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OVINE ILL-THRIFT IN NOVA SCOTIA
6. QUANTITATIVE DESCRIPTION OF THE FUNGAL FLORA OF SOILS OF PERMANENT PASTURE*

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Four hundred thirty-seven soil samples were collected from upland pasture at Nappan, Nova Scotia between April 24 1967 and October 28 1975. From these soil samples 5605 fungal isolates were established in culture. The mean value for the number of fungal propagules per g of dry soil was 8.2×10^5 and this value remained the same over the experimental period of 9 yrs. One hundred forty-two fungi have been classified, 137 belong to known genera, of which 54 are known species. The isolates classified into these 142 fungi constitute 35% of all isolates. The isolates collected in 1967, 1968, and 1970 were not available in 1978 mainly because of loss of viability. The remaining 2683 isolates, collected between May 5 1969 and October 28 1975 had characteristics similar to those of the whole collection. Methods of selecting random samples of 750 isolates were investigated. Tests were devised to compare these random samples with the total population and used to select a sample that appeared to be most representative. The mean number of fungal propagules/g of soil in this sample was 4.8×10^4 and this number remained unchanged throughout the experimental period. Sixty-seven fungi from the representative sample have been classified and these constitute 49% of the different isolates in the sample. All of these isolates (543) were tested for their ability to produce metabolites that inhibited the growth of *Sarcina lutea* and/or *Candida utilis*. The growth of both organisms was inhibited by 3% of the isolates; 24% of isolates inhibited the growth of only *S. lutea* and 3% affected only *C. utilis*.

Introduction

A number of difficulties attend a quantitative description of the fungal flora of a soil sample. The flora may be an interlaced web of mycelium on the one hand or a dense packet of spores on the other, but it is generally somewhere between these two extremes. It is therefore hard to assess whether 2 or more fungal colonies, isolated by any means, are derived from the same or from different organisms, unless they are different species. It has therefore been argued (Ainsworth 1968) that enumeration of a soil fungal flora is meaningless. To assess the population density of a fungal flora, the concept of a fungal propagule has arisen (Brierley et al. 1927) but the term is used differently by different workers. As it is essential to our attempts to describe the flora of permanent pasture, we define our use of the term. It is clear that dispersion of the soil matrix involves disruption of delicate living tissues such as root hairs, fungal mycelium, chains of bacteria, etc. If the cell contents are spilled in this shearing and grinding process, regeneration is unlikely. Hence, anything cultivated from the dispersed biomass arises from an essentially intact cell, be it spore, hyphal fragment, chlamydospore, etc., likely surviving structures are highly dependent on individual species. It might, therefore be expected that surviving viable entities will range from single cells, e.g. spores, to hyphal fragments (Kliejunas & Negata 1979) and further to intact conglomerations of cells, e.g. sclerotia, or even interwoven strands of hyphae from mycelial mats. Regardless of their morphology, such varied viable units may grow in cultivation and result in a discreet colony. We use the word "propagule" to delineate a fungal fragment isolated by a process such as that described; the process, method of dispersion,

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temperature of cultivation, and nutrient medium all being defined (Brierley et al. 1927). It is now possible to examine the questions: is the number of such propagules related to the density of the fungal flora of a soil sample; can this relation be determined, and if so, with what precision?

During the isolation procedure, provided normal sterility techniques are used, there are no additions to the flora. However, at the moment of isolation, metabolic and mechanical modifications of the flora start. These modifications result in the loss of propagules, by disruption, digestion, aeration, and/or dilution. The number of species in the soil defines the lowest possible number of fungal organisms therein. The greatest number of fungal organisms is also defined by the volume representing the difference between that of the hydrated ash and unit volume of soil divided by a hypothetical mean cell volume. The size of the interval thus defined is probably much smaller in practice. Many fungal organisms are present as single cells and at the other end of the scale much of the biomass may not be part of the fungal flora. It is evident that there is a probability function with a maximum value, that gives the chance of finding a number of fungal organisms in a particular soil sample. The position is similar to that of a fundamental particle whose position is described by a probability function, the uncertainty not precluding predictions about its role in the properties of matter. Similarly, the smallest possible number of fungal propagules that it is possible to isolate is the number of surviving species and the maximum number is the same as that defined above for the maximum number of fungal organisms present. The interval is therefore wider than that of fungal organisms but the probability distribution is displaced downwards because of the loss of fungal tissue in the isolation procedure. It follows that the probablility functions of the two concepts—fungal organisms and fungal propagules—will be similar, with a high probability that the maximum of the latter will lie at a smaller value than that of the former. A similar argument can be applied to any other concept of the fungal flora, e.g. the number of cells. The foregoing describes the fraction of the soil flora theoretically available for study by means of some dispersion and isolation process. There remain two other matters to be discussed before proceeding to report the results of our experimental work.

In the foregoing analysis, the concept of species has been used as a limit. Historically, classification of fungi has been based on their morphology with particular reference to structures elaborated during reproduction. In the case of parasites, and to a lesser extent symbionts, the discrimination of hosts or partners has played a role in fungal classification. This system of classification has its disadvantages when studying the ecology of fungi from a habitat in a more or less comprehensive fashion. Many fungi, with known teleomorphs, produce only their anamorph in culture and it is often difficult to relate the culture to the known teleomorph. It is also possible for the anamorphs of different teleomorph species to be grouped into the same species aggregate (Rifai 1969). The existence of the parasexual cycle (Pontecorvo et al. 1953) in the Deuteromycotina leads to the possibility of isolates of the same anamorph species having conspicuously different characteristics. Also, anastomosis of hyphae of supposedly different species provides new heterokaryons often difficult to assign. A further problem stems from the objective—to describe all the fungal isolates, large numbers of which may produce no morphological feature that is characteristic in a classical sense. These difficulties suggest that a numerical system of classification might be useful. However, numerical systems of taxonomy traditionally have been applied to relatively uniform groups, usually bacteria, but a wide diversity of organisms is found in the soil fungi. Hence, we have adopted a numerical key, each isolate having similar, recognizable characters being assigned a number. In some cases, these characters

were sufficient to classify the organism with respect to genus and in others, species. The system has the disadvantages that it has no logical or mnemonic properties.

