

Sampling Effort and Choice of Methods

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A number of studies have qualitatively assessed the efficacy of diverse methods for sampling rainforest ground-dwelling ants, including pitfall traps (e.g., Adis 1979), Winkler extraction (e.g., Olson 1991), baits (e.g., Fowler 1995), hand collection from quadrats (Room 1975), and “trapping” using artificial nesting sites (Young 1986). However, very few studies have addressed the question of how many samples need to be taken in order to obtain a reasonably complete census of an ant community (e.g., Fisher 1999a). This deficiency of the literature results in part from the fact that the goal of most past ant sampling studies has been to provide a general inventory of the ant fauna of a region (e.g., Wilson 1959; Cover et al. 1990; Veerhagh 1990) rather than a rigorous measure of the underlying

biodiversity in terms of species numbers and relative abundances.

The qualitative approach may well be adequate when the aim is merely to provide a list of the species present. However, if ants are to be used as bioindicators of some aspect of the environment, or if a rigorous census of species is desired, then richness and abundance measures must be described explicitly per unit area or per unit of sampling effort. In such cases, it is necessary to know whether the samples are or are not capturing a reasonably high proportion of the ant species present; if they are not, then it is necessary to estimate what proportion of the total ant fauna is being sampled.

A single sampling method is unlikely to capture all the ants present in the litter or in other

parts of the ground stratum. To overcome this problem, researchers often use a combination of complementary sampling procedures. Combinations that have been used to sample rainforest ground-dwelling ants include Winkler extraction of both litter and soil (Belshaw and Bolton 1994a); Winkler extraction plus pitfall traps (Olson 1991); Winkler extraction plus pitfall traps plus hand collecting (Andersen and Majer 1991; Fisher 1996a, 1998, 1999b); pitfall traps plus hand collecting (Jackson 1984); pitfall traps plus baits (Fowler 1995); and Berlese funnels plus baits (Levings 1983). In this chapter we use data from comprehensive studies of the ant communities in Brazilian cocoa plantations to address (1) the optimal combination of sampling methods for maximizing the species count of rainforest ground-dwelling ants; and (2) the relationship between the size of individual litter samples and the number of species obtained by Berlese funnel or Winkler extraction methods.

Methods

Field work was carried out on the grounds of the Centre for Cocoa Research (CEPLAC), Ilheus, Bahia (14°45'S, 39°13'W), Brazil. The CEPLAC site formerly consisted of primary Atlantic rainforest (Mata Atlantica), although most of it is now planted with cocoa. Cocoa plantations provide a habitat that retains many features of the original rainforest, and the ground-dwelling ant fauna retains a high degree of similarity to the fauna of the original habitat (Belshaw and Bolton 1993; Young 1986; Delabie et al. 2000).

Sampling Methods Experiment

A 1-ha plantation of regularly planted 20-year-old cocoa trees, shaded with *Erythrina fusca*, was divided into three rows of six cells, each measuring 23.5 m × 23.5 m. For each sampling method, three sample points were randomly selected in each cell, resulting in a total of 54

samples for each method for the entire plot. Sampling was originally designed to census the ant fauna from the soil, the litter, the ground surface, the tree trunks, and the canopy; the preliminary results have been reported in Delabie et al. (1994). Here we consider only those sampling procedures that are relevant to the soil and litter stratum. The 17 sampling methods used were as follows:

1. Small soil samples. Cubes of soil measuring 15 cm on a side were dug up, broken open, sieved, and then inspected on a white surface so that ants could be manually removed.
2. Large soil samples. As in (1), except that the sides of the cubes were 30 cm across.
3. Berlese funnel samples. Samples of litter measuring 1 m² were collected and placed in a funnel for 24 hours.
4. Winkler extraction samples. As in (3), except that the litter was sieved and then placed in Winkler sacks for 24 hours.
5. Pitfall traps (24-hour). A 75-mm-internal-diameter pitfall trap, containing water and a drop of detergent, was placed out for 24 hours.
6. Pitfall traps (7-day). As in (5), except that the traps contained a mixture of ethanol and glycerol and were placed out for 7 days.
7. Sardine bait (4-hour). Small pieces of sardine were placed on a square of tissue paper, and the ants that were attracted were collected after 4 hours.
8. Sardine bait (24-hour). As in (7), except that the baits were inspected after 24 hours.
9. Meat bait (24-hour). Small pieces of meat were placed on a square of tissue paper, and the ants that were attracted were collected after 24 hours.
10. Cassava flour bait (4-hour). A small pile of coarse cassava flour was placed on a

