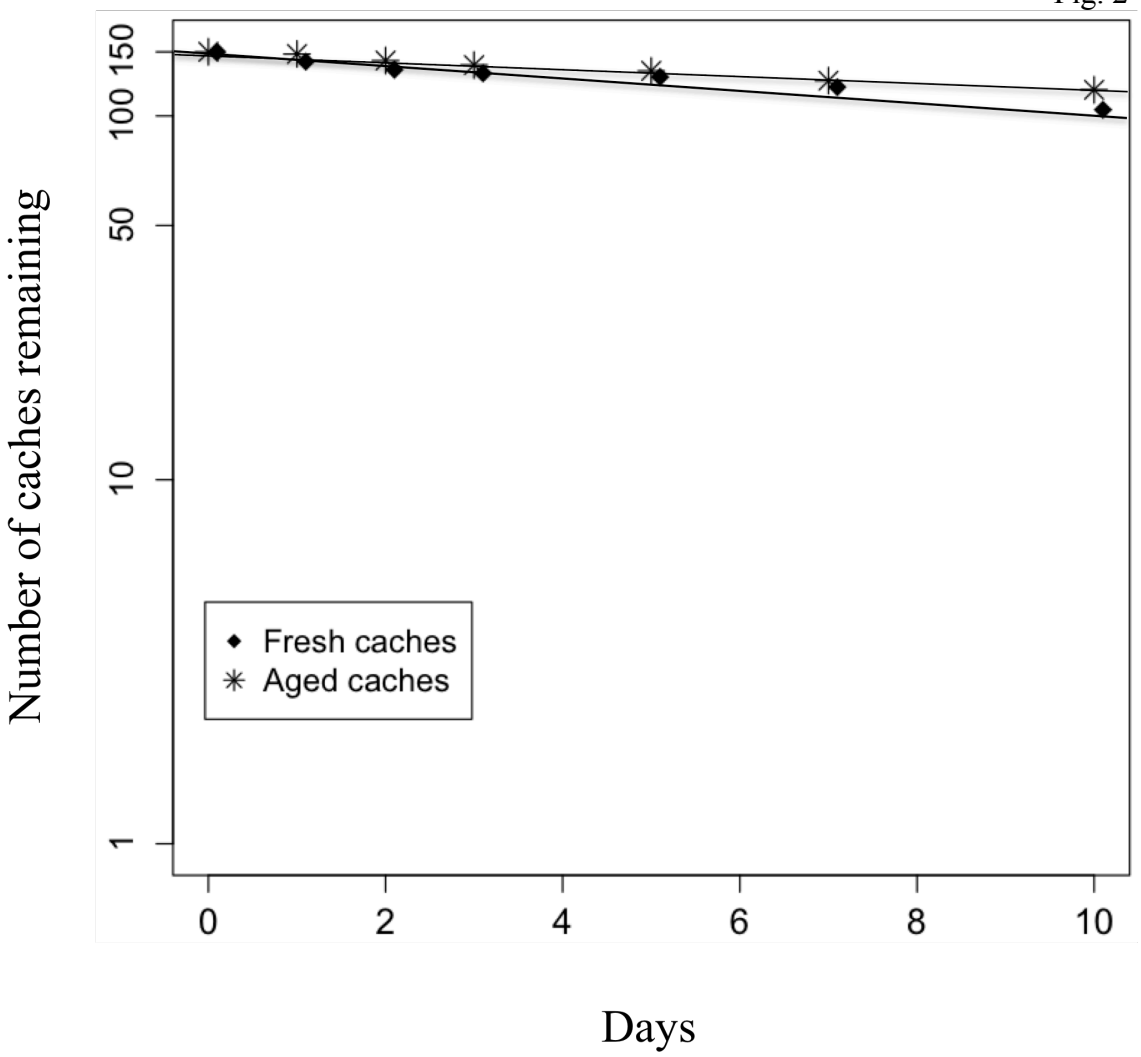

Number of caches remaining
150
100
50
10
1
0
2
4
6
8
10
Days
Fresh caches
Aged caches

Fig. 3

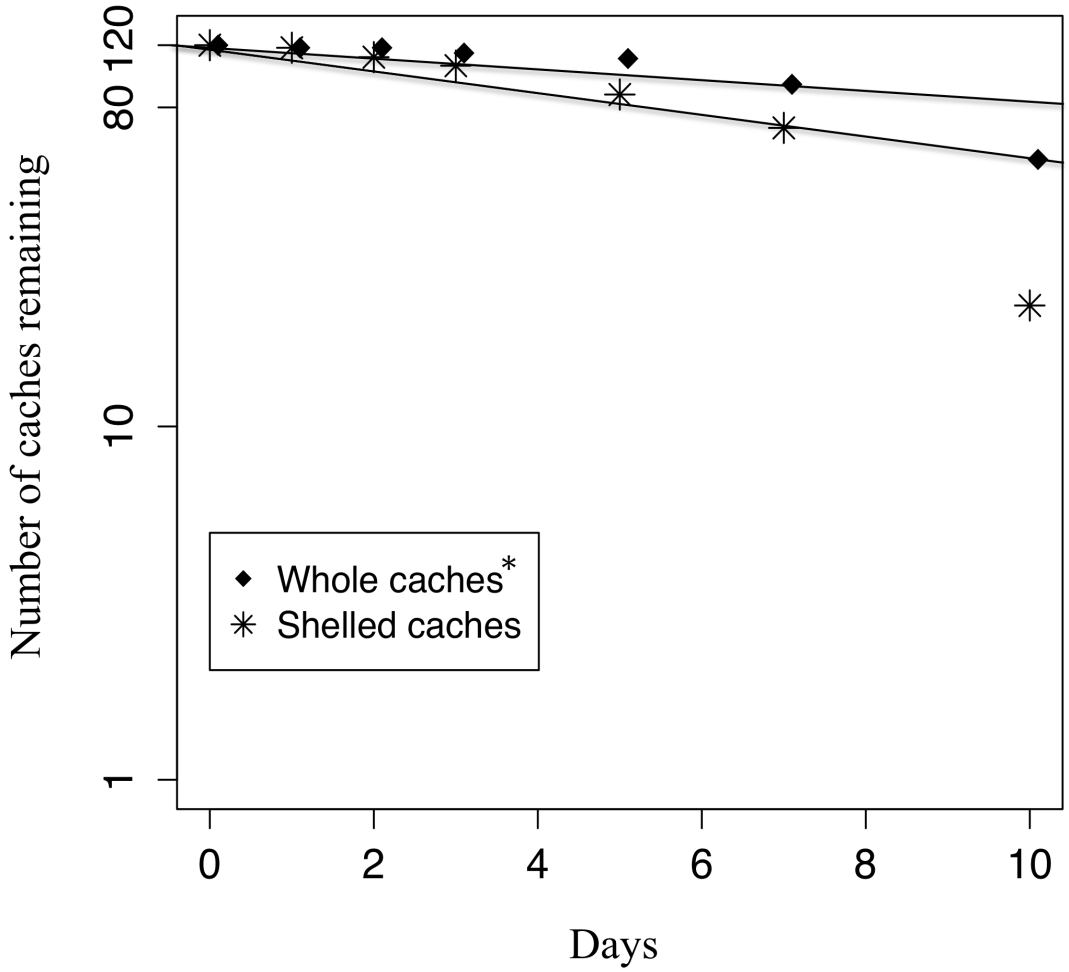

Number of caches remaining
80 120
10
1
0
2
4
6
8
10
Days
Whole caches*
Shelled caches

Fig. 4

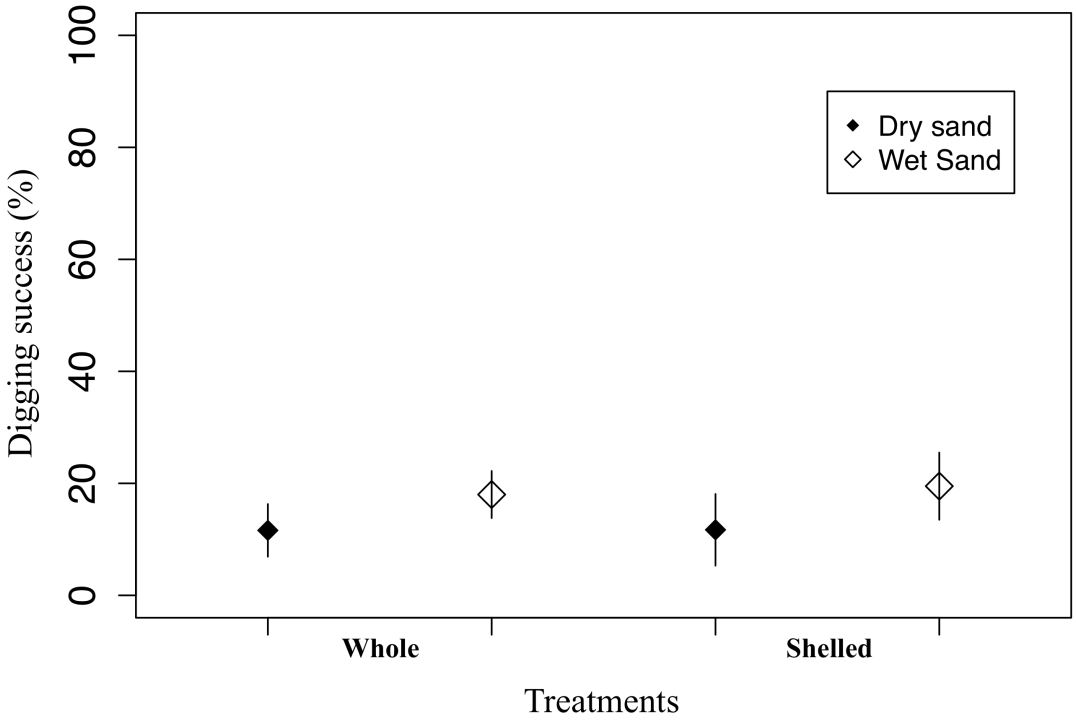

Digging success (%)
0
20
40
60
80
100
Dry sand
Wet Sand
Whole
Shelled
Treatments

Fig. 5

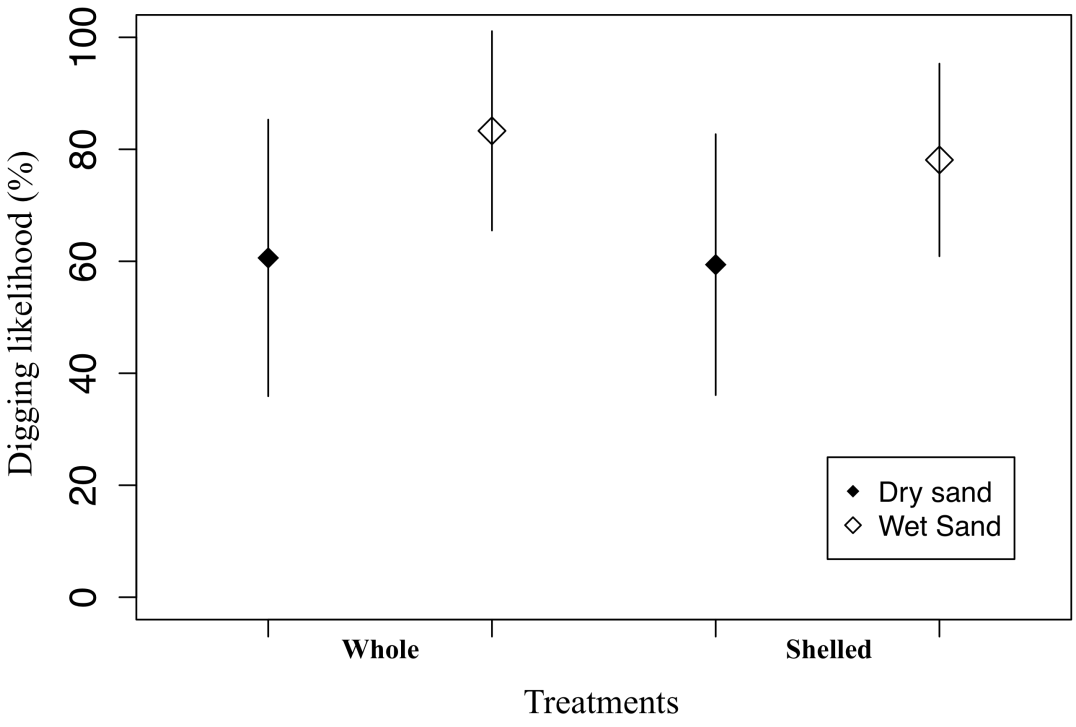

Digging likelihood (%)
0
20
40
60
80
100
Whole
Shelled
Treatments
Dry sand
Wet Sand