Faced with the object " . . . . . ", most would agree that it contained 5 periods, but if about 5000 periods were to be counted on 2 or more occasions, greater difficulty would be found in arriving at the same total. If, therefore, it was possible to disperse 1 kg of soil it would be unlikely that 2 or more mycologists would count the same number of fungal colonies grown therefrom in culture. But it follows from the analysis given, that a large number of soil samples must be counted to arrive at a reliable estimate of the number of fungal propagules in a given weight of soil. This Herculean task is evaded by counting the colonies grown from about 50 μg of soil, but then it is difficult to be sure that the soil samples are representative, and that distortions do not occur in extrapolation of the results to the soil of a useful area of land, e.g. a hectare. We have tried to overcome these problems by collecting a large number of soil samples in a random distribution over the experimental area. The samples have been collected over a period of 9 years and the fungi have been isolated by setting up 10 replicates of a dilution series of each soil sample (Montegut 1960).

Problems in counting and sorting have been overcome by the use of a computer, and this aid has also allowed the expansion of collections to reflect their frequency in the field. The algorithms used are simple and inelegant, but are given in the hope that other mycologists will use and develop them.

Methods

Experimental Plot

The experimental plot (P2, Brewer et al. 1971) was located at the Agriculture Canada Experimental Farm, Nappan, Nova Scotia. The boundaries of the plot in 1967, 1968, and 1969 were those described by Brewer et al. (1971), but only collections from the sectors specified below are included in the data used in this paper. From and including 1971 the plot was essentially sectors C and D in 1967 and 1969, and sector B in 1968, but extended eastwards towards the wooded area and westwards towards the bull-barn. A detailed map is given in Figure 1. The extensions of the plot did not introduce additional plant species, areas of different husbandry nor different soil types. For the collection periods 1973 to 1975 inclusive the plot was divided into 10 equal sectors as shown in Figure 1. In other years, when calculations required sector specification, a random number (1-10) was generated for each soil sample during the calculations. Collections were made approximately at noon, at random over the area of the plot, by generating random numbers for each collection date, the numbers referring to a section of the plot. Additional randomization was ensured by using about 20 people to collect the samples over the 9-year period. The method of collecting the soil cores was described by Brewer et al. (1971) where the number and times of collection in 1967, 1968, and 1969 are also given. In 1970, 28 soil cores were collected on 15 days at about weekly intervals between May 12 and August 20. In 1971, 7 soil samples were obtained at monthly intervals from April 27 to October 25. Sixty-four soil cores were collected in 1973 on 26 occasions, at roughly weekly intervals between May 2 and October 28. In 1974, 50 soil samples were obtained on a weekly basis between May 6 and June 3, and from June 17 to October 29. Fifty-two soil samples were collected in 1975 at 26 weekly intervals from May 6 to October 28. The total number of soil samples collected, from 1967 to 1975, was 437, on 167 collection dates.

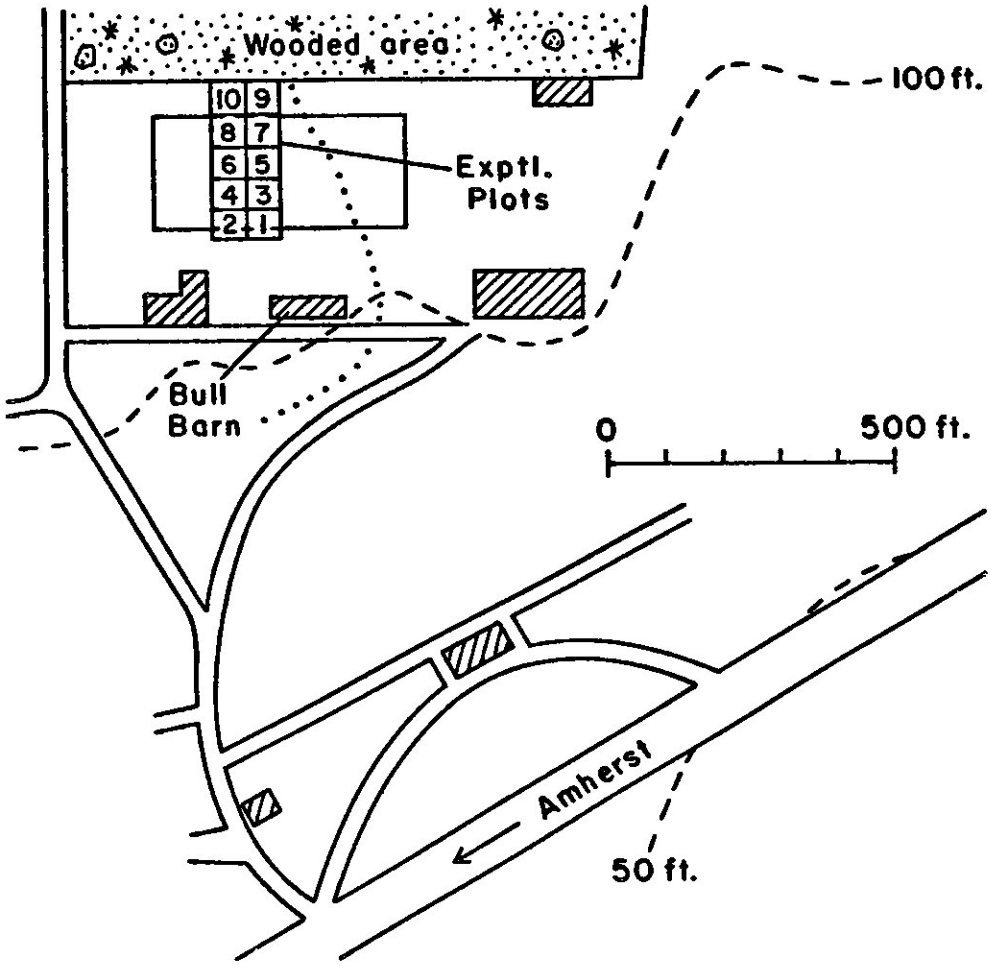

Fig 1. Location of the experimental plots. The area divided into 10 numbered sections is the plot used to collect soil samples described in this paper. The larger area in the same locality was the area used to collect soil samples described by Brewer et al. (1971).

Isolation of Fungal Propagules

In the years 1967 to 1972 inclusive, fungal propagules were isolated as described by Brewer et al. (1971). From 1973 to 1975, a laboratory was available adjacent to the experimental plot and dilution series were made within 1 hour of collection of the soil sample. In the 2 latter years, the medium used to cultivate the fungal propagules was augmented by addition of soluble starch (1 g l^{-1}) and cellulose (6 g l^{-1}, MN300, Nagel & Co.). The plates were incubated for 7 days at 25°C and the numbers of fungal colonies appearing were counted. A subculture onto 2% (w/v) malt agar was made of each of the colonies that appeared to have characteristics that differed from others on the plate. A record was made of the number of colonies of each type occurring on 10 plates from which it was isolated, the ability to hydrolyze starch and cellulose, the ability to reduce rose bengal, and the growth-inhibiting effect (if any) on adjacent fungal colonies. When doubt occurred as to whether or not one colony was truly different from another, subcultures were made from both. In 1979, those organisms collected in 1969, 1971, and 1973 that remained viable were sub-

cultivated onto the starch-cellulose medium, and their ability to reduce rose bengal and hydrolyze the polysaccharides was recorded.

