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Abstract

Selective foraging by granivores can have important consequences for the structure and composition of plant communities, and potentially severe consequences for rare plant species. To understand how granivore foraging behavior affects common and rare plant species, diet selection should be viewed relative to the availability of alternative seed options, and with consideration of the individual attributes of those seeds (e.g., morphology, nutrient content). We examined the foraging decisions of Owyhee harvester ants, *Pogonomyrmex salinus* [Olsen], in semiarid grassland dominated by two species of grass, *Poa secunda* and *Bromus tectorum*, and two species of mustard, *Sisymbrium altissimum* and *Lepidium papilliferum*. The latter is a rare plant endemic to southwestern Idaho, and its seeds are readily consumed by *P. salinus*. We examined the diets of *P. salinus* colonies in June and July over three years and compared these values to the weekly availability of seeds on the ground in a 3-12 m radius around individual ant colonies. Small seeded species (*P. secunda*, *S. altissimum* and *L. papilliferum*) were usually overrepresented in the diet of ants relative to their availability, whereas the large seeds of *B. tectorum* were largely avoided despite being abundant and nutritious. The reduced travel time associated with carrying small seeds may overshadow differences in nutritional content among seed types, except in times when small seeds are in short supply. *Lepidium papilliferum* appears particularly vulnerable to seed predation, likely in part because it grows in dense patches that are easily exploited by foragers.

Keywords: seed predation, foraging, harvester ant, *Pogonomyrmex salinus*, *Lepidium papilliferum*

Selective foraging by granivores can have important consequences for the structure and composition of plant communities (Brown et al. 1979, Reichman 1979, Inouye et al. 1980, MacMahon et al. 2000). In many arid and semiarid environments, seed harvesting ants impact plant demographics by collecting and consuming large numbers of seeds and by differentially selecting some seed species over others (Hobbs 1985, Crist and MacMahon 1992, Nicolai et al. 2007, Pirk and Lopez de Casenave 2011). Although harvester ants typically consume only a small proportion of the seed pool available to them, predation on preferred seed types is often intense and in some cases results in their complete removal (Andersen and Ashton 1985, Crist and MacMahon 1992, Ireland and Andrew 1995). Seed predation may have particularly severe consequences for rare plant species, where any effect of herbivory on seed recruitment could limit its recovery or drive the plant to extinction (Crawley 2000, Albert et al. 2005, Ancheta and Heard 2011). To understand how granivore foraging behavior may influence soil seed pools, preferences of ants for seeds need to be measured as a function of seed availability in the environment. Here we examine diet selection in the Owyhee harvester ant, *Pogonomyrmex salinus* [Olsen] (Hymenoptera: Formicidae), in an area of southwest Idaho dominated by grasses. Scattered within this habitat are patches of slickspot peppergrass, *Lepidium papilliferum* [(L. Henderson) A. Nels. & J.F. Macbr] (Brassicaceae), a rare mustard whose seeds are readily collected and consumed by harvester ants (White and Robertson 2009).

Pogonomyrmex ants are common seed predators throughout arid and semiarid regions of the Americas, including sagebrush-steppe and grassland habitats in the Great Basin of North America. Their large, conical nest mounds often dot the landscape, and in some cases occur in densities as high as 150 colonies/ha (MacMahon et al. 2000). In temperate climates, harvester ants forage diurnally from spring to autumn whenever surface temperatures are sufficiently warm. Although granivory largely defines harvester ants as a group (MacMahon et al. 2000), many species incorporate arthropods (living and dead), fungi, feces, and assorted vegetation into their diets (Hölldobler and Wilson 1990, Taber 1998, Belchior et al. 2012).

As single-load central place foragers (Stephens and Krebs 1986), the economics of seed gathering may influence diet selection of harvester ants (Holder Bailey and Polis 1987, Morehead and Feener 1998). Although harvester ants collect and consume a wide variety of seeds, many species exhibit marked preferences for certain seed types, with grass seeds often topping the list (Crist and MacMahon 1992, Detrain and Pasteels 2000, Pirk et al. 2009, Pirk and Lopez de Casenave 2011). Several seed traits relevant to the economics of foraging are known to influence diet in harvester ants, including relative availability (Whitford 1978, Briese and Macauley 1981, Mehlhop and Scott 1983, Hobbs 1985, Crist and MacMahon 1992, Willott et al. 2000, Pirk et al. 2009, Pol et al. 2011), morphology (Davidson 1977, Pirk and Lopez de Casenave 2010), and nutritional quality (Kelrick et al. 1986, Crist and MacMahon 1992, Kay 2004). For example, in both natural and controlled experiments, foraging ants preferred medium sized seeds with high caloric content and high percent soluble carbohydrates relative to the other seed types available (Crist and MacMahon 1992, see also Kelrick et al. 1986, Willott et al. 2000). Likewise, Crist and MacMahon (1992) found that *P. occidentalis* colonies displayed narrower diet breadth when preferred seed types were abundant than when they were scarce (see also Wilby and Shachak 2000). Thus, to understand the foraging decisions of harvester ants it is important to examine both the relative abundance and individual attributes of available seed types under natural conditions.

Our primary objective in the present study was to evaluate whether Owyhee harvester ants inhabiting semiarid grassland exhibit selectivity for certain seeds types, or whether their diet mirrors the relative availability of seeds on the ground. To help place the results in the context of foraging theory, we examined the nutritional content of available seeds and the travel speeds of ants carrying different seed types as they returned to their nests. We hypothesized that ants would prefer seeds that were higher in nutrition and easier to transport.

A secondary objective of our study was to examine the extent to which harvester ants incorporate the seeds of slickspot peppergrass into their diet. Within sagebrush-steppe habitat slickspot peppergrass is restricted to patchily distributed microsites known as "slick spots" (Moseley 1994) — shallow depressions of soil characterized by high levels of clay and minerals as well as by subsurface water retention that is higher than that of surrounding areas (Fisher et al. 1996). Reliance on these microsites produces localized concentrations of *L. papilliferum* that may increase the plant's vulnerability to seed predators (Reichman 1979, Wilby and Shachak 2000, Flanagan et al. 2012). Harvester ant nests are scattered throughout most of the 80 or so remaining populations of *L. papilliferum* (Kinter et al. 2013, Robertson 2015), and experiments have shown that the ants can consume upwards of 90% of the total seed production by individual plants (White and Robertson 2009). However, the extent to which *L. papilliferum* seeds are consumed by harvester ants when other naturally occurring seed types are available has not been investigated. Thus, the main objective of our study - to characterize and understand diet choices of *P. salinus* under natural conditions in semiarid grassland - also provided a unique opportunity to examine the extent to which harvester ants influence seed recruitment in a rare plant species.

Materials and Methods

Study Area and Selection of Ant Colonies

Our study was conducted from June through July 2009-2011 at a 10 ha site located near Melba, Idaho. This site was chosen because it supports a relatively large population of *L. papilliferum* and has an abundance of harvester ant colonies (~25.5 colonies/ha). In addition to *L. papilliferum*, vegetation at the study site consisted of a sparse overstory of big sagebrush (*Artemisia tridentata*), and an understory that consisted primarily of Sandberg's bluegrass (*Poa secunda*), cheatgrass (*Bromus tectorum*), and tumble mustard (*Sisymbrium altissimum*).

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In May 2009, we selected 10 harvester ant colonies for observation and analysis of diet over three consecutive years. Actual sample sizes varied across years (2009, N = 9; 2010, N = 10; 2011, N = 8) because in 2009 one of the colonies ceased activity for several weeks and was excluded from analysis, and in 2011 two of the colonies were incorporated into another study that involved manipulation of food availability. Each of the colonies we selected was located within 12 m of one or more slick spots that contained flowering *L. papilliferum*. A distance of 12 m was chosen because although harvester ants forage up to 20 m from their nests, most foraging activity occurs within 12 m (MacMahon et al. 2000, personal observations). The colonies we used were at least 40 m apart to ensure independence of samples.