- square of tissue paper, and the ants that were attracted were collected after 4 hours.
11. Cassava flour bait (24-hour). As in (10), except that the baits were inspected after 24 hours.
 12. Sugar bait (4-hour). A small amount of dilute sugar solution was placed on a square of tissue paper, and the ants that were attracted were collected after 4 hours.
 13. Sugar bait (24-hour). As in (12), except that the baits were inspected after 24 hours.
 14. Orange peel bait (4-hour). A small piece of orange peel was placed on a square of tissue paper, and the ants that were attracted were collected after 4 hours.
 15. Orange peel bait (24-hour). As in (14), except that the baits were inspected after 24 hours.
 16. Dead wood inspection. A rotting branch near the sampling point was cut open and the ants within were collected.
 17. Dried cocoa pod inspection. A rotting cocoa pod near the sampling point was cut open and the ants within were collected.

A number of additional sampling methods were used to collect ants from other strata in the plantation. These included chemical knock-down and beating of trees, meat and sardine baiting in trees, inspection of dried cocoa pods on trees, inspection of fallen epiphytic bromeliads, and direct observation over fixed time intervals. The ant collections resulting from these methods are outside the scope of this chapter and will be reported in a subsequent publication.

Extended Sampling Experiment

To investigate the effect of number of samples on the number of species obtained, an extended set of soil and litter samples were collected in a nearby plantation of 60-year-old cocoa. This area differs from the 20-year-old plantation described previously in two important ways:

(1) the cocoa is grown in a less “manicured” way, is shaded by around 15 species of native overstory rainforest trees, and thus constitutes a more structurally diverse habitat than the 20-year-old plantation; and (2) no pesticides have been applied to it for 30 years. In this plantation, 500 Winkler extractions were collected from 1-m² samples of litter taken from randomized points in a 0.87-ha area.

Sample Size Experiment, 60-Year-Old Brazilian Cocoa Plantation

To investigate the influence of litter sample size on the number of species obtained, a set of samples was taken from the same 60-year-old plantation described previously, but using different litter sample sizes. Samples of 0.01 m², 0.04 m², 0.25 m², and 1 m², each replicated 20 times, were collected and extracted by Berlese funnels over 24 hours. An identical sampling regimen using Winkler extraction methods was also undertaken, except that additional samples of 2 m² were also taken.

Data Analysis

All ants from each experiment were initially sorted to the level of morphospecies, then identified to genus and, where possible, to species. For each sampling method, we constructed a matrix of individual ant species by individual samples and determined the frequency of each species in the samples. Standard diversity analyses were then carried out on the resulting data sets. See Chapter 13 and Magurran (1988) for additional background on the statistical analyses described in this section.

The incidence-based coverage estimator (ICE) (Lee and Chao 1994; Chazdon et al. 1998) and the first-order jackknife estimator (Heltshe and Forrester 1983) are nonparametric approaches for estimating species richness in the local community from which the samples were taken, that is, for estimating how many

species (including those not collected) are present in the sampled community. Calculation of the ICE is based on the number of species found in ten or fewer sampling units, whereas calculation of the jackknife is based on the observed frequency of unique species. To estimate what proportion of the total species richness was collected by each of the methods employed in the current study, plots of cumulative species-per-sample curves were generated in which species accumulation was plotted as a function of the number of samples taken. Three values were plotted for each succeeding sample: the observed number of species, the ICE of the total number of species present, and the first-order jackknife estimate of the total number of species present. For species-accumulation curves, sample order was randomized 100 times and the means of the ICE and jackknife estimates were computed for each succeeding sample station using the program EstimateS (Colwell 1997; see also Colwell and Coddington 1994; Chazdon et al. 1998).