Fig. 6

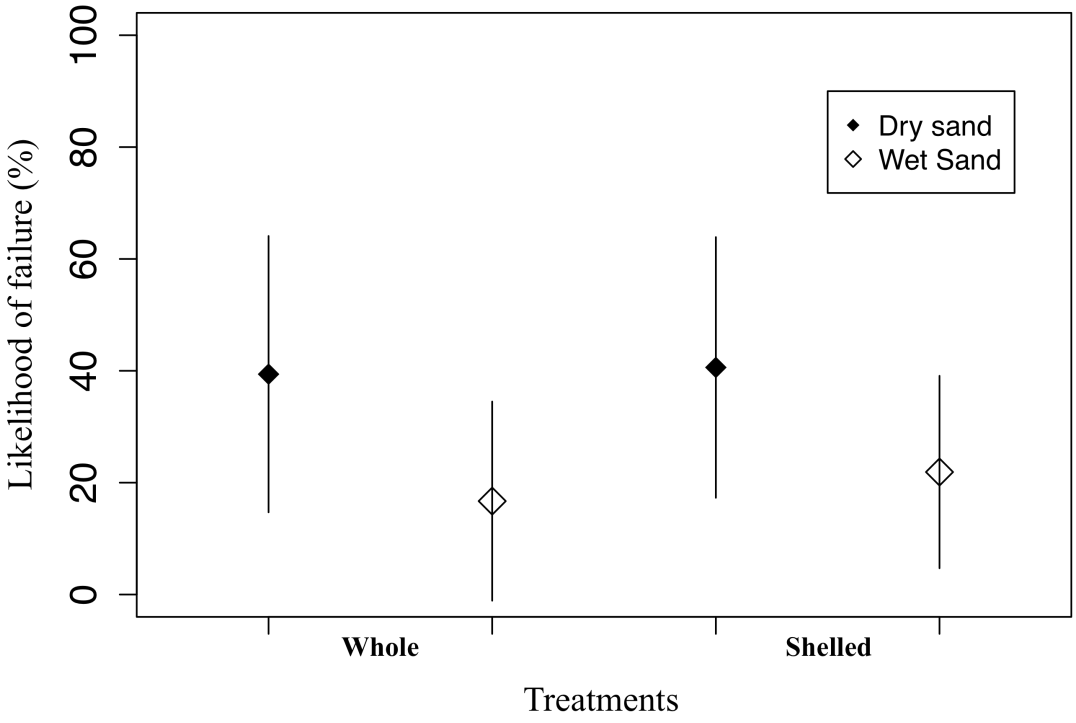

Likelihood of failure (%)
0
20
40
60
80
100
Dry sand
Wet Sand
Whole
Shelled
Treatments

Fig. 7

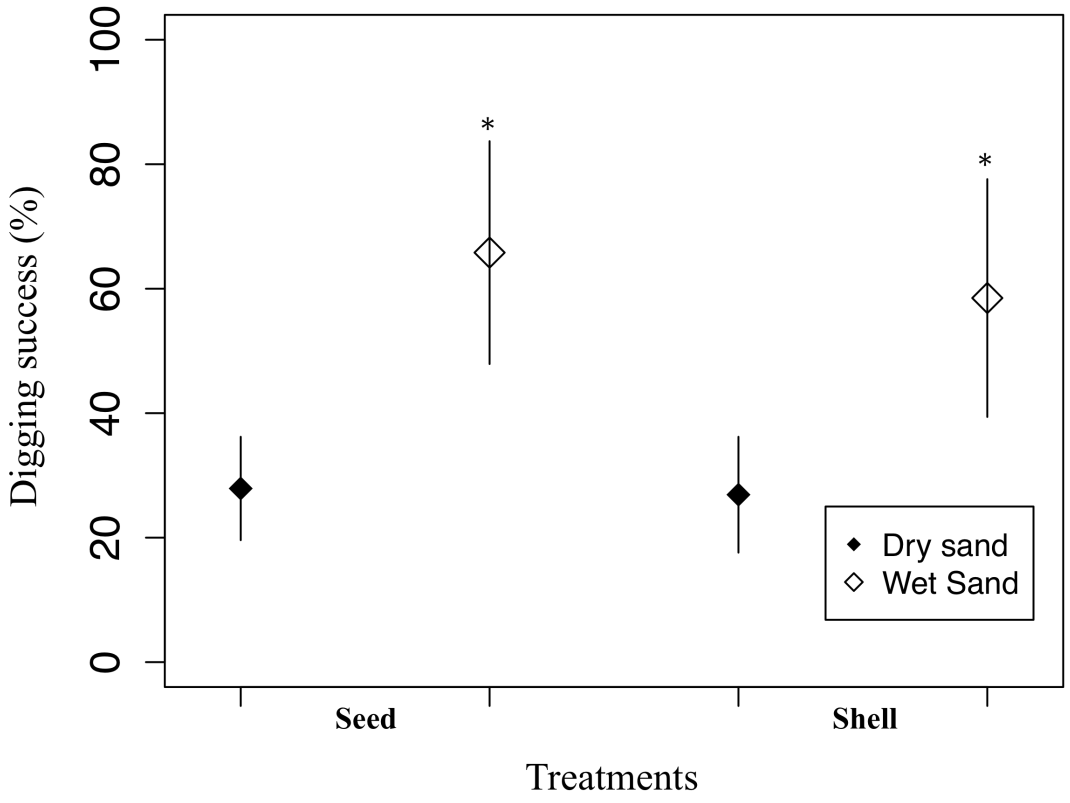

Digging success (%)
0
20
40
60
80
100
*
*
Dry sand
Wet Sand
Seed
Shell
Treatments

Fig. 8

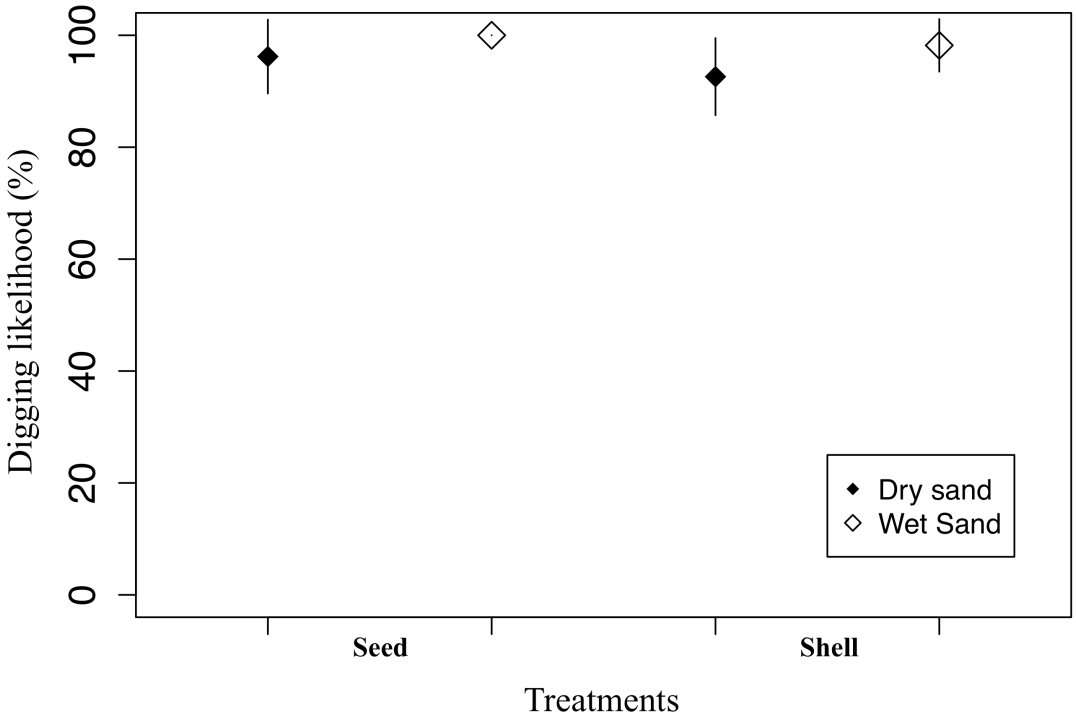

Digging likelihood (%)
0
20
40
60
80
100
Dry sand
Wet Sand
Seed
Shell
Treatments

Fig. 9

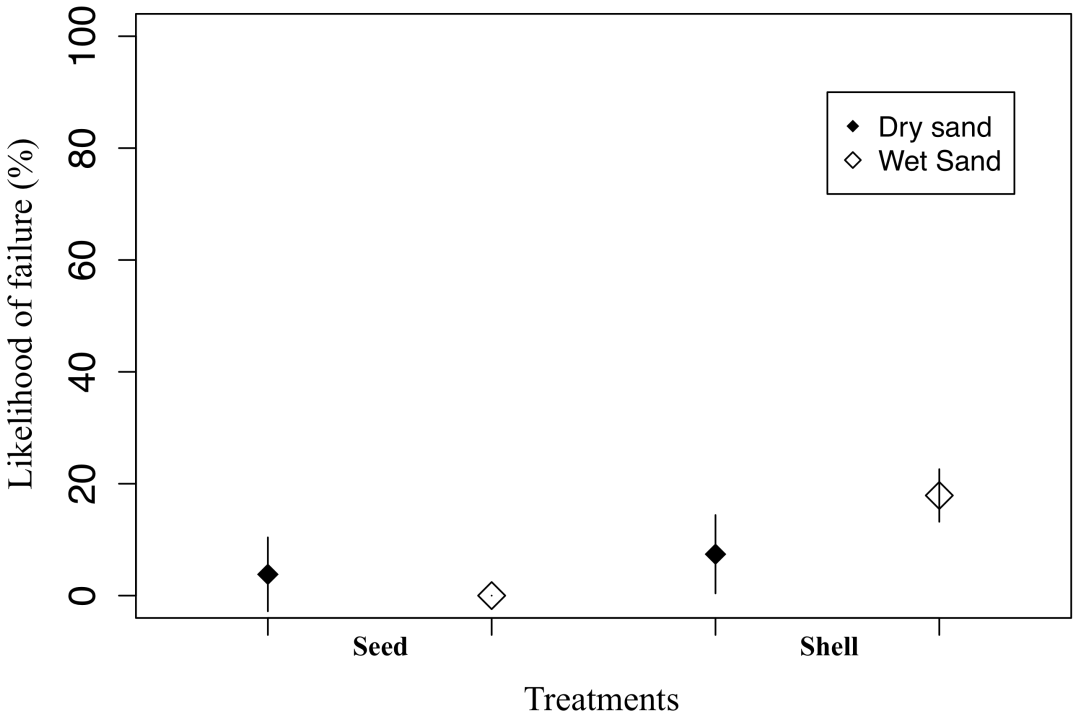

Likelihood of failure (%)
0
20
40
60
80
100
Dry sand
Wet Sand
Seed
Shell
Treatments

Fig. 10

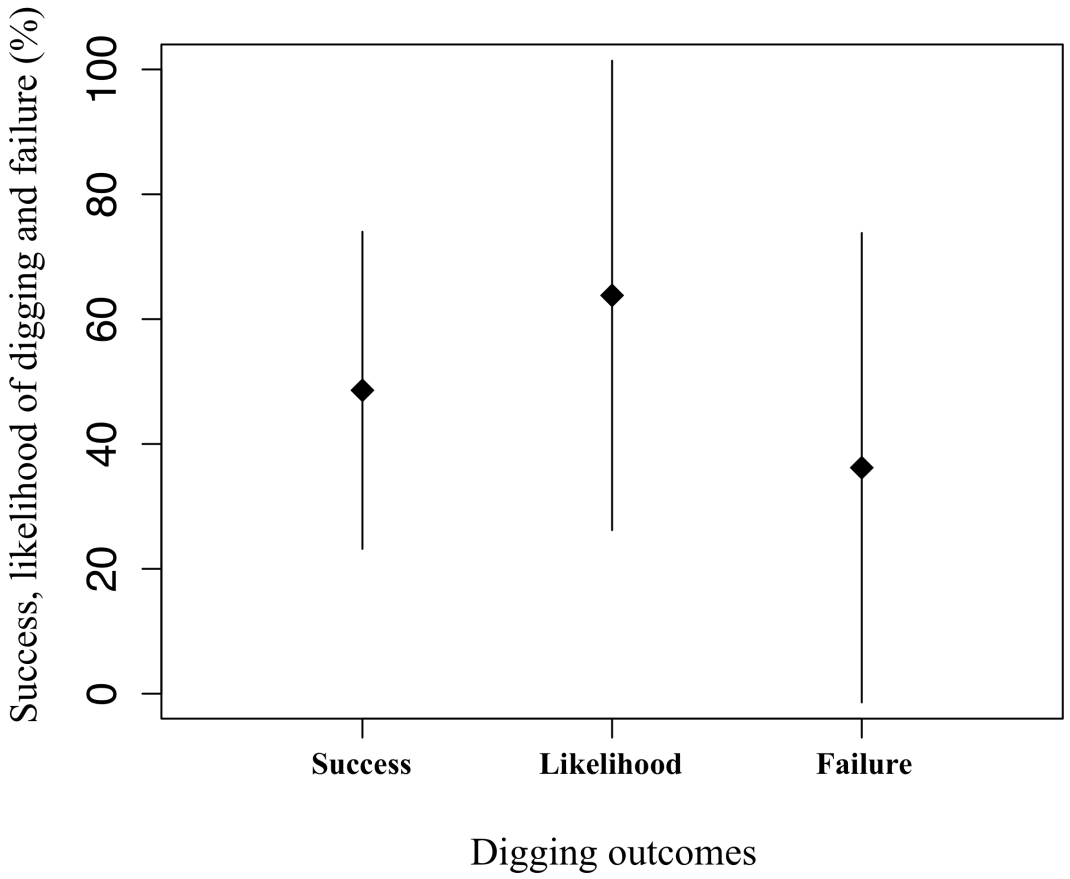

Success, likelihood of digging and failure (%)
0
20
40
60
80
100
Success
Likelihood
Failure
Digging outcomes

