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Behavior

Seed Selection by the Harvester Ant *Pogonomyrmex rugosus* (Hymenoptera: Formicidae) in Coastal Sage Scrub: Interactions With Invasive Plant Species

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Abstract

Harvester ants can be the dominant seed predators on plants by collecting and eating seeds and are known to influence plant communities. Harvester ants are abundant in coastal sage scrub (CSS), and CSS is frequently invaded by several exotic plant species. This study used observations of foraging and cafeteria-style experiments to test for seed species selection by the harvester ant *Pogonomyrmex rugosus* Emery (Hymenoptera: Formicidae) in CSS. Analysis of foraging behavior showed that *P. rugosus* carried seeds of exotic *Erodium cicutarium* (L.) and exotic *Brassica tournefortii* (Gouan) on 85 and 15% of return trips to the nest (respectively), and only a very few ants carried the native seeds found within the study areas. When compared with the availability of seeds in the field, *P. rugosus* selected exotic *E. cicutarium* and avoided both native *Encelia farinosa* (Torrey & A. Gray) and exotic *B. tournefortii*. Foraging by *P. rugosus* had no major effect on the seed bank in the field. Cafeteria-style experiments confirmed that *P. rugosus* selected *E. cicutarium* over other available seeds. Native *Eriogonum fasciculatum* (Bentham) seeds were even less selected than *E. farinosa* and *B. tournefortii*.

Key words: coastal sage scrub, harvester ant, *Pogonomyrmex rugosus*, preference, exotic

Coastal sage scrub (CSS) vegetation is an excellent model system for investigating harvester ant interactions with exotic plants; harvester ants are native to and abundant in CSS (Holway 2005), and this habitat type is currently severely impacted by exotic invasive plants (Minnich and Dezzani 1998). Exotic plants in CSS can displace native plants (O'Leary and Westman 1988) through altered fire frequencies (Keeley et al. 2005) and water competition (Eliason and Allen 1997, Wood et al. 2006), leading to declines in numerous plant and animal species (MacMahon et al. 2000, Diffendorfer et al. 2007). Harvester ants are often dominant seed predators through their collecting and feeding activities (Buckley 1982, Wolff and Debussche 1999, Sánchez et al. 2006). Seed selection by ants may affect plant community composition and plant species distribution (Whitford 1978, O'Dowd and Hay 1980, Mull and MacMahon 1996, Whitford et al. 2008, Brown et al. 2012). Additionally, seed selection by ants has been demonstrated to have the potential to affect the abundance and distribution of invasive exotic plants (Pearson et al. 2014).

The rough harvester ant, *Pogonomyrmex rugosus* Emery 1895 (Hymenoptera: Formicidae), is common in CSS and is also abundant in the areas invaded by exotic plants. *Pogonomyrmex rugosus* consumes seeds almost exclusively, and colony density on the landscape can reach 25 colonies per hectare, with 1,000 to over 22,000 adult workers per colony (McKay 1981). Foraging ants travel up to 40 m

from their nest (Hölldobler and Wilson 1990), and *Pogonomyrmex* spp. seed harvesting preferences can change relative abundances of plant species (MacMahon et al. 2000). The objectives of this study were to determine *P. rugosus* seed selection through observations of foraging and through cafeteria-style experiments. First, foraging *P. rugosus* were observed harvesting seeds under field conditions. Next, seeds of four plant species found in CSS (brittlebush (*Encelia farinosa* Torr. & A. Gray), buckwheat (*Eriogonum fasciculatum* Bentham), filaree (*Erodium cicutarium* L.), and mustard (*Brassica tournefortii* Gouan) were presented to *P. rugosus* in cafeteria-style experiments (after Hobbs 1985). Brittlebush and buckwheat are native plants commonly found within CSS, while filaree and mustard are prevalent invasive nonnative plant species (Robbins et al. 1970, Whitson 1992, Minnich and Sanders 2000). While the inclusion of more seed species might more accurately replicate natural foraging conditions, the cafeteria-style test is limited in that less-preferred seeds will not be selected whenever more-preferred seeds are present. Thus, it would be difficult to compare preference among the least-selected seeds (Raffa et al. 2002). Rather than strict rank orders of preferences, this study is concerned with simply discriminating between the most-selected and least-selected seeds.

