Evaluation of Three Techniques for the Study of Harvester Ant (*Pogonomyrmex* spp.) Diet

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ABSTRACT The estimation of an ant's diet is crucial in many ecological studies. Different techniques, which involve different assumptions and field procedures, have been used to estimate the composition of harvester ant diet. In this study, three techniques are compared for the estimation of the diet of *Pogonomyrmex rastratus* (Mayr), *Pogonomyrmex pronotalis* (Santschi), and *Pogonomyrmex inermis* (Forel) in the central Monte desert, Argentina: (1) hand collection of items brought back to the nest by foragers, (2) collection of items with a semiautomated device with pitfall traps, and (3) collection of the discarded material accumulated in middens. The hand collection technique and the collection of middens provided the lowest and the highest number of items, respectively. Midden samples and pitfall traps contained a higher proportion of nonseed items, probably coming from sources other than ants, than hand-collected items. The three techniques provided similar estimations of species richness but a bias against small seeds was detected for *P. pronotalis* and *P. inermis* with the hand collection technique, possibly because of the difficulty of collecting small items by hand. The percentage of seed species in the diet obtained with different techniques was positively correlated in the great majority of colonies. Overall, despite their intrinsic differences, the three techniques proved consistent, which constitutes a robustness test for the estimations obtained. In comparative ecological studies, the awareness that results depend on the techniques and their assumptions is particularly important.

La estimación de la dieta de las hormigas es de gran importancia para muchos estudios ecológicos. Diversas técnicas han sido empleadas para estudiar la composición de la dieta de hormigas granívoras, las cuales presentan diferentes supuestos y procedimientos. En este estudio se comparan tres técnicas para la estimación de la dieta de *Pogonomyrmex rastratus* (Mayr), *Pogonomyrmex pronotalis* (Santschi) y *Pogonomyrmex inermis* (Forel) en la región central del desierto del Monte, Argentina: (1) recolección manual de ítems acarreados por las obreras hacia los nidos, (2) recolección de ítems con un dispositivo semiautomático con trampas de caída, y (3) recolección del material de desecho acumulado en los alrededores de los nidos (basureros). En nuestro diseño experimental, con la recolección manual y la recolección del basurero se obtuvieron el menor y el mayor número de ítems respectivamente. El dispositivo semiautomático y la recolección del basurero colectaron una mayor proporción de ítems diferentes a semillas, probablemente no acarreados por las hormigas, que la recolección manual. La riqueza resultó similar con las tres técnicas, pero se detectó una menor proporción de semillas pequeñas con la recolección manual para *P. pronotalis* y *P. inermis*, probablemente debido a la dificultad de detectar y recolectar ítems pequeños manualmente. El porcentaje de las diferentes semillas en la dieta estuvo positivamente correlacionado entre técnicas en la mayoría de las colonias. Finalmente, las tres técnicas resultaron consistentes en la estimación de la dieta a pesar de sus diferencias intrínsecas, constituyendo una prueba de robustez. La conciencia sobre el grado en que los resultados dependen de las técnicas y sus supuestos, es particularmente importante en estudios comparativos.

KEY WORDS *Pogonomyrmex*, diet estimation, sampling techniques, Monte desert, Argentina

Harvester ants are an important component of arid ecosystems. Several studies have shown that, through their selective seed consumption, ants can inflict severe seed losses that can ultimately affect the composition and distribution of the vegetation (Brown et al. 1979, Reichman 1979, Inouye et al. 1980, Louda 1989, Mull and MacMahon 1996). Thus, the estimation of ants' diet composition is crucial to evaluate the role they play in arid ecosystems.

Different techniques have been used to estimate the composition of harvester ant diet. Because ants are central place-foragers, the most direct technique consists in the hand collection of items brought back to the nest by workers (Tevis 1958, Whitford 1978, Crist and MacMahon 1992, Gordon 1993, Wilby and Shachak 2000). In some studies, a semiautomated collecting

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device with pitfall traps placed around the nest entrance has been used to quantify forage intake (Skinner 1980, MacKay 1981, Crist and MacMahon 1991, Pirk et al. 2004, Pirk and Lopez de Casenave 2006). Another technique involves the collection of discarded seeds and seed remains accumulated in the middens near the nest entrance because midden composition is likely to reflect dietary composition (Gross et al. 1991, Steinberger et al. 1991, Andersen et al. 2000). Finally, seeds stored in granaries (underground chambers for seed storage) could also provide an estimation of the seeds consumed by these ants (Davidson 1982, Gross et al. 1991).

