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Inventory and bioindicator sampling: Testing pitfall and Winkler methods with ants in a South African savanna

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Abstract

The sampling efficiency and consistency of pitfall traps and Winkler samples for inventory, bioindicator and ecological studies in savanna habitats was compared using ants. Pitfall traps are often used for ant collecting while Winkler litter sampling has until now had rather limited use. We test Winkler sampling for the first time in a South African savanna. Pitfall traps were more efficient and productive than Winkler sampling for epigaeic ants, with a greater total species richness and higher abundance of ants recorded. Winkler samples contributed few additional species. The relative abundance of different sized ants was different with the two collection methods. Winkler sampling was found to catch greater numbers of smaller ants than pitfall trapping, whereas pitfall trapping caught more larger ants. The standard collecting Winkler quadrat size of $1\,m^2$ did not perform as well as $2 \times \frac{1}{2}\,m^2$ quadrats combined for one sample.

Introduction

Insects are recognised as important components of global biodiversity terms of their considerable biomass (Groombridge 1992), species richness (Erwin 1991; Gaston 1991), and significant role in ecosystem functioning (Kim 1993; Jones *et al*. 1994; Folgarait 1998; McGeoch 1998). In consequence, their conservation requirements are being increasingly investigated (e.g. Samways 1994). Although formal conservation areas clearly contribute to the conservation of insects, the extent to which these animals are represented in such areas is not well known (Koch *et al*. 2000). Likewise, investigations of the extent to which different kinds of habitat transformation affect insects outside protected areas are recent (Scholtz & Chown 1993; Samways 1994). Thus increasing attention is being paid to inventorying and understanding patterns of insect diversity both inside and outside of formal conservation areas (see Brown 1991; Van Rensburg *et al*. 1999; Lawton *et al*. 1998; Balmford & Gaston 1999). Like inventories for all other taxa, those undertaken for insects should be designed so that they are as realistic, rapid, repeatable, quantitative and cost-effective as possible (Margules & Austin 1991; Kim 1993; Oliver & Beattie 1996; Fisher 1999).

Insects are also being used increasingly as bioindicators in a variety of roles (Noss 1990; Cranston & Trueman 1997; McGeoch 1998; Hilty & Merenlender 2000). Not only are they being used as valuable indicators of the effects of changes in the environment on local communities (ecological indicators, e.g. Van Rensburg *et al*. 1999; McGeoch & Gaston 2000), but they may also be used to detect changes in environmental condition (environmental indicators), and more broadly as indicators of diversity as a whole (biodiversity indicators, see McGeoch 1998 for additional discussion). Initial quantitative studies are essential to establish the utility of insects in these roles. As with inventorying, the techniques employed for such work should ideally be realistic, rapid, repeatable, and cost-effective.

To address these inventory and bioindicator survey requirements, the most appropriate sampling technique (or techniques) should be identified for the area or taxon in question. In addition to the requirements mentioned above, this technique should also seek to reduce problems associated with both the analysis and interpretation of data with many zeros, and low species abundances (see Clarke & Warwick 1994; Hilty & Merenlender 2000). Often, when time and finances are limited, only one sampling method may be employed. Because methods differ in their efficacy, efficiency and repeatability, the optimum method for the study must therefore preferably be selected *a priori*.

For sampling epigaeic ant species, the most commonly employed method is pitfall trapping. Pitfall traps provide a relatively simple, quick and cost-effective sampling method for collecting epigaeic ants, and allow for continuous day and night sampling (Southwood 1978; Andersen 1991; Majer 1997). There are,however, several disadvantages to this method (Luff 1975). Pitfall trapping is most productive in open habitats because catch can be compromised by vegetation complexity (Greenslade 1964; Majer 1997; Melbourne 1999). Pitfall size influences catch in terms of species caught (e.g. small traps may undersample large ants (Abensperg-Traun & Steven 1995)), and abundances. The clumped nest distribution of ants may also pose problems for pitfall sampling because pitfalls adjacent to nests or foraging trails can lead to distorted results (Andersen 1983). Furthermore, ants may be differentially susceptible to capture (Marsh 1984). For example, fast moving ant species have been over-recorded in open habitats (Greenslade 1973; Andersen 1983). Certain species of ants may also be deterred from traps, while others may be attracted to them (Marsh 1984). Sampling with pitfall traps is also affected by 'digging in' effects (Greenslade 1973) where catches are generally greatest immediately after traps have been dug.