The asymptote of a species-accumulation curve (i.e., the value that the curve approaches as a limit) is interpreted as the total number of species present at the sample site, including those that were not collected. In the current study, the asymptote of the observed species-accumulation curve was calculated with EstimateS (Colwell 1997) using the two-parameter Michaelis-Menten (M-M) equation (Colwell and Coddington 1994) and the maximum likelihood method of Raaijmakers (1987), which is based on the Eadie-Hofstee transformation of the M-M equation. The observed number of ant species and the proportion of the M-M asymptote represented by this number were evaluated for different sample sizes for each of the sampling methods.

The numbers of species collected by different combinations of the various sampling methods have important implications for future sampling studies. To elucidate the optimal combination of

sampling methods tested in the sampling methods experiment, first a table was prepared listing the total number of species sampled by each method. The method that sampled the most species was identified, and combinations including one and two additional sampling procedures were assessed in order to elucidate the optimal combinations of two and three sampling methods for maximizing the species count.

To evaluate the influence of the size of the litter sample from which ants were collected by Berlese funnel or Winkler extraction, the mean number of individual ants and the number of species per extraction were calculated; the total number of species from 20 samples of a particular area was also calculated.

Results and Discussion

Interpretation of species richness estimates should take into account a number of factors. A species-accumulation curve is specific to the area of the survey, the season or year, and the collecting techniques employed. The use of additional collecting methods, or a survey in a different area or season at the same site, would most certainly collect additional species. The actual number of species in an area at a given time is of course finite, but, in most cases, exhaustive sampling is not physically or logistically possible. If an observed or estimated species-accumulation curve indicates a decrease in the rate of species accumulation across the number of samples collected, then, for the particular methods employed, that number of samples is arguably adequate for estimating the species richness in the area or transect surveyed. Conversely, if the curve continues to rise rapidly for the number of samples collected, then more intensive sampling may be necessary to obtain an adequate measure of the diversity at that site.

The number of samples sufficient for achieving a high level of species completeness is thus

practically defined as the point at which the accumulation curve shows an adequate decrease in species accrual. The principal problems with this “sufficient-sampling” definition are the lack of a single asymptote for diverse taxa and the difficulty in quantifying “an adequate decrease in species accrual.” One practical solution to the latter problem is to sample until a certain percentage—say 80%—of the estimated species are obtained, based on ICE and jackknife estimates of the total number of species that occur in the plot or transect. An alternative approach is to continue to sample until additional sampling effort is predicted to achieve a negligible increase in the number of species sampled. In this approach, the increase in species obtained with additional sampling must be weighed against both the cost of sorting and identifying additional specimens and the relative importance of identifying the full complement of the species at the site. Under either approach, species-accumulation curves can be calculated by randomizing sample accumulation order and using asymptotic or nonasymptotic functions (Colwell and Coddington 1994), and the predicted species richness values can be extrapolated using the ICE, jackknife, and M-M estimation techniques.

Sampling Methods Experiment

The 17 sampling methods yielded a total of 134 ant species. In the total of 918 samples (17 methods \times 54 samples per method), most of these ground-dwelling ant species are extremely rare, with 43 species collected only once, 18 species collected twice, and 34 species collected in up to 10 samples; only 39 species occurred in 11 or more samples.

The number of species sampled by the 17 different methods is shown in Table 10.1. Winkler extraction sampled the greatest number of species (63), followed by Berlese funnels (48), inspection of dead wood (45), small soil samples (42), and pitfall traps. Pitfall traps run for 7

Table 10.1 Actual Number of Ant Species Sampled by the 17 Methods Described in the Text

Sampling Method	Number of Species	Rank ^a
Winkler extraction samples	63	1
Berlese funnel samples	48	2
Dead wood inspection	45	3
Small soil samples	42	4
Pitfall traps (7-day)	40	5
Pitfall traps (24-hour)	27	6
Large soil samples	26	7
Sardine bait (24-hour)	20	8.5
Orange peel bait (24-hour)	20	8.5
Sardine bait (4-hour)	19	10.5
Orange peel bait (4-hour)	19	10.5
Sugar bait (4-hour)	18	12
Dried cocoa pod inspection	17	13
Cassava flour bait (4-hour)	16	14
Meat bait (24-hour)	15	15
Cassava flour bait (24-hour)	14	16
Sugar bait (24-hour)	11	17

^aMethods are ranked from 1 (most species sampled) through 17 (fewest species sampled).

days yielded more ant species than those run for 24 hours (40 versus 27 species). Large soil samples yielded only 26 species and dead cocoa pods only 17 species, while the species counts from the baiting methods never exceeded 20. Baiting for longer periods of time did not necessarily yield more species; indeed, with the cassava and the sugar baits, a lower number of species was obtained after the longest baiting period.