Fig. 11

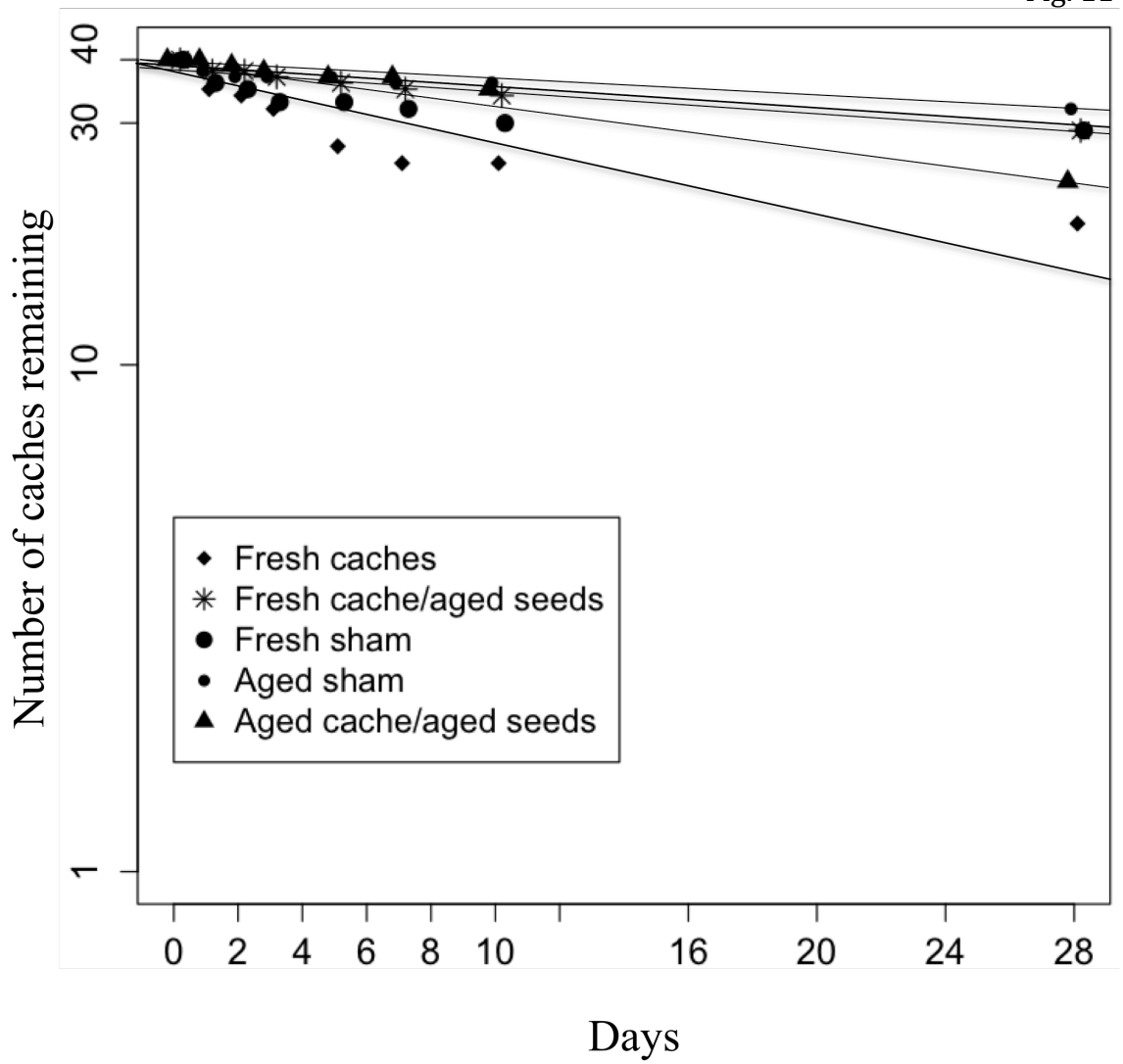

Number of caches remaining
40
30
10
1
0
2
4
6
8
10
16
20
24
28
Days
Fresh caches
Fresh cache/aged seeds
Fresh sham
Aged sham
Aged cache/aged seeds

Fig. 12

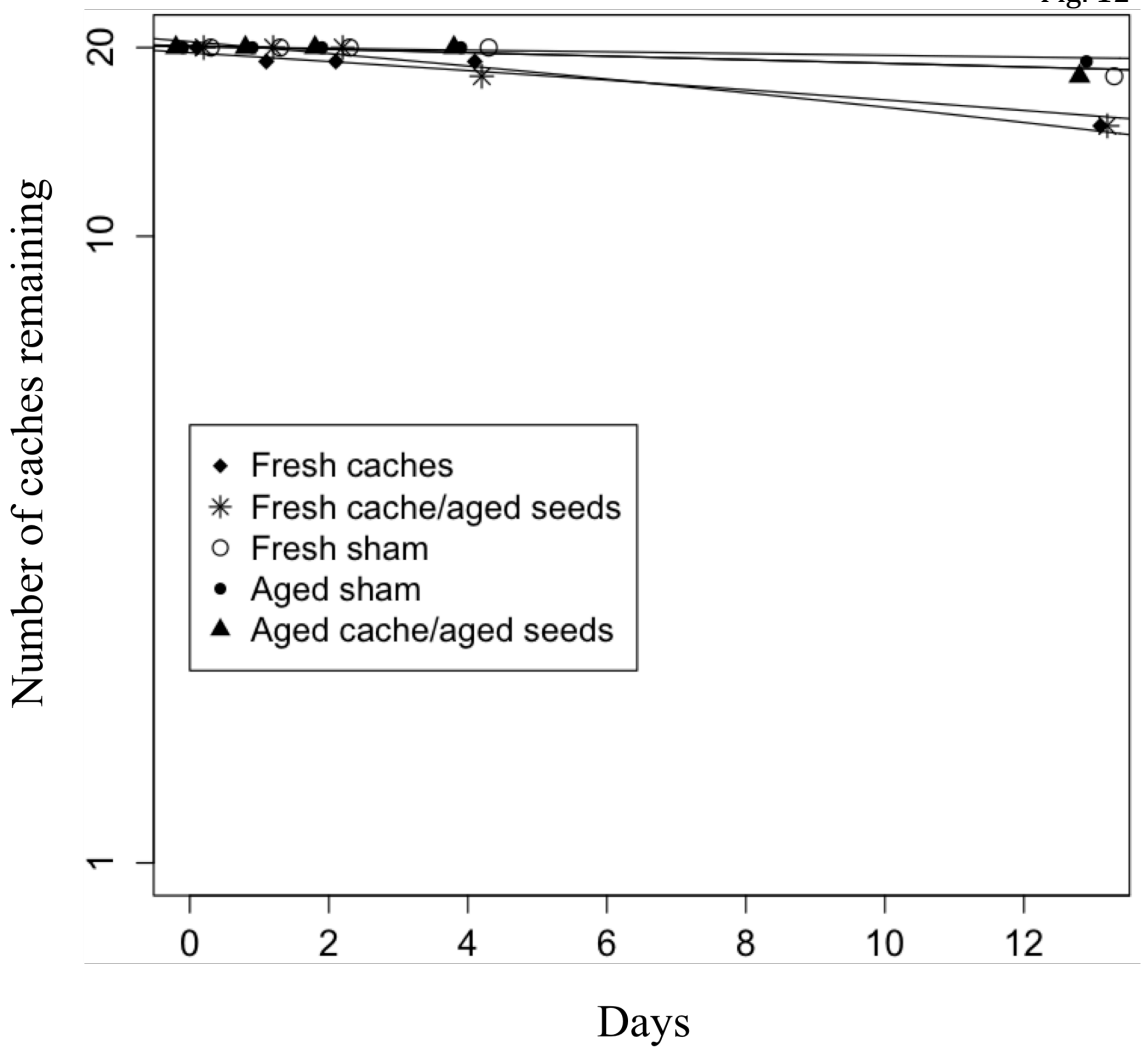
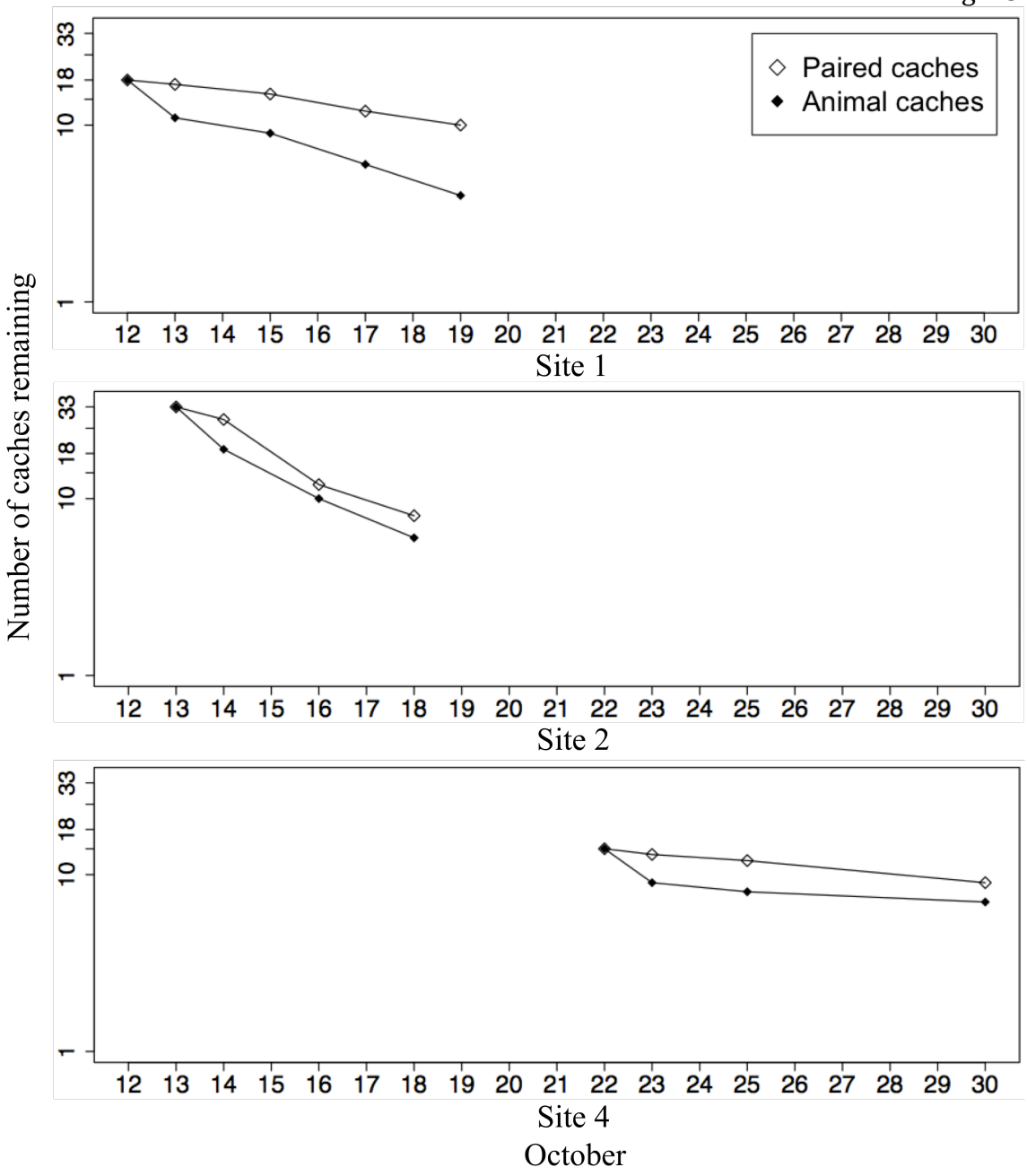

Number of caches remaining
20
10
1
0
2
4
6
8
10
12
Days
Fresh caches
Fresh cache/aged seeds
Fresh sham
Aged sham
Aged cache/aged seeds

Fig. 13


Paired caches
Animal caches
Number of caches remaining
33
18
10
1
12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Site 1
Site 2
Site 4
October

CHAPTER 2: Rodent interactions with volatile components of the Jeffrey pine (*Pinus jeffreyi*) seed and isolated macronutrients.

INTRODUCTION

During the long shared evolutionary history of seed-bearing trees and their biotic vectors of dispersal, fundamental shifts in diaspore morphology have occurred; seeds increased in size and nutritional quality, while developing increasingly durable seed-coats (Stiles 1989, Thayer & Vander Wall 2005, Vander Wall 2010). This trend has been especially apparent among pines (Pineaceae) that live in arid environments where there is often a suite of potential seed predators and dispersal agents (Vander Wall 2006). Granivorous rodents, the most ubiquitous and abundant mammals in arid ecosystems, function in both ecological roles, and it is thought that the coevolutionary processes functioning between pines and their rodent dispersers have been diffuse (Vander Wall 2001,Thompson 2006), such that plants rely upon multiple rodent species as dispersal agents (Vander Wall & Beck 2012). As highly effective density-dependent seed predators, rodents have the ability to impose selective pressures on their plant resources. Pressure from seed predators, along with the need to reward and attract dispersal agents, and be defended from pre-dispersal seed predators, pathogens and fungi (Stiles 1989, Jorgensen & Chesser 2000) are what have molded propagule characteristics.

In addition to adaptive seed morphology, plants may respond to selective pressures via the production of plant secondary metabolites (PSMs). These internally synthesized metabolites, or volatile organic compounds (VOCs), contain chemosensory

information pertinent to a sessile organism's ability to communicate and interact with its environment (Tholl et al. 2006). VOCs typically belong to a few classes of low-molecular weight, largely lipophilic, carbon-based compounds that easily diffuse into the atmosphere from a variety of internal and superficial plant tissues, including seeds (Dudareva et al. 2006, Tholl et al. 2006). Of the 1700 VOCs that have been isolated and described, the majority are compounds such as terpenoids, phenylpropanoids, benzoids, fatty acid derivatives, and animo-acid derivatives, although these types probably represent only about 1% of total plant secondary metabolites (Dudareva et al. 2006). Woody species are found to contain high levels of terpenoids, including hemiterpenes (C5), monoterpenes (C10), and sesquiterpenes (C15); all having a high vapor pressure (Dudareva et al. 2006).