Numerical Formulation of Ecological Data

The system developed to translate fungal ecological data into numerical code, had as its basic condition that one line of ciphers was assigned to each isolate. The following criteria were used in devising the code: each line had to be unique, with mnemonic properties, i.e. it could be deciphered without recourse to a key; the code should appear in groups of ciphers that were code for related phenomena, to ease sorting problems; and finally, the code should be compatible with readily available peripheral machinery associated with computers. It was also desirable, though not essential, that the code should be written in a way that made its use in computational devices as efficient as possible, thus reducing the time required for calculations. When (1970) we decided to translate our field observations into such a code the computer facilities available to us imposed the following restrictions. Only 6 digits could be used for input and output of integral numbers; no line could be more than 72 characters; integral numbers were most efficiently stored in groups of 4; and processing of numerical data was several orders of magnitude faster than text. Most of these restrictions have now disappeared, but only minor modifications of the code have been made, thus improvements in computers have merely increased the speed of processing. Each fungal isolate can be uniquely described by the date of collection of the soil sample from which it was cultivated, the geographical location of the collection site and a number assigned to each different isolate on a group of 10 dilution plates. As collections were made in the period 1967 to 1975, the year of collection can be represented by the last digit of the year number A.D., i.e. 7 to 5, and as collections were only made each year between April and October, these months can be indicated by the numbers 4, 5, 6 . . . 0. The number 224309416 thus uniquely describes fungal isolate number 16 cultivated from a soil sample collected from sector 4 of plot 9 on April 22 1973. The number had to be broken into the parts 224309 and 416 because of the restrictions mentioned. This division leaves 3 digits in the second group, i.e. 416xxx, and these have been used to indicate the frequency of this particular isolate in terms of numbers of propagules in 1 g of dry soil. This number was recorded to 2 significant figures, the last digit being used to record the power of 10 to which these significant figures should be raised. Thus the ciphers 274 indicate that the frequency is 27,000 g^{-1}. The third and fourth groups of ciphers have been used to record taxonomic, physiological and meteorological information concerning the isolate. Each of these subjects is discussed separately.

Taxonomy

No attempt has been made to devise a systematic numerical code for classified soil fungi. Initially it was considered unlikely that more than 9999 species would be found, thus the 4 left-hand digits in the third group were assigned for taxonomic classification. It was also thought that it might be possible to reserve the second and third ciphers from the left to indicate genus, and this has been done in several instances but, in general, has proved to be too complex to retain in mind. Rather, it was easier to construct and augment a separate file of species and their assigned numerical code (Brewer et al. 1978).

Physiology

Most of the physiological information observed on these fungal isolates was obtained after considerable development of computer machinery. In particular, the restriction of 6 digits disappeared. Thus, physiological data have been written in

code in the third group of digits. The data are of 2 kinds. First, there is information read directly from the isolation plates, viz., does the colony reduce rose bengal; is it surrounded by a clear zone in the opaque cellulose agar or is there a zone around the colony that does not give a blue colour with iodine? Finally, does the colony inhibit the growth of an adjacent colony and if so which? The code used to record these data is as follows (digital place numbers are counted from right to left): in the fifth place the zone of starch hydrolysis is given as the difference between the zone diameter and the diameter of the colony divided by 2. The sixth place is assigned the value of 1 or 0 according to whether rose bengal is reduced or not and the seventh place is given the same values (1 or 0) if cellulose hydrolysis is or is not observed. The eighth place is assigned the isolate number of the colony whose growth is inhibited, the value 0 indicating that no growth inhibition was observed (the cipher 9 was used to indicate growth inhibition of several isolates). Ciphers in the ninth (and greater) place indicate data of the second kind i.e. experimental results obtained on isolates after they had been admitted into the culture collection. Numbers in the ninth place indicate the following: 1, activity against *Sarcina lutea* (HLX 701*); 2, against *Candida utilis* (NRRL Y900); 3, against both; and 9 indicates activity in the screen reported by Brewer et al. (1972). Thus the ciphers 181130178 indicate an isolate of *Trichoderma hamatum* (0178) which hydrolyzes starch (zone radius 3 mm), reduces rose bengal, hydrolyzes cellulose, inhibits the growth of isolate 8 collected in the same soil sample and also of *S. lutea*.

Meteorology

Meteorological data were stored in the fourth group of ciphers. The 2 digits on the extreme right record the rainfall in millimeters in the 7 days prior to the collection of the soil sample, the third and fourth digits record the temperature of the soil at collection time 5 cm below the surface, and the fifth and sixth digits record the temperature of the soil 1 cm below the surface. The seventh place in this group of ciphers has been used to indicate whether or not the isolate has been selected in a random sample upon which taxonomic and/or physiological studies have been done. The number inserted in this seventh position indicates the number of times the isolate was selected in the frequency-weighted random sample. Thus the group of ciphers 2181604 means that there were 4 mm of rain in the week prior to collection, that the temperature of the soil at the collection site at the time of collection, 5 cm below the surface, was 16°, at 1 cm below the surface 18°, and that 2 entries of this isolate appeared in a random sample from which additional information has been obtained. To summarize, the line 224309 416274 181130178 2181604, taken from the examples given, uniquely describes the fungal propagule and condenses much information about it in a line of code easily deciphered.

Construction of Computer Files of Fungal Ecological Information

The conversion of field and laboratory observations into code described in the preceding section and the assembly of these data into files of information is a tedious job that has a high risk of human error. Several methods of doing this job were tried and the following was found to be susceptible to fewest errors. It is, therefore, described in detail. One page, divided into 16 columns, of an 8 x 11-in hard-backed book was used for each soil sample collected. The day, month, year, plot number and sector number were written into the first 5 columns from the left at the time of collection of the soil sample. At the same time, the temperature of the soil adjacent to the collection core at 1 cm and 5 cm was entered into the seventh

*Accession number to the collection of micro-organisms held at this laboratory.

and eighth columns and the rainfall into the sixteenth. When the cultures were examined after 7 days incubation, the different colonies were given an isolate number 1 to n, and this number was inserted into column 6, the combination of the numbers in columns 1-6 then being used to identify subcultures. These field books thus served as an accession catalogue to the growing collection of fungal isolates. At the time of isolation the following data were also entered into the field books: the number of identical colonies on the 10 plates was inserted into column 9 and the dilution of the soil sample represented by the 10 plates into column 10. The isolation number of another colony (if any) that was inhibited was entered into column 12, starch and cellulose hydrolysis in columns 13 and 14, and rose bengal reduction in column 15. If the taxonomic code was known, it was entered at this stage into column 11.