Understory Vegetation Measurements

We used the quadrat frame technique (Gauch Jr. 1982, Scheller and Mladenoff 2002) to estimate percent understory cover of each plant species surrounding the ant colonies included in our study. Ten randomly selected locations 3-12 m from the center of each ant nest were sampled. We did not sample within 3m of nests because harvester ants actively clear vegetation from within this area (MacMahon et al. 2000). We also avoided sampling within slick spots; an alternative methodology was used to quantify *L. papilliferum* coverage within each colony's foraging radius (see below). At each sampling location we estimated the percent cover of each plant species found within a 1-m^2 metal frame placed on the ground. We used the mean percent understory cover from the 10 samples to estimate total understory cover within the 12-m foraging radius of each ant nest. These estimates were adjusted to account for areas within the foraging radius that were taken up by slick spots.

The relative rarity of *L. papilliferum*, and its patchy distribution within slick spots and around ant colonies meant that the sampling protocol described above would not have effectively accounted for *L. papilliferum* coverage. Therefore, we used the quadrat frame to take a complete inventory of *L. papilliferum* coverage within slick spots located 3-12 m from each ant nest. These values represented total *L. papilliferum* coverage within the 12-m foraging radius of each ant nest.

Seed Availability

We sampled the foraging areas around harvester ant colonies weekly to determine the relative availability of *P. secunda*, *B. tectorum*, and *S. altissimum* seeds on the ground. We excluded the pre-dispersal seed pool from consideration, and instead considered only the seeds located in the upper 0.5 cm of soil (Crist and MacMahon 1992). Each week we took 10, 5.3 cm diameter soil cores at random locations 3-12 m from each ant colony, excluding areas within slick spots. We brushed loose surface debris, including seeds, from the upper 0.5 cm of each core sample into a zip-lock bag. We placed all 10 samples for a colony into the same bag. In the laboratory we sifted the samples through a series of increasingly finer sieves (1.4 mm, 850 μm, 710 μm, 500 μm, and 250 μm diameter mesh, Hogentogler & Co., Inc., Columbia, MD), and we identified and counted the seeds trapped by the mesh. We included only "sound" seeds (Crist and MacMahon 1992) in our analyses. A seed was considered "sound" if it withstood light pressure from forceps without breaking, and did not show signs of fungal infection (e.g., discoloration and/or malformation) when viewed under 10x magnification.

Taking soil cores within slick spots was not an acceptable method to quantify the availability of *L. papilliferum* seeds because the procedure would have compromised soil integrity and imperiled the *L. papilliferum* growing there. Instead, we used our measurements of *L. papilliferum* coverage, along with counts of the total number of seeds produced per unit coverage area of *L. papilliferum*, to model the number of *L. papilliferum* seeds available to ant colonies throughout the season. To quantify seed production by individual *L. papilliferum* we collected 10, 25-30 cm diameter plants from a different area of the study site. We selected only large, mature plants because we wanted to create liberal estimates of the total number of seeds produced per unit of overhead coverage area, and larger individuals produce larger seed sets. We used individual zip-lock bags to transport the specimens back to the laboratory, where we counted intact fruits as well as any seeds that had shaken free in the bags. Seed totals were calculated as the number of individual seeds collected plus two times the number of undehisced fruits (each fruit contains two seeds). In our model of *L. papilliferum* seed availability, seeds were distributed using a normal distribution bracketed by the first week we observed *L. papilliferum* seeds in the diet of ants and the end of the study period. (*n.b.*, A normal distribution approximated the plant's fruiting phenology as well as the observed pattern of seed intake by ants.) We assumed that the *L. papilliferum* population was composed entirely of large plants and that seed production was equal

to one standard error above the mean value we obtained from our counts (i.e., 18.62 seeds/cm^2). Inundating the system with a large yet biologically plausible amount of *L. papilliferum* seed allowed for a conservative estimate of the numbers of *L. papilliferum* seeds consumed by harvester ants relative to seed availability.

Seed Intake at Ant Colonies

We quantified seed intake by foragers from each colony once per week between 0900 and 1200, the period of the day when foraging activity peaked. Observations lasted five minutes per colony, during which time we recorded the type of seed carried by each forager as it returned to its nest. We randomized the order that we visited colonies each week to avoid any bias in foraging associated with time of day. Seed intake observations were made within 24 h of sampling for seed availability around each colony.

Early in the study we noticed a substantial number of foragers returning to their nests (seemingly) without seeds. Thereafter, to account for the possibility that small seeds were being returned to nests undetected by the observer, immediately following each 5-min observation we aspirated and placed into vials the first 20 ants returning to the nest without any obvious sign of a seed in their mandibles. From these samples we later established how many of the ants were truly seedless, how many were carrying seeds, and the identity of any seeds they carried.

Relative Seed Abundance and Diet Association

To relate relative seed abundance to diet selection, we calculated a concordance correlation coefficient (CCC, Carrasco et al. 2013) for each seed species included in the diet. To account for low sample size and repeated measures, we used non-parametric procedures (Carrasco et al. 2013) that utilize a U-statistic to determine a CCC for each seed species during each year of the study. A CCC value of 1 indicates that seeds were harvested in direct proportion to their relative availability in the environment. Values that deviate from 1 indicate selectivity, either positive (i.e., the proportion of a given type of seed in the diet was higher than its proportion in the environment) or negative (i.e., the proportion of a given type of seed in the diet was lower than its proportion in the environment).

Travel Speed While Carrying Seeds

On four consecutive mornings between 0900 and 1200 (when surface temperatures each day ranged from 27-30°C during the observation period) we located harvester ants that were carrying seeds to their nests. We noted the type of seed being carried, and measured the distance covered (cm) and time required (sec) to transport the seed to the nest entrance. We used a one-way ANOVA to test for an overall effect of seed type on travel speed, and a Tukey's Multiple Comparisons test for pairwise comparisons among seed types.

Nutritional Attributes of Seeds

We collected samples of the four seed types (*B. tectorum*, *P. secunda*, *L. papilliferum*, *S. altissimum*) to conduct species-specific analyses of their moisture content, protein content, water-soluble carbohydrate content, and energy content. We collected seeds by placing a zip-lock bag over randomly selected plants at the field site and shaking loose mature seeds. After removing fruit husks and other debris from the bags, we transferred the samples to vials and shipped them to DairyOne Forage Laboratories, Ithaca, NY, for analysis.

Results

Understory Vegetation Surrounding Harvester Ant Colonies

Bromus tectorum was the dominant understory species at our site, followed by *Poa secunda* (Table 1). *Sisymbrium altissimum* was present in 2009 and 2010, but was experimentally removed in 2011 as part of a separate experiment. *Lepidium papilliferum* covered only a small fraction of the foraging area around ant colonies. On average, no more than 1.9% of the foraging area around nests was covered by vegetation other than *P. secunda*, *B. tectorum, L. papilliferum* and *S. altissimum* (Table 1).

Seed Availability

In 2009, *B. tectorum* seeds were consistently abundant and available to foraging ants throughout the eight-week study period, whereas *P. secunda* seeds declined rapidly as the season progressed (Fig. 1a). Based on model estimates, *L. papilliferum* seed numbers were 5-fold lower than *B. tectorum* seed numbers, but comparable to *P. secunda* when *L. papilliferum* was at its peak (Fig. 1a). In 2010, *B. tectorum* and *P. secunda* seeds increased in availability throughout the season, and exceeded the availability of *S. altissimum* by 7-fold and (estimated) *L. papilliferum* seeds by 6-fold (Fig. 2a). In 2011, both *P. secunda* and *B. tectorum* seeds doubled in availability compared to the previous year. By contrast, *L. papilliferum* seeds appeared much later and saw a ~94% decrease in abundance compared to previous years (Fig. 3a).