Among many possibilities, ant selection for seed species may differ due to seed characteristics such as mass (Briese and Macauley

1981, Crist and MacMahon 1992), abundance (Crist and MacMahon 1992), nutrient content (Kelrick et al. 1986), olfactory cues (Nickle and Neal 1972, Gordon 1978), water content (Casazza et al. 2008), chemical defenses (Nickle and Neal 1972, Carroll and Janzen 1973, Janzen 1978), length of harvest window, the number of seeds deposited at a single time (Whitford 1978), ease of transport (Azcarate et al. 2005), and accessibility of the edible portion of the seed (Kauffman and Collier 1981), and ant characteristics such as worker size (Johnson 1991, Willot et al. 2000), colony food reserves (Reyes-López and Fernández-Haeger 2002), and previous exposure and memory (Johnson 1991, Johnson et al. 1994). Considering, briefly, some of these seed characteristics, seeds of the four plant species in this study vary in average mass (filaree ∼2 mg, and mustard, buckwheat, and brittlebush all ∼1 mg). Kelrick et al. (1986) suggest that *P. rugosus* prefers seeds weighing 3–30 mg. Thus, filaree may be selected over other seeds. *Pogonomyrmex* ants in another system prefer seeds with the highest abundance (Crist and MacMahon 1992), and filaree and mustard are likely to be most abundant in the invaded CSS in this study. The nutritional contents, olfactory cues, and water contents of this study's four seed species have not been examined. Some ants prefer seeds bearing nutrient-rich elaiosomes (Pemberton 1988, Smith 1989, Jensen and Six 2006), but this study investigates seeds that have no elaiosomes.

Chemical defenses have been characterized only for mustard seeds: they contain glucosinolates (Ulmer and Dosdall 2006), which can deter ants (Müller et al. 2002). Some approximations for defenses in the other seeds can come from studies within their respective families. Seed extracts from a member of Geraniaceae (*Silybum marianum*), the family of filaree, are toxic to some insects (Velcheva et al. 2001). Brittlebush plants contain defensive chromenes, including encecalin (Kunze et al. 1996), and another member of Asteraceae (*Centaurea cyanus*) contains four indole alkaloids (Sarker et al. 2001), which are known to deter insect feeding (Kutchan 1995). Another buckwheat in Polygonaceae (*Fagopyrum esculentum*) has seeds containing protein inhibitors of serine proteases (Tsybina et al. 2004), which interfere with insect digestion (Habib and Fazili 2007). Thus, all four seeds may have chemicals that deter ants.

Filaree and mustard seeds both have short harvest windows. Filaree seeds can use hygroscopic coiling to bury a third of a season's seeds within 10 d of maturation in optimal (i.e., rainy) conditions (Stamp 1989). The mustard's mucilagenous seed coat, when wetted, can adhere seeds to the soil (Bangle et al. 2008), reducing their collection by some ants (Engelbrecht and García-Fayos 2012). Repeated short harvest windows in the past may prioritize selection of filaree and mustard, even if no precipitation occurs to wet these seeds during the study period. Filaree and mustard seeds also mature on the plant within a short time period, and *P. rugosus* prefers seeds shed in large quantities in other systems (Whitford 1978). The propensity for filaree seed awns to become tangled in litter may make them less preferred. Ants carrying filaree seeds often exhibit halting movement as the ant stops to disentangle the seed (C. Briggs, personal observation). If ants are able to walk quickly while carrying a seed, they may be at a lower risk of predation and desiccation, and be able to complete more trips to a foraging patch within a certain amount of time. Lastly, refuse piles located near nests frequently possess a 2- to 3-cm-deep layer of discarded seed coats of filaree and mustard (C. Briggs, personal observation), yet the ease with which *P. rugosus* processes these seeds is not known.

By focusing on a single ant species, this study controls for some ant characteristics that could influence seed selection. Furthermore, this study did not attempt to tease apart any of the seed characteristics that could be factors in selection. Instead, results are discussed in the context of these possible criteria listed above, and the consequent speculations will have to be confirmed by a more extensive series of preference tests. Given the complexity of these considerations, we chose to test the null hypothesis of no preference, and thus predict that *P. rugosus* collects seeds in proportion to their availability.