The data in the field books were then recorded on magnetic tape using a computer terminal (e.g. Texas Instruments silent 700) equipped with a cassette recorder. Each line in the field book was typed as 1 line of data on the tape starting with a line number (2000 increasing by 1 for each line) and then the word "data". This method has the advantages that the data can be checked and errors corrected before transmission to the computer via acoustic coupler and telephone line, and the tapes are a valuable copy of the data in the field books. The computer program given in Appendix A can also be recorded on the tape and then all the information transmitted to the computer. The commands required to do this job, to execute the program and to store the program but not the data, differ from one computer center to another, but are simple and will be explained to potential users by the staff of such institutions. All computer programs used in this work were written in the beginners all-purpose symbolic instruction code (BASIC). An annotated listing on the program used for expanding a collection of isolates to reflect their frequency and for selecting a random sample of 750 of the expanded collection is given in Appendix B. Duplicate copies of files of field data were kept on standard 7-track magnetic tape. One of these was kept at the computer center and the other in the laboratory. As new information, e.g. taxonomic, became available it was added to the files on the 7-track tapes by using facilities available at the computer center. At the beginning of this work programs were written to edit these files; these programs and all others used in this work are available on cassettes from the Librarian of the Nova Scotia Institute of Science should they be thought to be of use to other mycologists. All computations were done using Control Data Corporation computers.

Antibiotic Assays of Cultures of Soil Fungi

Giant colonies of the fungi screened for the production of antibiotics were grown as described by Brewer et al. (1972). The assay procedure was as described by Brewer et al. (1974), employing *S. lutea* and *C. utilis*, except that the dishes inoculated with *C. utilis* were incubated at 25°C for 42 h.

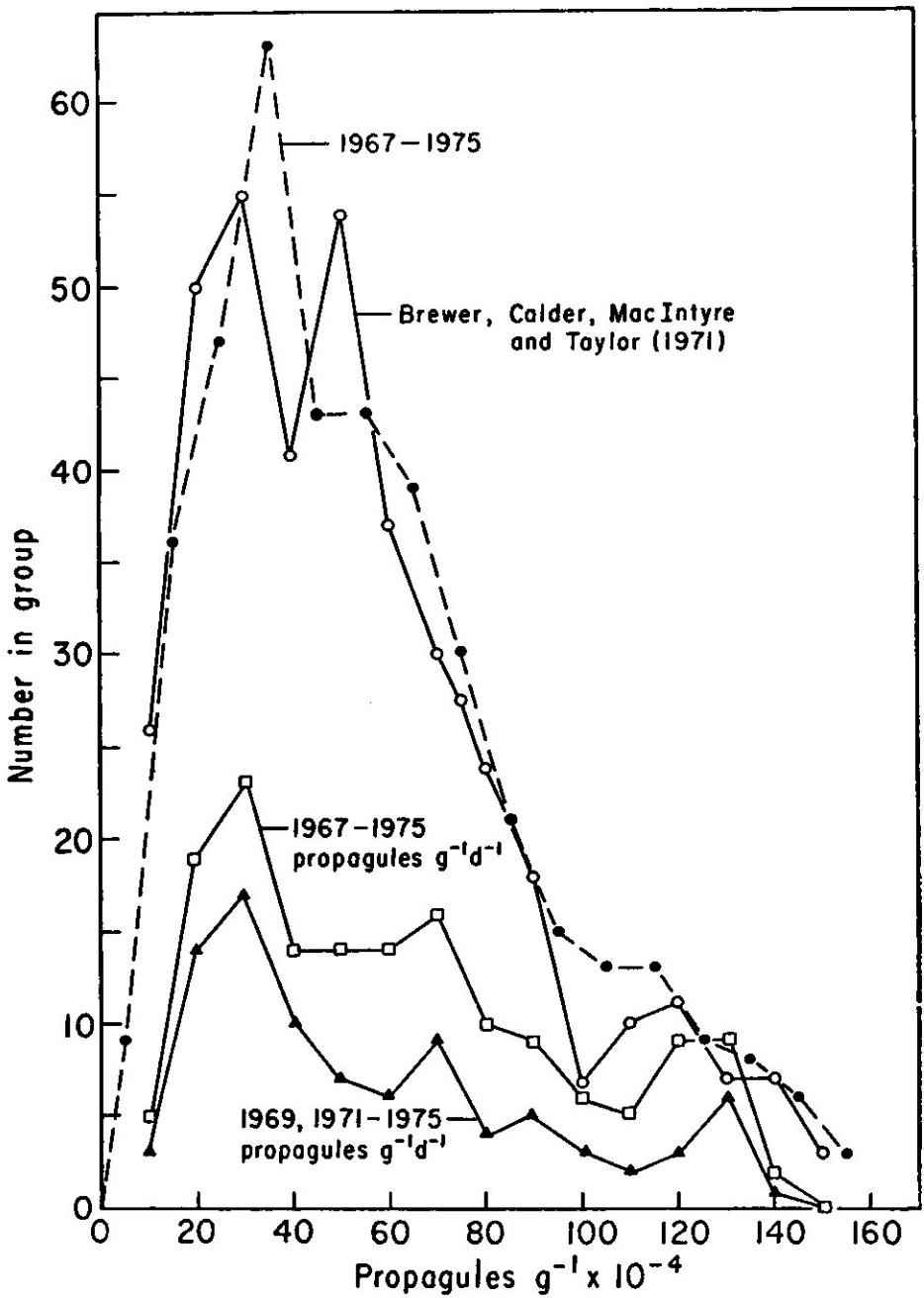

Fig 2. Numbers of soil samples collected from which fungal propagules were cultivated (groups of 10^5).
In the period 1967 to 1975 there were 39 samples collected for which values exceeding 1.55 x 10^6 propagules g^{-1} were obtained. In the period 1967 to 1975 there were 12 occasions when the mean value for the collection date exceeded 1.6 x 10^6 propagules g^{-1} and in the period 1969, 1971 to 1975 there were 8 occasions.

Table I Mean numbers of fungal propagules in 1 g of dry soil collected from different sectors of a plot of permanent pasture in 1973 to 1975.

