

Harvester Ants (Hymenoptera: Formicidae) Discriminate Among Artificial Seeds with Different Protein Contents

by

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ABSTRACT

Workers of colonies of the western harvester ant, *Pogonomyrmex occidentalis*, were recruited to patches of artificial seed of the same caloric value but different protein content. Rates of forager returns with artificial seeds containing five percent protein were nearly twice those of zero percent protein. Differences in foraging rates on artificial seeds of ten and twenty percent protein compared to foraging rates on zero percent protein seeds were not different from the comparison of foraging rates on five percent and zero percent protein seeds. There were no differences in foraging rates on zero percent protein seeds, 0.5 percent and one percent protein seeds. *P. occidentalis* presented with patches containing equal amounts of zero, five, ten, and twenty percent protein seeds returned equal amounts of each protein content seed type to the colonies. When presented with high quality seed patches in the same location for four successive days, the rates of forager returns increased over time. When seed patch locations were switched, *P. occidentalis*' rate of return of zero protein content seeds was the same as for the five percent protein seeds at that location the previous day. Seeds with protein content between one and two percent represent a threshold for seed quality that affects the foraging rate of *P. occidentalis* workers.

Key Words: Harvester ants, *Pogonomyrmex occidentalis*, protein, food choices.

INTRODUCTION

It has been hypothesized that for many, if not most, animals the most important factor limiting their abundance is the availability of nitrogen-rich

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food for the development of young (White 1978). Studies of diet composition and forage selection have shown that animals may optimize protein, nitrogen or other nutrients like essential amino acids rather than calories (Slansky and Feeny 1977, Greenstone 1979). Seed-harvesting ants are widely distributed in arid and semi-arid regions of the world. There is a rich literature on foraging behavior, seed selection, and diet composition of seed harvesting ants. These characteristics and others of the genus *Pogonomyrmex* have been recently reviewed by MacMahon *et al.* (2000).

Pogonomyrmex spp. collect seeds from many species of plants but exhibit a clear preferences for some species when available (Whitford 1978, MacMahon *et al.* 2000). Seed-harvesting ants can harvest up to 100% of the seeds of a preferred species. A number of variables have been shown to affect seed selection including caloric content, percent soluble carbohydrate, morphology, viability, and seed size (MacMahon *et al.* 2000). None of the studies of the properties of the seeds preferred by harvester ants have examined seed protein content in order to test the hypothesis that animals may optimize protein or other nutrients rather than calories.

MacMahon *et al.* (2000) report that some *Pogonomyrmex* spp. are opportunistic predators on termites and other arthropods but suggest that insect prey are not important for the success of the colony. One experimental study found that scarcity of food including insect carrion had no effect on reproductive output as measured by alate production (Munger 1992). However, the viability of a colony is a function of production of workers and the importance of protein in food items for worker production has not been tested. Termites are a consistent small fraction of the forage items carried to the nest by harvester ants (Whitford & Ettershank 1975, Whitford *et al.* 1976, Whitford 1978). *Pogonomyrmex* spp. collect seeds from high density patches. Subterranean termites on the surface are extremely sparse during most of the warm season (MacKay *et al.* 1989). The difference in availability of termites suggests that *Pogonomyrmex* spp. expend more effort to obtain these packets of protein than to collect the abundant low-protein content seeds.

Other studies provide indirect evidence that suggest that the availability of high-protein food items stimulates intensive foraging activity by harvester ant colonies. High densities of grass cicadas (*Beameria vanosa*) stimulated intense collection of these insects by *Pogonomyrmex rugosus* (Whitford & Jackson

2007). *P. rugosus* colonies were not active in adjacent areas where there were few or no emerging grass cicadas. Another seed harvester, *Aphaenogaster* (*Novomessor*) *cockerelli*, continued to collect bits of dry tuna from baits until soil surface temperatures reached lethal levels. Colonies of *A. cockerelli* that had no access to the tuna baits ceased foraging for seeds one to three hours earlier in the day (Whitford *et al.* 1980). Based on the observations of the efforts of seed harvesting ants to collect insects and their behavior in response to protein baits, we hypothesized that seed harvesting ants would detect differences in protein content of potential items and would select items of higher protein content. We tested this hypothesis by designing a study of forage selection by western harvester ants, *Pogonomyrmex occidentalis*, using artificial “seeds” of the same energy content but of different protein content.