Materials and Methods

Site Description

Foraging observations were conducted at field sites located on the southern portion of the University of California, Riverside campus (33° 57′ N, 117° 19′ W; hereafter the "UCR site"). Cafeteria-style experiments (described below) were conducted at both the UCR site and at the Motte Rimrock Reserve near Perris, CA (University of California Natural Reserve System; 33° 48′ N, 117° 15′ W). Tests for impact of ant foraging on seed-bank density were conducted at the UCR site. All tests took place in June through August of 2008.

Vegetation commonly found at the UCR site includes native brittlebush (*Encelia farinosa*), native *Amsinckia* spp. Lehm., exotic filaree (*Erodium cicutarium*), exotic mustard (*Brassica tournefortii*), and exotic *Schismus* spp. P. Beauv. Vegetation commonly found at the Motte Rimrock site includes native buckwheat (*Eriogonum fasciculatum*), native California sagebrush (*Artemisia californica* Less.), native black sage (*Salvia mellifera* Greene), and native brittlebush, and by the exotic filaree (*Erodium* spp.), exotic rat's tail fescue (*Vulpia myuros* L.), and exotic compact brome (*Bromus madtritensis* ssp. *rubens* L.). The exotic mustard *Brassica tournefortii* is not found at the Motte Rimrock site (Allen et al. 1998, Vourlitis et al. 2007).

Native buckwheat (*Eriogonum fasciculatum*) is not found at the UCR site (location of foraging observations), and ants were not observed carrying seeds of this species in the field under natural conditions. Buckwheat seeds were included in all cafeteria-style experiments because of the plant's presence at the Motte Rimrock Reserve and throughout other areas of CSS.

Foraging Selection With Respect to Seed Availability

Foraging *P. rugosus* were observed harvesting seeds under field conditions at the UCR site. *Pogonomyrmex rugosus* typically travels along a trunk trail, characterized by a cleared path directed outward from the colony entrance (Hölldobler 1976). Several meters from the nest the trunk trail broadens significantly, and foraging ants scatter over an area of a few square meters. This area is defined here as the foraging patch.

In total, 15 replicate colonies were selected, and for each colony a single foraging patch was observed. Data collection from each colony began when ants established a new trunk trail and began collecting seeds from a previously unvisited foraging patch. The newness of the trunk trail and the unvisited nature of foraging patch were confirmed by observing colonies regularly after the seasonal maturation of nearby seeds. No foraging had been observed in these patches since the time that nearby seeds matured. Assuming that *P. rugosus* uses seed-availability criteria to select where to forage (Gordon 1991, Detrain et al. 2000, Greene et al. 2013), and that the most-collected seeds are likely to become rarer over time in a single patch, observations from a fresh foraging patch were best able to detect uncompromised foraging selection by the ants.

For a given foraging patch, observations were made at the "convergence point," where ants carrying food items converged
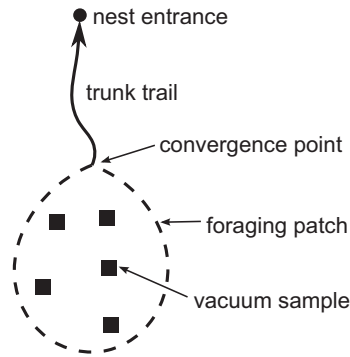
Fig. 1. Diagram of a characteristic *P. rugosus* foraging column, showing the trunk trail, colony entrance, convergence point, foraging patch (2–27 m²), and randomly located vacuum samples.

within the patch on their return to the nest (Fig. 1). The outermost convergence point was used to ensure that ants were sampled from a distinct foraging patch. (If returning ants were observed closer to the nest, it is possible that they were foraging in areas that were not clearly defined by the observer.)

Morning and evening observations were made between 0800–1030 hours and 1900–2100 hours during 19–27 June 2008. After dark, a headlamp was used to illuminate the convergence point, with no obvious effects on ant behavior in the foraging patch. Ants were inactive during the heat of the day (1030–1900 hours). One-minute observational periods were replicated 5–25 times (depending on ant activity) and separated by one minute. During observations, all individuals returning to the nest were counted and categorized according to the item they carried. In no case did any individual return with more than one seed.