Winkler sampling is an alternative collection method for surface and litter-dwelling ants, and has been shown to render pitfall sampling of epigaeic ants redundant in areas of high litter loads (Fisher 1999). The Winkler method involves collecting leaf-litter from within a 1 m^2 quadrat, and sifting this material through a coarse sieve (1 cm^2 mesh size). The collected leaf litter is then placed into a mesh bag, which is vertically suspended inside a cotton enclosure. The leaf litter usually amounts to approximately 2 litres. Where leaf litter is scarce, less may be used, and where it is extremely plentiful, a maximum of 2 litres is used. Over a period of at least 48 h, ants and other invertebrates work their way out of the drying litter, and are collected in a small cup partially filled with ethanol, suspended at the bottom of the bag (Besuchet *et al*. 1987; Nadkarni & Longino 1990; Fisher 1996, 1998, 1999; Didham *et al*. 1998).

The Winkler method is particularly useful for collecting litter and soil fauna that are not caught as readily with pitfall traps and is highly recommended for use in forested habitats where litter is plentiful (Nadkarni & Longino 1990; Olson 1991; Fisher 1996, 1999). Previous sampling using the Winkler method in rainforest habitats has proved very successful, yielding significantly better results than pitfall trapping alone (e.g. Olson 1991; Fisher 1999). Nonetheless, few studies have tested Winkler sampling in drier systems where litter levels are lower and more patchily distributed. Although traditionally used in forest systems, the Winkler method could be used in savannas where a litter layer also accumulates. The relative efficiency of Winkler extractors compared to other sampling methods in such habitats still requires investigation.

Furthermore, the rationale underlying the choice of 1 m^2 as the preferred quadrat size is not well documented. Most studies simply state that a 1 m^2 quadrat was chosen for the collection of leaf litter (e.g. Fisher 1996, 1999; Longino & Colwell 1997; Didham *et al*. 1998; Lawton *et al*. 1998; Bestelmeyer *et al*. 2000). Although this might be the most appropriate quadrat size for habitats with high litter loads (e.g. rainforests), it is not clear why this should be the most efficient quadrat size for determining the species richness and relative abundance of insect species in such habitats, or in those where litter is less abundant and more patchily distributed. Rather, it appears that this quadrat size was chosen because it yields sufficient, but not too much, litter for extraction. Thus, there appear to be considerable grounds for also assessing the relative merits of the quadrat size and number used for each Winkler sample to improve the efficiency of this method. In the case of ants, such an assessment is particularly important given the influence that clumped nest distributions are likely to have on the outcome of the sampling.

Hence, the aims of the current study were to test the relative merits of pitfall traps and Winkler sampling for assessing the diversity (richness and abundance, and size composition of catch) of ants in a savanna ecosystem, and to assess the effects of differences in quadrat size and number on the efficacy of the Winkler sampling method. In so doing, recommendations for the most appropriate sampling technique(s) for inventories and for other quantitative studies in savannas are made.

Study site

Sampling of ants was undertaken on a long-term field experimental area in the central Satara area of the Kruger National Park, South Africa. The study was carried out on an experimental control plot (7 ha) that constitutes part of a long-term burning experiment (Experimental Burn Plot Trial), and has remained unburned since 1945 (Trollope *et al*. 1999). As a consequence of no burning, the plot is densely vegetated, comprising moribund grass, bushes and trees. The vegetation of the area is classified as a Knobthorn (*Acacia nigrescens*)/Marula (*Sclerocarya birrea*) savanna in the Sweet Lowveld Bushveld (Low & Rebelo 1996). Litter load and cover on the plot was patchy, being thick (up to 20 cm deep) in some areas, while almost non-existent in other areas. Mean annual rainfall in this area is approximately 550 mm (Gertenbach 1983). The site is on basalt-derived clay soils.

Methods

A total of 30 pitfall traps (fifteen 62 mm diameter (large), and fifteen 18 mm diameter (small)) were set at 5 m intervals along six randomly placed transects, covering an area of approximately 2 ha. Each transect consisted of five traps, with large and small traps alternating. Each trap contained a 50% propylene glycol solution, and was open for three days from 4–7 December 1999. Propylene glycol is non-toxic to larger animals, and is not known to significantly attract or repel ants (Adis 1979). Vegetation around the pitfall traps was not cleared.

Following the pitfall trapping, Winkler litter samples were collected in the same area (13–19 December 1999). Fifteen samples were collected for each of the following Winkler quadrat combinations: 1 m^2 (total 15 m^2), $\frac{1}{2}$ m^2 (total $7\frac{1}{2}$ m^2), $\frac{1}{4}$ m^2 (total $3\frac{3}{4}$ m^2), $2\times\frac{1}{2}$ m^2 (total 15 m^2) and $4\times\frac{1}{4}$ m^2 (total 15 m^2). Collection quadrats were spaced at 2 m intervals along randomly placed transects. Where more than one quadrat was used per Winkler sample, the litter from all quadrats was mixed and sieved together, and then 2 litres taken for hanging. Each set of Winkler samples was suspended for 48 h; this being the hanging time in many other studies (e.g. Olson 1991; Fisher 1998, 1999). It is unlikely that earlier removal of ants with pitfall trapping would affect numbers caught with Winker sampling because the pitfall samples were not sufficiently numerous, or open for a prolonged period of time, and the collection area was large.