Being aware of the differences in the nature of techniques is essential as the results obtained from different sampling techniques or from variations in the execution of a particular technique may bias the data in different ways. For example, some diet items can be underrepresented or overrepresented in the samples compared with their actual proportion in the diet. The hand collection technique may be biased against small items, because they could become difficult to detect or collect. The semiautomated device could collect items from sources other than foragers (e.g., seed rain, falling invertebrates) because traps remain open during the sampling period without continuous checking. In the midden collection, which is an indirect measure of the diet, different seed species could vary in the number of discarded fragments that represent the original seed. This would lead to an overall bias in the quantification of the relative importance of seed species through the overestimation of some species (Andersen et al. 2000). Also, some material found in middens could not be related to ant activity (e.g., litter, seeds from the soil seed bank, invertebrates). Regarding granaries, not all seed species taken to the nest are stored in granaries and, if they are, their permanence inside the nest may vary because they are consumed gradually (Steinberger et al. 1991).

Other differences among techniques, which can also affect researcher's decisions, are related to the procedures themselves. Some techniques could disturb colonies, producing changes in the foraging behavior of harvester ants that may affect the reliability of the estimation. Hand collection involves the collection of foragers near the nest entrance with the aid of forceps or an aspirator. This manipulation not only affects the individual forager involved, but could trigger an alarm in the colony if not done carefully. The semiautomated device is an enclosure placed around the nest entrance and its presence may cause some disturbance. The study of the granaries causes the worst disturbance, because to reach the granaries, the nest needs to be excavated. It is a destructive technique, not desirable for ethical reasons or if the colony needs to be resampled.

Few studies have used different techniques on the same colonies simultaneously (Gross et al. 1991, Andersen et al. 2000, Pirk et al. 2004, Pirk and Lopez de Casenave 2006) and even fewer made a comparison of them (Andersen et al. 2000). The aim of this study was to compare three techniques for the estimation of the diet of three species of the genus *Pogonomyrmex* in the central Monte desert, Argentina: (1) hand collection of the items brought back to the nest by foragers, (2) collection of items with a semiautomated device with pitfall traps, and (3) collection of the discarded material accumulated in middens.

Three *Pogonomyrmex* species occur in the Biosphere Reserve of Ñacuñán located in the central Monte desert: *Pogonomyrmex rastratus* (Mayr), *P. pronotalis* (Santschi), and *P. inermis* (Forel) (Claver and Fowler 1993). They are active during daytime throughout spring and summer (Pol and Lopez de Casenave 2004). These species are granivorous (Pirk et al. 2004, Pirk and Lopez de Casenave 2006) as are most species of the genus studied in North America (Whitford 1978, Melhop and Scott 1983, Hölldobler and Wilson 1990, MacKay 1991, Taber 1998, Johnson 2000, MacMahon et al. 2000). Seeds account for >87% of the items carried to the nests by *P. rastratus* and *P. pronotalis,* and among them, 93% are grass seeds (Pirk and Lopez de Casenave 2006). Food-handling behavior differs among species: most caryopses carried by *P. pronotalis* and *P. inermis* (≈99%) bear bracts, whereas most caryopses carried by *P. rastratus* (≈60%) lack them (Pirk and Lopez de Casenave 2006, unpublished data). *P. inermis* is the only species that accumulates bracts and seed remains in conspicuous middens around the nest entrance.

Taking into account the existing differences between techniques and the features of the ant species studied here, we expect that (1) the semiautomated device and the middens will gather a higher proportion of nonseed items, (2) the hand collection technique will underestimate seed species richness, (3) the hand collection technique will underestimate the proportion of small seeds, and (4) despite their differences, the three techniques will prove consistent for diet composition estimations because the composition of the diet of *Pogonomymex* ants in the Monte desert consists mainly of seeds with a low proportion of small ones (Pirk et al. 2004, Pirk and Lopez de Casenave 2006).

