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A Comparison of Two Sampling Strategies to Assess Discomycete Diversity in Wet Tropical Forests

SHARON A. CANTRELL

Science & Technology, Universidad del Turabo, P. O. Box 3030, Gurabo, P. R. 00778
e-mail: scantrel@suagm.edu

ABSTRACT.—Most of the fungal diversity studies that have used a systematic collecting scheme have not included the discomycetes, so optimal sampling methods are not available for this group. In this study, I tested two sampling methods at each sites in the Caribbean National Forest, Puerto Rico and Ebano Verde Reserve, Dominican Republic. For a plot-based sampling method, 10 × 10 m plots were established and divided into one hundred 1 × 1 m subplots. For each sample, 12 subplots were selected at random with replacement. For a transect-based sampling method, 60 m long transects were established with twelve 1 × 1 m subplots randomly placed on either side of the transect line at 5 m intervals at the beginning of the study. The study was conducted from October 2001 to September 2002. For Puerto Rico, 46 and 51 morpho-species were identified in the transects and plots, respectively. There was a 32% overlap (68% complementarity) between sites. The Sorensen Similarity Coefficient between sites was 0.50 for both methods, and 0.55-0.63 between methods within sites. For the Dominican Republic, 25 and 26 morpho-species were identified in the transects and plots, respectively. There was a 24-31% overlap (69-76% complementarity) between sites. The Sørensen Similarity Coefficient between sites was 0.40-0.47 for transects and plots, respectively, and 0.40-0.70 between methods within sites. The species accumulation curve indicates that the minimum number of subplots needed is 10 per transect and 60-70 per plot to obtain between 70-80% of the species. In terms of sampling effort, I concluded that at least 12 samples distributed throughout a year but with shorter intervals during the rainy season are needed. There was no difference between using transects or plots based on the number of species and similarity indexes. Based on a Chi-Square analysis using the frequencies of species, however, transects were better that plots because the distribution of species is more homogeneous.

KEYWORDS.—discomycetes, Ascomycota, Fungi, sampling methods, diversity, tropical, Caribbean

INTRODUCTION

The Caribbean region is among the 25-biodiversity hotspots of the world with only 11% primary vegetation remaining containing 2.3% of the endemic plants and 2.9% of the endemic veterbrate of the world (Myers et al. 2000). This makes the region a high priority for conservation. Currently, approximately 70,000 species of fungi have been described, which represents only 5% of the estimated 1.5 million fungal species (Hawksworth 1991). The mycoflora of tropical regions is poorly known as pointed out by Hawksworth (1991, 1993), May (1996) and Raven and Wilson (1992). In most tropical surveys, the great majority of the species of fungi are new records for the region and about 15-30% represent new taxa (May 1996). Minter et al. (2001) compiled most of the published and unpublished fungi records for the fungi of the Caribbean. They reported 11, 268 fungi species for the Bahanmas, West Indies and islands off the coast of Venezuela, Colombia and Honduras. For the Greater Antilles, over 5000 collections of Basidiomycetes have been obtained in the past seven years and approximately 400 species have been classified (Cantrell et al. 2001; Lodge et al. 2002). Among these 400 species, 75 new species and varieties have been identified or described. The Ascomycetes of the Caribbean region are also poorly known and documented particularly the apothecial ascomycetes (cup fungi), referred as the discomycetes. The majority of published reports are found in general floras that include all fungi and little is known for Hispaniola (Dominican Republic and Haiti).