The importance of chemosensory information contained in VOC's is classically recognized for its role in mammalian predator recognition, social communication, and resource assessment (Vander Wall 2003). In addition, chemical cues exert a large influence on plant-pollinator and plant-herbivore relationships (Freeland & Janzen 1974, Tholl et al. 2006). Experimentation has shown that volatiles indirectly affect tri-trophic interactions in 23 separate plant families, often allowing an insect herbivore species to sequester chemicals toxic to predators (Dudareva et al. 2006). These compounds are not only effective against insect herbivores, but mammlian foliavore and granivores as well. A variety of rodent foragers are known to consume plant parts or seeds that contain compounds capable of reducing weight, impeding digestion, delaying estrus, causing hair loss, causing neurological disorders or causing death at high concentratios (Freeland & Janzen 1974). Wood rats, *Neotoma sp.*, that are foliavores of Juniper (*Juniperus*

monosperma), regulate their intake of foliage partially based on their ability to detoxify PSMs (Henderson 1990). Henderson (1990) also found that a combination of nutritional, PSM, and morphological seed traits interact to create patterns of seed preference exhibited by *Dipodomys ordii* (Ord's kangaroo rat). In addition, many VOC's act as organic precursors that organisms can metabolize into beneficial compounds. For example, pine bark beetles in the family Scolytidae (Coleoptera), are able to synthesize an aggregation pheremone partially by hydroxylating terpenoid precursors released from their pine host (Blomquist et al. 2010). In addition, some mammals are able use terpenoid precursors to synthesize vitamins A, E and K (Freeland & Janzen 1974).

In addition to secondary metabolites, plant reproductive structures contain amino acids, proteins, organic acids, sugars, lipids, and ions that contribute to a bouquet of odors foraging rodents perceive (Vander Wall 1998). Secondary compounds in seeds likely serve functions separate from the nutritional components, that are primarily derived to benefit seedling germination and establishment (Vander Wall & Beck 2012). Furthermore, it is probable that seed embryos and seed coats contain both different types and amounts of volatile compounds (Moïse et al. 2005, Dudareva et al. 2006). Regardless if plant VOCs are in the embryo or seed coat, their presence is thought to increase seed handling costs and extend the amount of time a seed disperser and plant resource interact. This handling cost hypothesis predicts that VOCs will increase with the level of threat propagules encounter in the environment and serve to increase the likelihood a seed will be buried and remain so (Vander Wall & Beck 2012).

Food-hoarding rodents rely on temporally and spatially structured resources to obtain energy reserves that allow them to survive periods of resource scarcity (Vander

Wall 1990). Although rodents initially become aware of annual seed resources because they are visually apparent upon ripening (either ripening on the branch, or falling with the wind), scatter-hoarding species subsequently bury them, eliminating the usefulness of these cues. While most rodents use a combination of spatial memory and microsite characteristics to locate seeds they have cached (Thayer & Vander Wall 2005), these methods are not available for use in locating food items that have been buried abiotically, or by sympatric species (Vander Wall 1998). While seed availability and abundance are known to influence rodent foraging behavior, in most cases the effects of individual internal or superficial seed odors on behavior are not known (Jorgensen 2001). It is generally assumed that the effects of a given resource odor are due to the number, type, and concentration of all volatiles combined, versus the individual compounds making up a given scent (Dudareva et al. 2006). However, rodents differentiate between chemicals very accurately, and in some cases, such as the pregnancy blocking response of mice exposed to non-stud males, hard-wired behaviors are linked to specific odor cues (Bargmann 2006).

Olfactory capabilities are expected to be an important aspect of an animal's foraging strategy that contributes to overall fitness (Smallwood & Peters 1986). Factors affecting chemosensations (tastes and smells), that rodents receive from the environment, influence caching, pilfering, and consumption behaviors. Inter and intraspecific responses to chemosensations are therefore expected to impact resource partitioning within a community, and contribute to competitive exclusion via unequal resource discovery and pilfering, which may limit a species' range and distribution (Johnson & Jorgensen 1981, Tarraborelli 2009). Using the locally abundant Jeffrey pine (*Pinus jeffreyi)* and its

associated granivore community as a study system, I propose tests for three hypotheses, 1) Jeffrey pine seeds contain volatile terpenoids (VTs) that contribute to seed odor and are separate from nutritional components, 2) VTs are used as cues when rodents forage for buried seeds, and 3) Rodents can detect individual macronutrients. In order to investigate these hypotheses, I addressed 6 questions: 1) Does the removal of organic lipophilic compounds from Jeffrey pine seeds lower the rate of cache detection? 2) Do individual VOCs found within Jeffrey pine seeds elicit digging behavior? 3) Can rodents detect protein, carbohydrate or lipid macronutrients without additional tactile or olfactory cues provided by seeds? 4) What volatile terpenoid compounds are present in the chemical profile of Jeffrey pine seeds? 5) Are the compounds in the shell the same as the seed? 6) does the chemical profile change under wet and dry conditions?

In the selected study system, Little Valley, NV, both sciurid and murid rodent species are dispersal agents of the locally abundant Jeffrey pine. Yellow-pine chipmunks (*Tamias amoenus*) are particularly efficient dispersers that provide high-quality dispersal of Jeffrey pine seeds: systematically caching seeds in non-random locations (Briggs et al. 2009), avoiding areas of dense litter where fitness is reduced, and thereby positively affecting pine seedling survival (Briggs et al. 2009). Yellow-pine chipmunks, lodgepole chipmunks (*T. speciosus*), golden-mantled ground squirrel (*Spermophilus lateralis*), Douglas squirrel (*Tamiasciuris douglasii*), California ground squirrel (*Spermophilus beecheyi*), and deer mouse (*Peromyscus maniculatus*), are all found at the study site, and many of them have been shown to use a combination of olfaction, random digging, and spatial memory to locate buried seeds (Vander Wall et al. 2009). Clark's nutcracker (*Nucifraga columbiana*) and Stellar's jay (*Cyanocitta stelleri*) also cache seeds within the

study site, but lack the ability to use olfaction when locating seeds (Vander Wall 1982, Thayer & Vander Wall 2005). Exploitation of Jeffrey pine seeds by diverse taxa within this study site creates an ideal system to begin exploring factors that affect the olfactory cues of buried seeds.

METHODS

General Methods

I studied the foraging behavior of four male and four female yellow-pine chipmunks between December 2010 and April 2012 in a laboratory at the University of Reno, Nevada. These eight experimentally naïve individuals, were captured, sexed, weighed, and ear-tagged in Little Valley, NV on 13 October 2009. Following capture, and while trials were conducted, I housed rodents at the Fleischmann Agriculture building in separate 48 x 27 x 20 cm plastic cages and provisioned them with quart-sized glass jars and cotton bedding for nesting, Sani-chips® on the cage floor, black-oil sunflower seeds, Hekklah® rodent pellets, and ad libitim access to water. Lighting in the room operated on a 12:12 hour light-dark cycle. I cared for animals according to University of Nevada, Reno's, Institutional Animal Care and Use Committee protocol that was consistent with guidelines set by the American Society of Mammalogists (Gannon & Sikes 2007).

Individual chipmunks participated in two trial types; training and experimental, all of which were conducted within a 2.4 x 3.6 m indoor arena located in the basement of the Fleischman Agriculture building. One wall of the arena had a door, a one-way glass observation window, and an opening leading to a nest chamber that

eliminated the need to handle animals when removing them from the arena. A water bottle was mounted on the opposing wall so animals were never water limited during trials, a condition with the potential to bias food choices or stress the animals. The arena floor contained 48 equal sized holes, spaced 26.5 cm apart in a 6 x 8 array. I placed cups made of PVC tubing (52 mm diameter x 110 mm deep) into each hole, flush with the plywood flooring, and completely filled them with clean dry sand. During all trials, I buried treatments ~1 cm deep in the center of the cup, and leveled the sand surface to obscure visual cues, which I did using forceps or a spoon to avoid contaminating treatments with human odors. I arranged natural objects, such as rocks and sticks haphazardly between cups. Their positions remained the same for all subjects in a trial, but I rearranged them before each new experiment. I placed a radio in the arena and played white noise during trials to mask potentially stressful sound. A CCD-VXS Sony Hi-8 Video Camcorder was mounted on the ceiling to record every individual's session during an experimental trial.

I randomized the order in which subjects participated in trials, and always completed one trial type before testing an individual again. Following each individual session, I immediately removed the animals from the arena and gave them access to food and water in their home cages. After all 8 individuals had completed a training or experimental trial, I removed the cups, swept, vacuumed, mopped the floor with hot water to remove odors and other markings, and refilled all cups with fresh sand. Between subjects in both training and experimental trials, I swept the arena and removed any excrement with hot water. During experimental trials, between subjects, I emptied the cups that had contained treatments and filled them with fresh sand.

Training trials

I acclimated subjects to the foraging arena and trained them to search the cups for buried items using whole Jeffrey pine seeds during 9 trials that were conducted between 20 December 2010 and 20 February 2011. Trials lasted up to 3 hours per individual. For the first three trials I placed one seed on the sand surface of 12 cups. During the next three trials, I placed 1 seed half-buried in 12 cups, and during the final three trials, I completely buried one seed 5-10 mm deep in 12 cups. I randomly selected cups to contain seeds for each individual, during all nine trials, and only used dry sand as a substrate.

Experimental trials

Subjects participated in a total of 16 experimental trials between 25 February 2010 and 29 March 2012. In the autumn of 2011, one individual died of unknown causes, leaving a total of 7 subjects in the spring of 2012. Prior to all experimental trials, we food-deprived chipmunks to standardize foraging motivation. Deprivation typically lasted for ~12 hours, most of which occurred during the night when animals often do not feed. During experimental trials, I allowed individuals one to two hours to forage, and I videotaped each trial.

Following an experiment, I watched the videos to note the order in which cups were visited and the action taken during each visit. I recorded actions as checking (the rodent's nose directly over a cup), successful dig (digging and exposing contents when a treatment is present), or unsuccessful dig (digging when no treatment is present, or

digging when a treatment is present and failing to locate the treatment). I also recorded the time of subsequent visits to cups containing treatments, and the action of all subsequent visits. I translated each rodent's session for a period of 1 hour after the individual began to forage, however, if less than half of the cups had been visited after an hour, I continued to watch the tape for another half-hour. If all cups and treatments had been dug up before the hour was over, the trial was considered complete.

Whenever I used Jeffrey pine seeds, I weighed them to eliminate unfilled seeds, and selected them to be within 2 SD of the mean mass (160.2 mg $\pm$ 54.2 mg) (Vander Wall 2008). When a trial required wet sand, I applied ~3 ml of distilled water directly to the sand after leveling the surface.

Experiment 1a: Lipid extraction

To test the prediction that removal of organic lipophilic compounds from Jeffrey pine seeds will lower foraging success, two experimental trials were conducted. I ran trials between 5 and 14 April 2011 using whole seeds and again between 19 and 20 May 2011 using shelled seeds. I used wet sand for both trials. For both trials I separated seeds into two treatment types: seeds lacking lipophilic compounds and control seeds. I extracted lipophilic compounds by soaking seeds in GC-grade pentane for 30 minutes during which time I soaked control seeds in distilled water. I then removed seeds from the liquid and air-dried them under a hood at ~25°C for 48 hours. During trials I buried 6 seeds of each treatment individually in randomly selected cups so that individuals had a 12.5% likelihood of finding each treatment by chance.

Experiment 1b: Lipid extraction in the field

To test the prediction that the removal of organic lipophilic compounds from Jeffrey pine seeds will lower the rate of cache detection in the field, I established wandering transects in five open Jeffrey pine sites in Little Valley, NV, at the University of Nevada's Whittell Forest and Wildlife Area in Washoe County. Each transect contained 40 cache sites, spaced 5-8 m apart, and I placed flagging after every fifth cache, approximately half of a meter to the side, to indicate the transect route. Cache locations were recorded through the use of inconspicuous, unmoved, natural objects, such as pine cones, rocks, or sticks, and their arrangement to surrounding vegetation and rock. I established transect and cache markers on 22 June 2011, 5 days prior to seed caching, and prepared seeds on 24 June in the lab. I separated 400 Jeffrey pine seeds into sets of 2 seeds and placed each set in a separate glass test tube. I removed the shells of 100 sets and then weighed all sets to obtain pre-treatment mass. I extracted lipophilic compounds from 50 shelled and 50 whole sets by adding ~10 ml of GC-grade pentane to each test tube and allowing seeds to soak for 30 minutes. At the same time, I soaked the remaining, 50 shelled and 50 whole, sets in distilled water. Afterwards, I removed the liquid, allowed seeds to air-dry under a hood at ~25°C for 48hr, and reweighed each pair of seeds.

10 sets of each treatment type were alternately buried ~1 cm deep along transects on 27 June 2011. I monitored cache detection each afternoon for the first seven days, and again on days 10, 14, 17, 22, and 31. On day 31, I inspected all

caches to verify presence/absence. A cache was considered detected when rodents 1**)** had removed seeds, 2) had exposed seeds, 3) had dug directly above the cache but not removed it, or 4) had eaten seeds (shells nearby).

Rodent Abundance

The abundance and species of small mammals were determined by trapping for four consecutive days, from 8 to 11 October 2011, at three open Jeffrey pine sites in Little Valley. 40 Sherman live traps were set in a 4 x 10 array with ~10 m spacing. Traps were covered with pine needles for shade, baited with black-oil sunflower seed and checked in the early morning and early evening. Captured rodents were identified to species, sexed, weighed (in grams), checked for reproductive status, and ear-tagged to establish minimum number present on grids.