METHODS

Artificial Seeds

Artificial seeds were prepared by mixing 200 g of corn-starch, the desired amount of casein, 200 ml of water, 50 ml of vegetable oil and one of four colors of vegetable dye in a blender. The addition of the vegetable oil served to mask the small differences in caloric value of the corn-starch and casein. The corn-starch casein mixture was poured in a thin layer and allowed to air dry. The dried mixture was crumbled and sieved through geological sieves to obtain uniform particle size (approximately 5 mm).

Seed Selection

The studies were conducted in a grassland on a private ranch located approximately 100 km ENE of Albuquerque, N. M. with abundant colonies of *Pogonomyrmex occidentalis* (between 20 and 30 colonies . ha⁻¹). In order to examine the effect of food coloring on seed selection, four lots of artificial seeds of zero percent protein were prepared with one of the vegetable dye colors: red, yellow, green, or blue. Equal amounts of seed of each color were mixed to obtain an artificial seed mix. The seed mixture was presented to six colonies of *Pogonomyrmex occidentalis* in a small mound of approximately 10 cm diameter at a distance of two meters from the nest disc in a random direction from the nest. The number of each color returned to the colony in 20 minutes was recorded. The mean rate at which the different colored

seeds were returned to the nest ranged from $16.4 \pm 6.2 \text{ min}^{-1}$ to $16.6 \pm 6.2 \text{ min}^{-1}$ with no differences among artificial seeds of different colors ($p > 0.8$). Therefore, we used the four colors of vegetable dye to identify the artificial seeds of varying protein content.

Seed protein selection

Ten grams of artificial seeds were placed in individual petri dishes for transport to the *P. occidentalis* colonies chosen for the experiment. Colonies selected at random were presented with four plates containing the zero percent protein seeds and four containing 0.5, 1.0, 2.0, 5.0, 10.0 or 20.0 percent protein seeds. Because *P. occidentalis* workers forage in one or two directions along trunk trails, we divided the petri plates into pairs, one with zero percent protein seeds and the other with one of the higher-percent protein seeds. The four pairs were placed two meters from the nest mound, one pair in each of the cardinal compass directions with one meter between the plates. The position of the plates with respect to the compass direction was determined by a coin toss. Each pair of zero percent protein and higher-percent protein seeds was tested at nine different *P. occidentalis* colonies. The petri plate contents were dumped into a shallow mound of approximately 10 cm diameter. We recorded the number of seeds of each color carried to the colony in twenty minutes using a mechanical counter. The data from these experiments were analyzed by Analysis of Variance with mean comparisons by Duncan's Multiple Range test.

Another experiment compared artificial seed patches of either two percent or 5 percent protein paired with twenty percent protein seed patches using the protocol described above. Paired low-protein percent seeds were paired with the twenty percent protein seeds placed at two meters in random direction from a *P. occidentalis* nest. We tested six colonies provided with two percent and 20 percent protein seeds and six colonies with five percent and 20 percent protein seeds.

Seed patch composition and location

In order to examine the ability of foragers to distinguish between seeds of different protein contents within a single patch, we presented six colonies with seed mixtures of equal parts of zero percent, five percent, ten percent and twenty percent protein seeds.

In the study designed to examine path fidelity memory of *P. occidentalis* colonies for preferred seeds, four colonies were presented with dishes containing zero percent protein seeds paired with 5 percent protein seeds following the same procedures as the original seed selection study. On day two, the location of the seed dishes within each quadrant was exactly reversed. On day three, the seed dishes were switched back to the locations of day one. The process of reversing locations was continued for two of the colonies and compared with two colonies where the location of the percent protein seed patches remained the same.