Systematic biodiversity studies that incorporate standardized methods and develop databases are particularly important (May 1996). Standardized systematic studies are important because they allow comparisons between places and can be used to monitor changes in community structure through time (Rossman 1994). The methods to study the diversity of fungi in a particular ecosystem will depend on the habitat, substrate and the group of interest. This is particularly important with the discomycetes because many species are substrate or habitat specific. Various systematic sampling schemes have been tested for different groups of fungi but none have included the discomycetes as target organisms, so their patterns of dispersion and the most efficient methods for sampling them are unknown (Lodge and Cantrell 1995a, b; Polishook et al. 1996; Schmit et al. 1999). These systematic studies provided information on number, frequency and abundance of species, distribution in time and space, and substrate or habitat specificity (Lodge and Cantrell 1995b; Rossman 1994). Depending on the group of fungi, sampling procedures usually involve the establishment of plots or transects (Lodge and Cantrell 1995a; Schmit et al. 1999). Within plots or along transects, small subplots are established varying in size from 1 × 1 m to 5 × 5 m (Lodge and Cantrell 1995a; Schmit et al. 1999). The subplots can be established at random or at a fixed distance. Some other methods include monitoring a specific substrate or habitat in a given area (Polishook et al. 1996; Schmit et al. 1999). Based on the lack of information for the discomycetes, I initiated a study to survey the diversity of this group of fungi comparing two sampling methods in two areas in the Caribbean National Forest in Puerto Rico and in the Dominican Republic.

The main objective of this study was to evaluate the efficiency of two sampling methods (transects and plots) in obtaining 70-80% of the discomycetes species in a given forest type with the least effort (Coddington et al. 1991). For fungi, particularly discomycetes and microfungi, it is important to evaluate the habitat emphasizing on plant community structure and species richness (Hammon 1992; Lodge and Cantrell 1995b). The concept of species richness represents the total number of species present in a given area. In diversity studies, only a portion of the species is sampled. Different estimators have been designed to calculate total richness in a given sample. Refer to Colwell and Coddington (1996) for more information on the different estimators of species richness. It is difficult to determine species richness for fungi because it is impossible to sample all the species in a given area. The great majority of the species are microscopic and for the macroscopic ones (basidiomycetes and ascomycetes), seasonality in the production and abundance of fruiting bodies represents a problem. Schmit et al. (1999) compared different species richness estimators for macrofungi in a temperate oak forest and concluded that the predictions of species richness obtained by the different estimators were too low and not stable. Estimating microbial diversity is a very difficult task and microbiologits should be careful about sampling methods and definitions of taxonomic units. Hughes et al. (2001) used different species richness estimators for different microbial communities and concluded that nonparametric estimators can be used for microbial data.

For this study the following questions were addressed: 1) Was the distance between the study areas appropriate?; 2) Which method is more efficient, plots or transects? Which method is better: fixed subplots at regular intervals or randomly selected subplots for each sample?; 3) What is the minimum number of subplots needed per plot or transect?; 4) What is the minimum number of samples and what is the optimal sampling frequency?

MATERIALS AND METHODS

In the eastern part of Puerto Rico, two study areas were established in the Caribbean National Forest, in the Luquillo Mts. One area was located near the Sabana Field Research Station (18°19′27″N, 65°43′48″W) and the other in the Bisley Watershed (18° 18′52″N, 65°44′42″W), both near Road 988.

The Sabana area is a secondary forest composed of *Cecropia schreberiana* Miq., *Guarea guidonia* (L.) Sleumer, *Inga vera* Willd., and *Syzygium jambos* (L.) Alst.. The Bisley area is a disturbed Tabonuco forest composed primarly by *Cecropia schreberiana* Miq., *Dacryodes excelsa* Vahl., *Prestoea montana* (R. Graham) Nichols and *Schefflera morototoni* (Aubl.) Maguire. In the Dominican Republic the study areas were located in the Ebano Verde Reserve in the Cordillera Central, one near the Arroyazo Station (19°1′ 57″N, 70°32′35″W) and the other near La Sal Station (19°3′31″N, 70°34′3″W). The Arroyazo area is characterized of broadleaf subtropical wet forest composed of *Schefflera tremula* (Krug & Urban) Alain, and *Prestoea montana* with understory ferns (*Dicranopteris* sp. and *Gleichenia* sp.). The La Sal area is a humid pine forest composed of the endemic *Pinus occidentalis* Schwartz, mixed with broadleaf vegetation dominated by *Syzygium jambos*. In each study area we established a 10 × 10 m plot and a 60 m long transect. Each plot (10 × 10 m) was divided into one hundred 1 × 1 m subplots, and a sample consisted of twelve subplots randomly selected with replacement. For each transect I established a 1 × 1 subplot every 5 m, placed randomly on either side of the line at the beginning of the study. The 10 × 10 m plots and the 60 m transects were also scanned for 15 min for discomycetes and all fungi possibly belonging to the discomycetes were collected and brought to the laboratory for study. If possible, all samples were taken the same day.