Relative Seed Abundance and Diet Association

The four types of seed returned by ants to their nests were distinguishable based on size (Table 2), shape, and color. On average, more than half of the ants we aspirated following each 5-min observation period were carrying either *L. papilliferum* (27.5 ± 0.01%) or *S. altissimum* (23.0 ± 0.01%) seeds, confirming that these contributions to diet were being partially overlooked during the 5-min observations. We used these data to adjust the seed intake values for ants that had been scored as carrying "nothing" during the preceding 5-min visual sampling period.

Distinct patterns of seed use emerged each year, but these patterns varied among years. In 2009, harvester ants initially focused their foraging efforts on *P. secunda* seeds, but as the season progressed these seeds gradually declined in their contribution to diet (Fig. 1b). *Lepidium papilliferum* seeds were represented in the diet more than any other seed type for several weeks mid-season (Fig. 1b), despite an overwhelming abundance of *B. tectorum* seeds on the ground at all times (Fig. 1a). It was only in the later stages of July, when other seed types were scarce, that *B. tectorum* seeds were regularly collected by ants (Fig. 1b). *Sisymbrium altissimum* seeds were never a large component of harvester ant diet in 2009 (Fig. 1b).

In 2010, harvester ants again focused their foraging efforts on *P. secunda* early in the season (Fig. 2b). Although *P. secunda* seed was consistently abundant throughout the season (Fig. 2a), seed intake by ants shifted from *P. secunda* to *S. altissimum* and *L. papilliferum* once those latter seeds became available, albeit in limited quantities (Fig. 2b). *Bromus tectorum* seeds were not well represented in the diet at any time in 2010 despite their abundance.

In 2011, ants collected *P. secunda* seeds almost exclusively (Fig. 3b). *Bromus tectorum* seeds were again avoided despite being available in large quantities. *Lepidium papilliferum* seeds were represented only in small amounts late in the season (Fig. 3b). The late and limited appearance of *L. papilliferum* seeds in the diet of ants mirrored the late emergence, flowering, and fruit production of this species in 2011 (Fig. 3a). Periodic observations conducted later in the season (September – October) revealed that harvester ants continued to collect *L. papilliferum* seeds after the study had concluded.

CCC analysis confirmed that the diet of harvester ants did not mirror the relative availability of seeds within the foraging areas of ant colonies (all $P > 0.05$, range $0.1 – 0.8$, indicating that there was no significant agreement between seed selection and availability [Zar 2010]). Regardless of seed type or year of study, CCCs did not consistently approach a value of 1 in any of the species evaluated (Fig. 4). *Bromus tectorum* seeds were almost always underrepresented in the diet of harvester ants despite the dominance of these seeds in terms of their relative availability on the ground (Fig. 4a). During the first half of each season *P. secunda* seeds were usually collected in disproportionately higher numbers than their availability, whereas in the second half of the season the pattern of use became more variable. For example, during the second half of both 2009 and 2011 (open circles in Fig. 4b, 2009 and 2011), *P. secunda* seeds were usually overrepresented in diet relative to their availability. By contrast, *P. secunda* seeds were widely underrepresented in the diet during the second half of the 2010 season (open circles in Fig. 4b, 2010), a period during which both *L. papilliferum* and *S. altissimum* seeds were available in higher numbers (Fig. 2a). In general, *S. altissimum* seeds were overrepresented in the diet (Fig. 4c). Similarly, despite *L. papilliferum*'s rarity relative to other plant species (Table 1), and the liberal seed production we assumed in the model, its seeds were consistently overrepresented in the diet (Fig. 4d). Lower, more realistic values of seed production in the model would only have bolstered the conclusion that *L. papilliferum* seeds were overrepresented in the diet of ants. Moreover, the results of the analysis were similar, and conclusions the same, when we assumed that *L. papilliferum* seeds dropped to the ground uniformly rather than following a normal distribution.

Travel Speed and Nutritional Quality of Seeds

Seed length differed significantly across the four seed types (ANOVA: $F = 475.00$; df = 3,56; $P < 0.0001$), although the difference in mean length between *L. papilliferum* and *S. altissimum* seeds was not statistically significant (Table 2). The travel speeds of ants carrying seeds back to their nest also differed significantly across seed types (ANOVA: $F = 75.78$; df = 3,153; $P < 0.0001$). Ants travelled faster while carrying smaller seeds; however, as with seed length, the difference in travel speed was not statistically significant between *L. papilliferum* and *S. altissimum* (Table 2). Throughout the three-year study we noted that ants carrying *B. tectorum* seeds often experienced difficulty handling the seeds, and in some cases received assistance from other ants before reaching the nest. On numerous occasions ants discarded *B. tectorum* seeds while in transit, a behavior that was never observed for the other seed types (see also White and Robertson 2009).

Table 3 summarizes the nutritional attributes of the seeds collected by ants. *Lepidium papilliferum* and *S. altissimum* both had significantly higher crude protein and caloric content by weight than either *B. tectorum* or *P. secunda.* By contrast, *P. secunda* and *B. tectorum* seeds contained significantly more moisture than seeds from the two types of mustard. On a per seed basis, *B. tectorum* seeds contained 7 to 12 times the energy of *P. secunda*, *L. papilliferum*, and *S. altissimum* seeds. *Bromus tectorum* seeds also had a significantly higher percent water-soluble carbohydrate content than the other seeds.

Discussion

Consistent with some *Pogonomyrmex* species (Crist and MacMahon 1992, MacMahon et al. 2000, Pirk et al. 2009) but not others (Whitford 1978), Owyhee harvester ants did not gather seeds in direct proportion to the abundance of seeds on the ground. Instead, they exhibited marked preferences for certain seed types over others. Although *B. tectorum* seeds were abundantly available throughout the study, *P. salinus* largely ignored these seeds in favor of *P. secunda* seeds early in the season and *L. papilliferum* and *S. altissimum* seeds later in the season, at least when these latter seed types were available. Avoidance of *B. tectorum* seed has been documented in *Pogonomyrmex occidentalis*, as has the preference for seeds of *Poa* and various mustards (Crist and MacMahon 1992). However, *B. tectorum* seeds figured prominently in the diet of ants in July 2009. This shift in diet coincided with a decline in the availability of alternative seed types, which suggests that foragers were making the best of a bad situation by collecting *B. tectorum* seeds.

Sisymbrium altissimum and *L. papilliferum* seeds were collected preferentially over *P. secunda* seeds during the second half of the season of 2010; this was the only time of the study when *P. secunda* seeds were frequently underrepresented in the diet of *P. salinus*. Although we cannot rule out the possibility that *P. secunda* seeds on the ground in July 2010 were lower quality than those on the ground in June – i.e., selective foraging by ants might lead to a decline in seed quality over time if higher quality seeds become picked over - nutritional analyses of *P. secunda* seeds collected weekly in 2011 revealed no significant changes in nutritional content over time (unpublished data). Unfortunately, the appearance of *L. papilliferum* seeds in the diet of ants in 2009 coincided with a drop in the availability of *P. secunda*, making assertions about preference for *L. papilliferum* seeds over *P. secunda* seeds difficult. Nevertheless, both types of mustard seed were almost uniformly overrepresented in the diet of ants, indicating that they were considered high value. The overrepresentation of *L. papilliferum* seeds occurred even though we assumed unusually high seed production in the model. By intentionally overestimating the number of *L. papilliferum* seeds available to harvester ants, we created conservative estimates of selectivity for their seeds based on the number returned to nests by foragers.