Ant samples collected with both techniques were stored in 80% alcohol. All ant samples were sorted and identified to morphospecies level on the basis of characters previously established to be important at the species level for each genus. Where possible, species names were assigned. Voucher specimens were mounted, and a representative set stored at the South African Museum, Cape Town.

The maximum head width of each species was measured to 0.01 mm with an ocular micrometer mounted on a dissecting microscope. Head width provides a standard and accurate measure of overall body size (Hölldobler & Wilson 1990; Kaspari 1993). For each species, and where possible, several individuals were measured. In order to provide an indication of the body size frequency distribution for each sampling method, ant species were placed into 0.2 mm size classes based on head size: 1 = 0.30–0.50 mm, 2 = 0.51–0.70 mm, 3 = 0.71–0.90 mm, 4 = 0.91–1.10 mm, 5 = 1.11–1.30 mm, 6 = 1.31–1.50 mm, 7 = 1.51–1.70 mm, 8 = 1.71–1.90 mm, 9 = 1.91–2.1 mm, 10 = 2.11–2.30 mm. The mean abundance of ants/sample and standard error for each size class were calculated.

To assess and compare pitfall and Winkler sampling completeness, species accumulation curves for each sampling method were plotted. Species accumulation curves were extrapolated to estimate expected species richness with different sample numbers. Since the shape of species accumulation curves is influenced by the order in which samples are added to the total (Colwell & Coddington 1994), sample order was randomised 100 times with the program EstimateS to produce smooth curves (R.K. Colwell 1997, Version 5, http://viceroy.eeb.uconn.edu/estimates).

The incidence-based coverage estimator (ICE) provided in EstimateS is a robust measure for indicating completeness of sampling. However, where the number of rare species does not decrease with increased sampling effort, ICE does not perform well (Colwell & Coddington 1994). In this study, singletons (species that only occur in one sample) did not decrease with sampling for all sampling methods, and in consequence ICE could not be reliably used. Therefore, smoothed, observed species accumulation curves were fitted using a logarithmic model: $S(t) = \ln(1 + zat)/z$, where t is the measure of sampling effort (sample number), and z and a are curve-fitting parameters (Fisher 1999, see also Soberón & Llorente 1993). Non-asymptotic functions were used for the extrapolation. Although extrapolation may result in enhanced bias of data (Colwell & Coddington 1994), this method provides a suitable alternative to a non-parametric estimator such as ICE.

Results

A total of 34 ant species comprising 16 genera was recorded with pitfall traps and Winkler samples combined (Appendix 1). Species richness was greatest with pitfall trapping. A total of 25 ant species was recorded from pitfall trapping alone, with the richest genera being *Monomorium* (5 species), *Pheidole* (4 species), and *Tetramorium* (3 species). The most abundant species was *Pheidole* sp. 1, representing 74.0% of all ants found in pitfall traps. A total of 22 ant species was recorded from Winkler samples, with the richest genus being *Tetramorium* (5 species). The most abundant species were *Plagiolepis* sp. 1, representing 38.7% of all ants found in Winkler samples, and *Pheidole* sp. 1 representing 37.2%. Not all Winkler samples contained ants (11 of the 75 samples did not; Table 1).

In all of the Winkler sampling protocols, with the exception of the 1 m^2 quadrats, the mean number of ants per sample was lower than that found in both the large and small pitfalls (Table 1). Total species richness was greatest for large pitfall samples (20 species), and lowest for Winkler quadrat size $\frac{1}{4}$ m^2 (7 species). Moreover, there were 12 species of ants unique to pitfall sampling, and 7 species unique to Winkler sampling. The mean number of ants per sample and mean number of species per sample was highest for large pitfall traps (44.0 ± 10.0, mean $\pm$ S.E., and 5.2 ± 0.4, respectively) (Table 1).

Comparisons of extrapolated species accumulation curves as a function of the number of stations sampled (Figure 1) showed that large pitfall traps had a higher rate of species accumulation than did small pitfall samples, and all Winkler samples. For Winkler samples, $2\times\frac{1}{2}$ m^2 quadrats showed the highest rate of species accumulation (Figure 1). Completeness of collection methods was assessed by estimating the effect of additional sampling. Based on the extrapolation of observed curves using the logarithmic model, a doubling of sampling effort (an additional 15 samples) would achieve a 18–22% gain in species richness at sites with an initial 15 samples (Table 2). The addition of 15 samples from 45 to 60 samples would achieve a 7–8% gain in species richness.

Table 1. Species richness and abundance (total and mean with standard error (SE)) for pitfall traps (62 mm and 18 mm diameter) and Winkler samples.