RESULTS

Seed protein selection

When presented with the artificial seed patches, the workers from a colony quickly recruited to the artificial seeds and ceased gathering native seeds. The counts of seeds by color represent the proportion of the forager population that was recruited to the different nutrient content seed patches. The rates of collection of zero percent protein seeds were significantly different among the paired comparisons ($F_{5,48} = 11.9, p < 0.0001$) and the rate of collection of protein content seeds were also different among paired comparisons ($F_{5,48} = 3.4, p < 0.009$). The rates of collection of the zero percent protein paired with the 0.5, 1.0, and 2.0% protein were significantly higher than the rates of foraging on the zero percent protein seeds paired with the 5, 10, and 20% protein seeds (Table 1). There were no differences in the rate at which *P. occidentalis* collected zero percent protein seeds and seeds of 0.5, 1.0, and 2.0% protein ($p > 0.05$). The rate of collection of five percent protein seeds and higher protein content seeds was considerably higher than of the zero

Table 1. Comparison of the average rate of collection of artificial seeds of different protein contents with artificial seeds with no protein by harvester ants, *Pogonomyrmex occidentalis*.

Comparison percent protein	Control (No. · min ⁻¹)	Protein (No. · min ⁻¹)	p value
0.5 percent	32.7 ± 11.6	32.7 ± 12.1	> 0.5
1.0 percent	27.7 ± 12.1	27.9 ± 12.1	> 0.5
2.0 percent	27.2 ± 8.5	34.6 ± 9.8	<0.05
5.0 percent	12.1 ± 4.3	21.1 ± 4.9	<0.001
10.0 percent	13.0 ± 4.8	21.7 ± 14.3	< 0.02
20.0 percent	11.0 ± 5.0	19.3 ± 9.3	< 0.003

percent protein seeds from reference patches (Table 1). *P. occidentalis* workers recruited to five percent protein seed patches at nearly twice the rate of recruitment to zero percent protein seed patches. However, the rate of recruitment to ten percent and twenty percent protein seed patches was not different from the rate of return of seeds from the five percent protein seed patches (Table 1).

The results of the first experiment suggested an upper threshold of five percent protein seeds to modify the rate of recruitment of *P. occidentalis* workers. In the comparison of two percent protein seed patches and five percent protein seed patches, *P. occidentalis* workers recruited at a significantly higher rate to the twenty percent protein seed patches than to the two percent protein seed patches (Table 2). There was no difference in rates of recruitment to the five percent protein and twenty percent protein seed patches ($p > 0.7$).

Seed patch composition and location

If *P. occidentalis* foragers are not capable of distinguishing among the different protein content seeds in a patch, then the expected ratios of seeds taken by foragers should be 25% zero protein seed and 75% higher protein seeds. There were no significant differences between observed and expected ratios of seeds returned to the colonies. Zero percent protein seeds accounted for 24.1% of the seeds returned to the colonies. The higher protein content

Table 2. Comparison of mean rate of collection of 2 percent and 5 percent protein seeds with collection rates of 20 percent protein seeds by six colonies of *Pogonomyrmex occidentalis*.

Comparison pair	Control (No. . min-1)	20% (No. . min-1)	p value
2.0% vs. 20.0%	20.2 ± 10.2	30.4 ± 15.7	< 0.06
5.0% vs. 20.0%	22.9 ± 7.3	15.3 ± 7.6	>0.5

Table 3. Comparison of mean rates of collection of zero percent and 5.0 percent protein seeds by four colonies of *Pogonomyrmex occidentalis* where location was switched on day 2 and returned to the original location on day 3. On days 4 and 5 location of seed patches were switched for two colonies (switch) and location remained the same for two colonies (same). Comparisons of rates of collection that are significantly different at $p < 0.05$ are indicated by different letters.