TABLE 1. Comparison between different species richness estimators.

	S=	CU=	Chao 1	Chao 2	Jack 1	Boot	LN
Bisley Transect	31	180	34	36	40	35	32
Bisley Plot	34	275	75	58	48	40	44
Sabana Transect	30	127	50	50	45	36	31
Sabana Plot	33	182	44	48	46	39	37

S represents number of species; CU represents collecting units.

The areas were monitored from October 2001 to September 2002, with more frequent samples during the rainy season.

To answer the questions addressed in this study, I plotted species accumulation curves, and calculated the percent of species overlap and complementarity, and Sorensen Similarity Coefficients. Chi-Square analyses were also performed using the species frequencies. Two species accumulation curves were constructed, one using the cumulative number of species per subplot and the other the cumulative number of species per sample. The percent of overlap and complementary were calculated based on Colwell and Coddington (1996). Sorensen's Similarity Coefficient and Chi-Square Goodness of Fit Analysis were calculated based on Krebs (1998). The program EstimateS 6.0 developed by Robert K. Colwell (www.viceroy.eeb.uconn.edu/estimates) was used to calculate species richness using different estimators (Chao 1, Chao 2, Jackknife and Bootstrap). The Coleman Curve (similar to rarefraction curve) was constructed using the same program. The species abundance data set was fitted to the log normal distribution following Krebs (1998).

RESULTS AND DISCUSSION

Species richness.—The results obtained using the discomycete data from transects and plots in Puerto Rico indicate that both the Bootstrap and the Log Normal estimates were closer to the observe number of species in each transect and plot (Table 1). These species richness estimates as well as the Coleman Curve (Figs. 1 & 2) supports the present study is a fairly thorough sampling of the total number of species present. This means that between 77-97% of the species were observed during the course of the study. This result is in congruence to what was observed by Schmit et al. (1999) for macrofungi.

Comparison between sites.—Vegetation– The two study sites in Puerto Rico (Sabana and Bisley) are classified as tabonuco forest, but the tabonuco tree (*Dacryodes excelsa*) has disappeared from the Sabana area. Based on a vegetation study conducted by