Sector	No. of soil samples collected	Propagules g^{-1} ($\times 10^{-4}$)			
		Max.	Min.	Mean	Standard deviation
1	15	33	2.7	12	25
2	10	26	2.3	9.4	24
3	18	130	2.2	14 (6.7*)	37
4	18	22	1.2	8.2	15
5	13	29	0.6	9.0	21
6	18	63	0.9	11.	27
7	22	15	0.7	5.6	8.5
8	16	17	1.3	5.0	10
9	19	11	0.9	4.5	0.8
10	11	32	2.1	9.4	23

*Omitting one collection of 10^7 g^{-1}

Results

Characteristics of the Fungal Flora of the Soils of Permanent Pasture

Brewer et al. (1971) reported that the mean value of fungal propagules g^{-1} for 423 upland (forest) soil samples collected in spring, summer and fall of 1967, 1968, and 1969 was 6.04×10^5. They gave a histogram of the numbers of soil samples collected from which fungal propagules (in groups of 10^5) were cultivated. This histogram is reproduced in Figure 2. A very similar number of soil samples (437) was collected from part of the same plot (Fig 1) but over a longer period of time (1967-1975). The mean number of fungal propagules g^{-1} of dry soil in this set was 6.9×10^5 if one collection of 1.3×10^7 is omitted. A histogram of these 437 soil samples is given in Figure 2; both groups of data are clearly similar. The mean numbers for each month of the growing season in the 1967-1975 data were as follows (soil samples collected each month in parentheses): April, 5×10^5 (17); May, 6.9×10^5 (73); June, 6.8×10^5 (73); July, 6.9×10^5 (92); August, 6.5×10^5 (65); September, 7.5×10^5 (55); October, 7.3×10^5 (62), omitting a single value (1.3×10^7) collected in July. The standard deviation of the means is 8.2×10^4 and hence, as in earlier work (Brewer et al. 1971), there was no significant difference in the number of fungal propagules cultivated from soil samples collected in the annual period April to October inclusive. In general, more than one soil sample was collected on each of the 167 collection dates in the period 1967-1975. The mean value of propagules g^{-1} obtained on each of these collection dates was calculated, and the numbers obtained sorted into groups of 10^5. The resulting histogram is drawn in Figure 2. The group containing the maximum number of collections ($3\text{-}4 \times 10^5$) is the same as in the other histograms in Figure 2. The mean value for all 167 collection dates was 8.2×10^5. The dates of collection were converted into a time scale—April 24 1967 = day 1, October 28 1975 = day 3108 and the mean propagules g^{-1} for each of the collection days used as 167 data points to calculate a linear least mean squares fit. The gradient of the fitted line was 22 and the difference, therefore, between day 1 and day 3108 was 6×10^4. Hence no significant change in propagules g^{-1} isolated occurred in the 9 years. No differences in the

Table II. List of fungi isolated from soil samples.

Organism		Mean no. of propagules /g soil	No. of soil samples	No. of isolates in collection	Observed optimum temperature range* (°C)	Calculated optimum temperature (°C)
Absidia		62,000	2	2	17-20	
Absidia coerulea Bainier		22,000	7	8	25-28	
Absidia corymbifera (Cohn) Sacc. & Trotter		38,000	1	1	25-28	
Absidia glauca Hagem		27,000	20	21	17-20	18 (17)
Absidia spinosa Lendner		15,000	2	2	17-20	
Acremonium		6,000	1	1	5-8	
Alternaria		32,000	1	1	17-20	
Alternaria alternata (Fr.) Keissler	(461)	7,000	1	1	21-24	
Alternaria alternata	(462)	19,000	3	3	21-24	
Aspergillus		125,000	2	2	25-28	
Aspergillus chevalieri (Mang.) Thom & Church		5,000	1	1	13-16	
Aspergillus chevalieri var. *intermedius* Thom & Raper		34,000	3	3	25-28	
Aspergillus fumigatus Fresenius		7,000	2	2	21-24	
Aspergillus sydowi Bain. & Sart.		36,000	1	1	9-12	
Chaetomium		17,000	7	7	9-12	
Chaetomium funicolum Cooke		12,000	1	1	13-16	
Chaetomium umbonatum Brewer		19,000	11	13	17-20	(17)
Circinella		10,000	1	2	13-16	
Cladosporium cladosporioides (Fresen.) de Vries		78,000	10	10	21-24	
Cladosporium herbarum (Pers.) Link ex S.F. Gray		31,000	9	9	21-24	(21)
Cunninghamella		28,000	8	11	17-20	
Cunninghamella elegans Lendner		71,000	1	1	25-28	
Curvularia protuberata Nelson & Hodges		20,000	1	1	21-24	
Cylindrocarpon destructans (Zins.) Scholten		17,000	3	3	13-16	

Organism	Mean no. of propagules /g soil	No. of soil samples	No. of isolates in collection	Observed optimum temperature range* (°C)	Calculated optimum temperature (°C)
Diheterospora chlamydosporia (Kamyschko) Barron & Onions	68,000	7	7	25-28	
Eladia saccula (Dale) Smith	26,000	5	5	13-16	
Fusarium	26,000	64	74	17-20	19 (18)
Fusarium avenaceum (Corda ex Fr.) Sacc.	31,000	2	3	17-20	
Fusarium culmorum (W.G. Smith) Sacc.	61,000	1	1	17-20	
Fusarium oxysporum Schlecht.	15,000	13	13	17-20	
Fusarium poae (Peck) Wollenweber	45,000	4	5	25-28	
Fusarium solani (Mart.) Sacc.	29,000	38	44	17-20	20 (18)
Gliocladium	32,000	2	2	17-20	
Gliocladium atrum Gilman & Abbot	21,000	3	3	13-16	
Gliocladium deliquescens Sopp	13,000	4	4	29-32	
Gliocladium roseum Bain.	58,000	114	149	17-20	19 (18)
Gongronella butleri (Lendn.) Peyronel & Dal Vesco	91,000	122	129	13-16	19 (17)
Gymnoascus roseus (Raillo) Apinis	15,000	6	6	9-12	
Humicola grisea Traaen	26,000	6	6	25-28	
Metarrhizium anisopliae (Metsch.) Sorok.	4,013,000	3	3	25-28	
Microthecium retisporum Udagawa & Cain	7,000	1	1	21-24	
Mucor attenuatus Linn.	36,000	6	6	17-20	
Mucor hiemalis Wehmer	11,000	4	4	21-24	
Myrothecium	10,000	1	1	21-24	
Myrothecium cinctum (Corda) Sacc.	16,000	2	2	25-28	
Oidiodendron	64,000	6	7	9-12	
Oidiodendron tenuissimum (Peck) Hughes	52,000	48	58	25-28	21 (19)
Oidiodendron truncatum Barron	21,000	5	5	9-12	