Materials and Methods

Study Site. The study was carried out at the Biosphere Reserve of Ñacuñán (34°03′ S, 67°54′ W), located in the central portion of the Monte desert, Mendoza Province, Argentina. The main habitat of the reserve, where this study was carried out, is the open woodland of *Prosopis flexuosa,* where individuals of this species and of *Geoffroea decorticans* are scattered within a matrix of perennial tall shrubs (>1 m height, mostly creosotebush *Larrea divaricata,* but also *Condalia microphylla, Capparis atamisquea, Atriplex lampa* and *Larrea cuneifolia*), low shrubs (*Lycium* spp., *Junellia aspera* and *Acantholippia seriphioides*), and perennial grasses (*Trichloris crinita, Pappophorum* spp., *Sporobolus cryptandrus, Aristida* spp., *Digitaria californica, Setaria leucopila*). Annual forb cover (*Chenopodium papulosum, Phacelia artemisioides, Parthenium hysterophorus*) is highly variable from year to year. Ñacuñán's climate is dry and temperate with cold winters. Mean annual temperature is 15.6°C (1972–2004), and mean annual rainfall is 333.5 mm (1972–2004), with high interannual variation. Seventy-five percent of the annual rainfall occurs in spring and summer (October–March), and seed production of almost all plants is restricted to summer months.

Sampling Techniques. The diet of the three species was evaluated using three simultaneous techniques: (1) hand collection of items brought back to the nest by foragers, (2) collection of items with a semiautomated device with pitfall traps, and (3) collection of middens. Samples were taken during three activity seasons (October–April 2000–2001, 2001–2002, and 2002–2003) on four occasions (October, December, February, and April), with two or three techniques at one to eight colonies of each species per occasion.

The hand collection technique consisted in picking up at least 20 returning foragers with their loads at each nest entrance with the help of forceps and a teaspoon. Once a forager and its load were taken apart, the forage was kept and the ant was returned to the nest.

The semiautomated collecting device consisted in the collection of returning foragers by means of a special device similar to others used previously (Skinner 1980, MacKay 1981, Crist and MacMahon 1991). Our version is a circular plastic enclosure (33 cm diameter, 10 cm tall) placed around the nest entrance, low enough to allow air circulation and transparent so it never shaded the nest. Each enclosure had four pairs of openings 90° apart and 3 cm above the ground, consisting of one "entrance" and one "exit" each. Soil ramps allowed ants to access these ports on the corresponding side of the enclosure. Because these ants are bad climbers (like other species of the same genus; Gordon 1999), they were unable to climb the 3 cm vertical rise on the opposite side of the "entrance" or "exit" and thus they were prevented from using the exit port as an entrance or vice versa. Pitfall traps (plastic containers 3 cm diameter, 5 cm deep) with removable covers were set into the ground inside the enclosure, flush with the entrance 3 cm above the ground. The device was placed the day before the sampling to minimize disturbance on the sampling day and to allow ants to learn the appropriate paths in and out the enclosure. On each of 2 sampling d, trap covers were removed during the periods of high activity in the morning and the afternoon (according to Pol and Lopez de Casenave 2004), totaling 2 h of sampling per colony a day. Incoming ants captured during the sampling were counted and released near the nest entrance, and their forage was kept. Covers were placed again on the traps after each sampling period, allowing normal forage activity during nonsampling periods.

For the midden collection, a teaspoon-full sample ($\approx$3 cm^3) was taken of dense areas of *P. inermis* middens at the end of the sampling periods of April and February 2002 and 2003, the only occasions when middens became conspicuous. During this procedure, special care was taken to avoid the collection of soil.

Items collected from foragers or middens were identified and counted in the laboratory. Each item was assigned to a category: seeds or nonseed items (flowers, fruits, vegetative plant parts, invertebrates, and other items). To estimate the number of harvested seeds that gave place to the fragments in the middens, vegetative bracts of different grass species were identified and counted. Because a single caryopsis can give place to several separated bracts (i.e., a pair of glumes and a pair of palea and lemma), the most numerous type was taken as the estimator of seed number (e.g., if 50 pairs of glumes and 60 pairs of palea and lemma were found, 60 was considered as the number of seeds that gave place to these fragments). When single bracts were found (i.e., single glumes, palea, or lemma), they were considered as half a seed because one whole seed bears two of them. When the estimator was not a whole number, it was rounded up. Complete seeds found in samples were not considered, because they were not consumed and could have been wind-dispersed seeds (i.e., not brought to the nest by workers).