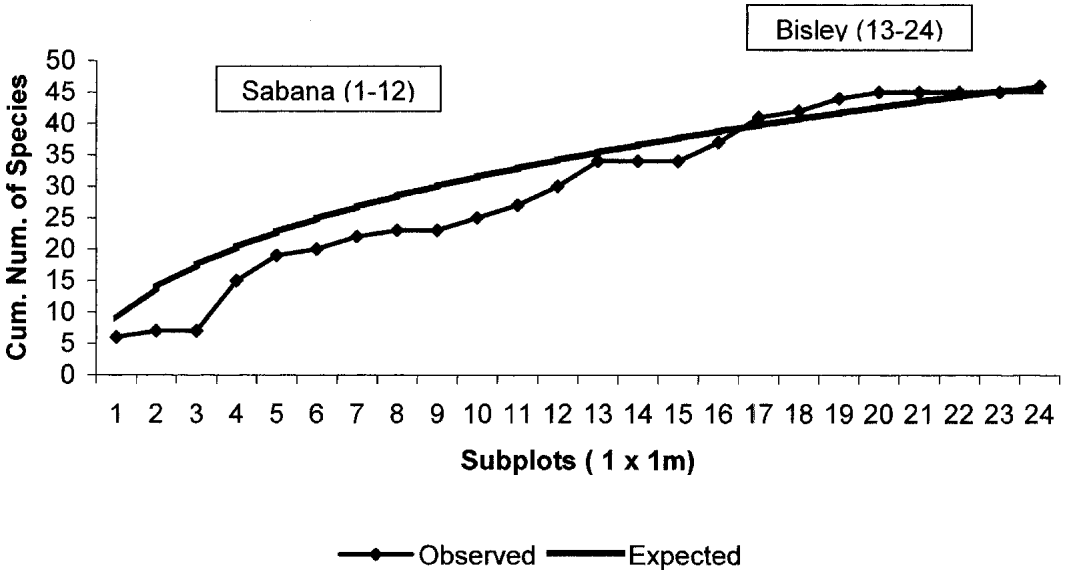

FIG. 1. Species accumulation curves showing the cumulative number of expected and observed species for each subplot in transect. The expected number of species was calculated using Coleman Curve.

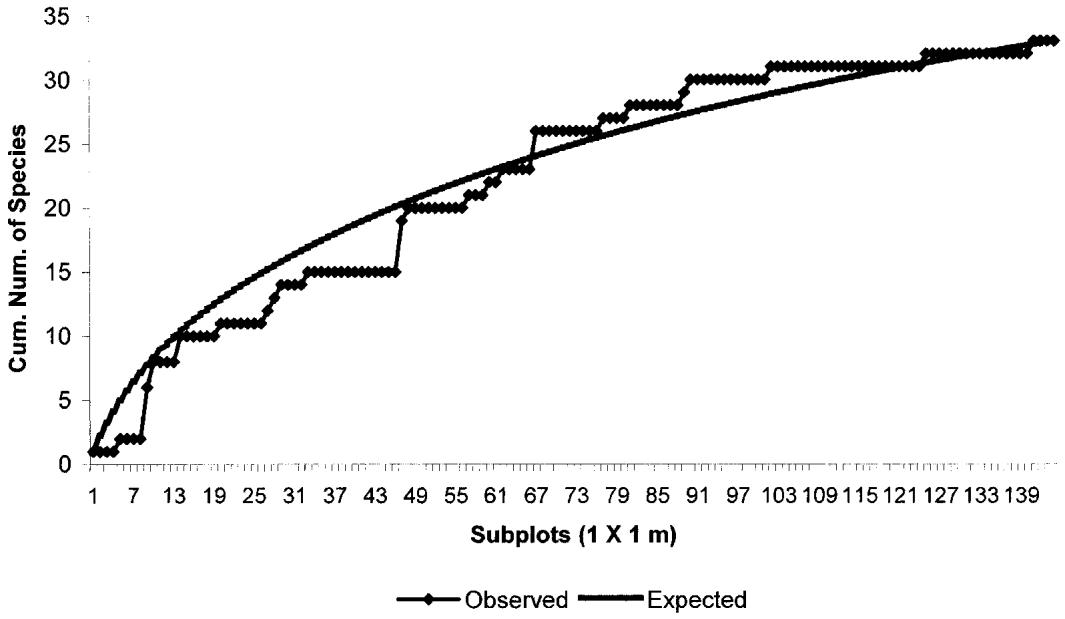

FIG. 2. Species accumulation curves showing the cumulative number of expected and observed species for each subplot for the Sabana plot. The expected number of species was calculated using Coleman Curve.

two undergraduate students, the Sorensen Similarity Coefficient was 0.38, and there was a 24% species overlap (76% of complementarity) between sites in Puerto Rico. *Discomycetes* – Between the two transects and the two plots at Sabana and Bisley in Puerto Rico a 32% overlap (68% complementarity) and a Sorensen's Similarity Coefficient of 0.50 were observed. In the Dominican Republic, there was a 24-31%

overlap (69-76% complementarity) and Sorensen's Similarity Coefficients ranged from 0.40-0.47 between the two transects and the two plots in Arroyazo and La Sal. It is difficult to determine the optimal spacing between transects and plots in order to best cover the area to be studied. The percentage of complementarity (generally falling between one- and two thirds) and the similarity coefficients (falling above 0.40) suggest that the sites were established at appropriate distances and are close enough spatially to represent independent samples of the same community. Similar results in complementarity were obtained by Bills and Polishook (1994) and Polishook et al. (1996) studying microfungi from decaying leaves, and by Lodge and Cantrell (1995a) studying litter agarics.