Organism	Mean no. of propagules /g soil	No. of soil samples	No. of isolates in collection	Observed optimum temperature range* (°C)	Calculated optimum temperature (°C)
Pachybasium niveum Rostr.	103,000	74	87	17-20	22 (19)
Paecilomyces	70,000	22	23	9-12	16 (15)
Paecilomyces carneus (Duché & Heim) Brown & Smith	60,000	55	72	21-24	19 (18)
Paecilomyces elegans (Corda) Mason & Hughes	6,000	3	3	9-12	
Paecilomyces marquandi (Massee) Hughes	37,000	74	91	17-20	19 (18)
Paecilomyces striatisporus Onions & Barron	8,000	5	5	21-24	
Penicillium (11)	107,000	54	67	13-16	19 (18)
Penicillium (12)	55,000	11	13	21-24	
Penicillium (13)	91,000	24	37	17-20	18
Penicillium (14)	96,000	2	2	21-24	
Penicillium (15)	14,000	1	1	13-16	
Penicillium (16)	40,000	7	7	13-16	
Penicillium (17)	60,000	5	5	17-20	
Penicillium (18)	55,000	6	7	21-24	
Penicillium (19)	54,000	11	12	9-12	
Penicillium (20)	40,000	2	2	13-16	
Penicillium (21)	35,000	3	5	13-16	
Penicillium (22)	117,000	3	3	17-20	
Penicillium (23)	12,000	1	1	25-28	
Penicillium (24)	40,000	5	6	13-16	
Penicillium (26)	376,000	4	5	13-16	
Penicillium (27)	42,000	5	5	9-12	
Penicillium (28)	55,000	3	3	9-12	
Penicillium (30)	36,000	4	4	21-24	
Penicillium (33)	394,000	4	5	9-12	
Penicillium (34)	32,000	3	3	21-24	
Penicillium (36)	20,000	1	1	17-20	
Penicillium (38)	10,000	1	1	21-24	
Penicillium (39)	73,000	7	9	9-12	
Penicillium (40)	21,000	1	1	9-12	
Penicillium (41)	78,000	7	8	13-16	
Penicillium (42)	21,000	1	1	9-12	
Penicillium (46)	67,000	3	4	21-24	
Penicillium (47)	142,000	2	2	17-20	
Penicillium (48)	24,000	1	2	13-16	
Penicillium (50)	39,000	5	5	13-16	

Organism		Mean no. of propagules /g soil	No. of soil samples	No. of isolates in collection	Observed optimum temperature range* (°C)	Calculated optimum temperature (°C)
Penicillium	(52)	38,000	1	1	9-12	
Penicillium	(55)	20,000	1	1	17-20	
Penicillium	(56)	81,000	1	1	9-12	
Penicillium	(57)	6,000	1	1	13-16	
Penicillium	(59)	110,000	1	1	21-24	
Penicillium	(64)	44,000	3	3	13-16	
Penicillium	(66)	10,000	1	1	21-24	
Penicillium	(68)	71,000	1	1	13-16	
Penicillium	(69)	10,000	1	1	21-24	
Penicillium	(75)	16,000	6	7	21-24	
Penicillium	(76)	680,000	1	1	25-28	
Penicillium	(82)	5,000	1	1	21-24	
Penicillium	(87)	37,000	1	1	25-28	
Penicillium	(94)	103,000	3	3	21-24	
Penicillium	(95)	58,000	1	1	21-24	
Penicillium	(96)	114,000	2	2	21-24	
Penicillium	(100)	22,000	1	1	21-24	
Penicillium	(711)	200,000	1	1	13-16	
Penicillium	(720)	11,000	1	1	21-24	
Penicillium	(726)	51,000	1	1	21-24	
Penicillium	(810)	14,000	1	1	17-20	
Penicillium	(814)	2,000	1	1	17-20	
Penicillium	(817)	76,000	1	1	21-24	
Penicillium	(820)	113,000	3	4	21-24	
Penicillium	(823)	14,000	1	1	17-20	
Penicillium	(825)	40,000	1	1	17-20	
Penicillium brefeldianum Dodge		22,000	1	1	17-20	
Penicillium lanosum Westling		131,000	2	2	13-16	
Penicillium notatum Westling		13,000	1	1	17-20	
Penicillium steckii Zaleski		27,000	6	7	21-24	
Penicillium waksmani Zaleski		4,000	2	2	13-16	
Pestalozzia		12,000	1	1	17-20	
Phialocephala		25,000	10	10	17-20	
Phialophora		10,000	1	1	17-20	
Phoma	(531)	16,000	1	1	17-20	

Organism		Mean no. of propagules /g soil	No. of soil samples	No. of isolates in collection	Observed optimum temperature range* (°C)	Calculated optimum temperature (°C)
Phoma	(532)	1,000,000	1	1	21-24	
Phoma	(533)	20,000	1	1	13-16	
Pseudeurotium zonatum van Beyma		7,000	1	1	17-20	
Pyrenochaeta		105,000	4	4	9-12	
Thysanophora penicillioides (Roum.) Kendrick		15,000	1	1	25-28	
Trichocladium opacum (Corda) Hughes		25,000	1	1	9-12	
Trichoderma	(170)	39,000	183	238	17-20	19 (18)
Trichoderma	(192)	34,000	5	5	21-24	
Trichoderma	(193)	22,000	1	1	13-16	
Trichoderma hamatum (Bonn.) Bain	(178)	42,000	112	151	13-16	18 (17)
Trichoderma hamatum	(179)	62,000	1	1	21-24	
Trichoderma harzianum Rifai	(174)	39,000	4	4	21-24	
Trichoderma harzianum	(198)	1,000	1	1	25-28	
Trichoderma harzianum	(199)	13,000	1	1	21-24	
Trichoderma koningii Oud.		75,000	89	121	21-24	20 (19)
Trichoderma polysporum (Link ex Pers.) Rifai		7,000	1	1	21-24	
Trichoderma viride Pers ex S.F. Gray		14,000	5	6	17-20	
Volutella		19,000	1	1	25-28	
Zygorhynchus moelleri Vuillemin		30,000	135	148	25-28	21 (19)
Unknown	(450)	44,000	1	2	21-24	
Unknown	(571)	241,000	59	68	17-20	20 (19)
Unknown	(851)	21,000	1	1	25-28	
Unknown	(861)	16,000	1	1	17-20	

*The temperature range and the calculated optimum temperatures are those taken 1 cm below the surface. Temperatures in parentheses are similar calculations for measurements made 5 cm below the surface.