Data Analysis. For each colony, the percentage of seeds and nonseed items, the percentage of each seed species and the number of seed species (species richness) obtained with each technique were calculated. Only colonies where at least two different diet-sampling techniques were used simultaneously and where >10 items were collected with all techniques were considered for data analysis. One- or two-tailed Wilcoxon matched pair tests were performed to compare techniques for each ant species, the former when one particular technique was expected to have lower or higher value than the other, and the latter for cases where no particular trends were expected (Zar 1996). Thus, the number of items obtained with two different techniques were compared with two-tailed Wilcoxon matched pair tests, whereas the percentage of nonseed items and species richness between the hand collection technique and the other techniques were compared with one-tailed Wilcoxon matched pair tests because the estimates were expected to be lower with the hand collection technique. For the same comparison but between the semiautomated device and the collection of middens, a two-tailed test was performed because these techniques were expected to show similar values. Replicates were colonies whose diet was estimated with two different techniques on the same sampling occasion. Colonies sampled on different occasions were included as replicates in the analyses because this study focused on differences between techniques, which are assumed not to vary among occasions. Because the sampling effort usually differed between techniques and thus the total number of items obtained was not the same, the rarefaction method was performed before the species richness analysis (Simberloff 1972). In this way, comparisons of species richness estimated with each technique were based on the same number of items (which corresponded to the colony with fewer items). To address if the tech-

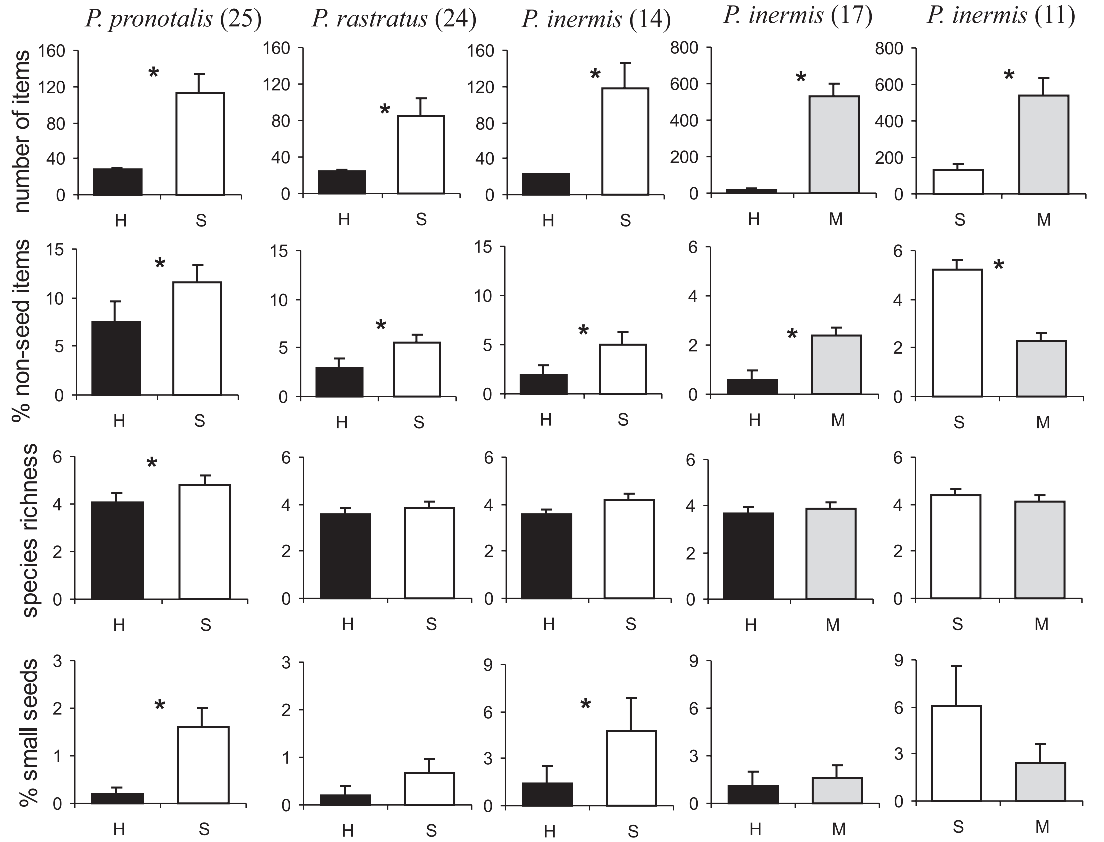

Fig. 1. Mean number of items (±SE), mean percentage of nonseed items (±SE), mean seed species richness (±SE), and mean percentage of small seeds (i.e., <0.1 mg; ±SE) collected in colonies of *P. pronotalis*, *P. rastratus*, and *P. inermis* with three techniques: hand collection (H), a semiautomated collecting device (S), and midden collection (M). Number of colonies used for the analysis are given between brackets. Differences between techniques were evaluated with Wilcoxon matched pair tests (*significant after the sequential Bonferroni adjustment, except for species richness, $P < 0.05$). Rarefactions were performed prior for the species richness comparison.