Comparison between methods within a site.—For the sites in Puerto Rico, 46 and 51 morpho-species of Discomycetes were identified in Sabana and Bisley, respectively (Table 2). Twenty-five and 26 morpho-species of Discomycetes were identified in Arroyazo and La Sal in the Dominican Republic, respectively (Table 3). Between methods, the Sorensen's Similarity Coefficients were O.65 for Sabana, 0.55 for Bisley, 0.40 for Arroyazo and 0.70 for La Sal. Both methods in each site yielded approximately the same number of species, but the similarity coefficients demonstrated that some species were missed either by transects or the plots. The main objectives of this study was to determine which method was more efficient in obtaining a good representation of the Discomycetes species at a given forest type or habitat. In order to answer this question, Chi Square Analysis was conducted using species frequencies to determine if they were randomly distributed or were more likely to occur in an adjacent subplot than in a more distant one. These analyses were conducted for the data in Puerto Rico and the results are presented in Table 4. Based on the Chi Square Analysis, along a transect the species distribution is random and the species are likely to occur at a distant subplot rather than in an adjacent one. But in a plot species are more likely to occur in an adjacent subplot than in a more distant one and the distribution of species tend to be patchier. Similar conclusion was obtained by constructing a distribution map of the more frequent species. This result suggests that it is better to use subplots along transects than subplots within a plot. Krebs (1998) explained that long thin quadrants are better than circular or square ones because in the former, the area is never uniform, habitat is heterogeneous, and the organisms are distributed patchily within the area. Also, the result suggests that it is better to used fixed rather than randomly selected subplots each sampling time. Krebs (1998) emphasized the importance of using random sampling, but also explained that using a systematic (fixed) sampling has the advantage of sampling evenly across an area.

Number of subplots and frequency of samples.—There is always a concern on the size of the plot or transect to be establish in a given study area and the amount of effort needed to obtain the greatest number of species. Coddington et al. (1991) explained several criteria that should be considered at the moment of defining a sampling protocol. One of these criteria is the sampling unit, which should be large enough to obtain the greatest number of species and suitable for statistically analysis, but small enough as not to waste effort. This study was not designed to determine the optimal plot or transect size and shape. For more detailed information please refer to Krebs (1998) who discussed size and shape of the sampling area. Instead, this study was designed to determine the optimal sampling effort needed. Species accumulation curves were constructed to determine the minimum number of subplots and samples needed.

For a study that involves subplots along transects a minimum of 20 subplots are needed (Fig. 1) to sample 90% of the species. These subplots should be divided among transects of equal length. In this study, I used a 60 m transect with subplots every 5 m. There were no significant differences in the Sorensen's Similarity Coefficients for pairs of plots at different distances (0.44 ± 0.19 at 5 m; 0.46 ± 0.16 at 10 m; 0.44 ± 0.11 at 35 m). This suggests that

TABLE 2. List of species in the transects and plots in the sites of Puerto Rico.