Experiment 2: Volatile compounds

To determine if individual terpenes, found within Jeffrey pine seeds, can illicit digging behavior, laboratory trials were run on 14 and 15 June 2011 using beta-pinene ((-)ß-pinene) and during 26 and 29 March 2012 using limonene ((-)R-limonene). I used dry sand as a substrate for both trials and presented compounds and controls to individuals using .5 x .5 cm pieces of filter paper (hereafter squares) prepared in glass Petri dishes ~15 minutes before releasing animals into the arena. I applied 2-µL of distilled water to all 12 squares and added .2-µL of (-)ß-pinene to 6 squares in the first trial so that animals had a 14.3% likelihood of finding a square by chance. In the second trial I applied .2-µL of distilled water to 16 squares and added .2-µL (-)R-limonene to 8, so that animals had a

20.0% likelihood of finding a square by chance. In both trials, immediately after preparing treatments, I buried them ~1 cm deep in randomly selected cups. In preliminary trials, filter paper was determined not to affect treatment removal rates. In addition to data regularly taken from videotapes, I recorded the amount of time spent digging at each treatment during each visit.

Experiment 4: Macronutrients

To determine whether subjects could detect individual macronutrients isolated from other chemosensory or tactile information provided by seeds, a series of 4 experimental trials were run between 20 January and 29 March 2012. I presented chipmunks with pure protein, pure carbohydrate, and pure lipid during three, non-choice, dry sand trials, and presented them with pure carbohydrate and halved Jeffrey pine seeds (containing shells) during the fourth, choice, trial. I used wet sand in the later trial, to test for a possible increase in macronutrient detection under wet conditions, and to ensure rodents found food rewards necessary for maintaining their motivation to forage. I used 1 x 1 cm square pieces of filter paper to present animals with food material. Treatments were prepared ~15 minutes before I released an animal into the arena. To prepare protein and carbohydrate treatments, I briefly submerged 16 squares in distilled water and placed them directly into petri-dishes containing pure rice protein (Nutribiotic® pure vegan rice protein) or pure starch (Bob's Red Mill® all natural corn starch), until ~100 mg had adhered. To prepare lipid treatments, I briefly submerged 16 squares into pure lipid (Mazola® Corn oil) until ~50 mg had absorbed. During each non-choice trial, 16/48 cups contained treatments, giving individuals a 33 % likelihood of finding a cache by chance,

and during the fourth trial, treatments were present in 8/40 cups, so that the likelihood of success was 20 %.

Experiment 5: Gas chromatography

I measured the volatile compounds released from Jeffrey pine seeds under five conditions; 1) whole dry seeds, 2) whole wet seeds, 3) dry seeds without shells, 4) dry shells without seeds, and 5) dry parasitized seeds (seeds with apparent exit holes or frass). Solid phase micro-extraction (SPME) paired with gas chromatography/mass spectrometry (GC/MS) headspace analysis techniques were used under all conditions. I collected Jeffrey pine seeds in Little Valley, NV during September 2012 and stored them in a refrigerator over winter. During the spring, I placed six groups of 20 seeds into separate 20 ml headspace vials and recorded their mass. I added distilled water to three of the vials until the mass had increased ~1 g, enough so that seeds would fully imbibe. I then sealed all vials with Perkin Elmer® crimping aluminum caps and silicone seals, and allowed them to sit for 24 hours. Headspace samples were collected using a 100 µm polydimethylsiloxane coated SPME fiber that I placed in the vial for 20 minutes prior to GC/MS analysis. The GC contained a DV-5 capillary column, carrier gas Helium, a spitless mode, flow-rate was 1.4 ml min^{-1}; the column initial temperature was 35°C for 4 minutes followed by an increase to 275°C at 10°C min^{-1} and 275°C was held for 5 minutes. Transfer line temperature was 180°C and total acquisition time was 33 minutes. Volatiles were scanned using an Agilent 5973 *Network* Mass Selective Detector.

Data Analysis

I analyzed data from the laboratory trials using three measures of digging success; success rate (number of treatments found/number of cups dug in; first visits only), likelihood of digging in a filled cup (number of filled cups dug in/ number of filled cups visited), and the likelihood of failing to find a treatment when one was present (number of treatments not removed/number of cups with treatments dug in). Probabilities were acrsine square-root transformed to normalize the data, and I used one-way ANOVAs to compare success, likelihood, and likelihood of failure. Chi-square goodness of fit analyses was used to compare digging success to chance values. Data from field removal transects was analyzed using multi-sample survival analysis in Program R (*package-survival*). Intervals were censored due to sampling on non-consecutive days 1,2,3,5,7,10, and a Weibull distribution was used. Cache survival was analyzed using last day present and first day absent as parameters. Log-transformed daily number remaining were used to fit decay lines.

RESULTS

Experiment 1a: Lipid extraction— In the first of two laboratory trials testing whether rodents were able to detect whole Jeffrey pine seeds lacking lipophilic compounds, chipmunks removed control and extracted seeds at similar rates; 23.7 ± 7.5 % for control seeds and 24.3 ± 7.1 % for extracted seeds ($F_{1,12} = 0.028$, $P = 0.870$). Extracted seeds had been soaked in pentane to remove lipophilic compounds, while control seeds had been soaked in distilled water. Digging success for both seed types did not statistically differ from the expected rate of 14.3 % (Table 1). When shells were removed from the seeds during the second laboratory trial, chipmunks dug for both control (35.1 ± 28.5 %) and extracted treatments (43.1 ± 29.4 %) more than expected (Table 1), although again,

extracted and control seeds were found with similar success ($F_{1,14} = 0.147$, $P = 0.707$) (Table 1). Although success rates were higher during the shelled trial, chipmunks' likelihood of digging remained high during both trials (Figure 2, Table 1). Chipmunks failed to find control seeds more often than extracted seeds during both trials (Figure 3, Table 1), although in no case was the difference significant.

Experiment 1b: Lipid extraction in the field— The rate of removal for control and extracted Jeffrey pine seed caches in the field did not follow the predicted pattern. Both whole control and shelled control seeds, those not treated with pentane for lipid extraction, were removed at lower rates than seeds that were treated for extraction. Whole control seeds were found at 0.59% per day, compared to a rate of 1.3% for whole extracted seeds, and shelled control seeds were removed at 7.3% per day compared to extracted shelled seeds at 8.9% (Figure 4)(Table 2). Mean survival of the different cache treatments was estimated to be 72.6, 42.3, 30.0, and 22.8 days, respectively (Table 2). This range in values resulted from the presence of a significant interaction between seed shell removal and lipid extraction when Chi-square survival analysis was conducted on pooled site data ($\chi2 = 99.83$, df = 3, $P < 0.001$). There were no differences between sites' cache survival ($\chi2 = 7.96$, df = 4, $P = 0.093$). When treatment effects were isolated, survival was most strongly impacted by the presence of the seed coat ($\chi2 = 95.25$, df = 1, $P < 0.001$), and lipid removal through pentane extraction did not significantly decrease detection ($\chi2 = 2.66$, df = 1, $P = 0.100$) (Table 3).

During the study a high number of small rodents were found in the areas used. Trapping at three sites between 8 and 11 Oct 2011 yielded a total of 56 yellow-pine

chipmunks, 14 deer mice, 3 long-eared chipmunks, 8 golden-mantled ground squirrels and 1 jumping mouse. There was an average of 18.7 ± 8.4, 4.7 ± .6, 1, and 2.7 ± 1.5 animals, per site, respectively. The relative abundance of species is similar to past years in this study site (Vander Wall 1992).

Experiment 2: Volatile Compounds—To test whether yellow-pine chipmunks can detect (-)β-pinene, treatments were present in 6/42 cups in the foraging arena, so that chipmunks had a 14.3% chance of finding treatments randomly. Chipmunks removed control, distilled water, treatments with 17.3 ± 10.5 % success and found β-pinene treatments 33.6 ± 11.5 % successfully (Figure 5). The higher removal of β-pinene treatments ($F_{1,12} = 7.46$, $P = 0.018$) was also statistically higher than random removal ($\chi 2 = 25.32$, df=6, $P < 0.001$) (Table 4). Differences in the likelihood of digging up each treatment, and the likelihood of failing to dig up each treatment, were also significant ($F_{1,12} = 7.46$, $P = 0.018$, and $F_{1,12} = 7.46$, $P = 0.018$, respectively) (Figure 5). Chipmunks were much more likely to dig at a cup, when β-pinene was encountered (Table 4).

I used 16 cups to test if chipmunks detect and dig for (-)R-limonene, so that each treatment was present in 8/40 cups, and individuals had a 20.0 % chance of finding treatments randomly. Chipmunks removed control treatments with 19.8 ± 10.2 % success, and R-limonene treatments 27.8 ± 13.5 % successfully (Figure 6). Success in finding both treatments followed a random pattern (Table 4), and neither was found more successfully ($F_{1,12} = 1.617$, $P = 0.228$). Chipmunks' likelihood of digging and likelihood of failure was also similar for both treatments ($F_{1,12} = 0.900$, $P = 0.362$ and $F_{1,12} = 0.743$, $P = 0.406$, respectively) (Figure 6).

Experiment 4: Macronutrients—During all non-choice tests using isolated macronutrients, 16 cups contained treatments so that individuals had a 33.3 % probability of finding a treatment at random. When detecting protein, chipmunks dug more frequently than random (53.8 ± 20.0 %, $\chi 2 = 32.19$, df=6, $P < 0.001$). They dug at carbohydrate treatments in a random pattern (38.4 ± 18.2 %, $\chi 2 = 6.94$, df=6, $P = 0.327$), and at lipid treatments, more frequently than random (68.2 ± 13.4 %, $\chi 2 = 44.51$, df=6, $P < 0.001$) (Table 5) (Figure 7). Chipmunks were more likely to dig at protein treatments (82.7 ± 22.6 %) and lipid treatments (80.5 ± 18.3 %) than carbohydrates (53.7 ± 18.7 %) when a treatment was present (Figure 7), although direct statistical comparisons are not appropriate, because trials were carried out on different dates. Chipmunks failed to dig up carbohydrate treatments most often (55.5 ± 13.9 %), followed by lipid treatments (19.5 ± 18.3 %), and then protein treatments (17.3 ± 22.6 %) (Figure 7). When carbohydrate treatments were presented to chipmunks in a separate trial using wet sand, following low detection under dry conditions, 8 Jeffrey pine seeds and 8 carbohydrate treatments were presented so individuals had a 20.0% of finding a treatment by chance. Chipmunks removed Jeffrey pine seed treatments with 68.1 ± 23.5 % success, and were only 16.1 ± 19.1 % successful at locating carbohydrates under wet conditions (Figure 8). Again digging rates for carbohydrates were not greater than chance ($\chi 2 = 4.90$, df=5, $P = 0.428$). The high success rate for Jeffrey pine seed, was however, significantly greater than random ($\chi 2 = 80.04$, df=5, $P < 0.001$)(Table 5), and statistically greater than carbohydrate success ($F_{1,10} = 12.09$, $P = 0.005$).

In all experiments using filter paper to present chipmunks with macronutrients, I recorded evidence of treatments being handled with individuals forepaws and mouths. In some cases the entire contents of a single treatment had been licked from the paper, however in other cases the paper was ignored. Only four animals handled carbohydrate treatments, although one did so extensively (longer than 20 seconds) during a single visit. Animals handled lipid treatments most often.

Experiment 5: Gas chromatography— There were 16 compounds that regularly appeared in chromatographs of Jeffrey pine seeds. Wetted whole seeds showed the greatest number of compounds (13), followed by the shells alone (11), whole dry seeds (10), parasitized seeds (9), and seeds only (5) (Table 6). All of the compounds found in dry seeds were also found in wet seeds with the exception of α-campholenal and wet seeds had an additional four compounds. The seeds alone had the fewest compounds, yet were the only from which γ-caprolactone was recorded. Only two compounds were found in all sample types: 1R α- pinene and β-pinene. Limonene was absent in parasitized seeds and seeds without shells.

DISCUSSION

The treatment of Jeffrey pine seeds with pentae for the removal of lipophilic compounds did not greatly alter the behavior of foraging rodents. During laboratory trials, whole control and extracted seeds were removed with similar success, and although success was higher when the shells of both treatments types were removed, and the experiment was repeated, pentane extraction did not significantly decrease detection rates

(Figure 1). In fact, in all cases, extracted seeds were removed at slightly higher rates (Table 1). Values for digging likelihood, and the likelihood of failure were similar between treatments during both trials. This outcome runs counter to the prediction, and when the removal rates of extracted and control Jeffrey pine seeds were compared in the field, the same trend appeared even more strongly. When site data were combined, shelled seeds that had been treated with pentane to remove lipophilic compounds were removed most rapidly (8.9 %), followed by shelled control seeds, whole seeds treated with pentane, and whole control seeds (Figure 4)(Table 2). The unexpectedly high removal of extracted seeds may be due to the presence of residual pentane odors, which presented foraging rodents with a unique stimulus. Additionally, the removal of lipophilic compounds may have increased the hygroscopic character of seeds, causing them to imbibe larger quantities of water, from the surrounding soil, thereby increasing the ease of detecting seeds using olfaction. Differences between treatment removal rates were enough to show significance in survival analyses, and they were the result of a strong interaction between seed coat removal and lipid extraction. When explanatory variables were separated, shell removal proved to be the major contributing factor in lowering cache survival (Table 3).