All observed items were identified and counted separately. Items were identifiable by eye, by observing how the ants moved, the shape and color of the item, and how the item was held in the ants' mandibles. Items being returned to the nest were categorized into one of four groups: brittlebush seed, filaree seed, mustard seed, and nonseed. Nonseed items included vegetation, arthropod bodies, bird droppings, rocks, and ants returning to the nest without an item. Because we could not determine the abundance of nonseeds, this category was excluded from subsequent analyses. Individual ants were not observed to consume items prior to returning to the nest. The total number of seeds collected by a colony during the observation periods was determined by adding the counts of brittlebush, filaree, and mustard seeds carried by ants. This total enabled us to calculate the proportion of the harvested total comprised by each seed type.

After collecting observational data, two separate estimates of seed availability were made in each of the 15 replicate foraging patches. The first estimate was made 36 h after the ants began foraging in a patch. This initial estimate of availability of each seed type allowed us to compare our observations of ant foraging to the relative abundances of each seed type in the foraging patch. For instance, half of all seeds collected by a colony may have been brittlebush seeds, and these seeds may have comprised only a 10th of all seeds in the patch.

Because the observations of foraging ants and the observations of seed availability were made on the same colonies, we had to negotiate a methodological problem. It was not possible to assess true availability within a patch prior to the onset of foraging, as we were restricted by requiring that we first allow the ants to select the patch. No method would have allowed us to quantify all naturally available seeds in all potential foraging areas, and then present the same

undisturbed seeds to ants. Disturbance during seed-availability estimation was minimized by working during the heat of the day when ants were not foraging. The 36-h delay allowed morning and evening periods of observations before potentially disturbing the ants by estimating seed availability within the patch. The second estimate of availability was conducted after the ants stopped foraging within the selected patch—a time determined by monitoring the foraging patches for up to an additional 16 d. This second estimate allowed us to determine whether ant foraging altered the existing seed bank within a foraging patch. Again, it was not possible to determine true initial seed availability for these colonies, since ants foraged for 36 h before the initial availability estimate was made. Our data thus miss any small change to the seed bank that the ants may have made in the first 36 h. However, foraging activity appeared to involve the same number of ants from day to day for a given colony (not overwhelmingly vigorous at first), and foraging patches were utilized by ants for a long period of time (12–16 d). Thus, we assume that the overall impact of the 36-h delay on this comparison of initial and final seed availability is minimal.

As ants foraged both on the ground and on plants, determining seed availability required estimates of seed availability both on the ground and within the canopy of plants in which ants were found foraging. While overall colony activity appeared to be consistent over time, the relative rate of foraging on the ground versus in the canopy appeared to vary among foraging patches and over time. We did not attempt to adjust our estimates of ground and canopy seed availability for individual colonies.

To estimate seed availability on the ground, five random locations were chosen within each patch (Fig. 1). At each location, 100 sq. cm (10 by 10 cm²) of ground surface was vacuumed (number 2820B battery operated vacuum, BioQuip Products, Inc., Rancho Dominguez, CA). All seeds of three plant species (brittlebush, filaree, and mustard) were counted and their mass determined; no other species appeared in a sufficient number of samples to be included in the analysis. These five samples within each patch were subsequently averaged and converted to a square meter basis, to provide an estimate of seed availability per patch at the time when ants began foraging.

As filaree plants lacked structures that retained seeds above the ground, vacuum samples were sufficient to determine overall filaree seed density. The densities of seeds in the canopies of mustard and brittlebush plants were estimated using the methods described in Nicolai et al. (2007) and Whitford (1978). In an adjacent area of similar vegetation to the above foraging studies, the average number of siliques per mustard plant was determined by counting the total number of siliques per plant ($n = 21$ plants). Within the same field, the average number of seeds per silique was determined by randomly collecting 29 siliques from across the field and counting the total number of seeds per silique. Then, within each foraging patch, the number of mustard plants was determined as well as an estimate of the percentage of intact siliques per plant. On a square meter basis, canopy seed density for mustard was then estimated as:

$$(number\ of\ seeds\ per\ silique) \times (number\ siliques\ per\ plant)$$
$$\times\ (number\ of\ plants\ per\ area\ of\ foraging\ patch)$$
$$\times\ (percentage\ of\ intact\ siliques\ per\ plant)$$

Canopy seed density of brittlebush within a foraging patch was determined similarly. In an adjacent area, the number of seeds per inflorescence was calculated by randomly collecting inflorescences and counting the number of seeds per inflorescence ($n = 8$ inflorescences). Then, within each foraging patch, the number of

inflorescences was counted. On a square meter basis, canopy seed density of brittlebush was then estimated as:

(*number of seeds per inflorescence*)

$\times$ (*number of inflorescences per area of foraging patch*)

Seed densities of mustard and brittlebush canopies were recorded at the same time as vacuum sampling of the ground was conducted. We assumed that all mustard and brittlebush seeds in siliques and inflorescences were equally available to ants. While not all seeds on these plants mature simultaneously, the siliques and inflorescences in the foraging patches were not obviously variable in their maturity at the time that *P. rugosus* started foraging.

Density estimates of seed on the ground and within the plant canopy were combined to yield an overall estimate of seed abundance per square meter within each of the 15 foraging patches, determined at both the beginning and the end of ant foraging in a patch.

Foraging Selection Under Equivalent Seed Availability

Seeds of four plant species found in CSS (brittlebush, buckwheat, filaree, and mustard) were presented to *P. rugosus* in cafeteria-style experiments (after Hobbs 1985) at both the UCR and Motte Rimrock field sites. These selection experiments at the UCR and Motte Rimrock sites were conducted over five weeks from July to August. Each experiment involved 60 arbitrarily selected *P. rugosus* colonies (120 colonies total) that were not already used in other parts of this investigation. This period coincided with mature seed availability for all four species being evaluated. In preparation, seeds of filaree and mustard were collected from field sites near UCR. Seeds of brittlebush and buckwheat were relatively rare in the field and were purchased from S&S Seeds (Carpinteria, CA).

Seeds were presented to foraging ants in clear plastic Petri dishes (9 cm diameter, 2 cm height), with a separate dish used for each species of seed. To allow access by foraging ants, eight holes (1.5 cm wide by 1 cm high) were cut through the sidewalls of each Petri dish with a hot soldering iron. Dishes were then covered with their standard lids to prevent access to the seeds by vertebrates.

To prepare for testing, active *P. rugosus* colonies were identified and the divergence point of each main foraging trail was marked (Fig. 2). The following morning, four dishes (each containing 70.0 ± 3.0 mg of seeds of a single species, with the mass of seeds recorded to nearest 0.01 mg) were placed at the divergence point of the main foraging trail of each colony, as identified the previous day. The dishes were ordered randomly in a semicircle, with each dish 50 cm from the divergence point. *P. rugosus* individuals were soon observed foraging in all four dishes. Because of seed placement within the foraging patch, and the ants' characteristic territorial defense of their foraging patch (Hölldobler 1976), other insects were unlikely to remove seeds from the dishes. During roughly six hours of casual observations, *P. rugosus* was the only species observed entering the dishes. Indeed we witnessed no other animals foraging within our observation patches during the duration of the experiment. However, some wildlife did turn over a few dishes, while we were absent from the field. After excluding disturbances by wildlife, the UCR and Motte Rimrock sites yielded results from 50 and 58 replicate colonies, respectively. After 48 h, the mass of seeds remaining in each dish was determined to the nearest 0.01 mg. Measurements of initial mass, remaining mass, and the colony's total mass removed were used to determine the proportion removed from each seed dish.
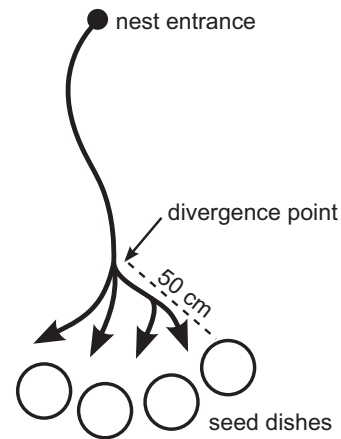

Fig. 2. Diagram of a characteristic *P. rugosus* foraging column, showing the colony entrance, divergence point, and four randomly ordered dishes for four seed species, each located 50 cm from the divergence point.

Control dishes placed outside the foraging patch were used to account for mass changes unrelated to ant foraging. Each control dish contained 70.0 ± 3.0 mg of an individual species of seed. Three control dishes for each seed species (for a total of 12) were placed in an area adjacent to the cafeteria tests. Dish openings were covered by fine mesh cloth to prevent animal access.