niques differed in the percentage of small seeds collected, the percentage of small seeds (seeds weighting <0.1 mg: *Neobouteloua lophostachya*, *Sporobolus cryptandrus*, *Conyza* spp., and *Descurainia* sp.) (Marone et al. 1998) obtained with pairs of techniques was compared with one-tailed Wilcoxon matched pair tests and two-tailed for midden collection versus semiautomated device.

Because several tests were performed with the same data sets, the sequential Bonferroni adjustment was applied for the Wilcoxon matched pair tests with number of items, percentage of nonseed items, and percentage of small seeds (Holm 1979, Rice 1989).

Spearman correlations were carried out to compare the percentage of seed species estimated with two techniques for each colony. The total number of species found in the diet of the corresponding ant species was considered for each analysis (24, 18, and 16 species for *P. pronotalis*, *P. rastratus*, and *P. inermis*, respectively). Because a high number of correlations was performed, the significance level was adjusted using the sequential Bonferroni adjustment.

Results

Under the sampling design of this study, the semiautomated device provided a higher number of items than the hand collection technique for the three ant species (an average of 112.1 versus 27.9, 85.2 versus 24.9, and 117.9 versus 22.0 items for *P. pronotalis*, *P. rastratus*, and *P. inermis*, respectively; $P < 0.01$ in all comparisons, Wilcoxon matched pair test; Fig. 1). In *P. inermis* colonies, midden samples provided significantly more items than the hand collection (530.3 versus 21.3) and than the semiautomated device (536.1 versus 130.5; $P < 0.01$ in both cases, Wilcoxon matched pair test; Fig. 1).

The percentage of nonseed items in the diet was higher when estimated with the semiautomated device than with the hand collection technique (11.6 versus 7.5, $P = 0.01$; 5.5 versus 3.0, $P = 0.04$; and 5.1 versus 1.9, $P = 0.02$; percentage of nonseed items with the semiautomated device versus the hand collection technique for *P. pronotalis, P. rastratus* and *P. inermis,* respectively; Wilcoxon matched pair test; Fig. 1) and

Table 1. Spearman correlation coefficients of *P. pronotalis* diet estimations with the hand collection technique and the semiautomated device

Colonies	Oct. 2000	Dec. 2000	Feb. 2001	April 2001	Dec. 2001	Feb. 2002
P1	0.40	0.75[a]	0.72[a]	0.53[a]	0.85[a]	—
P8	0.82*	—	—	—	—	—
P4	—	0.75[a]	0.67[a]	—	—	—
P10	—	0.77[a]	0.78[a]	—	—	—
P12	—	0.82[a]	—	—	—	—
P15	—	—	0.83[a]	—	—	—
P17	—	—	0.90[a]	—	—	—
PP1	—	—	0.62[a]	—	—	—
PP2	—	—	0.82[a]	—	—	—
PP3	—	—	0.63[a]	—	—	—
P16	—	—	—	0.58[a]	—	—
P21	—	—	—	0.68[a]	0.57[a]	0.68[a]
P5	—	—	—	—	0.72[a]	0.74[a]
P18	—	—	—	—	0.75[a]	—
P23	—	—	—	—	0.80[a]	0.66[a]

[a] Significant correlations after sequential Bonferroni adjustment.

also higher than midden samples for *P. inermis* (5.2 versus 2.3, $P = 0.01$). For this species, a greater proportion of nonseed items was found in midden samples than in hand collection samples (2.4 versus 0.6, $P < 0.01$; Wilcoxon matched pair test; Fig. 1).

The three techniques estimated similar seed species richness when based on the same number of items (i.e., after rarefaction; Fig. 1). The only exception was *P. pronotalis,* for which the semiautomated device provided a higher estimation of species richness than the hand collection technique (4.8 versus 4.1, $P = 0.01$; Wilcoxon matched pair test; Fig. 1).