Winkler and pitfall sizes	Number of samples	Mean no. ants/sample (±SE)	Total no. of species (unique species)	Mean no. species/sample (±SE)
1 m^2	15	34.5 ± 7.1	16	4.5 ± 0.5
1/2 m^2	11	4.8 ± 1.3	9	2.4 ± 0.4
1/4 m^2	11	12.2 ± 4.6	7	1.9 ± 0.2
$2\times(1/2)$ m^2	13	11.8 ± 2.7	16(2)	3.9 ± 0.5
$4\times(1/4)$ m^2	14	17.6 ± 4.4	14	3.2 ± 0.4
62 mm	15	44.0 ± 10.0	20(5)	5.2 ± 0.5
18 mm	15	32.0 ± 7.3	16(4)	3.9 ± 0.4

Frequency distributions for abundance and species richness per sample (Figures 2a and b) were used to compare the collecting efficiency and consistency of pitfall and Winkler sampling (data from the different protocols were combined). Mann–Whitney U tests performed on frequency distribution data for both abundance and species richness revealed significant differences between sampling methods (two-tailed test, $p = 0.001$, and $p < 0.001$ respectively).

Pitfall sampling was more consistent and less variable than Winkler sampling for both number of ants/sample (Coefficient of Variation (CV%) for pitfalls was 90% while Winklers was 130%), and number of species/sample (CV% $=$ 39% for pitfalls, and 73% for Winkler samples). The majority of pitfall samples had >11 ants/sample, while the Winkler samples were highly inconsistent (Figure 2a). Fifty percent of Winkler samples contained five or fewer ants, while there were no pitfall samples with five or fewer ants. This emphasises the relative inefficiency of Winkler sampling where a high effort yields low output. Pitfalls were highly productive and efficient with 70% of samples containing >21 ants/sample.

In the case of species richness/sample there were large differences between the sampling methods (Figure 2b). More than a quarter of the Winkler samples (28%) contained one or no species, and more than 50% only had two or fewer species. No pitfall samples contained fewer than two species. Conversely, $>50\%$ of pitfall samples were found to contain more than four species, whereas this was the case in only 22% of the Winkler samples.

The body size frequency distributions suggested that the mean number of ants/sample for different size classes differed with sampling method. The mean number of ants/sample for size classes 1 and 3 was greatest for 1 m^2 quadrat Winklers, while pitfall traps had the highest mean number of ants/sample for size class 4. Only pitfall traps and Winkler $2\times\frac{1}{2}$ m^2 quadrats