Transects	Plots
Acervus flavidus (Berk. & M. A. Curtis) Pfister[S]	*Acervus flavidus*[SB]
Bisporella sp. 1[S]	*Bisporella* sp. 1[S]
Bisporella citrina (Batsch:Fr.) Koft & S. E. Carp.[S]	*Bisporella citrina*[S]
Callycelina sp.[SB]	*Callycelina* sp.[SB]
Ciboria sp. 1[S]	*Ciboria* sp. 1[SB]
Ciboria sp. 2[B]	*Ciboria* sp. 3[S]
Coccomyces sp. 1[SB]	*Cistella* sp.[B]
Coccomyces sp. 2[SB]	*Coccomyces* sp. 1[SB]
Coccomyces sp. 3[SB]	*Coccomyces* sp. 2[SB]
Cookeina speciosa (Fr.:Fr.) Dennis[S]	*Coccomyces* sp. 3[SB]
Hyalorbilia inflatula (P. Karst.) Baral & Marson[SB]	*Cookeina tricholoma (Mont.) O. Kuntze*[B]
Hyaloscyphaceae sp. 1[SB]	*Crocicreas* sp.[B]
Hyaloscyphaceae sp. 2[S]	*Hyalorbilia inflatula*[SB]
Hymenoscyphus sp. 1[SB]	*Hyaloscyphaceae* sp. 1[S]
Hymenoscyphus sp. 2[B]	*Hyaloscyphaceae* sp. 3[B]
Lachnum euterpes S. A. Cantrell & J. H. Haines[B]	*Hymenoscyphus* sp. 1[S]
Lachnum fimbiiferum (Berk. & M. A. Curtis) J. H. Haines[B]	*Hymenoscyphus* sp. 3[SB]
Lachnum sp. 1[S]	*Lachnum brasiliense (Mont.) J. H. Haines & Dumont*[B]
Lambertella sp. a[SB]	*Lachnum euterpes*[B]
Lambertella sp. 2[S]	*Lachnum* sp. 1[S]
Lambertella sp. 3[S]	*Lachnum* sp. 2[B]
Lambertella sp. 4[S]	*Lachnum* sp. 3[B]
Lambertella sp. 5[B]	*Lambertella* sp. 1[SB]
Lambertella sp. 6[B]	*Lambertella* sp. 3[S]
Lophodermium sp. 1[SB]	*Lambertella* sp. 4[S]
Lophodermium sp. 2[B]	*Lambertella* sp. 5[S]
Lophodermium sp. 3[B]	*Lambertella* sp. 6[S]
Moellerodiscus sp. 1[B]	*Lanzia* sp.[B]
Moellerodiscus sp. 2[B]	*Lophodermium* sp. 1[SB]
Mollisia sp. 1[S]	*Lophodermium* sp. 2[B]
Mollisia sp. 2[B]	*Moellerodiscus* sp. 1[SB]
Orbilia sp. 1[SB]	*Mollisia* sp. 1[B]
Orbilia sp. 2[S]	*Mollisia* sp. 2[B]
Orbilia sp. 3[S]	*Orbilia* sp. 1[SB]
Orbilia sp. 4[B]	*Orbilia* sp. 2[B]
Orbiliaster sp. 1[SB]	*Orbilia* sp. 4[B]
Orbiliaster sp. 2[B]	*Orbilia* sp. 5[S]
Orbiliopsis sp.[SB]	*Orbilia* sp. 6[S]
Ostropa sp.[B]	*Orbilia* sp. 7[B]
Phillipsia domingensis (Berk.) Berk.[S]	*Orbilia* sp. 8[B]
Pulvinula globifera (Berk. & M. A. Curtis) Le Gal[B]	*Orbiliaster* sp. 1[SB]
Rhizodiscina lignyota (Fr.) Hafellner[SB]	*Orbiliaster* sp. 2[B]
Stictis radiata (L.) Pers.[SB]	*Orbiliopsis* sp.[SB]
Stictis sp. 1[SB]	*Ostropa* sp.[S]
Trichoglossum sp.[B]	*Phillipsia domingenesis*[S]
Unguicularia[S]	*Rhizodiscina lignyota*[B]
	Stictis radiata[SB]
	Stictis sp. 1[SB]
	Stictis sp. 2[B]
	Strossmayera sp.[S]
	Vibrissea sp.[S]

[S]Sabana; [B]Bisley

TABLE 3. List of species in the transects and plots in the sites of Dominican Republic.