†The identity of these isolates is not certain.

propagules g^{-1} isolated from different geographical locations of the pastures supported by forest soils were detected in the seasons 1967 to 1969 (Brewer et al. 1971) and the results in Table I show that the same conclusion is valid for the 1973 to 1975 seasons. The mean value for all the results given in Table I is 8.2×10^5 and the standard deviation of the 10 means is 2.9×10^5 (omitting the value 1.3×10^7 from the calculations).

A list of the 142 classified fungi that have been isolated from soil samples collected between 1967 and 1975 is given in Table II. Of these fungi, 137 have been assigned to a genus and 54 to species. Of these 54, one, *Aspergillus chevalieri* var *intermedius* (Taxonomic code 111), is a distinct variety, the 2 strains of *Trichoderma hamatum* are clearly separable, as are the 3 of *Trichoderma harzianum* and the 2 of *Alternaria alternata*. It is possible that those isolates now classified with respect to genus only (e.g., *Circinella*) are a group of several different species, and contrariwise, those now separated as numbered isolates within a genus, (e.g., *Penicillium* 34, 36) will prove to be the same species. Isolates of these 142 fungi constitute 35% of all the isolates in the collection. The total number of propagules cultivated from all soil samples was $6.9 \times 10^5 \times 437 = 3.02 \times 10^8$. The number of propagules in the list of fungi in Table II is 1.15×10^8, or 38% of the total. In Table II the number of isolates of each organism in the collection is given and the number of soil samples from which each was isolated. The most common organism, *Zygorhynchus moelleri*, was found in 135 soil samples, or about 30% of those collected. The mean number of propagules g^{-1} soil of classified organisms is given in Table II. There are only 23 organisms in Table II that have been isolated from 10 or more soil samples, thus the frequencies given in the other cases are uncertain. Only 3 organisms—*Penicillium* sp. 11, *Pachybasium niveum* and the unknown species 571—have been found in numbers greater than 10^5 in more than 20 soil samples.

In the 9-year period, soil samples were collected when the soil temperature lay in the range 0-28°C. When the number of soil samples collected in the 4-degree temperature ranges 0-4°C, 5-8°C, etc. were calculated, the results fitted the equation:

$$\textit{numbers of collections} = -7.12 + 5.33x - 0.17x^2$$

where x is the maximum of the soil temperature range, with an index of fit of 0.9. Such a curve has a maximum at 16°C and variations from this maximum might be a characteristic of a particular species. The numbers of propagules of each of the fungi in Table II collected in soil temperature ranges of 4 degrees, between 0°C and 32°C at collection time was therefore calculated, and the range in which the maximum numbers were found at 1 cm depth is given. Where organisms occurred in more than 20 soil samples and at least 5 temperature ranges, the data were used to calculate a least mean squares fit to an equation of the second degree. The maximum value for temperature in the calculated relationship was then found by differentiation, in the usual way. The result gives the apparent optimum temperature for growth in the field for a particular species. This temperature is given in appropriate cases in Table II. The results of similar calculations are also given for soil temperatures measured 5 cm below the surface. The results in the majority of cases are greater than 16°C and in the instance of *Pachybasium niveum* the optimum temperature for isolation is apparently 22°C.

In 1974 and 1975 an attempt was made to obtain information about some aspects of the physiology of the soil isolates. This was done by incorporating the polysaccharides starch and cellulose in the medium, the hydrolysis of these polymers could be detected directly on the isolation plates. Similarly, reduction by a colony of the

antibacterial dyestuff rose bengal, normally incorporated into the medium, could be easily detected. Finally, inhibition by a colony of another colony was readily seen. These 4 tests provide a simple assessment of some aspects of the physiology of the flora, and in practice proved so successful that they were retrospectively applied to such viable isolates as were in the collection for the years 1969, 1971, and 1973. Two hundred and three soil samples were collected in this period on 98 different occasions, and from these samples, 2683 isolates were obtained in culture. The mean value for the frequency (propagules g^{-1}) for each collection date was calculated and the results obtained, sorted as before into intervals of 10^5 g^{-1}. The resulting histogram is drawn in Figure 2. Its similarity to the corresponding histogram for the whole experimental period is clear. The mean value for the 98 collection dates was 8.1×10^5 propagules g^{-1} and a linear least means squares fit of the data gave a line with gradient 81; the calculated value for the first day (April 22 1969) was 7×10^5, and for the last day (October 28 1975), 8.7×10^5. Though this difference is greater than the difference calculated for the period 1967 to 1975, it is obviously insignificant. None of the classified isolates collected in this group were observed to hydrolyze cellulose. Such isolates that hydrolyzed starch, reduced rose bengal and inhibited the growth of other fungal colonies are found in a list in Table III. The mean values given in Table III are number of propagules of the species having the designated activity, that were found in 1 g of soil. The number of soil samples from which such species were isolated is also given in Table III. Of the 17 most common species (Table II), only one—*Absidia glauca*—is absent from Table III. The activity of certain species is striking; thus 20% of *Penicillium* sp. 11 hydrolyzed starch, and 6% inhibited the growth of other fungi. Ten percent of all *Trichoderma* species reduced rose bengal and 6% inhibited the growth of other fungal colonies. Isolates of two very common species—*Gliocladium roseum* and *Paecilomyces marquandi*—were active in all 3 tests.

Information on the physiological activity of unidentified isolates was also obtained, but it is not possible to present these data in a way that permits them to be related to a soil unit. The number of isolates that have not been classified in the 1969 and 1971 to 1975 periods is 1512, or 56%. One of these isolates hydrolyzed cellulose and 114 (4%) hydrolyzed starch. Rose bengal reduction was observed around colonies of 21 of these unclassified isolates and 45 (2%) inhibited the growth of other fungi. Activity in one or more of the 4 tests was observed in 11% of the isolates, classified and unclassified.

From the foregoing description of the soil fungal flora, a number of characters to which a numerical value can be assigned emerge. These numerical values allow comparisons to be made of the soil floras from two or more geographical locations. They can also be used to test how closely a sample of the flora corresponds to the whole. Here only the latter utility is discussed.

Selection of Random Samples from a Collection of Soil Fungi

A random sample of the estimated 3×10^8 propagules collected in this work was obtained by the use of dilution series (Montegut 1960). This random sample was then distorted by placing only 1 isolate of several colonies of the same organism found at a particular 10-fold dilution, in the culture collection. This distortion can be rectified by construction of a theoretical collection by multiple entry of each isolate in the real collection, the multiplication factor being a function of the isolate frequency. There are 2 extremes of this multiplication factor—a maximum case where the frequencies are divided by 1 giving an expanded collection of 3×10^8, and a minimum where the frequencies are divided by themselves to give 2683 cultures. In both cases the numbers are large enough to deter an examination of all. It is clear

Table III. Physiological properties of species of fungi isolated from soils of permanent pasture at Nappan, Nova Scotia.