Statistical Analyses

All analyses were conducted using SAS (Ver. 9.1, SAS Institute, Cary, NC) or Microsoft Excel (Redmond, WA).

Foraging With Respect to Seed Availability

To detect ant selection of seed with respect to availability in the field, three resource types were defined: filaree, mustard, and brittlebush seeds. We compared the proportion of available seed type to the proportion of seed type harvested by the ants (after Lele et al. 2013). The proportion of available seed was determined by dividing the density of seeds of a particular species (number per sq. m) by the density of all seeds in a foraging patch (number per sq. m). The proportion of a species of seed harvested by the ants was determined by dividing the number of seeds of a particular species collected by the total number of seeds retrieved by ants from the foraging patch. If ants exhibited no selection preference, the proportion of each species harvested would not be significantly different from the proportion of each species available. The Wilcoxon signed-rank test (Wilcoxon 1945) was used to compare the set of available proportions with the set of harvested proportions of seed types, with foraging patch considered as the replicate.

Paired *t*-tests with Bonferroni adjustment (Benjamini and Hochberg 1995) were used to determine whether ant foraging affected the available seed bank within a foraging patch. The seed bank was characterized by two parameters for each of the following species: mustard, brittlebush, and filaree. The Bonferroni adjustment corrected for these simultaneous tests. For each species, density of seeds found in the patch (number per sq. m; ground plus canopy), and the average mass of a single seed on the ground within a patch (number of seeds of a particular species divided by their total mass) were determined. Data from which these parameters were derived originated from the vacuum samples and canopy estimates of available seed determined prior to and after ant foraging in a patch. Data

Table 1. Seeds collected by *P. rugosus*, and seed availability at the UC Riverside site (means ± SEM; $n = 15$ foraging patches)

Seed	No. carried per min	Equivalent mass of seeds carried per min (mg)	Proportion of total no. of seeds carried	Initial proportion of available no. of seeds	Initial number of seeds per m²	Final number of seeds per m²	Initial mass of a seed on the ground (mg)	Final mass of a seed on the ground (mg)
Filaree	16 ± 3	~23	0.85 ± 0.06a	0.44 ± 0.09b	2,200 ± 400	1,600 ± 300	1.42 ± 0.04	1.44 ± 0.05
Mustard	3.3 ± 1.4	~3	0.15 ± 0.06a	0.55 ± 0.09b	7,000 ± 2,200	5,900 ± 2,300	0.99 ± 0.09	0.90 ± 0.05
Brittlebush	0.018 ± 0.010	~0.01	0.00090 ± 0.00051a	0.011 ± 0.003b	72 ± 21	55 ± 20	0.68 ± 0.11	0.62 ± 0.11

Columns show information regarding three seed species collected by *P. rugosus*. Filaree and mustard are exotic species. Density values include seeds from the ground and the plant canopy.

Different lowercase letters in the same row represent significant differences ($P < 0.005$; Wilcoxon signed-rank tests [Wilcoxon 1945]). No significant difference was detected between initial and final proportion of available seeds for each species, nor between initial and final density for each species, nor between initial and final mass of a single seed on the ground for each species ($P \geq 0.06$; paired *t*-tests with Bonferroni adjustment [Benjamini and Hochberg 1995]).

from vacuum samples were averaged for each patch before analysis. Foraging patch was considered as the replicate ($n = 15$).

Foraging Selection Under Equivalent Seed Availability

A multiple-choice feeding preference test (Lockwood 1998) was used to determine whether *P. rugosus* was collecting the four species of seed (brittlebush, buckwheat, filaree, and mustard) in different proportions; colony observed was considered as the replicate. This test accounts for variability in colony activity (some colonies were more active than others), while assessing differences in foraging preference among the four species of seed. If ants did not collect all seeds equally, pairwise comparisons, as detailed in Lockwood (1998), determined whether there were significant differences in preference for each seed type.

Results

### Foraging Selection With Respect to Seed Availability

Across all foraging patches in this study, *P. rugosus* carried mustard seed and filaree seed on the majority of return trips (Table 1). Ants harvested only a very few brittlebush seeds (7 out of the 9,600 ants observed). Two other seed types (*Schismus barbatus* and *Amsinckia* spp.) were detected and were excluded from analysis, because fewer than three of these seeds were carried by the ants during the experiment. Roughly 14% of all observed ants carried nonseed items, and about 26% returned to the nest carrying nothing.