The species-accumulation curve plots are shown for the individual methods in Figs. 10.1a–q, and for all methods combined in Fig. 10.1r. These graphs also show jackknife and ICE estimators of species richness based on successively larger numbers of samples. The agreement between the predicted M-M asymptotic values and the actual maximum value encountered in the 54 samples is shown in Table 10.2. This table

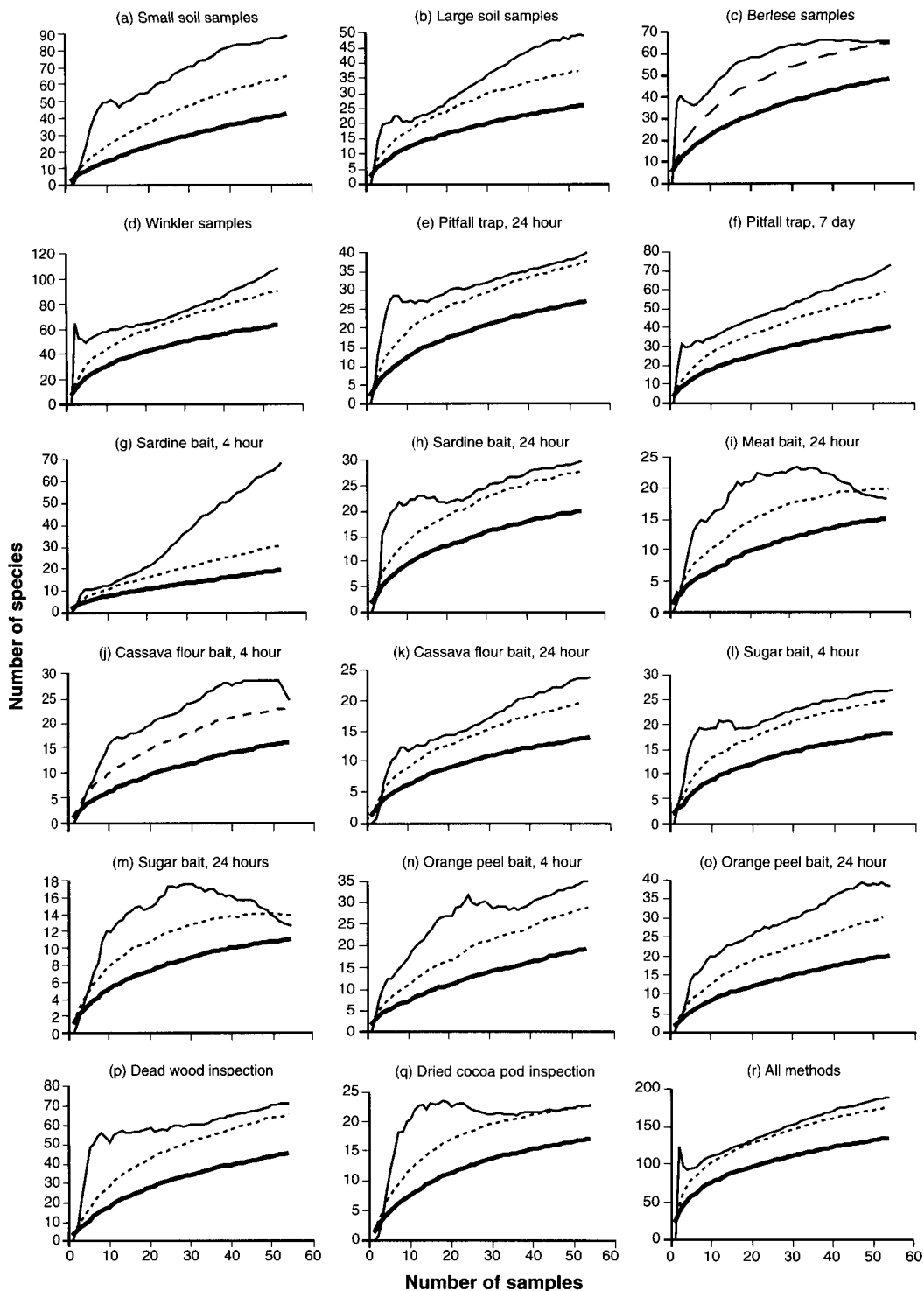


Figure 10.1. Assessment of each of 17 leaf litter ant sampling methods in Brazil. The lower (thick) species-accumulation curve plots the observed number of species as a function of the number of stations sampled. The upper curves display the nonparametric first-order jackknife estimator (dashed) and ICE (solid). The estimated total species richness is based on successively larger numbers of samples from the data set (Heltshel and Forrester 1983; Lee and Chao 1994). Curves are plotted from the means of 100 randomizations of sample accumulation order.

Table 10.2 Observed Number of Ant Species Evaluated at Different Sample Sizes for Each of the 17 Sampling Methods^a

Methods	Observed Species Richness after:					Estimated Species Richness ^b		
	10 Samples	20 Samples	30 Samples	40 Samples	All (54) Samples	ICE	Jack-knife	M-M
Small soil samples	14.0 (20.9)	22.4 (33.5)	25.3 (37.9)	29.6 (44.3)	42 (62.8)	88.5	64.6	66.9
Large soil samples	11.9 (39.1)	16.4 (53.9)	20.0 (65.8)	22.7 (74.5)	26 (85.5)	49.0	37.8	30.4
Berlese funnel samples	21.8 (39.4)	31.2 (56.4)	37.8 (68.4)	42.4 (76.7)	48 (86.8)	65.7	64.7	55.3