Time	D 1	D 1	D 2	D 2	D 3	D 3	D 4	D 4	D 5	D 5
Protein	0 %	5%	0%	5%	0%	5%	0%	5%	0%	5%
Switch	15.1a	24.3b	46.7a	35.9a	22.5a	26.2a	43.6a	44.1a	44.9a	52.1a
Same							29.9a	52.0b	17.1a	44.8b

seeds made up 75.9% of the items returned to the colonies by foragers ($X^2 = 2.2$, $p > 0.01$).

When the location of the zero percent protein seed patches and five percent protein seed patches were switched, the rate of collection of seeds from the zero percent protein patch was greater than from the five percent protein seed patch ($p < 0.001$, Table 3). When the location of the five percent protein seed patch and zero percent protein seed patch were not switched, *P. occidentalis* foragers collected five percent protein seeds at a higher rate (Table 3).

DISCUSSION

This study adds seed protein content to the list of seed attributes that contribute to seed selection by *Pogonomyrmex* spp., along with abundance, caloric content, soluble carbohydrate content, morphology, viability and size (MacMahon & Crist 2000). *Pogonomyrmex* spp. preferentially collect seeds from high-density patches (Reichman 1979). By providing large patches containing equal quantities of artificial seeds, our experiments eliminated seed abundance as a variable affecting rate of seed collection. The oil content of the artificial seeds masked small differences in caloric content of the other ingredients. Morphology, size, and viability of the artificial seeds were the same for all of the seeds used in the experiments because all were produced by the same protocol.

The recruitment of *P. occidentalis* workers to five percent protein seed patches at nearly twice the rate as to zero percent protein seed patches clearly demonstrates that these ants detect protein differences and select for higher protein seeds. However, this appears to be a threshold response because the ants did not exhibit differential foraging rates on seeds of higher protein content than the five percent protein seeds. There is also a lower threshold of between one percent and two percent protein content seeds where *P. occidentalis* failed to distinguish between seeds with low protein content and those with zero percent content.

The failure of *P. occidentalis* foragers to distinguish between zero percent and high percent protein seeds in patches where zero percent protein seeds were mixed with equal amounts of five, ten, and twenty percent protein seeds suggests that the workers distinguish between monospecific seed patches of uniformly low or uniformly high protein but not in mixed protein content

seed patches. This result suggests that workers returning to the colony from mixed seed patches do not elicit a differential chemical or tactile communication from nest mates that would stimulate collection of seeds of different quality (Hölldobler & Wilson 1990).

The differences in foraging rates among the separate experiments resulted from the weather conditions at the time of the experiment. Colony activity changes seasonally with changes in food availability and soil surface temperature (Whitford & Ettershank 1975, Crist & MacMahon 1991a). The differences in foraging rates in this study were attributable to differences in soil surface temperatures because *P. occidentalis* foraged exclusively on the artificial seeds within two to three minutes after the artificial seed patches were presented. Soil surface temperatures in the study area are affected by cloud cover in the summer monsoon season when this study was conducted. Experiments conducted after rains cooled the soil surface resulted in lower foraging rates on the low protein patches and high patches that were recorded in three of the zero-protein / high-protein pairs. Soil surface temperature was reported to affect forager departure rates in 70% of the sampled colonies across months in a two year study of *P. occidentalis* foraging (Crist & MacMahon 1991a).

The rapid change in foraging of *P. occidentalis* from the dispersed native seeds to the rich patches of artificial seeds supports the finding that forager departure rates are dependent on the return of successful foragers (Schafer *et al.* 2006). The behavior of *P. occidentalis* in our experiment is consistent with the simple behavioral rule that foraging intensity should be adjusted to current food availability (Gordon *et al.* 2008). However, the higher rates of foraging on high-protein content seeds demonstrates that food quality is communicated by foragers. Our study demonstrates that the rate at which successful foragers return to the nest varies as a function of food quality when food availability is a constant. This result suggests that communication among workers of a colony conveys more information about items collected by foragers than just food availability.