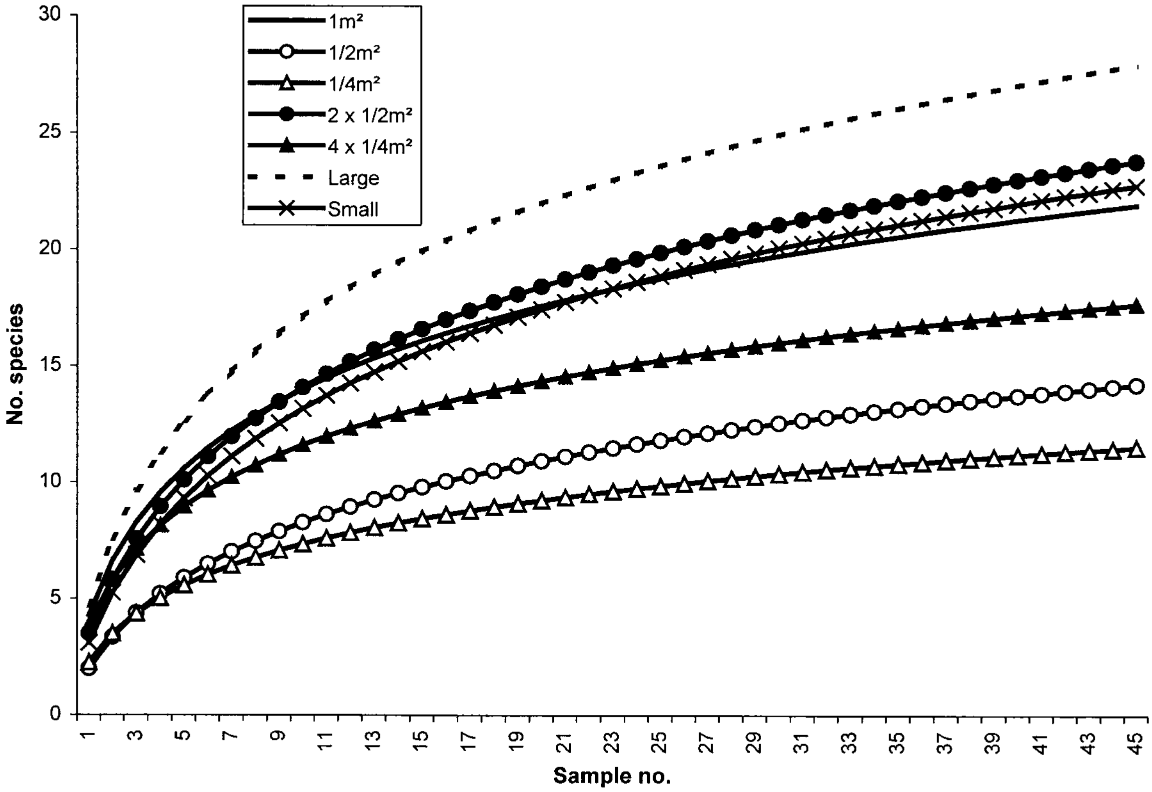

Figure 1. Projection of species accumulation curves for 45 samples using the logarithmic model (Soberón & Llorente 1993) for each sampling technique ($r^2 > 0.996$). Winkler quadrat sizes: 1 m^2, $\frac{1}{2}$ m^2, $\frac{1}{4}$ m^2, $2\times\frac{1}{2}$ m^2, $4\times\frac{1}{4}$ m^2. Pitfall sizes: large (62 mm), small (18 mm).

Table 2. The observed number of species for 15 samples and projected species richness at 30 and 45 samples. Projected species richness is based on extrapolation of observed species accumulation curves using the logarithmic model. Pitfall traps: 62 mm and 18 mm diameter.

Winkler and pitfall sizes	Observation at 15 samples	Extrapolation at 30 samples (*% increase from 15 samples*)	Extrapolation at 45 samples (*% increase from 30 samples*)
1 m^2	16	19.7 (*18.6*)	21.9 (*10.0*)
1/2 m^2	9	12.5 (*21.9*)	14.2 (*11.7*)
1/4 m^2	7	10.4 (*18.5*)	11.5 (*9.9*)
$2\times(1/2)$ m^2	16	21.1 (*21.4*)	23.8 (*11.4*)
$4\times(1/4)$ m^2	14	16.0 (*17.5*)	17.6 (*9.4*)
62 mm	20	24.9 (*20.0*)	27.9 (*10.7*)
18 mm	16	20.1 (*22.2*)	22.7 (*11.8*)

collected ants in size class 7, and the largest ants (classes 8 and 10) were found exclusively in large pitfalls (Table 3). Nonetheless, frequency-size distributions were not significantly different when tested in a pairwise fashion for distributions (Kolmogorov Smirnov two sample test, $p > 0.05$ in all cases), although this may be a result of small sample sizes.

The number of species in each size class was also determined for each sampling method. Large pitfall traps performed most efficiently; catching a wider range of sizes, and generally more species per class than did the other sampling techniques (Figure 3). Trends for pitfall sampling and Winkler $2\times\frac{1}{2}$ m^2 quadrats were similar, with species richness being greatest in size classes 1–3. From class 4 onwards, ant species richness was low. Although the mean abundance of ants/sample for class 1 was greatest with 1 m^2 quadrat Winklers (see above), when the number of species/size class is considered, pitfall samples contain more species in class 1 than did any of the Winkler samples.

Discussion

In this savanna system, pitfall traps proved more efficient and consistent than Winkler sampling in collecting epigaeic ants. Even with a relatively short pitfall sampling period (3 days), dense ground cover on the control plot, and fewer samples (Winkler samples: $n = 75$, pitfall samples: $n = 30$) pitfall traps produced a higher total species richness. More pitfall samples contained ants than Winkler samples, and a greater proportion of pitfall samples contained higher ant abundances, and species richness than Winkler samples. With extrapolation and equal sample sizes, large pitfalls were consistently more effective than all other sampling methods. The $2 \times \frac{1}{2}$ m^2 quadrat Winklers performed more efficiently than all other Winkler combinations. The fact that large pitfalls performed better than small pitfalls indicates that although pitfall traps may be used in preference to Winkler samples in savanna habitats, they must be of a sufficiently large diameter to allow for good catch.