Although the patterns of seed selection provide information about diet choices of ants relative to the options available, they do not provide insight into the factors that determine these choices. From optimal foraging theory (MacArthur and Pianka 1966, Stephens and Krebs 1986), foragers are expected to capture and consume food items that maximize calorie intake while minimizing the amount of time needed to do so. Although our study was not designed to test whether Owyhee harvester ants forage optimally (this would require detailed knowledge of energy gained and expended by ants while foraging - e.g., Schilman and Roces' [2006] study of the nectar-feeding ant, *Camponotus rufipes*), we were able to collect data on the nutritional attributes of the seed types collected by harvester ants, as well as travel speeds of ants carrying seeds to their nests. Together these pieces of information help place the foraging preferences of *P. salinus* into the economic context of foraging theory.

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Considered in isolation, the nutritional attributes of *B. tectorum*, *P. secunda*, *S. altissimum*, and *L. papilliferum* seeds provide no clear explanation for the foraging preferences exhibited by *P. salinus*, including why *B. tectorum* seeds were all but ignored as a food source. On a per seed basis, *B. tectorum* seeds contained 11 to 12 times the caloric content of *P. secunda* and *S. altissimum* seeds and 7 times that of *L. papilliferum* seeds. However, on a gram for gram basis, *L. papilliferum* and *S. altissimum* seeds had higher caloric content than *P. secunda* seeds, followed by *B. tectorum* seeds. In terms of percent water-soluble carbohydrate content, *B. tectorum* seeds exceeded the other three seed types by a significant margin. Granivorous ants living in arid environments are known to prefer seeds that are higher in water-soluble carbohydrates (Kelrick et al. 1986, Crist and MacMahon 1992), possibly because such seeds increase metabolic water yield and improve accessibility to assimilated energy (Christian and Lederle 1984). However, percent water-soluble carbohydrate and total caloric content per seed were clearly not sufficient to drive seed choice in *P. salinus*.

Protein rich foods are important to colony brood production in ants (Hölldobler and Wilson 1990, Portha et al. 2002, Dussutour and Simpson 2009), and thus may have influenced diet selection by *P. salinus* given that these ants were likely rearing offspring throughout the summer (Lavigne 1969, MacKay 1981, Kwapich and Tschinkel 2013). Both types of mustard seed had significantly higher crude protein content (*L. papilliferum* 23.3 ± 0.6%; *S. altissimum* 28.9 ± 0.7%) than either *P. secunda* (10.4 ± 0.1%) or *B. tectorum* (7.1 ± 0.1%), which may help explain why both mustards were overrepresented in the ant's diet when available. However, caution in such an interpretation is warranted because according to Whitford and Steinberger (2009) in a study of *Pogonomyrmex occidentalis*, foragers cannot discriminate between differences in crude protein content once those levels exceed 5%.

The large size of *B. tectorum* seeds may preclude them from being included regularly in the diet of *P. salinus* despite their favorable nutritional qualities (Kelrick et al. 1986). It was clear from observations conducted over the three years of the study that harvester ants have difficulty transporting these seeds, primarily because of the seed's large, bristled awn. By contrast, ants had little difficulty transporting smaller seeds to their nests, and it was smaller seeds that constituted the bulk of each colony's diet. Studies have shown that granivorous ants often match seed size to body size, perhaps in an effort to reduce transport times while maximizing energy returns (Davidson 1977, Kaspari 1996). From a foraging economics point of view, smaller seeds may be a more suitable food source for *P. salinus* as long as these seeds are available in sufficient numbers. Like many other harvester ants (Fewell 1988, Morehead and Feener 1998, Heredia and Detrain 2005, Pirk and de Casenave 2010), *P. salinus* foragers may adopt a time minimization strategy rather than a net energy gain per item returned strategy. However, such a strategy is not universal: when *Pogonomyrmex californicus* ants were provided with three different sizes of oat seed (*n.b.* larger seeds had higher caloric values and higher handling times), ants maximized net energy intake over time by preferentially taking larger seeds (Holder Bailey and Polis 1987). For *P. salinus*, the opportunity cost associated with handling large and cumbersome *B. tectorum* seeds at the expense of other seed types may make them unprofitable under most circumstances (Kelrick et al. 1986, Crist and MacMahon 1992). However, when search times for smaller seeds increase due to lack of availability, the opportunity cost associated with longer search times may prompt harvester ants to increase their uptake of *B. tectorum* seeds, as they did late in the summer of 2009. Similarly, seasonal shifts toward carnivory in the Neotropical species *Pogonomyrmex naegelii* may be explained by reduced availability of grass seeds during the wet/warm season (Belchior et al. 2012).

Further study is needed to sort out the interplay between nutrient content, handling times, and diet selection among the smaller seed types regularly consumed by *P. salinus*. Some studies indicate that seed size does not predict variation in seed selection by granivorous ant species (Whitford 1978, Briese and Macauley 1981), whereas others show that seed size is a significant predictor of preference (Crist and MacMahon 1992). When *P. secunda*, *S. altissimum*, and *L. papilliferum* seeds were available concurrently in 2010, ants consumed disproportionately large numbers of *S. altissimum* and *L. papilliferum* seeds (the two smallest seed types), perhaps in response to the rapid travel speeds associated with carrying each type. However, without detailed information about the spatial arrangement of these seed types relative to ant nests, as well as the specific nutrient requirements of colonies, it is impossible to draw conclusions about preferences. Ants may have been choosing specific seed types to provide the appropriate balance of nutrients required by the colony (Kay 2004), or they may have simply been exploiting profitable and easily accessible seed patches (Gorb and Gorb 2000, DeFalco et al. 2012, Flanagan et al. 2012), or both. Our study shows that *P. secunda*, *S. altissimum*, and *L. papilliferum* seeds are readily collected by Owyhee harvester ants, and are often overrepresented in the diet relative to the availability of other seeds on the ground. There is some indication that *L. papilliferum* and *S. altissimum* seeds are both taken preferentially over *P. secunda* seeds, although follow-up studies are needed to address alternative explanations.

There is growing recognition that selective foraging by seed-harvesting ants can alter the composition of plant communities, as well as add to the pressures facing rare plants within those communities (Crawley 2000, Albert et al. 2005, Ancheta and Heard 2011). Given the limited distribution and rarity of *L. papilliferum* (USFWS 2009), the affinity *P. salinus* has for the seeds of *L. papilliferum* (White and Robertson 2009, present study), and the prevalence of *P. salinus* within *L. papilliferum* populations (Robertson 2015), from a conservation perspective there is cause for concern regarding this plant and its relationship with harvester ants. The unique habitat requirements of *L. papilliferum* create dense localized concentrations of plants within the foraging ranges of ant colonies. Rich patches of seed deposited on the relatively unfettered surfaces of slick spots may facilitate rapid removal by ants because of the capacity of ants to recruit to profitable patches (Brown and Gordon 2000, Gorb and Gorb 2000, Guarino et al. 2005, Flanagan et al. 2012). Nevertheless, there may be conditions that mitigate seed loss to harvester ants. For example, although ants consume large numbers of *L. papilliferum* seeds soon after they drop to the ground, satiation effects (Andersen 1987) in productive years may allow seed banks within slick spots (Meyer et al. 2005) to replenish. Another possibility is that some seeds find refuge from ants by falling in cracks in the soil or being sheltered alongside lichens, rocks and other debris scattered on the soil surface. Further study is needed to test whether satiation, or some other mechanism, explains the persistence of *L. papilliferum* in the face of intense seed predation by harvester ants.

Acknowledgments

References Cited

Albert, M. J., A. Escudero, and J. M. Iriondo. 2005. Assessing ant seed predation in threatened plants: a case study. Acta Oecol. 28: 213-220.

Ancheta, J., and S. B. Heard. 2011. Impacts of insect herbivores on rare plant populations. Biol. Conserv. 144: 2395-2402.

Andersen, A. N. 1987. Effects of seed predation by ants on seedling densities at a woodland site in SE Australia. Oikos 48: 171-174.