The higher rates of forager return from the same patch after the location of zero percent and five percent protein seed patches were switched provides experimental evidence of path and seed patch fidelity of *P. occidentalis* (Crist & MacMahon 1991b). The higher rates of foraging on high-protein seed patches located in the same place for three successive days provides additional evidence that food quality affects the rate of forager return with the proviso that the

site remain the same. Forage site fidelity is a more important determinant of foraging rate than food quality.

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REFERENCES

- Crist, T.O. & J.A. MacMahon 1991a. Foraging patterns of *Pogonomyrmex occidentalis* (Hymenoptera: Formicidae) in a shrub-steppe ecosystem: the roles of temperature, trunk trails and seed resources. *Environmental Entomology* 20: 265-275.
- Crist, T.O. & J.A. MacMahon 1991b. Individual foraging components of harvester ants: movement patterns and seed patch fidelity. *Insectes Sociaux* 38: 379-396.
- Gordon, D. M., S. Holmes & S. Nacu 2008. The short-term regulation of foraging in harvester ants. *Behavioural Ecology* 19: 217-222.
- Greenstone, M.H. 1979 Spider feeding behaviour optimizes dietary essential amino acid composition. *Nature* 282: 501-503.
- Hölldobler, B. & E. O. Wilson 1990. *The Ants*. Belnap Press, Cambridge, MA
- MacKay, W.P., J.C. Zak & W.G. Whitford 1989. The natural history and role of subterranean termites in the northern Chihuahuan Desert. Pages 53 - 77 *In*: Schmidt, J.O. (ed.), *Special Biotic Relationships in the Arid Southwest*. University of New Mexico Press, Albuquerque, N.M.
- MacMahon, J.A., J. F. Mull & T.O. Crist 2000. Harvester ants (*Pogonomyrmex* spp.): Their community and ecosystem influences. *Annual Review of Ecology and Systematics* 31: 265-295.
- Munger, J. C. 1992 Reproductive potential of colonies of desert harvester ants (*Pogonomyrmex desertorum*): effects of predation and food. *Oecologia* 90: 276-282.
- Reichman, O.J. 1979. Desert granivore foraging and its impact on seed densities and distributions. *Ecology* 60: 1085-1092.
- Schafer, R.J., D.M. Gordon & S. Holmes 2008. Forager activation and food availability in harvester ants. *Animal Behaviour* 71: 815-822.
- Stansky, F. (Jr.) & P. Feeny 1977. Stabilization of the rate of nitrogen accumulation of larvae of the cabbage butterfly on wild and cultivated plant foods. *Ecological Monographs* 47: 209-228.
- White, T.R.C. 1978. The importance of relative shortage of food in animal ecology. *Oecologia* 33: 71-86.
- Whitford, W.G. & G. Ettershank 1975. Factors affecting foraging activity in Chihuahuan desert harvester ants. *Environmental Entomology* 4: 689 - 696.

- Whitford, W.G. 1976. Foraging behavior of Chihuahuan desert harvester ants. *The American Midland Naturalist* 95:455 - 458.
- Whitford, W.G. 1978. Foraging in seed harvester ants *Pogonomyrmex* spp. *Ecology* 59: 185-189.
- Whitford, W.G., E. DePree & P. Johnson 1980 Foraging ecology of two Chihuahuan Desert ant species: *Novomessor cockerelli* and *Novomessor albisetosis*. *Insectes Sociaux* 27:148- 156.
- Whitford, W.G. & G. Ettershank 1985. Factors affecting foraging activity in Chihuahuan Desert harvester ants. *Environmental Entomology* 4: 689-696.
- Whitford, W.G. & E. Jackson 2007. Seed harvester ants (*Pogonomyrmex rugosus*) as “pulse” predators. *Journal of Arid Environments* 70:549-552.