Shell removal appears to have a large effect on detection rates of buried seeds. A removal study I conducted the previous summer (Chapter 1), comparing survivorship of whole and shelled caches, again demonstrated that seed coat removal lead to significantly lower survival rates for Jeffrey pine seed caches (10.9% per day versus 5.7%). It seems that in addition to acting as protective barrier for a developing embryo, the seed coat acts as a barrier to the diffusion of volatile organic compounds. In addition the pericarp may

act as a moisture barrier, effectively controlling the release of odorant molecules. Becwar et al. (1982) found the removal of silver maple (*Acer saccharinum*) testa strongly influences both water imbibition and the loss of seed electrolytes over time. Seeds retaining their shells showed only a slight electrolyte loss. They found that electrolyte leakage was related to stress induced changes within the seeds' cellular membranes, potentially resulting from excessive seed dehydration or very rapid water imbibition. It is possible that removal of shells causes buried seeds to rapidly imbibe water, damaging the seeds' membranes and increasing electrolyte or odorant leakage. An increase in the detection of extracted seeds during my experiments was unexpected, as I had hypothesized that lipophilic volatile compounds were at least in part responsible for producing a seeds olfactory cue. However, removal of lipophilic compounds through pentane extraction may, as does the removal of shells, increase the liberation of other VOC's, possibly by making seeds more hydrophilic. Whether the amount and type of lipophilic compounds found in the seed and or seed coat influences water uptake remains to be investigated for Jeffrey pine seeds. A further possibility is that seeds retained an odor from the pentane extraction process, creating the presence of a novel odor that stimulated exploratory digging.

When presented with isolated terpenoids, chipmunks' digging behavior was stimulated by the presence of (-)β-pinene (Figure 5). Chipmunks dug up (-)β-pinene treatments more frequently than predicted by chance (Table 4) and were significantly more likely to remove them, than control treatments, when encountered. This strong trend was not seen when using (-)R-limonene (Figure 6). Only when considering total number of visits was chipmunk foraging affected by the presence of (-)R- limonene, in that they

made more repeated visited to (-)R-limonene treatments than the control. Although it is expected that a given resource odor is due to the make-up and concentrations of all volatiles combined (Dudareva et al. 2006), individual compounds, or classes of compounds have been found to affect foraging behavior. Tannins, or polyphenols, are two classes of secondary metabolites whose influence on rodent foraging behavior has been extensively studied. Increased tannin concentrations have been implicated in increasing the likelihood a seed will be cached, and in decreasing the digestion efficiency of the consumer (Smallwood & Peters 1986). Estell et al. (1996) found that increased concentrations of α-pinene in tarbush (*Flourensia cernua*, family Asteraceae) lowered levels of plant defoliation. They also found that the presence and concentration of β-pinene and 3-carene helped to distinguish between plants that had experienced middle and low levels of defoliation, implying that specific toxins and toxin concentration both influence resource selection by mammals.

In addition to being able to detect secondary compounds, animals make foraging decisions based on the nutritional composition of resources. As predicted, Yellow-pine chipmunks were able to detect isolated macronutrients in the lab. They were more successful at locating pure protein and lipids than carbohydrates, and were most successful when locating lipids (Figure 7). This is consistent with the idea that rodents forage in order to increase net energy gain. Both protein and lipid yield more energy per gram than carbohydrate, and adequate protein is necessary to sustain growth and reproduction. In selection experiments where animals are not water limited, they often chose items with higher fat or protein content. Jenkins (1993) found rodents had a preference for bitterbrush (*Purshi tridentata*) seeds over Indian rice grass

(*Oryzopsis hymenoides*) seeds, that contain 35.7% and 14.3% dry mass protein and 49.6% and 10% of crude fat respectively. Jeffrey pine seeds are also a highly preferred food, containing 31.5% protein, 47.8% fat, and 8.0% soluble carbohydrates (Vander Wall 1995). Smallwood & Peters (1982) also found that the addition of fat to seed sources increased squirrel preference for foods. Net energy intake is, however, limited for granivorous rodents in arid ecosystems based on their need to regulate metabolic water losses. Animals differ in their abilbity to conserve water under higher ambient temperatures while still consuming high-protein and high lipid diets; some will loose too much water on such diets (Jenkins 1993). Species diet limitations, paired with variation in the nutritive quality of available seed resources may create an opportunity for animals to partition resources based on their nutritional characteristics.

Resource nutrient content and secondary compounds both appear to have the ability to affect the detection of buried seeds, and the interaction between the two components is becoming increasingly apparent. Both Henderson (1990) and Wang & Chen (2009) documented foraging effects caused by the combined influences of plant secondary metabolites, seed nutrients, and seed morphology. Secondary plant compounds have the ability to reduce the digestibility of plant material by adding metabolic, nutritional, and handling costs to the feeding process. Typically the greater the toxin concentration in a food item, the less can be eaten in one feeding period, forcing animals to forage in shorter bouts or to consume food more slowly (McArthur et al. 2012). Toxin concentration is known to vary within plants, both between locations and

within locations creating an opportunity for foragers to select for high-quality, low toxin food.

We detected compounds in Jeffrey pine seeds that are capable of imparting handling costs on foraging rodents and that are typical of other pines (e.g. α-pinene, β-pinene, 3-carene and limonene). In addition, I detected 13 other compounds using SPME headspace techniques. There was variation between the sample types as expected (Table 6), and consistency was found between sample spectra, suggesting successful injection techniques. However, compounds found need to be verified against standards. Jorgensen 2001 also identified α-pinene, β-pinene, and limonene in lodgepole pine seeds (*Pinus contorta*), and the presence of all three compounds was greater in wet seeds. This trend was only seen for (+) Longifolene in my sample, and not for the majority of the compounds found in Jeffrey pine seeds, although our SPME methods were very similar to those used by Jorgensen (2001). As with their results, only relative abundance of compounds could be determined and not absolute composition. α-pinene, β-pinene, 3-carene and limonene have all been found previously in steam distillations of the sapwood and heartwood of Jeffrey pine (Anderson et al. 1969). In regards to foliavores, the abundance of some of these very compounds has been correlated with plant defoliation. Total terpenoid concentration has been negatively correlated with diet selection in deer, Abert squirrels, voles and sheep (Estell et al. 1996).

As suggested by Moïse et al. (2005) and Dudareva et al. (2006), whole Jeffrey pine seeds contain different amounts and types of volatiles than either the edible portion or the seed coat alone. The majority of volatile terpenoids (11) were found in the shell, rather than the edible portion (only 5). A wider diversity of compounds in the seed coat is

consistent with the idea that VOC's are present primarily in order to deter pre-dispersal seed pathogens, fungi and parasites, which would encounter the seed coat first. It is inconsistent with the idea that the seed coat acts as a diffusion barrier to VOC's within the seed. However, it is possible, as suggested by Becwar et al (1982) that imbibition or dehydration of the seeds contributes to disrupt the cellular membrane of the shell allowing leakage of seed substances. It is unclear how the location of compounds within the seed and seed coat tissues will affect rodent foraging behavior, as many species handle seeds parts in different manners.

The characterization of essential oils in plants seeds is rapidly expanding due to improved techniques for volatile collection in the field. Many seeds' essential oils have just recently been identified, such as those from, *Consolida Delphinium elatum, Nigella hispanica, Nigella nigellastrum*, family ranunculaceae (Kokoska et al. 2012), and *Tylosema esculentum,* family Fabaceae (Holse et al. 2012), while some are being characterized for the first time, such as *Rhododendron tomentosum*, wild rosemary, family Ericaceae, (Judzentiene et al. 2012), and *Asystasia gangetica*, family Acanthaceae (Olufunke 2011). As far as I am aware this is the first description of Jeffrey pine seed terpenes, and as the production of volatile compounds represents a mechanism plants can exploit in plant-animal interactions, volatile analyses are providing important insight into how plants, as sedentary organisms, use secondary compounds to interact with their environment.

CONCLUSION

Animals appear able to detect both individual terpenoids and individual macronutrients when foraging for buried items using olfaction. The presence of protein,

lipid and α-pinene seemed to stimulate digging behavior, while carbohydrates and limonene did not. As both nutritional and secondary qualities of the seed influence rodents' interactions with seed resources, these traits are likely to be important characteristics that can be selected for during coevolutionary interactions. It is becoming increasingly apparent that the chemosensory information available to rodents may be as important in making foraging decisions as they are in social and predator contexts. As effective density dependent predators, rodents pressure their plant resources to balance the selective costs of dispersal with loss to seed predators, pathogens, and fungi. The high preference of rodents for Jeffrey pine seeds and the presence of a variety of terpenoid compounds within them suggest that these balancing mechanisms have already been at work shaping plant-animal interactions in an animal-dispersed pine.

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Table 1. Foraging success (number of seeds found/number of cups dug in), digging likelihood (number of cups containing seeds dug in/ number of visits to cups containing seeds), likelihood of failure (number of seeds missed/ number of visits to cups containing seeds), and chi-square goodness of fit tests for two laboratory trials testing for effects of lipid extraction on the detection of Jeffrey pine seeds. All digging rates are expressed as percentages. When using whole seeds df = 6, and when using shelled seeds, df=7.

	Whole Seeds		Shelled Seeds	
	Not Extracted	Extracted		
Percent success (mean ± sd)	23.7 ± 7.5	24.3 ± 7.1	35.1 ± 28.5	43.1 ± 29.4
Digging likelihood (mean ± sd)	80.5 ± 17.8	89.3 ± 10.4	75 ± 35.6	85.4 ± 27.4
Likelihood of failure (mean ± sd)	19.5 ± 17.8	10.7 ± 10.4	25 ± 35.6	14.6 ± 27.4
$\chi 2$	10.95	10.97	26.07	50.34
P value	0.090	0.090	<0.001	<0.001

Table 2. Rates of cache removal pooled between five sites in Little Valley, NV, June-July 2011, during an experiment testing for effects of lipid extraction on rodents' ability to detect, whole and shelled, buried Jeffrey pine seeds. Mean cache survival was estimated using chi-square survival analysis. * Indicates an interaction between the presence of seed shells and the presence of lipid compounds that significantly affected cache removal rates ($\chi 2 = 99.83$, df = 3, $P < 0.001$).

	Whole seeds		Shelled seeds	
	Not Extracted	Extracted	Not Extracted	Extracted
Number of caches removed / total number available	9/50	17/50	45/50	47/50
Removal rate (% per day)	0.59	1.3	7.3	8.9
Mean cache survival (days)*	72.6	42.3	30.0	22.8

Table 3. Mean survival (days), estimated by Chi-square survival analysis, of cache removal rates pooled between five sites in Little Valley, NV, June-July 2011, during an experiment testing for effects of lipid extraction on rodents' ability to detect buried Jeffrey pine seeds. Control seeds were not treated with pentane to extract lipids. Chi-square and *P* values compare cache survival for separated explanatory variables after a significant interaction effect between seed shell removal and pentane extraction was seen (Table 2).

	Explanatory variables			
	Lipid Extraction		Cache type	
	Control	Extracted	Whole	Shelled
Mean cache survival (days)	95.0	47.5	128.1	14.3
$\chi 2$	2.66		95.25	
P value	0.100		>0.001	

Table 4. Foraging success (Number of treatments found/number of cups dug in), likelihood (number of cups containing treatments dug in/ number of visits to cups containing treatments), likelihood of failure (Number of treatments missed/ number of visits to cups containing treatments), and chi-square goodness of fit analyses of foraging success for laboratory trials testing for effects of isolated terpenes on digging behavior. During the (-) β-pinene trial, each treatment was present in 6/42 cups so that animals had a 14.3% chance of finding one at random. During the (-)R-limonene trial, each treatment was present in 8/40 cups so that the chance detection was 20.0%.

	(-)β-pinene		(-) R-Limonene	
	2 µl DiH_20	2 µl β-pinene	2 µl DiH_20	2 µl R-limonene
Percent success (mean ± sd)	30.0 ± 18.1	33.6 ± 11.5	19.8 ± 10.2	27.8 ± 13.5
Digging likelihood (mean ± sd)	70.9 ± 2.5	26.7 ± 17.0	39.3 ± 19.7	49.7 ± 26.1
Likelihood of failure (mean ± sd)	73.3 ± 17.0	29.0 ± 23.5	60.7 ± 19.7	50.3 ± 26.1
$\chi 2$	4.44	25.32	1.80	6.05
P value	0.617	<0.001	0.940	0.418

Table 5. Digging success (Number of treatments dug/number of cups dug in) and Chi-square goodness of fit values for the digging success of chipmunks foraging for isolated macronutrients. During protein, carbohydrate, and lipid trials 16/48 cups contained treatments giving individuals a 33.3 % chance of finding treatments at random. During the wet carbohydrate and JP trial, treatments were present in 8/40 cups giving individuals a 20.0 % of finding treatments at random. Differences in degrees of freedom occurred when an animal failed to forage or videotapes malfunctioned.

Treatment	Percent success (mean ± sd)	$\chi 2$	df	*P*
Protein	53.8 ± 19.9	32.19	6	<0.001
Carbohydrate	38.4 ± 18.2	6.94	6	0.327
Carbohydrate(wet)	16.1 ± 19.1	4.90	5	0.428
JP seed (wet)	68.1 ± 23.5	80.04	5	<0.001
Lipid	68.24 ± 13.4	44.51	6	<0.001

Table 6. Compounds detected using SPME GC/MS techniques to sample vial headspace of Jeffrey pine seeds. Samples were allowed to equilibrate in conditioned vials for 24 hours prior to sampling and headspace was sampled for 20 minutes. Three samples of each type were averaged however only one sample of parasitized seeds was obtained. Values relate peak mass spectrum with parent masses, with a possible high value of 1000.