Andersen, A. N., and D. H. Ashton. 1985. Rates of seed removal by ants at heath and woodland sites in southeastern Australia. Aust. J. Ecol. 10: 381-390.

Belchior, C., K. Del-Claro, and P. S. Oliveira. 2012. Seasonal patterns in the foraging ecology of the harvester ant *Pogonomyrmex naegelii* (Formicidae, Myrmicinae) in a Neotropical savanna: daily rhythms, shifts in granivory and carnivory, and home range. Arthropod-Plant Inte. 6: 571-582.

Briese, D. T., and B. J. Macauley. 1981. Food collection within an ant community in semi-arid Australia, with special reference to seed harvesters. Aust. J. Ecol. 6: 1-19.

Brown, M. J. F., and D. M. Gordon. 2000. How resources and encounters affect the distribution of foraging activity in a seed-harvesting ant. Behav. Ecol. Sociobiol. 47: 195-203.

Brown, J. H., O. J. Reichman, and D. W. Davidson. 1979. Granivory in desert ecosystems. Annu. Rev. Ecol. Syst. 10: 201-207.

Carrasco, J. L., B. R. Phillips, J. Puig-Martinez, T. S. King, and V. M. Chinchilli. 2013. Estimation of the concordance correlation coefficient for repeated measures using SAS and R. Comput. Meth. Prog. Bio. 109: 293-304.

Christian, D. P., and P. E. Lederle. 1984. Seed properties and water balance in desert granivores. Southwest Nat. 29: 181-188.

Crawley, M. J. 2000. Seed predators and plant population dynamics, pp. 167-182. *In*: M. Fenner M (ed.), Seeds: The Ecology of Regeneration in Plant Communities, 2nd Edn. CABI Publishing, University of Southampton, U.K.

Crist, T. O., and J. A. MacMahon. 1992. Harvester ant foraging and shrub-steppe seeds: interactions of seed resources and seed use. Ecology 73: 1768-1779.

Davidson, D. W. 1977. Foraging ecology and community organization in desert seed-eating ants. Ecology 58: 725-737.

DeFalco, L. A., T. C. Esque, M. B. Nicklas, and J. M. Kane. 2012. Supplementing seed banks to rehabilitate disturbed Mojave desert shrublands: where do all the seeds go? Restor. Ecol. 20: 85-94.

Detrain, C., and J. M. Pasteels. 2000. Seed preferences of the harvester ant *Messor barbarus* in a Mediterranean mosaic grassland (Hymenoptera: Formicidae). Sociobiology 35: 35-48.

Dussutour, A., and S. J. Simpson. 2009. Communal nutrition in ants. Curr. Biol. 19: 740–744.

Fewell, J. H. 1988. Energetic and time costs of foraging in harvester ants, *Pogonomyrmex occidentalis*. Behav. Ecol. Sociobiol. 22: 401-408.

Fisher, H., L. Eslick, and M. Seyfried. 1996. Edaphic factors that characterize the distribution of *Lepidium papilliferum*. Bureau of Land Management, Idaho State Office, Technical Bulletin 96-6.

Flanagan, T. P., K. Letendre, W. R. Burnside, G. M. Fricke, and M. E. Moses. 2012. Quantifying the effect of colony size and food distribution on harvester ant foraging. PLoS ONE 7: e39427. doi:10.1371/journal.pone.0039427.

Gauch Jr., H. G. 1982. Multivariate Analysis and Community Ecology. Cambridge University Press, Cambridge, U.K.

Gorb, E. V., and S. N. Gorb. 2000. Effects of seed aggregation on the removal rates of elaiosome-bearing *Chelidonium majus* and *Viola adourata* seeds carried by *Formica polyctena* ants. Ecol. Res. 15: 187–192.

Guarino, R., B. Ferrario, and L. Mossa. 2005. A stochastic model of seed dispersal pattern to assess seed predation by ants in annual dry grasslands. Plant Ecology 178: 225-235.

Heredia, A., and C. Detrain. 2005. Influence of seed size and seed nature on recruitment in the polymorphic harvester ant *Messor barbarus*. Behav. Process. 70: 289-300.

Hobbs, J. R. 1985. Harvester ant foraging and plant species distribution in annual grassland. Oecologia 67: 519-523.

Holder Bailey, K., G. A. Polis. 1987. Optimal and central-place foraging theory applied to a desert harvester ant, *Pogonomyrmex californicus*. Oecologia 72: 440-448.

Hölldobler. B., and E. O. Wilson. 1990. The Ants. Springer, Berlin.

Inouye, R. S., G. S. Byers, and J. H. Brown. 1980. Effects of predation and competition on survivorship, fecundity, and community structure of desert annuals. Ecology 61: 1344-1351.

Ireland, C., and M. H. Andrew. 1995. Ants remove virtually all western myall (*Acacia papyrocarpa* Benth.) seeds at Middleback, South Australia. Aust. J. Ecol. 20: 565-570.

Kay, A. 2004. The relative availabilities of complementary resources affect the feeding preferences of ant colonies. Behav. Ecol. 15: 63-70.

Kaspari, M. 1996. Worker size and seed size selection by harvester ants in a Neotropical forest. Oecologia 105: 397-404.

Kelrick, M. I., J. A. MacMahon, R. R. Parmenter, and D. V. Sisson. 1986. Native seed preferences of shrub-steppe rodents, birds, and ants: the relationships of seed attributes and seed use. Oecologia 68: 327-337.

Kinter, C. L., J. R. Fulkerson, J. J. Miller, and D. L. Clay. 2013. Habitat integrity and population monitoring of *Lepidium papilliferum* (slickspot peppergrass): 2012. Idaho Natural Heritage Program, Idaho Department of Fish and Game, Boise, ID.

Kwapich, C. L., and W. R. Tschinkel. 2013. Demography, demand, death, and the seasonal allocation of labor in the Florida harvester ant (*Pogonomyrmex badius*). Behav. Ecol. Sociobiol. 67: 2011-2027.

Lavigne, R. J. 1969. Bionomics and nest structure of *Pogonomyrmex occidentalis* (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 62: 1166-1175.

MacKay, W. P. 1981. A comparison of the energy budgets of three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). Oecologia 66: 484-494.

MacArthur, R. H., and E. R. Pianka. 1966. On optimal use of a patchy environment. Am. Nat. 100: 603-609.

MacMahon, J. A., J. F. Mull, and T. O. Crist. 2000. Harvester ants (*Pogonomyrmex* spp.): their community and ecosystem influences. Ann. Rev. Ecol. Syst. 31: 265-291.

Mehlhop, P., and N. J. Scott (Jr.). 1983. Temporal patterns of seed use availability in a guild of desert ants. Ecol. Entomol. 8: 69-85.

Meyer, S. E., D. Quinney, and J. Weaver. 2005. A life history study of the Snake River plains endemic *Lepidium papilliferum* (Brassicaceae). West. N. Am. Naturalist 65: 11-23.

Morehead, S. A., D. H. Feener (Jr.). 1998. Foraging behavior and morphology: seed selection in the harvester ant genus, *Pogonomyrmex*. Oecologia 114: 548-555.

Moseley, R. K. 1994. Report on the conservation status of *Lepidium papilliferum*. Idaho Department of Fish and Game, Conservation Data Center, Boise, ID.

Nicolai, N., J. L. Cook, and F. E. Smeins. 2007. Grassland composition affects season shifts in seed preference by *Pogonomyrmex barbatus* (Hymenoptera: Myrmicinae) in the Edwards Plateau, Texas. Environ. Entomol. 36: 433-440.

Pirk, G. I., J. Lopez de Casenave, R. G. Pol, L. Marone, and F. A. Milesi. 2009. Influence of temporal fluctuations in seed abundance on the diet of harvester ants (*Pogonomyrmex* spp.) in the central Monte desert, Argentina. Austral. Ecol. 34: 908-919.