Winkler extraction samples	29.3 (42.2)	41.1 (59.1)	49.1 (70.6)	55.0 (79.1)	63 (90.6)	108.1	90.5	69.5
Pitfall traps (24-hour)	12.2 (35.9)	17.4 (51.1)	20.8 (61.1)	23.9 (70.2)	27 (79.4)	40.0	37.8	34.0
Pitfall traps (7-day)	17.3 (36.5)	24.5 (51.7)	30.0 (63.2)	34.4 (72.4)	40 (84.3)	72.8	59.6	47.5
Sardine bait (4-hour)	7.3 (34.6)	10.5 (49.4)	13.2 (62.2)	15.8 (74.6)	19 (89.8)	68.5	30.8	21.2
Sardine bait (24-hour)	9.1 (37.3)	13.0 (53.4)	15.8 (64.7)	17.8 (73.2)	20 (82.1)	29.8	27.9	24.4
Cassava flour bait (24-hour)	6.1 (36.4)	8.9 (52.8)	10.8 (64.1)	12.2 (72.7)	14 (83.2)	23.8	19.9	16.8
Cassava flour bait (4-hour)	6.2 (27.5)	9.3 (41.8)	11.8 (52.8)	13.9 (62.2)	16 (71.6)	24.6	22.9	22.4
Meat bait (24-hour)	6.5 (35.0)	9.7 (52.4)	11.8 (64.2)	13.4 (72.6)	15 (81.3)	18.3	19.9	18.4
Sugar bait (4-hour)	8.7 (41.0)	12.0 (56.7)	14.3 (68.0)	16.0 (75.9)	18 (85.4)	27.0	24.9	21.1
Sugar bait (24-hour)	5.2 (39.3)	7.3 (54.9)	8.9 (66.9)	10.0 (75.5)	11 (83.0)	12.7	13.9	13.3
Orange peel bait (4-hour)	7.1 (31.0)	10.8 (47.1)	13.8 (60.4)	16.1 (70.0)	19 (82.9)	34.9	28.8	22.9
Orange peel bait (24-hour)	8.1 (31.8)	12.0 (47.2)	14.8 (58.1)	17.2 (67.5)	20 (78.5)	38.2	30.8	25.5
Dead wood inspection	17.6 (27.9)	27.6 (43.6)	34.1 (54.0)	39.2 (62.0)	45 (71.3)	71.0	65.6	63.1
Dried cocoa pod inspection	7.1 (32.7)	11.2 (51.2)	13.6 (62.4)	15.2 (69.8)	17 (77.9)	22.8	22.9	21.8
All methods	75.0 (55.0)	95.8 (70.3)	110.1 (80.7)	121.5 (89.1)	134 (98.3)	188.9	176.2	136.4

^aNumber of species represents the mean of 100 randomizations of sample pooling order.