The Wilcoxon signed-rank tests showed that *P. rugosus* collected filaree seeds in a greater proportion than what was available ($W_{filaree} = -120$; $Z = -3.39$; $P < 0.001$). Additionally, ants collected mustard and brittlebush seed in lower proportions than what were available ($W_{mustard} = 118$; $Z = 3.34$; $P < 0.001$) and ($W_{brittlebrush} = 81$; $Z = 2.81$; $P < 0.005$). These results suggest that *P. rugosus* preferentially collects filaree, in a proportion roughly double to what is available in the field. Paired *t*-tests showed no change to the seed bank as a result of ant foraging, and the changes were nonsignificant even without the Bonferroni adjustment (Table 1).

Foraging Selection Under Equivalent Seed Availability

Results from the UCR site indicated that that ants selected filaree over mustard and buckwheat. Selection of brittlebush was not different from either mustard or filaree (Fig. 3; $F = 2.80$; df = 3, 47; $P < 0.05$). Results from the Motte Rimrock site demonstrated a similar pattern, with ants selecting brittlebush, filaree, and mustard over buckwheat seeds (Fig. 3; $F = 2.77$; df = 3, 55; $P < 0.05$).

Summary of cafeteria-style experiments
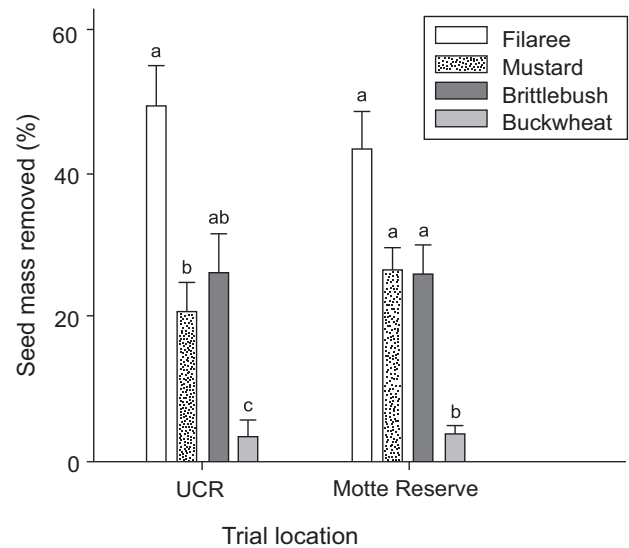
Fig. 3. Summary of cafeteria-style experiments on *P. rugosus*. Groups of four columns show data from the UC Riverside site ($n = 50$ replicate colonies) and from the Motte Rimrock site ($n = 58$ replicate colonies). Error bars show standard errors. The proportion of seed mass removed is reported here as a percentage of the total, so all columns for a site sum to 100%. Columns sharing letters within a field site location are not significantly different, as determined by a multiple-choice feeding preference test (Lockwood 1998), with colony as a replicate (UCR: $F = 2.80$; df = 3, 47; $P < 0.05$; Motte Rimrock: $F = 2.77$, df = 3, 55; $P < 0.05$).

In summary of all of the results, the foraging patch observations showed selection of filaree over mustard and brittlebush. At one field site, the cafeteria tests showed selection of filaree over mustard and buckwheat, and at the second field site, a selection of brittlebush, filaree, and mustard over buckwheat. Thus, filaree was always in the most-selected group, buckwheat was least-selected in the two cafeteria tests when it was used, and results for both mustard and brittlebush were mixed between the most- and least-selected.

Discussion

Foraging Selection With Respect to Seed Availability

Filaree and mustard seeds were collected on the majority of *P. rugosus* foraging trips, in a habitat where these two plants are introduced exotic species. Previous investigators observed *P. rugosus* collecting

filaree seeds (MacKay 1991), but this is the first report of *P. rugosus* collecting this exotic mustard (*B. tournefortii*).