Pirk, G. I., and J. Lopez de Casenave. 2010. Influence of seed size on feeding preferences and diet composition of three sympatric harvester ants in the central Monte Desert, Argentina. Ecol. Res. 25: 439-445.

Pirk, G. I., and J. Lopez de Casenave. 2011. Seed preferences of three harvester ants of the genus *Pogonomyrmex* (Hymenoptera: Formicidae) in the Monte desert: are they reflected in the diet? Ann. Entomol. Soc. Am. 104: 212-220.

Pol, R. G., J. Lopez de Casenave, and G. I. Pirk. 2011. Influence of temporal fluctuations in seed abundance on the foraging behavior of harvester ants (*Pogonomyrmex* spp.) in the central Monte desert, Argentina. Austral. Ecol. 36: 320-328.

Portha, S., J. L. Deneubourg, and C. Detrain. 2002. Self-organized asymmetries in ant foraging: a functional response to food type and colony needs. Behav. Ecol. 13: 776-781.

Reichman, O. J. 1979. Desert granivore foraging and its impact on seed densities and distributions. Ecology 60: 1085-1092.

Robertson, I. C. 2015. Habitat associations and dynamics of Owyhee harvester ant colonies located within slickspot peppergrass populations. U.S. Fish & Wildlife Service report, Boise, ID.

Scheller, R. M., and D. J. Mladenoff. 2002. Understory species patterns and diversity in old-growth and managed northern hardwood forests. Ecol. Appl. 12: 1329-1343.

Schilman, P. E., and F. Roces. 2006. Foraging energetics of a nectar-feeding ant: metabolic expenditure as a function of food source profitability. J. Exp. Biol. 209: 4091-4101.

Stephens, D. W., and J. R. Krebs. 1986. Foraging Theory. Princeton University Press, NJ.

(USFWS) U.S. Fish & Wildlife Service. 2009. Listing *Lepidium papilliferum* (slickspot peppergrass) as a threatened species throughout its range; Final Rule. Federal Register 74(194): 52014-52064.

Taber, S. W. 1998. The World of the Harvester Ants. Texas A&M University Press, College Station, TX.

White, J. P., and I. C. Robertson. 2009. Intense seed predation by harvester ants on a rare mustard. Écoscience 16: 508-513.

Whitford, W. G. 1978. Foraging in seed-harvester ants *Pogonomyrmex* spp. Ecology 59: 185-189.

Whitford, W. G., and Y. Steinberger. 2009. Harvester ants (Hymenoptera: Formicidae) discriminate among artificial seeds of different protein contents. Sociobiology 53: 549-558.

Wilby, A., and M. Shachak. 2000. Harvester ant response to spatial and temporal heterogeneity in seed availability: pattern in the process of granivory. Oecologia 125: 495-503.

Willott, S. J., S. G. Compton, and L. D. Incoll. 2000. Foraging, food selection and worker size in the seed harvesting ant *Messor bouvieri.* Oecologia 125: 35-44.

Zar, J.H. 2010. Biostatistical Analysis, 5th Edn. Pearson Prentice Hall, Upper Saddle River, NJ

Table 1. Mean percent coverage (± SEM) of understory vegetation and bare ground within the 12-m foraging radius of harvester ant colonies.

Understory Coverage	2009	2010	2011
Bromus tectorum	13.7 ± 2.7	15.4 ± 2.1	39.7 ± 2.7
Poa secunda	5.1 ± 1.0	12.6 ± 1.0	14.6 ± 3.1
Lepidium papilliferum	0.1 ± 0.04	0.3 ± 0.1	0.2 ± 0.06
Sisymbrium altissimum	3.2 ± 1.1	11.3 ± 1.6	-- [†]
Other vegetation	1.0 ± 0.3	1.6 ± 0.2	1.9 ± 0.9
Bare ground	76.9 ± 2.2	58.8 ± 2.1	43.6 ± 6.2

[†] *Sisymbrium altissimum* was experimentally removed from the site in 2011 as part of a separate experiment.

Table 2. Mean lengths (± SEM) and travel speeds (± SEM) of seed types returned by harvester ants to their nests. Sample sizes are in square brackets. Different letters indicate significant differences between samples within columns (Tukey's Multiple Comparisons Test, $P < 0.05$).

Seed Type	Length (mm)	Travel Speed (cm/sec)
Bromus tectorum[†]	18.40 ± 0.75 [15][a]	2.06 ± 0.29 [19][a]
Poa secunda	3.83 ± 0.07 [15][b]	7.64 ± 0.26 [36][b]
Lepidium papilliferum	1.38 ± 0.01 [15][c]	9.06 ± 0.26 [59][c]
Sisymbrium altissimum	0.98 ± 0.03 [15][c]	8.81 ± 0.31 [43][c]

[†] Length measurement includes awn. Results were unchanged when awn was not included, although mean length shortened to 6.89 ± 0.27 mm.

Table 3. Nutritional attributes of the seeds collected by harvester ants. Means (±SEM) are reported (N = 14 samples[†] for *P. secunda*, N = 14 for *B. tectorum*, N = 3 for *L. papilliferum*, N = 5 for *S. altissimum*). Wilcoxon rank sum pairwise comparisons were considered significant if $P \leq 0.0125$ after Bonferroni correction. Different letters indicate significant differences between samples within columns.

			Nutritional Attribute		
Seed Type	% Moisture	% Crude Protein	% Water-soluble Carbohydrate	Gross Energy (Calories/mg)	Gross Energy (Calories/seed)
B. tectorum	7.1 ± 0.3^a	7.1 ± 0.1^a	9.5 ± 0.2^a	3.88 ± 0.01^a	12.22 ± 0.28^a
P. secunda	$7.2 \pm 0.3^{a,b}$	10.4 ± 0.1^b	3.4 ± 0.1^c	4.07 ± 0.01^b	0.99 ± 0.03^c
L. papilliferum	4.4 ± 0.8^c	23.3 ± 0.6^c	7.2 ± 0.6^b	5.49 ± 0.05^c	1.64 ± 0.01^b $1.11 \pm$
S. altissimum	$5.8 \pm 0.1^{b,c}$	28.9 ± 0.7^c	3.8 ± 0.1^b	6.17 ± 0.07^c	0.01^b
Statistical Significance:	$H = 13.13$, $P = 0.0044$	$H = 30.86$, $P < 0.0001$	$H = 29.64$, $P < 0.0001$	$H = 27.52$, $P < 0.0001$	$H = 29.33$, $P < 0.0001$