Transects	Plots
Arachnopeziza sp. 1[S]	*Arachnopeziza* sp. 1[S]
Arachnopeziza sp. 2[S]	*Arachnopeziza* sp. 2[S]
Bisporella citrina[S]	*Bisporella citrina*[A]
Bisporella pallescens (Pers. Ex S. F. Gray) S. E. Carp. & Korf[A]	*Bisporella* sp.[S]
Bisporella sp.[AS]	*Ciboria* sp.[A]
Ciboria sp.[A]	*Coccomyces clusiae*[AS]
Coccomyces clusiae (Lév.) Sacc.[A]	*Colpoma* sp.[S]
Colpoma sp.[A]	*Cyathicula* sp.[A]
Cyathicula sp.[A]	*Dicephalospora rufocornea*[AS]
Dicephalospora rufocornea (Berk. & Broome) Spooner[S]	*Dicephalospora* sp.[S]
Hyalorbilia inflatula[AS]	*Geoglossum* sp.[A]
Hyaloscypha sp.[S]	*Hyalorbilia inflatula*[AS]
Hymenoscyphus sp.[AS]	*Hymenoscyphus* sp.[S]
Lachnellula sp.[AS]	*Lachnellula* sp.[S]
Lachnum brasiliense[A]	*Lachnum euterpes*[A]
Lachnum pterydophyllum[A]	*Lachnum pterydophyllun*[AS]
Lachnum sp. 1[A]	*Lachnum* sp.[S]
Lachnum sp. 2[AS]	*Lachnum virgineus* (Batsch:Fr.) P. Karst.[A]
Orbilia sp.[AS]	*Lambertella* sp.[A]
Orbiliaster sp.[S]	*Mollisia* sp.[AS]
Orbiliopsis sp.[S]	*Orbilia* sp.[AS]
Propolis sp.[S]	*Orbiliaster* sp.[AS]
Rhizodiscina lygniota[A]	*Orbiliopsis* sp.[S]
Sclerotinia sp.[S]	*Propolis* sp.[S]
Stictis radiata[A]	*Sclerotiniaceae* sp.[AS]
	Stictis radiata[S]

[S]La Sal; [A]Arroyazo.

subplots established at 5 to 10 m intervals, along a transect, are representative of random samples and are not significantly influenced by spatial aggregation (contagious distribution).

Using a plot method, 60-70 subplots are needed to obtain most of the species (Fig. 2). There is a problem with establishing subplots within a square plot i.e., how close or dispersed these subplots need to be. Krebs (1998) discussed this problem and emphasized the importance of knowing how the species are distributed. Fungi, as well as plants and other organisms, tend to have a patchy distribution and sometimes will be linked to the substrate. In this study, I have tried to overcome this problem and balanced time and effort by randomly selecting the subplots for each sampling period with replacement. The results obtained from a Chi Square Analysis indicate that the distribution of species within the plots was not homogeneous in comparison to the distribution of species along transects (Table 4). This indicate that it is better to use transects and to determine the distance intervals between subplots based on the scale of patchiness of the species being sampled.

Another important point of concern is the number and frequency of samples. In order to answer these questions, a species accumulation curve was constructed using the cumulative number of species per sample. Based on the location of the shoul-

TABLE 4. Chi square analysis using the frequency of species in the transects and plots for the sites in Puerto Rico.

	Sabana	Bisley	Between
Transect[a]	11.65	11.40	23.05*
Plot[b]	43.71*	33.80*	77.51*

*Significant
[a]$\chi^2_{0.05}$ = 15.5, d.f. = 8
[b]$\chi^2_{0.05}$ = 12.6, d.f. = 6.