The seed bank remained stable during the period of foraging observations. The relative proportions of the most-selected seeds did not significantly decline during ant foraging, nor did the average seed mass significantly decline from the collection of the heaviest seeds. Our finding of seed-bank stability is at odds with other studies that have found changes in the number of available seeds after foraging by ants (White and Robinson 2009, Westerman et al. 2014). One explanation for our finding is that *P. rugosus* left foraging patches due to nonseed factors, before the seed bank was significantly depleted (Crist and Wiens 1994, Mull and MacMahon 1997, Gordon 2013). For example, *P. rugosus* will shift its trunk trails to avoid competition with other ants (Hölldobler, 1976); however, here, no competing ants were observed. It appears that ant foraging during this period had a minimal impact on the seed bank simply due to the large of amount of seed present on the soil.

This does not mean, however, that foraging by *P. rugosus* has no impact on seed dynamics. Numerous investigators report impacts of ant foraging on seeds, such as through density- and distance-dependent predation (Janzen 1970, Connell 1971), changes in seed and plant density (Harmon and Stamp 1992, Avgar et al. 2007), seed dispersal (O'Dowd and Hay 1980, Mull 2003, Retana et al. 2004, Sanchez et al. 2006), and changes in plant productivity near ant mounds (Wight and Nichols 1966, Rissing 1986, Whitford 1988, Danin and Yom-Tov 1990, Nowak et al. 1990, Bossard 1991).

Foraging Selection Under Equivalent Seed Availability

Direct tests showed that *P. rugosus* selected seeds of filaree over brittlebush and mustard, and that buckwheat seeds were avoided (Fig. 3). This supports results from the foraging observations, where filaree was selected in greater proportions than mustard and brittlebush. In other areas, *Pogonomyrmex* ants are known to prefer seeds of *Eriogonum* spp. (buckwheat; Costa 1991)—a finding not supported by these experiments.

Seeds of the four plant species in this study vary in average mass, and Kelrick et al. (1986) suggest that *P. rugosus* prefers heavy seeds up to 30 mg. This could explain why *P. rugosus* selected the heavy filaree over the relatively light mustard, brittlebush, and buckwheat seeds. *Pogonomyrmex* ants in another system prefer seeds with the highest abundance (Crist and MacMahon 1992), but in this system, *P. rugosus* avoided the mustard seeds, even though mustard seeds were present in roughly double the number of the filaree, per square meter (Table 1).

We have already described our speculations regarding potential chemical defenses in these seeds. More generally, chemical defenses in seeds are likely to reflect specific challenges to reproduction from a local set of antagonists (Levin 1976). Defenses in filaree and mustard may have been effective within their native ranges, but did not evolve to deter feeding by ants in Southern California. However, observations within the native range of several filaree species suggest that harvester ants found in the native habitat also collect large numbers of *Erodium* seeds (Moggridge 1873), suggesting that chemical defenses do not play a major role in selection preference by harvester ants for filaree.

Filaree and mustard seeds only have short harvest windows when there is precipitation (Stamp 1989, Bangle et al. 2008), and filaree was selected by ants even though no precipitation fell during the study period. Lastly, filaree was selected even though it is the most-obviously difficult seed for *P. rugosus* to transport. Additional study is required to further elucidate the contributions of all of these seed selection criteria.

It is clear that *P. rugosus* is foraging extensively on seeds of exotic mustard and filaree. Our data suggest that foraging over a period of two weeks had limited (nonsignificant) impact on the seed bank of these two plant species. However, *P. rugosus* evidently prefers harvesting filaree seeds, and harvests large numbers of mustard seeds. It would not be unreasonable for this foraging activity to affect the distribution (and possibly abundance at a microsite level) of these two exotic plants. For instance, dyszoochory, or the accidental dispersal of seeds by seed-eating ants (Wolff and Debussche 1999), can occur, and escaped seeds may even experience increased germination rates (Rissing 1986) and greater mineral resource availability near ant nests (Wagner et al. 1997). In laboratory experiments, for example, *Pogonomyrmex badius* (Latreille) can alter distributions and resulting seed production of *E. cicutarium* (Harmon and Stamp 1992). Further investigations may be able to determine whether *P. rugosus* seed collection constitutes a significant predation pressure on filaree and mustard populations in the field.

While impacts on the CSS plant community remain unclear, *P. rugosus* colonies may be benefiting from the presence of exotic filaree and mustard in CSS, as this study confirms that *P. rugosus* can exploit these abundant sources of exotic seed. Future studies may elucidate the impact of plant invasions on colony nutrition. These harvester ants may prove to be resilient and resourceful seed predators.

Acknowledgments

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