	Formula	Dry seeds	Wet seeds	Seeds only	Shells	Psits
1 R α-pinene	$C_{10}H_{16}$	948.5	940.5	927.3	932.5	951
m β-cymene	$C_{10}H_{14}$	928.5	903.5		920	900
β-pinene	$C_{10}H_{16}$	953.5	947.5	931	932	917
3-carene	$C_{10}H_{16}$	918	914	926		947
D-limonene	$C_{10}H_{16}$	915.5	916		927	
α-campholenol	$C_{10}H_{16}O$	951			936	919
s-(cis) verenol	$C_{10}H_{16}O$	937	921.5		924.3	922
verbenone	$C_{10}H_{14}O$	953	913		950.7	946
(+) longifolene	$C_{15}H_{24}$	942	959		921	
camphene	$C_{10}H_{16}$	946	952		920.5	
pinocamphone	$C_{10}H_{16}O$		945			
estragole	$C_{10}H_{12}O$		926.5			
isopropenylphenol	$C_9H_{10}O$		937.5	926	923.5	906
bergamotene	$C_{15}H_{24}$		926.5			
ϒ caprolactone	$C_6H_{10}O_2$			955		
myrtenol	$C_{10}H_{16}O$				932.5	915

FIGURE LEGENDS

Figure 1: Foraging success (number of seeds found/number of cups dug in) during two laboratory trials testing for effects of pentane lipid extraction on the detection of Jeffrey pine seeds. Point estimates are mean ± sd. When using whole seeds df = 6 and while using shelled seeds df = 7. There were no differences in success during either trial, however * indicates shelled treatments were found more frequently than random ($\chi 2 = 26.07$, $P < 0.001$, and $\chi 2 = 50.34$, $P < 0.001$ for both control and extracted treatments, respectively.

Figure 2: Likelihood of digging (number of cups containing seeds dug in/ number of visits to cups containing seeds) during two laboratory trials testing for effects of lipid extraction on the detection of Jeffrey pine seeds. Point estimates are mean ± sd. When using whole seeds df = 6 and while using shelled seeds df = 7.

Figure 3: Likelihood of failure (number of seeds missed/ number of visits to cups containing seeds) during two laboratory trials testing for effects of lipid extraction on the detection of Jeffrey pine seeds. Point estimates are mean ± sd. When using whole seeds df = 6 and while using shelled seeds df = 7.

Figure 4: Rates of cache removal pooled between five sites in Little Valley, NV, June-July 2011. The open triangles and open circles are whole and shelled JP seeds, respectively, that have had their lipids removed through pentane extractions. For all

treatments *n*=50 caches. * Indicates that the effect of shelling JP caches significantly decreased survival rate ($\chi 2 = 95.25$, df = 1, $P < 0.001$). The extraction of lipophilic compounds alone did not significantly decrease detection in any case, however, there was also a significant interaction affect ($\chi 2 = 99.83$, df = 3, $P < 0.001$).

Figure 5: Foraging success (Number of treatments found/number of cups dug in), likelihood (number of cups containing treatments dug in/ number of visits to cups containing treatments), and likelihood of failure (number of treatments missed/ number of visits to cups containing treatments) during a laboratory trial testing for effects of (-) β-pinene on digging behavior. Points are mean ± sd. * Indicates that chipmunks were more successful when digging up (-) β-pinene treatments ($F_{1,12} = 7.46$, $P = 0.018$), more likely to dig at (-) β-pinene ($F_{1,12} = 10.74$, $P = 0.007$,) and that they failed to dig up (-) β-pinene treatments less often than controls, when they were encountered ($F_{1,12} = 18.08$, $P = 0.001$).

Figure 6: Foraging success (Number of treatments found/number of cups dug in), likelihood of digging (number of cups containing treatments dug in/ number of visits to cups containing treatments), and likelihood of failure (number of treatments missed/ number of visits to cups containing treatments) during a laboratory trial testing for effects of (-)R-limonene on digging behavior. Points are mean ± sd. Chipmunks detected treatments similarly by all measures of detection.

Figure 7: Foraging success (Number of treatments dug/number of cups dug in), likelihood (number of cups containing treatments dug in/ number of visits to cups containing treatments), and likelihood of failure (number of treatments missed/ number of visits to cups containing treatments), during three separate, non-choice, laboratory trials testing whether yellow-pine chipmunks can detect isolated macronutrients. Points are mean ± sd. Protein and carbohydrate were tested using 100mg and lipids were tested using 50mg. * Indicates that chipmunks successfully found protein and lipid treatments at higher than chance rates ($\chi 2 = 32.19$, df=6, $P < 0.001$ and $\chi 2 = 44.51$, df=6, $P < 0.001$, respectively).

Figure 8: Foraging success (Number of treatments dug/number of cups dug in) of yellow-pine chipmunks during a laboratory trial testing whether yellow-pine chipmunks can detect carbohydrates under wet conditions. Jeffrey pine seeds (JP in figure) were used to maintain foraging motivation and 100 mg of carbohydrate were used. Points are mean ± sd and $n = 6$. *Iindicates chipmunks dug for JP seeds more often than chance ($\chi 2 = 94.38$, df=6, $P < 0.001$), and significantly more often than carbohydrate treatments ($F_{1,10} = 12.09$, $P = 0.005$).

Fig. 1

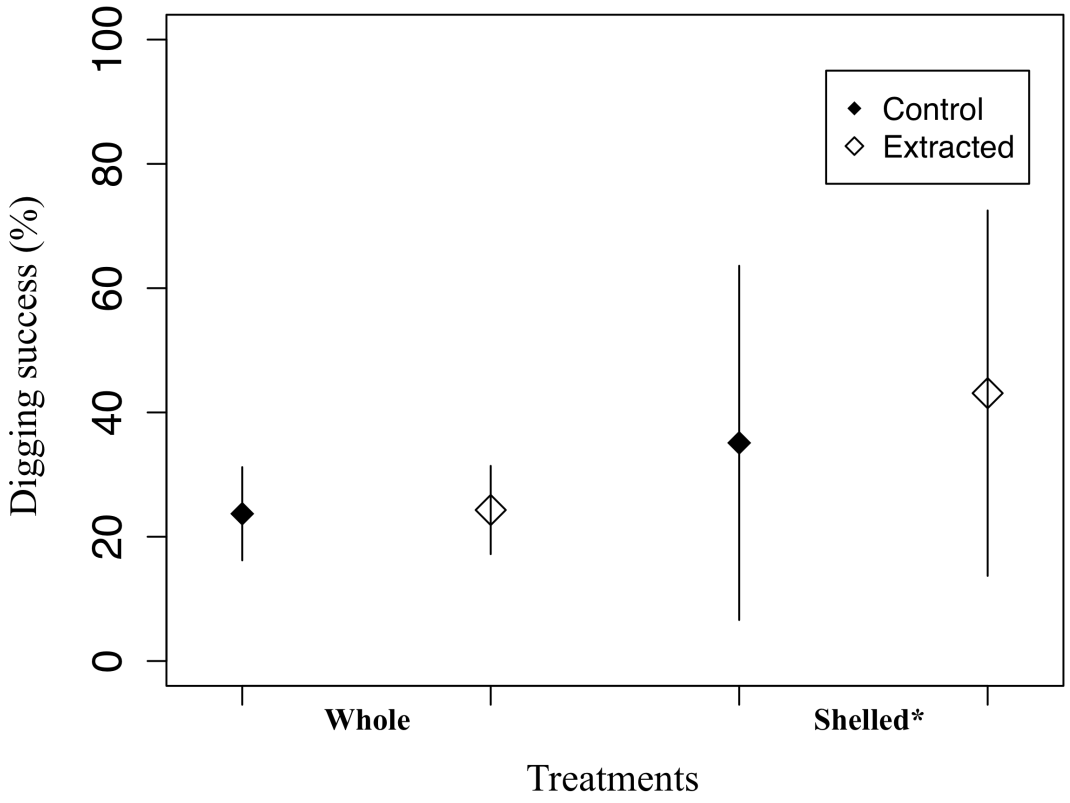

Digging success (%)
0
20
40
60
80
100
Control
Extracted
Whole
Shelled*
Treatments

Fig. 2

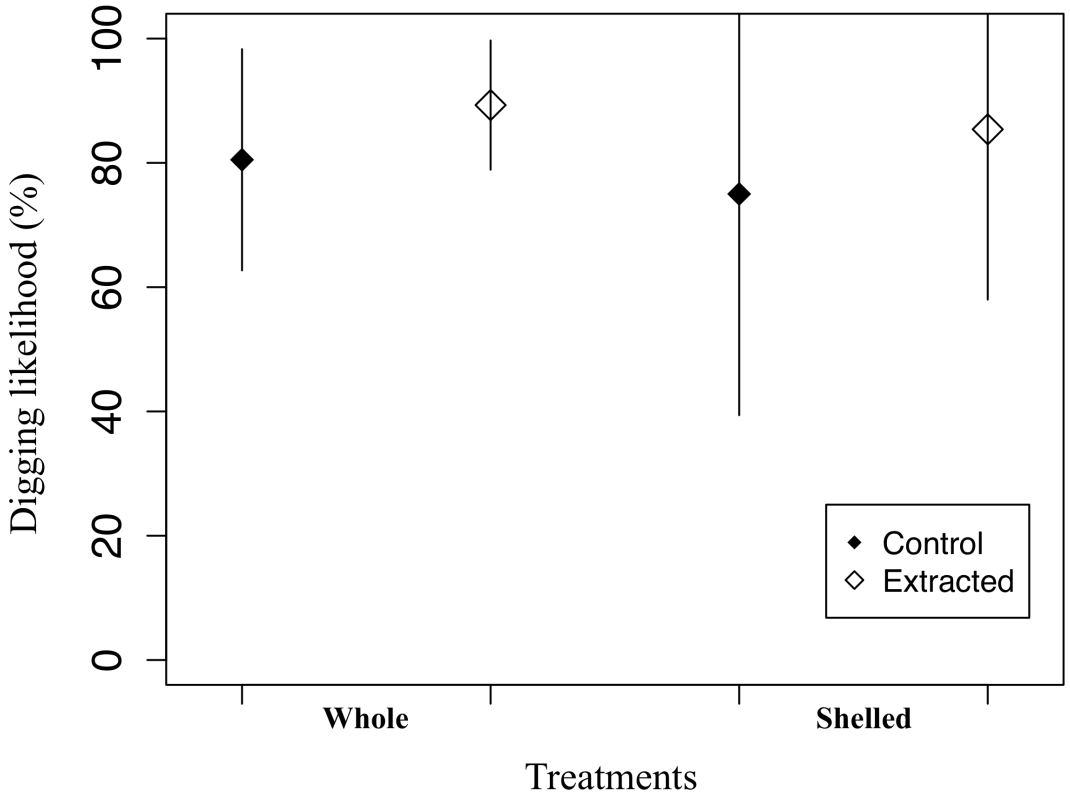

Digging likelihood (%)
0
20
40
60
80
100
Control
Extracted
Whole
Shelled
Treatments

Fig. 3

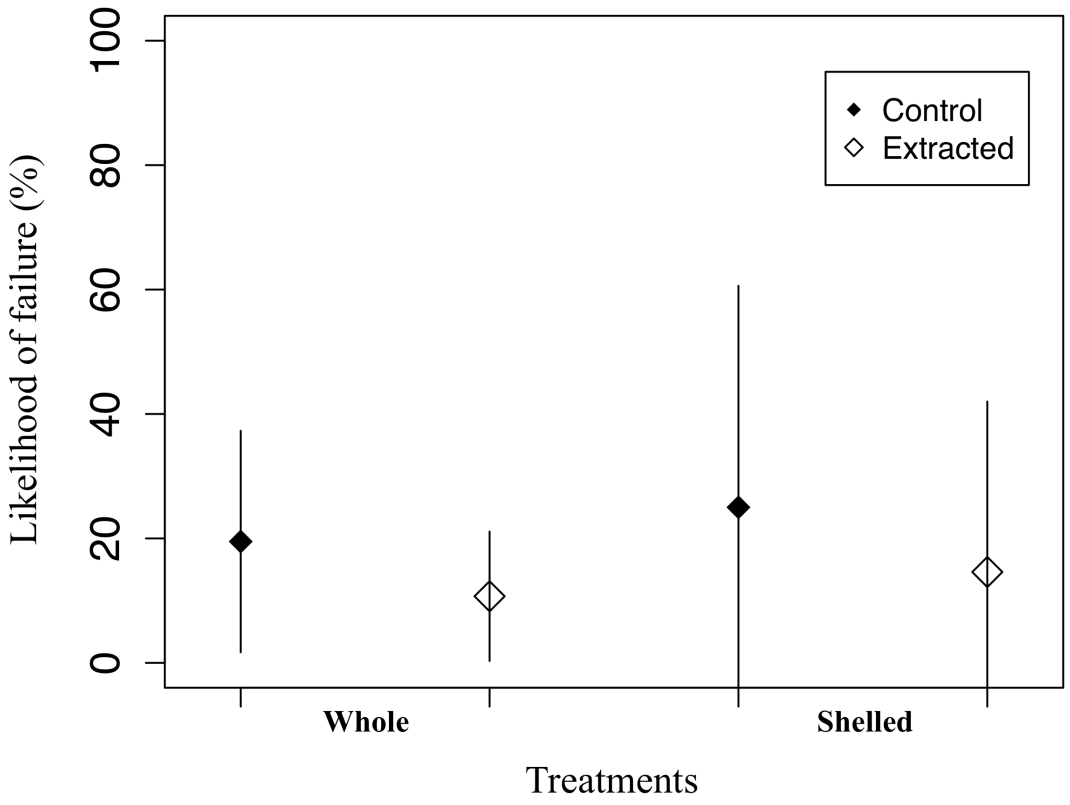

Likelihood of failure (%)
0
20
40
60
80
100
Control
Extracted
Whole
Shelled
Treatments