The proportion of small seeds in the diet obtained with the semiautomated device was higher than that obtained with the hand collection technique for *P. pronotalis* (1.6 versus 0.2, $P < 0.01$) and *P. inermis* (4.8 versus 1.4, $P = 0.02$; Wilcoxon matched pair test; Fig. 1). No difference was detected for *P. rastratus* between these techniques or between middens and hand collection or middens and the semiautomated device for *P. inermis* (Fig. 1).

The percentage of the different seed species in the diet estimated with the semiautomated device and the hand collection technique were significantly correlated in 24 of 25 studied *P. pronotalis* colonies (Table 1), 23 of 24 *P. rastratus* colonies (Table 2), and 13 of 14 *P. inermis* colonies (Table 3). For *P. inermis,* diet estimations with the semiautomated device and the midden collection were significantly correlated in all colonies sampled with both techniques (11 colonies; Table 3). For this species, when diet estimations with the hand collection technique and midden samples were compared, 14 of 17 colonies showed significant correlations (Table 3).

Discussion

Studies that use only one sampling technique are very common in ecology. However, the use of a single technique could introduce important biases in the estimations, sometimes without researchers being aware of it. Thus, it might be better to test the assumptions of the chosen technique and/or to compare several approaches to make sure the estimations obtained are reliable. When different techniques are used, results obtained could either be different or

Table 2. Spearman correlation coefficients of *P. rastratus* diet estimations with the hand collection technique and the semiautomated device

Colonies	Dec. 2000	Feb. 2001	April 2001	Dec. 2001	Feb. 2002	April 2002
R10	0.86[a]	0.69[a]	—	0.63[a]	0.58[a]	—
R13	0.71[a]	—	—	—	—	—
R26	0.63[a]	—	—	—	—	—
R27	0.75[a]	0.90[a]	—	—	—	—
R11	—	0.90[a]	1.00[a]	—	—	—
PR1	—	0.89[a]	—	—	—	—
PR2	—	0.89[a]	—	—	—	—
PR3	—	0.99[a]	—	—	—	—
R40	—	—	—	0.77[a]	—	1.00[a]
R41	—	—	—	0.85[a]	—	—
R42	—	—	—	0.42	0.73[a]	—
R43	—	—	—	0.73[a]	0.62[a]	—
R31	—	—	—	—	0.85[a]	—
R45	—	—	—	—	0.83[a]	1.00[a]
R49	—	—	—	—	—	0.91[a]

[a] Significant correlations after sequential Bonferroni adjustment.

Table 3. Spearman correlation coefficients of *P. inermis* diet estimations with the hand collection technique and the semiautomated device (H versu S), the semiautomated device and the midden collection (S versus M), and the hand collection technique and the midden collection (H versus M)

Colonies	H versus S			S versus M			H versus M		
	Dec. 2001	Feb. 2002	April 2002	Feb. 2002	April 2002	Feb. 2002	April 2002	Feb. 2003	April 2003
I5	0.60[a]	—	—	—	—	—	—	—	—
I9	0.64[a]	—	—	—	—	—	—	—	—
I6	—	0.70[a]	—	0.60[a]	—	0.86[a]	—	—	—
I10	—	0.87[a]	0.94[a]	—	0.70[a]	—	0.77[a]	—	—
I11	—	0.48	—	0.79[a]	—	0.32	—	0.98[a]	0.90[a]
I12	—	1.00[a]	—	0.70[a]	—	0.70[a]	—	0.83[a]	—
I13	—	0.74[a]	0.66[a]	0.94[a]	0.69[a]	0.75[a]	0.78[a]	—	—
I15	—	0.82[a]	0.82[a]	0.76[a]	0.88[a]	0.47	0.71[a]	—	—
IP1	—	0.64[a]	—	0.67[a]	—	0.31	—	—	—
IP3	—	0.86[a]	—	0.97[a]	—	0.89[a]	—	—	—
I17	—	—	0.99[a]	—	0.74[a]	—	0.75[a]	—	—
IP4	—	—	—	—	—	—	—	0.83[a]	—
IP5	—	—	—	—	—	—	—	—	0.92[a]
IP6	—	—	—	—	—	—	—	—	0.70[a]

[a] Significant correlations after sequential Bonferroni adjustment.

approximately equivalent. In the former case, some techniques (or all of them) may be introducing important biases, and thus, the use of only one of them could result in a poor estimation. Researchers should explore the possible sources of the differences found and analyze the reliability of the estimations. In the latter case, when several techniques provide similar estimations, as long as the techniques are related to a particular and different set of assumptions, the apparently redundant use of the techniques becomes a robustness test for the results obtained (Johnson 2002). Taking into account that results obtained in any ecological study could depend on the techniques, their assumptions are particularly important in comparative studies (Marone et al. 2000). Any careless comparison of results obtained with different techniques could be overestimating to an unknown degree the differences between samples, which will have negative consequences on the testing of ecological hypotheses (Marone 2006).