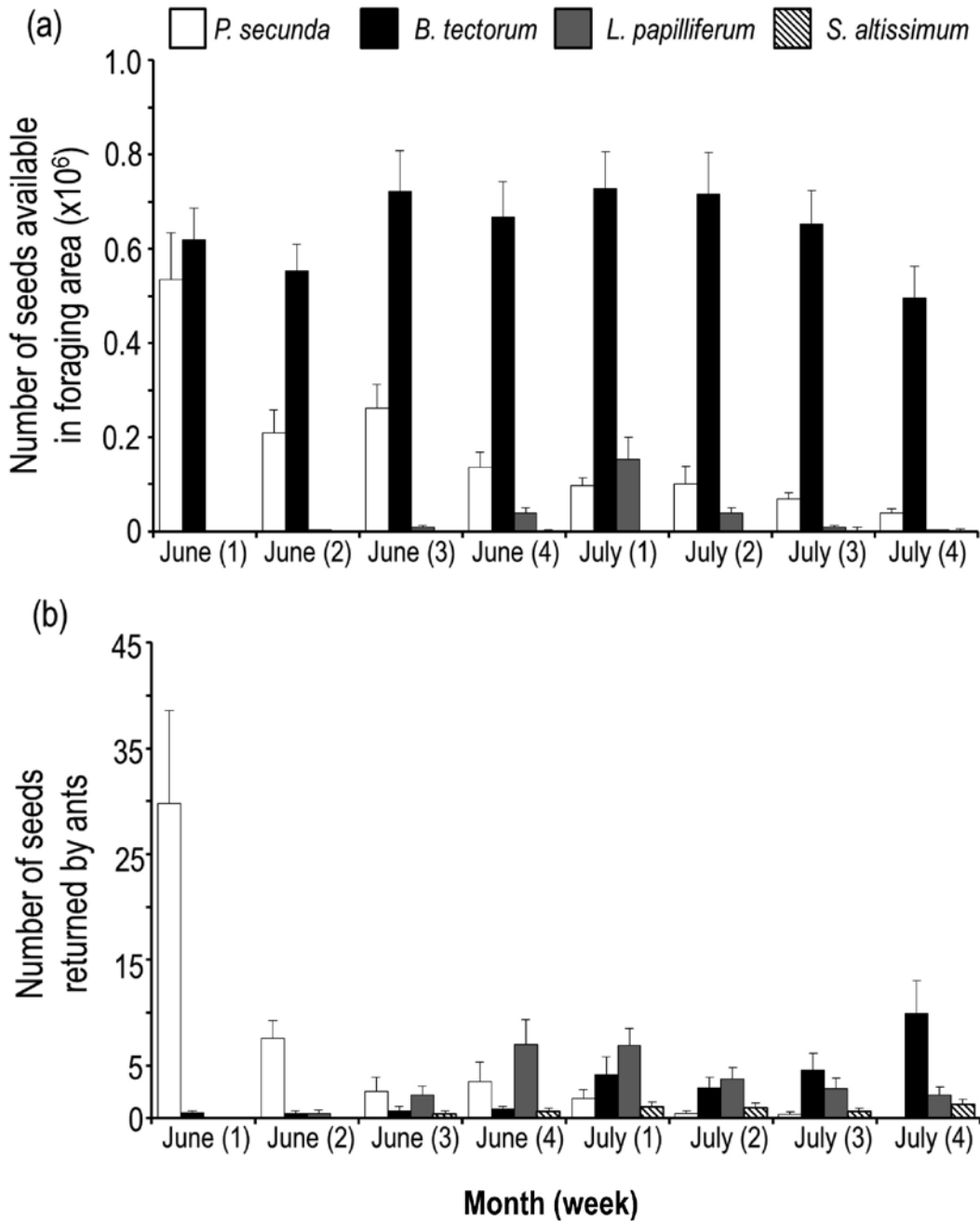[†] Each sample consisted of 3-4 g of seed taken from discrete patches of plants