Fig. 4

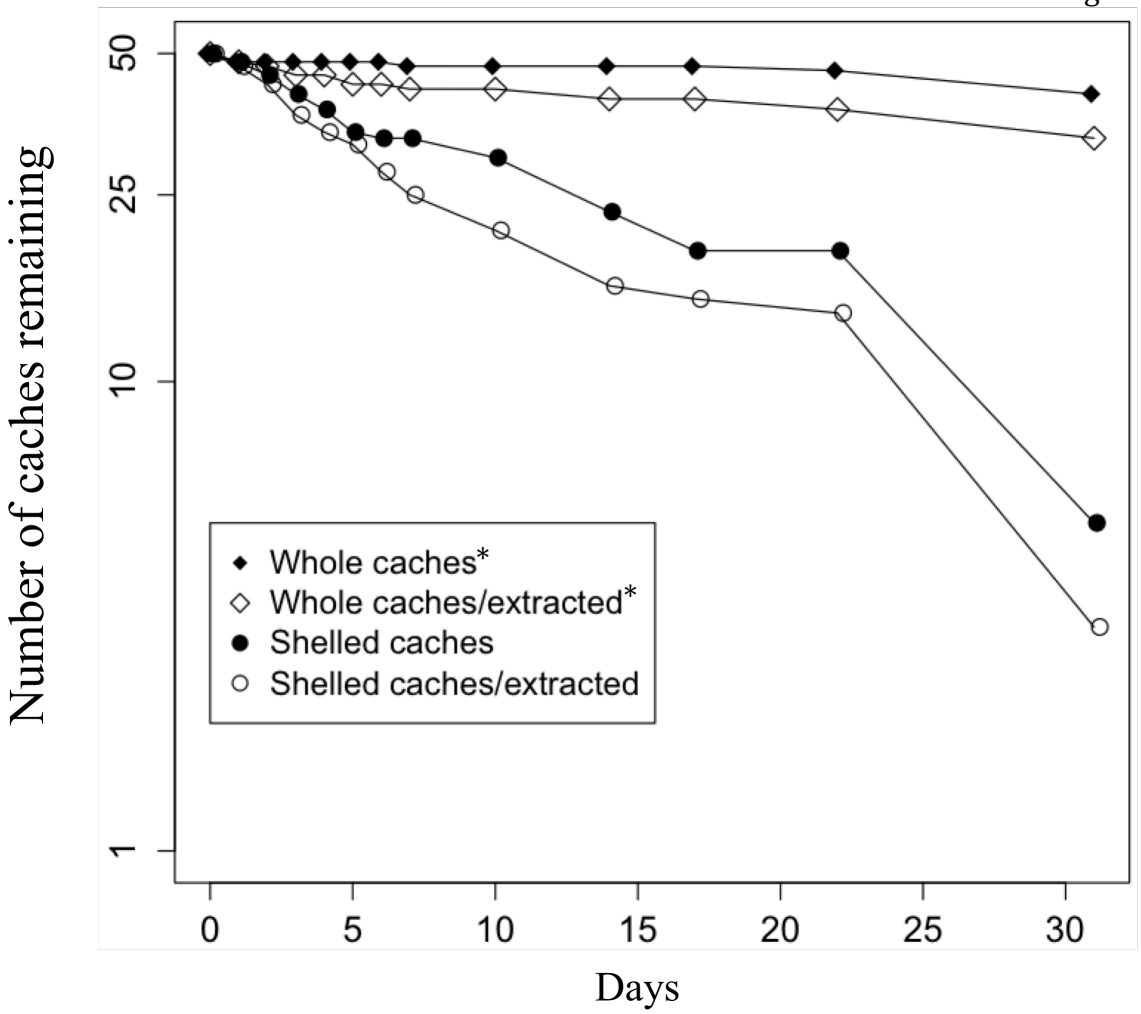
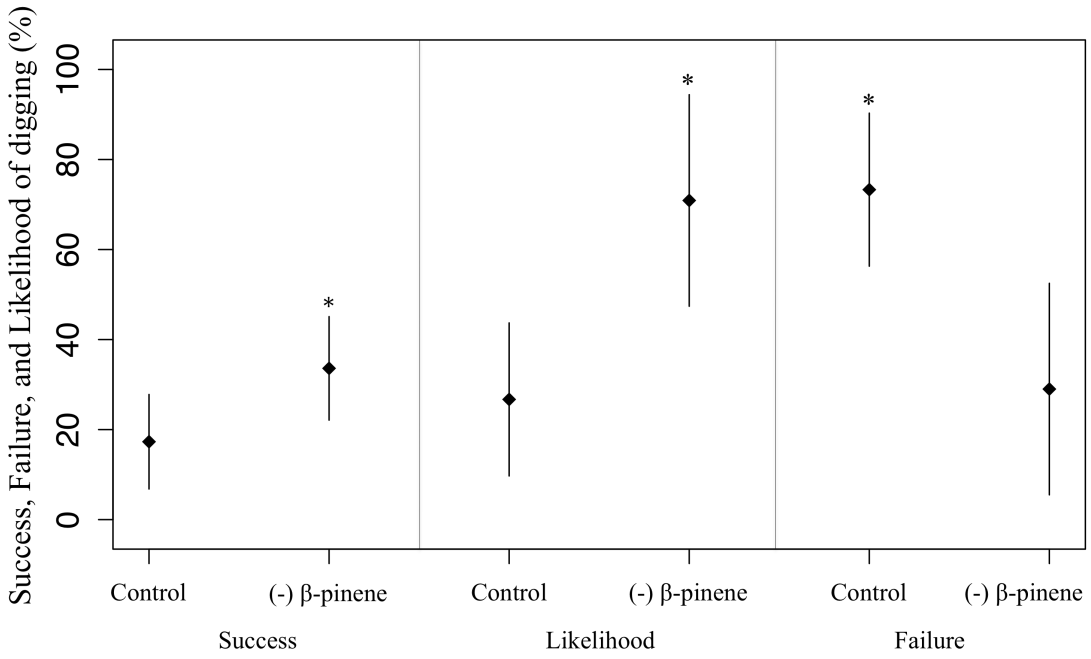
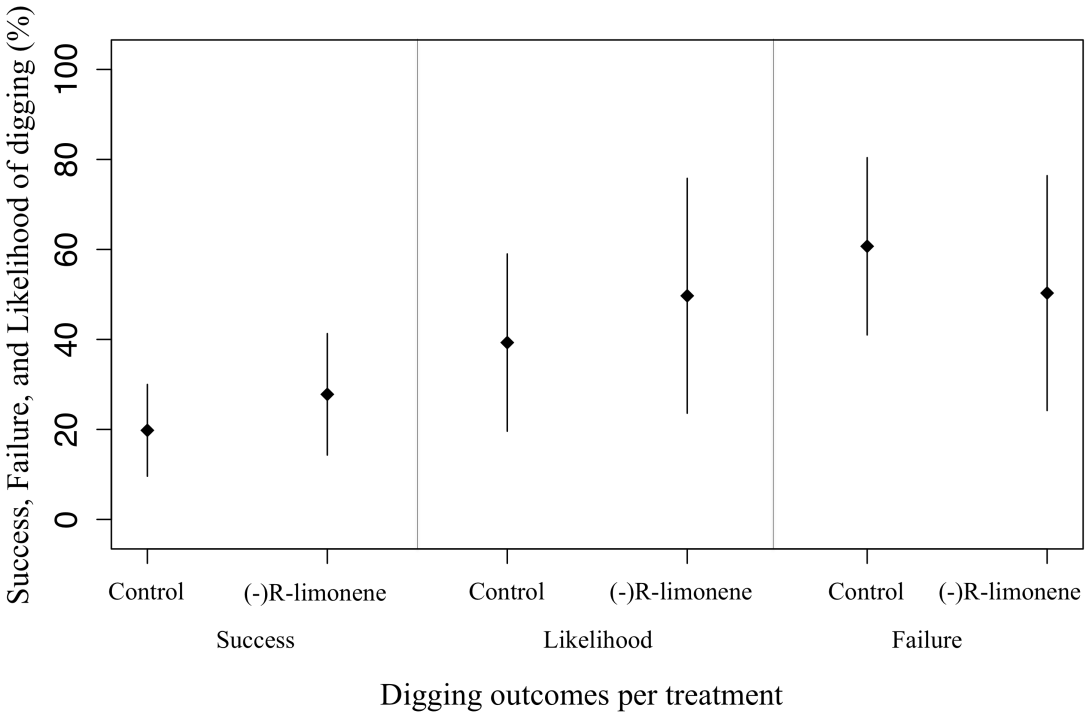

Number of caches remaining
50
25
10
1
0
5
10
15
20
25
30
Days
Whole caches*
Whole caches/extracted*
Shelled caches
Shelled caches/extracted

Fig. 5



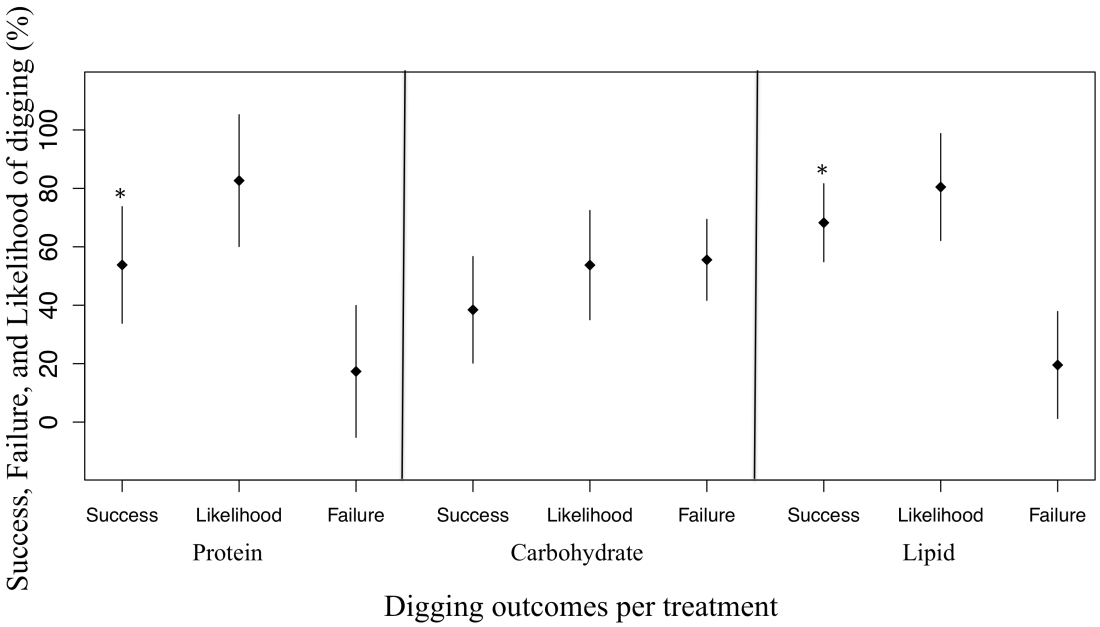
Digging outcomes per treatment

Fig. 6



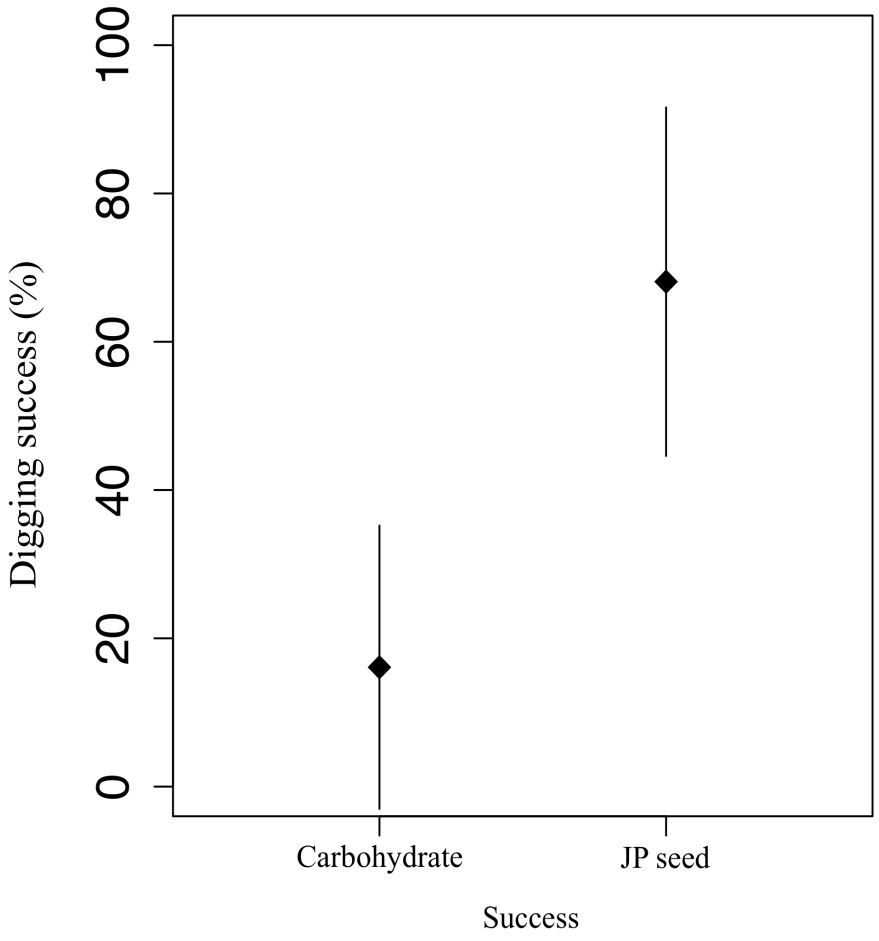
Digging outcomes per treatment

Fig. 7



Digging outcomes per treatment

Fig. 8


Digging success (%)
0
20
40
60
80
100
Carbohydrate
JP seed
Success