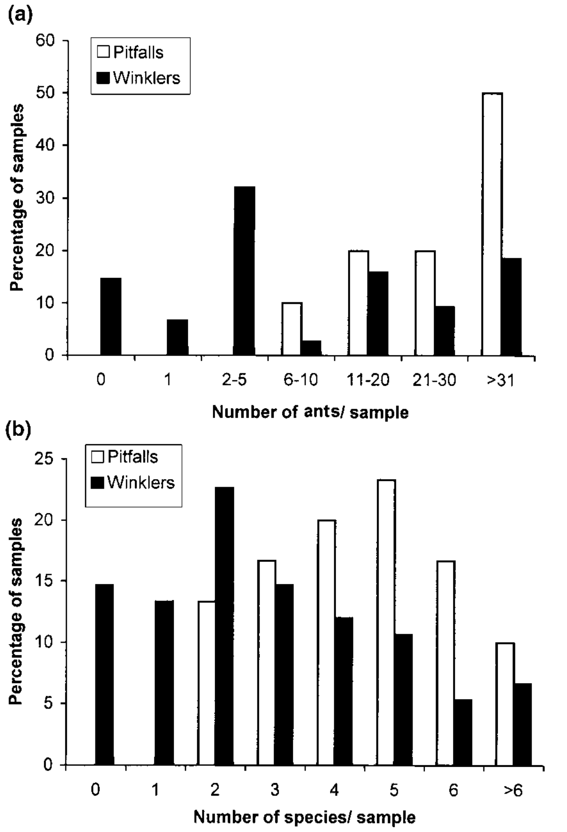

Figure 2. Frequency histogram for the number of ants/sample for pitfall and Winkler sampling as a percentage of the total samples (Mann–Whitney U two-tailed test): (a) $p = 0.001$. (b) $p < 0.001$.

Pitfall trapping is thought to favour large, mobile ants, while Winkler sampling should be more efficient at collecting smaller, more cryptic ants. This is because small ants are likely to be slower moving due to the nature of their foraging habits (Kaspari & Weiser 1999), and hence more likely to be caught with Winkler litter sampling. This study showed that although there was no significant difference in ant size frequency distribution with the different sampling techniques, some trends could be discerned. Thus, in terms of relative abundances of ants, Winkler sampling catches greater numbers of smaller ants than pitfall trapping, and pitfall trapping catches more, larger ants (particularly, in this case, size class 4). Olson (1991) also found that litter samples contained a greater proportion of smaller species.

Winkler sampling was much more time-consuming, and labour intensive than pitfall trapping since samples had to be collected in the field, and processed to extract the ants. Collection of the 75 Winkler samples took approximately six to seven times longer than the digging-in of the 30 pitfall traps. Although the hanging time for the Winklers (48 h) was less than that for the pitfall sampling (72 h), it took a total of 6 h to prepare the bags before hanging. It was expected that the Winkler method would yield different ant species to

Table 3. Number of ants/sample (mean and SE) for each size class for pitfall (62 mm and 18 mm diameter) and Winkler samples.

Size class	Pitfall and Winkler sizes						
	62 mm ($n = 15$)	18 mm ($n = 15$)	1 m^2 ($n = 15$)	1/2 m^2 ($n = 11$)	1/4 m^2 ($n = 11$)	2×(1/2) m^2 ($n = 13$)	4×(1/4) m^2 ($n = 14$)
1	4.13 ± 0.92	2.07 ± 0.56	13.73 ± 5.8	1.47 ± 0.65	6.07 ± 2.65	3.13 ± 1.22	7.53 ± 2.65
2	1.80 ± 0.66	1.20 ± 0.48	0.53 ± 0.4	0	0	0.33 ± 0.16	0.40 ± 0.27
3	4.00 ± 0.95	2.13 ± 0.38	8.67 ± 3.2	0.40 ± 0.34	0.33 ± 0.16	3.80 ± 1.35	1.27 ± 0.33
4	31.40 ± 9.77	25.47 ± 7.28	11.20 ± 2.4	1.07 ± 0.38	2.53 ± 1.19	2.60 ± 0.78	7.07 ± 2.64
5	0.67 ± 0.60	0	1.87 ± 0.6	0.53 ± 0.53	0	0.27 ± 0.15	0.20 ± 0.14
6	0.13 ± 0.09	0.20 ± 0.14	0.13 ± 0.1	0.07 ± 0.07	0	0.07 ± 0.07	0
7	0.13 ± 0.13	0	0	0	0	0.07 ± 0.07	0
8	1.87 ± 0.66	1.40 ± 0.57	0	0	0	0	0
9	0	0	0	0	0	0	0
10	0.13 ± 0.09	0	0	0	0	0	0