Fig. 1. (a) Estimated mean (± SEM) number of seeds available each week within the foraging area of each ant colony (N = 9) in June and July of 2009. *L. papilliferum* seed availability is based on model predictions. Values for other seed types are based on core samples, extrapolated to the entire foraging area of a colony. (b) Mean (± SEM) seed intake by individual colonies per 5-min visual observation period. Intake values were adjusted using data collected from individual ants that were aspirated immediately following each visual observation period.
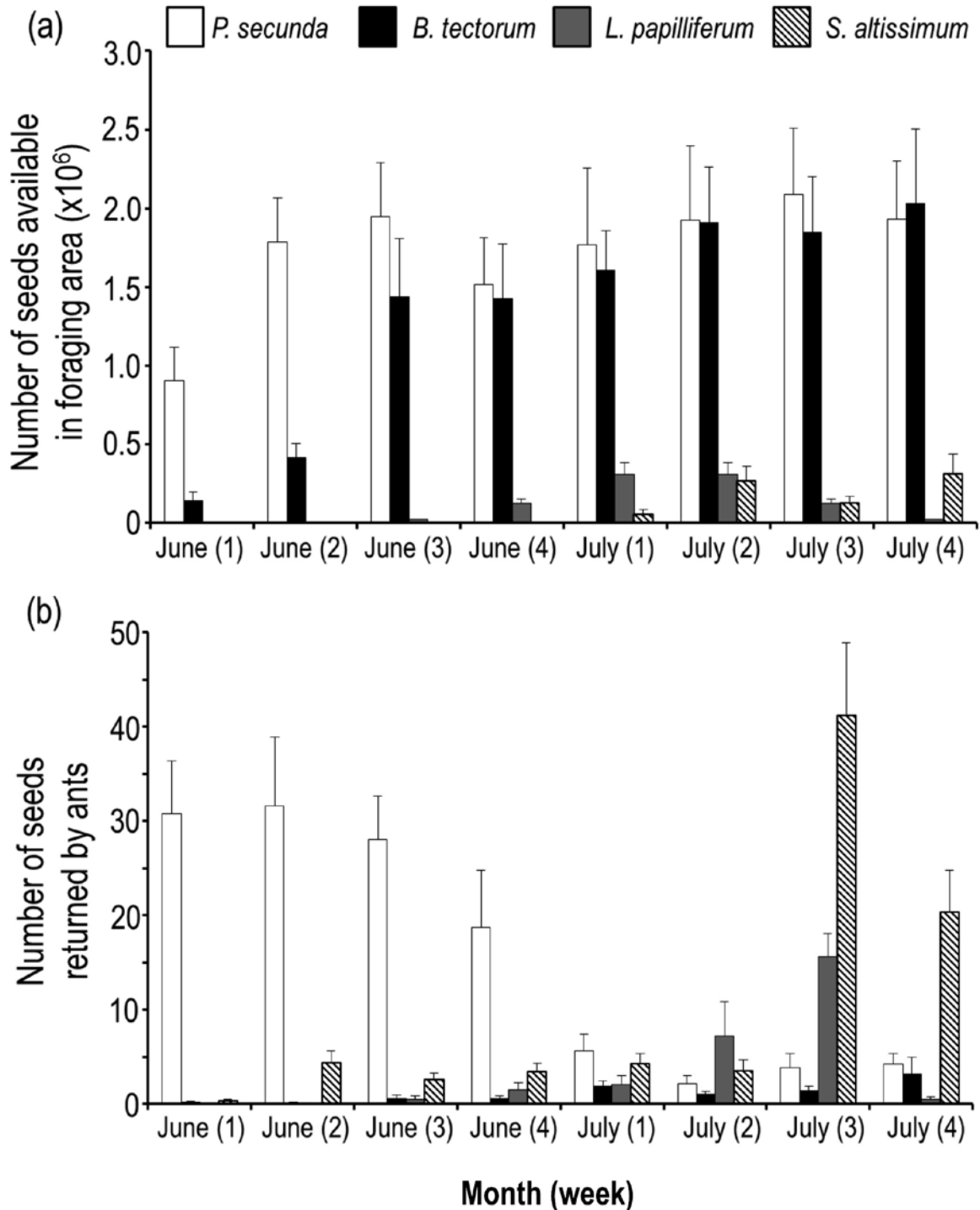
Fig. 2. (a) Estimated mean (± SEM) number of seeds available each week within the foraging area of each ant colony (N = 10) in June and July of 2010. *L. papilliferum* seed availability is based on model predictions. Values for other seed types are based on core samples, extrapolated to the entire foraging area of a colony. (b) Mean (± SEM) seed intake by individual colonies per 5-min visual observation period. Intake values were adjusted using data collected from individual ants that were aspirated immediately following each visual observation period.
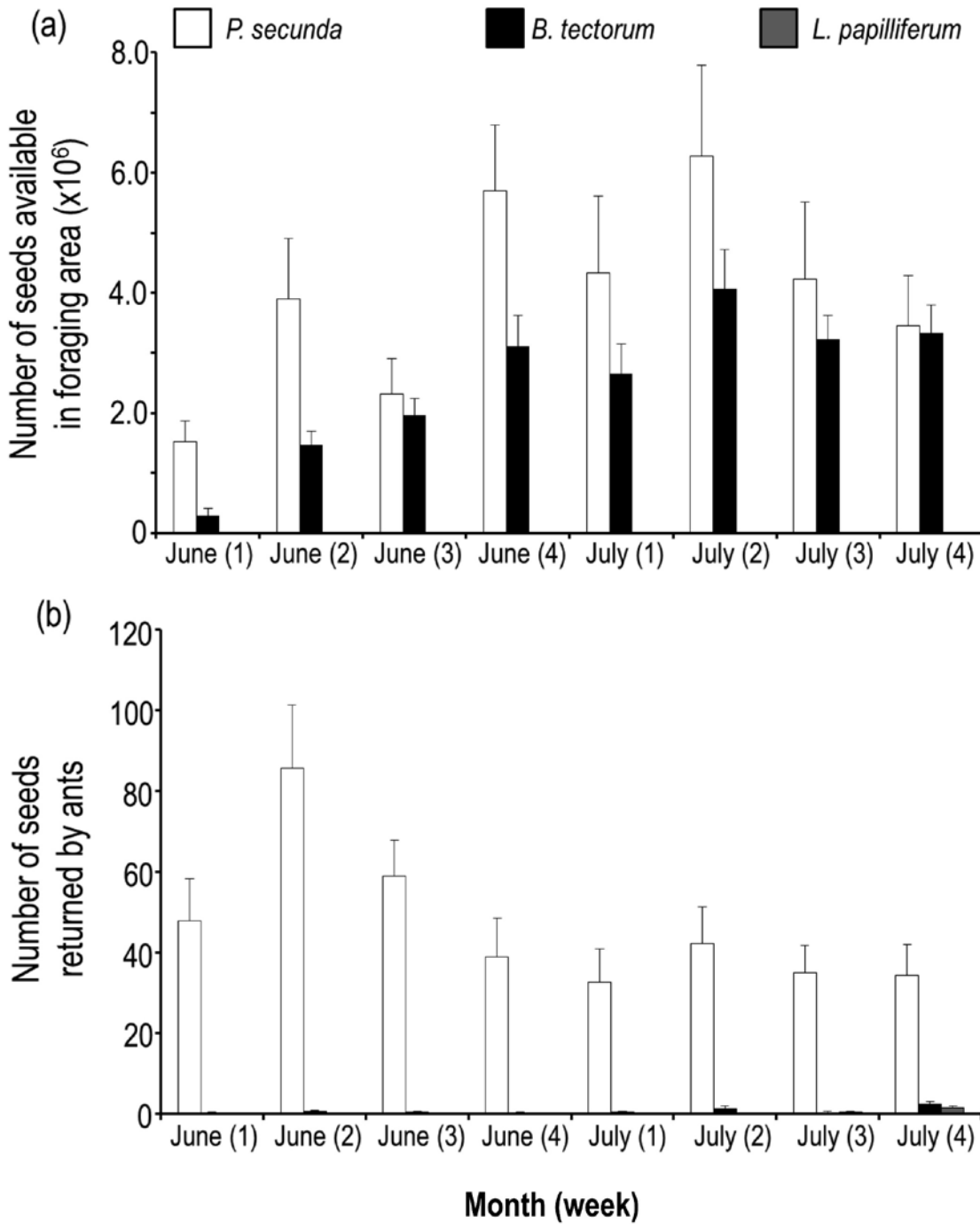
Fig. 3. (a) Estimated mean (± SEM) number of seeds available each week within the foraging area of each ant colony (N = 8) in June and July of 2011. *L. papilliferum* seed availability is based on model predictions. Values for other seed types are based on core samples, extrapolated to the entire foraging area of a colony. (b) Mean (± SEM) seed intake by individual colonies per 5-min visual observation period. Intake values were adjusted using data collected from individual ants that were aspirated immediately following each visual observation period.
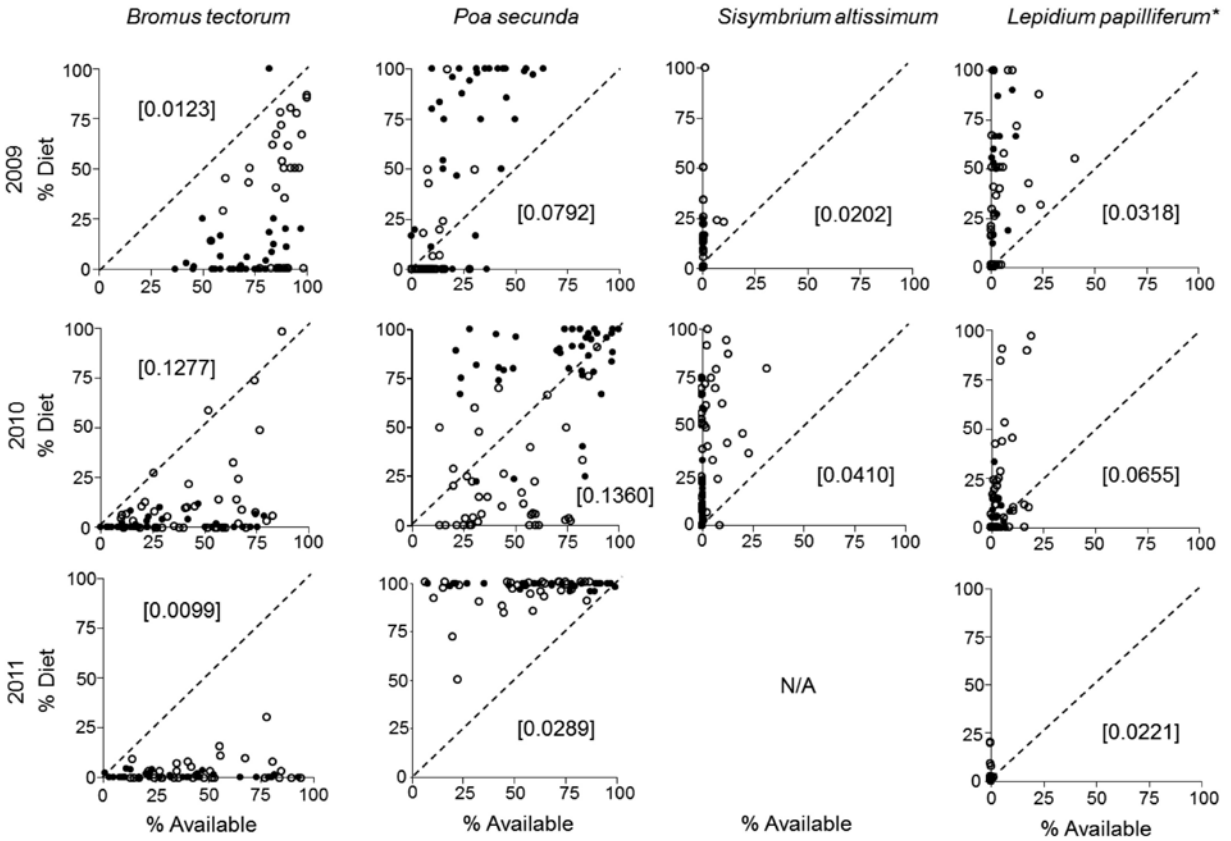
Fig. 4. Percentage of seeds consumed by ants as a function of each seed type's relative availability throughout June and July in each year of study. Dotted lines represent seed intake values that are directly proportional to seed availability. Individual points represent individual colonies during a specific week of sampling. Filled circles represent samples collected in June; open circles represent samples collected in July. CCC values are shown in brackets. Departures from 1 indicate selectivity, either positive (above dotted line) or negative (below dotted line). * *L. papilliferum* seed availability is based on model predictions.