In this study, the collection of middens and the hand collection technique provided the highest and lowest number of items, respectively, for the estimation of harvester ant diet composition in our sampling design. It should be taken into account, however, that with each technique, we could potentially have collected more items by increasing collection time (semiautomated device and hand collection technique) or by taking bigger samples (middens).

The techniques differed in the percentage of nonseed items collected (flowers, fruits, vegetative plant parts, and invertebrates). As expected, the hand collection technique provided the lowest estimation of these items, and pitfall traps of the semiautomated device collected a higher percentage of these items than the middens. These differences could be because leaves or twigs carried by the wind or invertebrates could have fallen into the traps of the semiautomated device because it remained open. Similarly, items that are not part of the discarded material could have been accumulated in middens, resulting in an overestimation of the number of nonseed items in the diet. However, because estimations with all techniques showed that >90% of the diet of the studied species consisted of seeds, this possible bias may not be relevant in this context (Fig. 1; see also Pirk et al. 2004, Pirk and Lopez de Casenave 2006).

In general, given a fixed number of items, the three techniques provided similar estimations of species richness. The semiautomated device, however, provided a higher estimation than the hand collection technique only for *P. pronotalis.* This could be because of a bias against some species, which could become difficult to collect by hand. However, this was detected only for one species, and the three of them have very similar diets (Pirk et al. 2004, Pirk and Lopez de Casenave 2006). Particular behavioral features of *P. pronotalis* could explain this difference: because their colonies present high activity levels with respect to the other two species (Pol and Lopez de Casenave 2004), hand collection samples were taken in a shorter period of time than in the other species, covering a shorter foraging period and probably a smaller forage variety. If this explanation holds, this bias could be reduced by changing the sample design so that items are collected on a time basis instead of setting the number of items to be collected before the sampling. Despite the similar species richness estimation among techniques, it should be taken into account that an accurate estimation will depend on the number of items collected. The optimal number could be calculated through richness versus sample size curves for each particular sampling situation.

In this study, a bias against small seeds was detected for *P. pronotalis* and *P. inermis* when the hand collection technique was used. With this technique, small items could have been missed because they are difficult to spot and collect by hand (Skinner 1980). Thus, there could be an overall underestimation of these types of items, which could have a great effect on diet estimation when they are common in the diet. This is not the case for the *Pogonomyrmex* species in this study (small seeds account for <6% of the diet as seen with all techniques; Fig. 1) so the impact of this bias on the overall estimation might not be important.

Percentages of seed species in the diet obtained with different techniques were highly correlated in the great majority of the colonies. This means that, despite their intrinsic differences, the three techniques proved consistent in their estimations. The fact that the results did not change with the techniques used makes the estimation of these species' diets highly robust.

It is important to consider that the reliability of the different sampling techniques could change depending on the ecological context. In some studies, the assumptions of a certain technique may not be fulfilled. Thus, for each particular context (i.e., the objective of a particular study or the studied species or sites involved), there can exist techniques that could be more or less appropriate than others (Marone 2006). Regarding the techniques evaluated here, one of the assumptions of the semiautomated device, for example, is that it should not affect colony behavior. Some species may be more sensitive to this kind of disturbance than others, resulting in less accurate estimations caused by behavioral changes. The hand collection technique could have a greater bias against small items for fast moving species because they become more difficult to catch when carrying small items. Some techniques simply cannot be applied for certain species, as the midden collection in our study, which could not be used in *P. rastratus* and *P. pronotalis* colonies because these species do not accumulate the discarded material around their nests. Thus, researchers should pay attention to their particular context when choosing among techniques, and if more than one is chosen, they should compare them for possible biases. In our context, the three techniques showed a high consistency in their estimations, but the semiautomated device seems to be the most efficient one: it can be used on different sampling occasions and for the three species (whereas midden samples can only be taken from *P. inermis* colonies from mid-season on), and it can gather a higher number of items per unit time because it can be used simultaneously in several colonies (whereas the hand collection technique requires the permanent presence of the researcher at each colony during the sampling period).

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