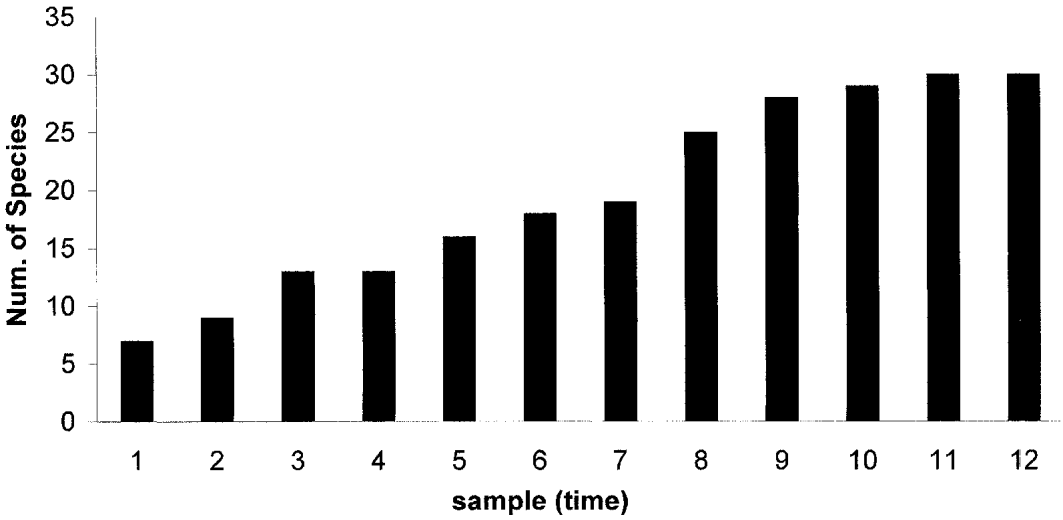

FIG. 3. Species accumulation curve showing the cumulative number of species observed in each sample for Sabana's transect.

der in the distribution in Fig. 3, the number of samples needed is 10-12. The samples should be distributed throughout a year as indicated by the occurrence of unique species in almost every month, with shorter intervals during the rainy season when more species fruit (Fig. 4).

In summary, in order to conduct a study of discomycetes diversity the study areas should be selected based on the diversity of plant species and their distribution. Most of the discomycetes species are substrate and habitat specific. From this study, I conclude that using several transects is a better technique than using plots. These transects should have a minimum of ten 1 m^2 subplots at 5-10 m intervals. Also the study should be conducted for at least a year with shorter sampling intervals during the rainy season. While the results of this study provide recommendations for tropical discomycetes, this protocol cannot be applied directly to the study of diversity of other fungi since each group behaves in a differ-

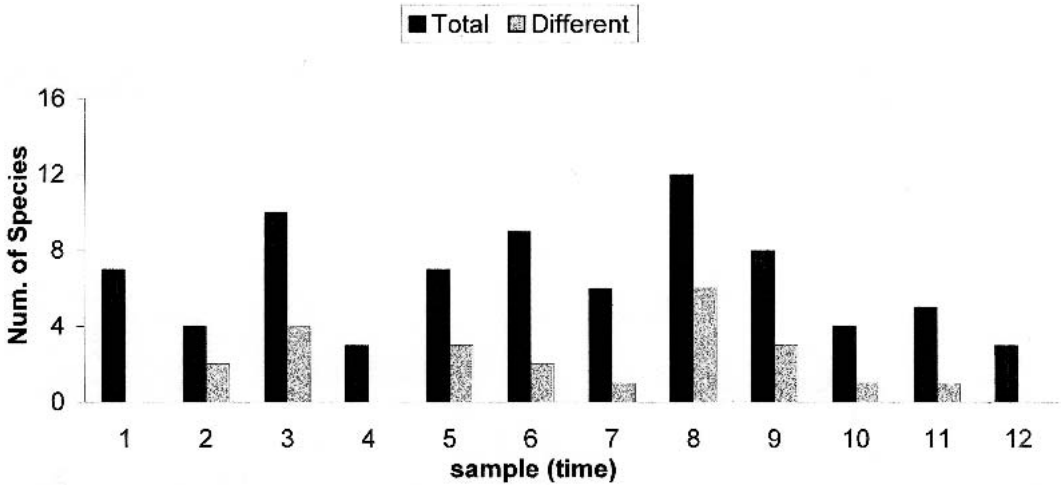

FIG. 4. Total species number and number of different species in each sample for the Sabana transect.

ent way, and many species are seasonal and tend to be substrate-and habitat-specific. The methods that were used in this study, however, can be emulated for designing optimal sampling strategies for other fungal groups.

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