^bICE, incidence-based coverage estimator; jackknife, first-order jackknife estimator; M-M, Michaelis-Menten asymptote (the percentage of the M-M asymptote is given in parentheses in the first five columns).

also shows the ICE and jackknife asymptote values, as well as the predicted proportions (as percentages) of the M-M asymptotic value that would be obtained if 10, 20, 30, 40, and all 54 samples were taken.

Depending on the sampling method, the percentage of the M-M asymptote value that was obtained varied from 20.9 to 42.2, 33.5 to 59.1, 44.3 to 70.6, 37.9 to 79.1, and 62.8 to 90.6 when 10, 20, 30, 40, and 54 samples were collected, respectively. In all cases, Winkler extraction obtained the highest percentage of the asymptote value and small soil samples the lowest.

There is reasonable agreement between the percentages of the asymptotes obtained for the various sampling methods, which were on average 34.6, 50.4, 61.8, 69.8, and 81.0 for 10, 20, 30, 40, and 54 samples, respectively.

For all methods combined, 70% of the asymptote value was collected by 20 samples, whereas for each individual method, a much lower percentage (33.5–59.1%) was collected by 20 samples, with some methods obviously performing much better than others. Thus, in the context of the total survey, increasing the number of samples from each individual

method did not necessarily add new species to the total census, although for particular methods, species that were rarely collected by one method were common in another.

Considering that the study area was sampled by 17 different methods and that a relatively large number of samples was taken, the total species count probably represents a nearly complete census. This conclusion is additionally supported by an asymptote value of 136.4 species (Fig. 10.1r) for the curve for all methods combined, which implies that the combination of 54 samples from all 17 methods obtained 98.3 percent of the asymptote.

Extended Sampling Experiment

The species-accumulation curves for the 500 Winkler extraction samples are shown in Fig. 10.2. As above, this graph also shows ICE, jackknife, and M-M estimators of species richness based on successively larger numbers of samples. The agreement between the predicted M-M asymptotic value and the actual maximum value encountered in the 500 samples is shown in Table 10.3. This table also shows the ICE and jackknife asymptotes, as well as the predicted percentages of the M-M asymptotic value that would be obtained if 10, 20, 30, 40, 100, 200, 300, 400, and 500 samples were taken.

If we were to compare the M-M asymptote value of 136.4 species for this extended sampling experiment (carried out in the 60-year-old cocoa plantation) with the asymptote value of 69.5 species obtained for only the Winkler extraction samples from the sampling methods experiment (carried out in the 20-year-old cocoa plantation), we might be led to conclude that ant species richness is higher in the 60-year-old cocoa plantation. In fact, this disparity is probably an artifact of the greater number of Winkler extraction samples that were taken in the extended sampling experiment, in which an average of 67.7 species was collected with 54 pooled samples. This value is quite close to the

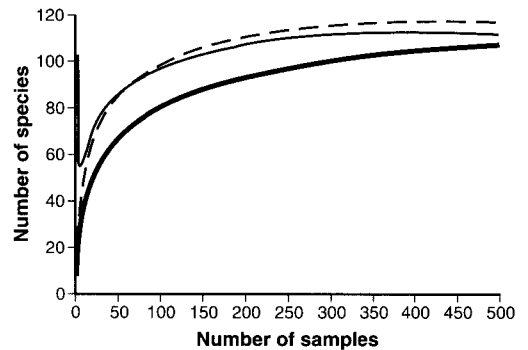


Figure 10.2. Assessment of Winkler sampling methods in Brazil. Lines follow the same convention as in Fig. 10.1.

observed total of 63 species for 54 Winkler sack samples in the sampling methods experiment. When the species-accumulation curve for the 54 Winkler sacks in the sampling methods experiment is extrapolated out to 500 samples using the logarithmic equation of Soberón and Llorente (1993), 109.9 species are predicted. This is also quite close to the 107 species observed in the extended sampling experiment. But perhaps the most telling argument against a significant difference in species richness between the 20- and 60-year-old plantations is the similarity in richness estimates of 134.6 species for the former (based on all sampling methods combined) and 136.4 for the latter (based on the full complement of 500 Winkler extraction samples). It is particularly noteworthy that the first 100 samples yielded over three-quarters of the species that were ultimately sampled by this procedure, and that the last 200 samples yielded very few additional species.