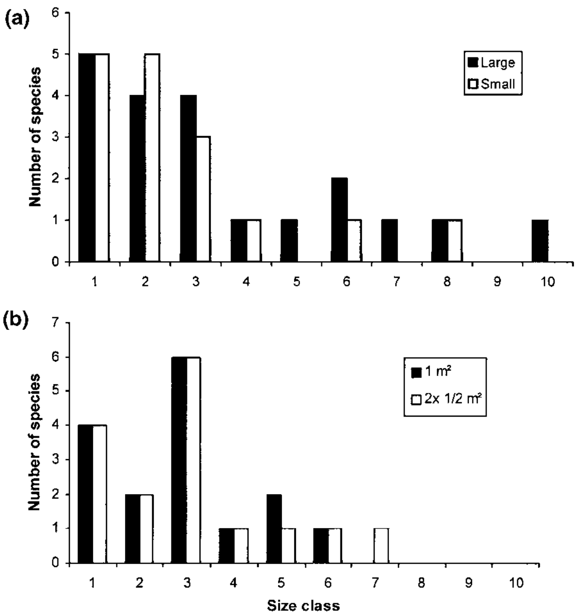

Figure 3. Ant species richness for different size classes: (a) Pitfall traps (62 mm and 18 mm diameters), (b) Winkler sampling. Size classes: 1 = 0.30–0.50 mm, 2 = 0.51–0.70 mm, 3 = 0.71–0.90 mm, 4 = 0.91–1.10 mm, 5 = 1.11–1.30 mm, 6 = 1.31–1.50 mm, 7 = 1.51–1.70 mm, 8 = 1.71–1.90 mm, 9 = 1.91–2.1 mm, 10 = 2.11–2.30 mm. Results from Winkler sampling with $\frac{1}{2}$ m^2, $\frac{1}{4}$ m^2 and $4\times\frac{1}{4}$ m^2 quadrats are not shown.

pitfall sampling because the former method is designed primarily for surveying litter and soil-dwelling species. It was, thus, expected that Winkler sampling would contribute additional information to pitfall sampling through the sampling of different ant communities. However, this was not the case. Firstly, species richness was greater using pitfall traps, even though the total number of pitfall samples was less than the total number of Winkler samples. Secondly, although Winkler sampling is designed primarily for litter sampling and not exclusively for epigaeic ants, the catch did not reflect a greater proportion of litter and soil species. There was significant overlap in species caught with the two methods: the number of unique species being much higher with pitfall traps. It is possible that more soil/litter dwelling and cryptic species could be recorded in pitfall traps given a longer sampling period.

When considering Winkler sampling alone, the standard 1 m^2 quadrat size was not the most efficient and productive (in terms of species richness). Instead, the $2\times\frac{1}{2}$ m^2 quadrat collection per Winkler sample was the most productive. By using litter from two quadrats per Winkler sample some of the problems associated with the clumped nest dispersion of ants are addressed. Although the total litter collecting area is kept constant ($2\times\frac{1}{2}$ m^2 = 1 m^2), a wider sampling area is covered and spatial variation is incorporated. The wider range of ant sizes (classes 1–7) collected with $2\times\frac{1}{2}$ m^2 quadrats (Table 3) compared to other Winklers lends further support to the use of this Winkler quadrat combination. The $4\times\frac{1}{4}$ m^2 quadrat collection method did not perform as well as the $2\times\frac{1}{2}$ m^2 quadrat collection, possibly due to edge effects when collecting litter; total quadrat edge increases as more smaller quadrats are used which could result in incomplete litter collection.

In addition to the number and size of quadrat used, Winkler sampling could be improved by increasing the number of samples. Furthermore, there appears to be some confusion with the Winkler technique; some studies did not disturb the bags during the hanging period (Didham *et al.* 1998; Fisher 1998), while others particularly emphasise that contents of the bag should be mixed on at least a daily basis (Besuchet *et al.* 1987). Mixing and disturbance of the litter material could be important to ensure all ants are active, and improve chances of them falling into the collecting cup, and not remaining within the litter. In this study, the samples were not disturbed and re-mixed daily. The extraction efficiency for Winkler samples should also be determined for the typical hanging period of 48 h. It is possible that extraction efficiency is affected by length of the hanging period, and other factors such as litter moisture (see Southwood's 1978 discussion on behavioural extraction methods).

Finally, it is worth considering that while Winkler sampling is typically viewed as a qualitative 'relative' method of sampling, useful primarily for inventory-type studies (e.g. Didham *et al.* 1998; Fisher 1999), it can also be used to produce quantitative results once the extraction efficiency has been determined. Although the present method of collecting Winkler samples cannot be used directly for quantitative purposes, it could be modified slightly to produce quantitative results. Estimates of local densities (animal numbers expressed as a density per unit area of the ground of the habitat (Southwood 1978)) are often used in ecological studies of species abundances (Gaston *et al.* 1999). Since quadrats of a known size are used to collect litter, Winkler sampling could be used to provide an indication of ant density.

One way that this could be done is to use all the litter collected within the quadrat, instead of only using a maximum of 2 litres of litter in each sample. This would provide an absolute measure of density, since the quadrat size is fixed. There are some practical problems with this approach though. For example, some vegetation types produce vast quantities of leaf litter, and fitting it all in a Winkler bag would be impossible. This could be overcome by increasing the bag size, or putting the litter into several Winkler bags for hanging. An alternative approach is to measure all the leaf litter collected in each quadrat, and calculate what proportion the 2 litres used in the Winkler bag is of this total. Results from the 2 litres could then be used to extrapolate back to determine total ants in the whole litter sample, and density of ant species. This method assumes that the litter is mixed and homogeneous; something which is particularly difficult to ensure. Since this method depends on evenly mixed litter, it could potentially be quite unreliable.