Sample Size Experiment

For both the Berlese funnel and Winkler sack samples, number of individual ants, total number of ant species, and mean number of ant species per sample generally increased with increasing litter sample size. However, although

Table 10.3 Observed Number of Ant Species Evaluated at Different Sample Sizes for the Extended Winkler Sampling Experiment^a

Observed Species Richness after Following Number of Samples:									Estimated Species Richness ^b		
10	20	30	40	100	200	300	400	All (500)	ICE	Jackknife	M-M
36.5 (35.2)	50.0 (48.2)	56.3 (54.3)	62.1 (60.0)	79.5 (76.7)	92.7 (89.5)	100.1 (96.6)	104.5 (100.8)	107 (103.3)	112.0	117.0	103.6

^aNumber of species represents the mean of 100 randomizations of sample pooling order.

^bICE, incidence-based coverage estimator; jackknife, first-order jackknife estimator; M-M = Michaelis-Menten asymptote (the percentage of the M-M asymptote is given in parentheses in the first nine columns).

the number of individuals increased quite dramatically with increasing sample size, the increase in mean number of species per sample was far less pronounced. Increasing the sample size from 0.25 m² to 1 m², and certainly from 1 m² to 2 m², is associated with a very limited return for a costly additional investment in effort.

Based on the results of the sample size experiment, when sufficient sampling devices are available it is probably generally more efficient to take a greater number of smaller samples than to take a lesser number of larger samples. Since a single Winkler sack can generally hold the sieved litter from a 1-m² sample, this is probably the most appropriate sample size for most situations.

Complementarity of Sampling Methods

Table 10.4 lists the combinations of two and three sampling methods that produced the largest numbers of ant species in the sampling methods experiment. In each case, only combinations that produced the four highest species counts are shown. Winkler extraction is an element in all combinations, along with inspection of dead wood, small soil samples, pitfall traps (7-day), and Berlese funnels. The various two-

method combinations captured species totals ranging from 59 to 65% of the M-M asymptote calculated for all methods combined (Table 10.2). Species totals for three-method combinations ranged from 73 to 77% of the M-M

Table 10.4 Combinations of Two and Three Sampling Methods That Obtained the Maximum Number of Ant Species in the Sampling Methods Experiment

Combination of Sampling Methods	N ^a
Winkler sack samples + small soil samples + inspection of dead wood	105
Winkler sack samples + inspection of dead wood + pitfall traps (7-day)	104
Winkler sack samples + pitfall traps (7-day) + small soil samples	103
Winkler sack samples + small soil samples + Berlese funnel samples	99
Winkler sack samples + inspection of dead wood	88
Winkler sack samples + small soil samples	87
Winkler sack samples + pitfall traps (7-day)	84
Winkler sack samples + Berlese funnel samples	80
Total number of litter species sampled by all methods	134
Total number of species from all strata	167

^aN, number of species.

asymptote calculated for all methods combined. A similar exercise (results not shown) was performed with combinations of four sampling methods. Generally speaking, the combinations that maximized species counts are simply permutations of the methods listed in Table 10.4. The combination of methods that produced the maximum species count (117 species) was Winkler sack samples + small soil samples + inspection of dead wood + pitfall traps (7-day). This value of 117 species is 86% of the asymptote value calculated for all methods combined (Table 10.2).

Conclusion

The choices of what collecting methods to use and how many samples to collect are dependent

on the intended species completeness of the proposed inventory, that is, on what proportion of the ant fauna the inventory intends to survey. The results presented here demonstrate that Winkler extraction is the most efficient method for surveying leaf litter ants and therefore that this method should be included in all ground-dwelling ant inventory protocols. If a second method is also to be used, we recommend pitfall traps. The total number of samples to collect should be determined both by the alpha diversity of the inventory site and by the level of species completeness that is necessary to achieve the project's goals, but, based on the results reported here, in most situations we recommend taking 20 1-m² Winkler samples for areas comparable in size to the 1-ha 20-year-old cocoa plantation.



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