Many studies using Winkler sampling have been carried out in forests, particularly rainforests (see Olson 1991; Didham *et al*. 1998; Fisher 1999) where leaf litter typically forms a very thick and continuous ground layer. In such areas, vegetation complexity is likely to influence pitfall catch negatively (Greenslade 1964; Majer 1997; Melbourne 1999), making Winkler sampling the more efficient method of collection for both surface-active and cryptic, hypogaeic ant species. Some rainforest studies have demonstrated that Winkler sampling can replace pitfall trapping entirely (e.g. Fisher 1999). In contrast, in more open, patchy and less complex environments, that have variable litter loads, such as savannas, it appears that pitfall traps perform better, and reduce the usefulness of Winkler sampling in adding new species. It is also worth noting that studies comparing pitfall and Winkler sampling efficiencies (e.g. Olson 1991; Fisher 1999) use only small pitfall sizes (18 mm diameter). It is highly probable that with a larger pitfall diameter, sampling efficiency would have increased. Moreover, if the sampling period for pitfalls is short (e.g. 48 h, Olson 1991), catch will be affected.

In summary, pitfall sampling alone is more efficient, productive and consistent than Winkler sampling for epigaeic ants in this savanna system. Where constraints limit sampling to one technique, the most appropriate method for ant collection in such habitats is pitfall trapping. When using Winkler sampling alone, we suggest collecting litter from several quadrats for each sample (e.g. $2\times\frac{1}{2}\,m^2$) instead of $1\,m^2$ may reduce the problem with clumped ant dispersions. Winklers may be used quantitatively to provide some indication of density, although it must be recognised that extraction efficiency and the exact number of quadrats required for a particular area should be determined in a pilot study.

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Appendix 1. List of species collected in pitfall traps and Winkler samples. Data are numbers of ants per species for each sampling method.

Species	Pitfall samples		Winkler samples				
	Large	Small	$1m^2$	$1/2m^2$	$1/4m^2$	$2\times(1/2)m^2$	$4\times(1/4)\,m^2$
Dolichoderinae							
Tapinoma sp. 2	—	1	—	—	—	—	—
Technomyrmex sp. 1	3	1	7	1	—	—	—
Technomyrmex sp. 2	1	—	—	—	—	1	2
Formicinae							
Anoplolepis sp. 1	13	10	—	—	—	—	—
Camponotus cinctellus (Gerstäcker)	10	—	2	—	—	—	1
Camponotus vestitus (F. Smith)	2	—	—	—	—	—	—
Camponotus sp. 2	1	—	—	—	—	1	—
Lepisiota sp. D	1	—	—	—	—	—	—
Lepisiota sp. 1	—	1	—	—	—	—	—

Appendix 1. (*Continued*.)

Species	Pitfall samples		Winkler samples				
	Large	Small	1m^2	1/2m^2	1/4m^2	2×(1/2)m^2	4×(1/4) m^2
Plagiolepis sp. 1	1	1	185	17	60	20	100
Plagiolepis sp. 2	—	—	—	—	—	1	—
Myrmicinae							
Meranoplus sp. 1	—	—	—	1	2	—	1
Messor sp. 1	2	—	—	—	—	—	—
Monomorium albopilosum Emery	10	3	2	—	—	—	—
Monomorium sp. 2	40	21	—	—	1	—	—
Monomorium sp. 3	6	7	—	—	—	—	—
Monomorium sp. 4	1	—	—	—	—	—	—
Monomorium sp. 5	14	1	6	—	—	1	6
Monomorium sp.8	—	—	6	—	—	4	6
Ocymyrmex sp. 1	28	21	—	—	—	—	—
Oligomyrmex sp. 1	—	—	14	5	28	25	5
Pheidole sp. 1	471	382	168	16	38	39	106
Pheidole sp. 3	4	—	—	—	—	—	—
Pheidole sp. 5	—	2	—	—	—	—	—
Pheidole sp. 6	—	2	—	—	—	—	—
Tetramorium frigidum Arnold	3	—	62	3	3	10	10
Tetramorium sericeiventre Emery	53	30	29	—	—	2	2
Tetramorium sp. 4	—	1	4	1	—	1	—
Tetramorium sp. 5	—	—	2	8	—	4	2
Tetramorium ?setigerum Mayr	—	—	26	—	—	42	3
Ponerinae							
Hypoponera sp. 1	—	—	1	—	2	—	2
Pachycondyla sp. 1	1	3	2	1	—	—	—
Pachycondyla sp. 2	—	—	2	—	—	2	1
Polyrachis sp. 1	—	—	—	—	—	1	—
Total no. of ants	665	487	518	53	134	154	247

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