Organism (code no.)	Starch hydrolysis		Rose bengal reduction		Antifungal activity	
	Mean*	No. of samples	Mean*	No. of samples	Mean*	No. of samples
Alternaria alternata (461)	7,400	1	0	0	0	0
Aspergillus chevaliere	4,800	1	0	0	0	0
Aspergillus fumigatus	0	0	0	0	9600	1
Aspergillus sydowi	0	0	0	0	36,000	1
Cladosporium cladosporioides	160,000	1	0	0	0	0
Fusarium (270)	32,000	3	0	0	8300	1
Fusarium oxysporum	0	0	0	0	19,000	1
Fusarium solani	16,000	1	0	0	0	0
Gliocladium atrum	13,000	1	0	0	0	0
Gliocladium roseum	13,000	1	21,300	3	41,500	2
Gongronella butleri	6350	2	0	0	0	0
Gymnoascus roseus	12,000	1	0	0	0	0
Myrothecium cinctum	13,000	1	0	0	0	0
Oidiodendron tenuissimum	50,400	11	0	0	0	0
Pachybasium niveum	20,000	1	0	0	130,000	1
Paecilomyces (180)	10,000	1	0	0	160,000	1
Paecilomyces marquandi	24,000	4	5100	1	45,500	2
Paecilomyces carneus	27,500	4	0	0	64,000	1
Penicillium (11)	168,000	11	0	0	41,000	3
Penicillium (12)	0	0	0	0	4800	1
Penicillium (13)	29,000	2	0	0	0	0
Penicillium (16)	11,000	1	0	0	37,000	1
Penicillium (18)	23,300	2	0	0	0	0
Penicillium (19)	197,000	1	197,000	1	0	0
Penicillium (26)	730,000	1	0	0	0	0
Penicillium (27)	0	0	0	0	60,500	2
Penicillium (30)	23,000	1	0	0	0	0
Penicillium (33)	930,000	1	0	0	0	0
Penicillium (34)	79,000	1	0	0	0	0
Penicillium (46)	51,000	1	0	0	0	0
Penicillium (48)	0	0	0	0	4800	1
Penicillium (720)	11,000	1	0	0	0	0
Penicillium brefeldianum	0	0	0	0	22,000	1
Penicillium notatum	0	0	0	0	13,000	1
Phoma (533)	20,000	1	0	0	0	0
Trichoderma (170)	0	0	50,700	5	39,000	21
Trichoderma hamatum	0	0	15,000	5	0	0
Trichoderma harzianum	0	0	13,000	1	0	0
Trichoderma koningii	0	0	72,000	28	14,000	1
Zygorhynchus moelleri	0	0	7400	2	28,000	2
Unknown (431)	56,000	1	0	0	0	0
Unknown (571)	750,000	10	0	0	39,000	1

*Number of propagules capable of the given activity.

that a further selection of a random number of isolates is needed. There are 3 questions concerning the way this random number is obtained: (1) How many cultures selected at random should be made from the expanded collection? (2) By what frequency factor should the culture collection be expanded? (3) Which of an infinite number of random samples fulfilling the first 2 criteria should be used for study? The answer to the first question was dictated by the human resources available and was set at 750. The last 2 questions can be settled on the basis of criteria developed from the description of the flora.

Size of Expanded Collection

The culture collection from 1969, and 1971 to 1975 was converted into 4 expanded collections. The expansions were made by dividing the frequency of each isolate by itself, 2×10^4, 10^4, and 10^3, and using the quotients to determine the number of times the isolate was entered into the expanded collection. Two conditions were imposed on this process. One was that every isolate must be entered at least once, and the other condition, applied to the last 3 collections, was that the maximum number of entries of a single isolate was, respectively, 28, 55, and 550. The resulting 4 collections contained 2683, 8047, 14,950, and 132,396 isolates. Random samples of 750 different entries from each of these 4 collections were then made. Random samples from each of these expanded collections showed no significant differences in the variation of the mean frequencies of all soil samples collected in each month of the 5 seasons. The standard deviations of the monthly means was about 30% of the overall mean and thus similar to that calculated for the collection as a whole (12%). Some other characters of the random samples are given in Table IV. It is evident that only small changes occur in the random sample by a 50-fold increase in the size of the expanded collection from which they were selected. Three examples of random samples from one expanded collection (14,950 entries) are also given in Table IV. Similar variability was found in random samples from the other expanded collections. In one respect none of the random samples resemble the total collection. This dissimilarity is the proportion of species, or isolates of unknown genus that inhibit the growth of other fungi. The ratios of the number of species that hydrolyze starch, inhibit growth and reduce rose bengal is 3.6:2.4:1 and the same ratios for these activities in the unknown isolates is 5.4:2.1:1, but in the case of the random samples they are about 4:0.5:1 and 5:0.4:1. The data in Table IV also show that there are many species known to be in the flora that are not selected in the random samples. Despite these problems, the information in Table IV allows a choice to be made of the size to which the collection should be expanded before taking a random sample that reflects the frequency of the isolates. Thus as the collection is expanded, the number of soil samples collected that are represented in the random sample decreases, and the gradient of the linear least mean squares fit of the frequency with respect to time increases. The number of different isolates selected, and hence the sample of the individuals in the flora, also decreases. Thus, expansion to 132,396 entries involves distortion of the flora in the random sample. The random sample taken from the smallest collection (2683) is, of course, unacceptable because no weighting has been given to those species that are predominant in the flora. It is interesting to note that the figures in Table IV also illustrate its unsuitability. Thus, the gradient of the linear least mean squares fit deviates from that of the total collection by the greatest magnitude of all the random samples given in Table IV. The ratio of the numbers of species that hydrolyze starch to those that inhibit other fungi is 13:1 in the random sample from the unexpanded collection, as compared to 3:2 for the collection as a whole. The choice between the expanded collections of 8047 and 14,950 entries was made on the basis of the greater similarity of the ratios

Table IV. Characteristics of random samples of isolates (750) selected from the culture collection, expanded by various factors related to frequency.

	Total collection	f	Random samples (750) of expanded collection Frequencies (f) divided by: $2x10^4$	10^4			10^3
No. of collection days	98		96	96	97*	96	95
Gradient of linear least mean squares fit	81	-33	112	113*	124	118	144
No. of soil samples	193	193	189	185	191*	181	176
Geographical distribution; S.D. of % isolates selected for each sector		3.3	4.1	4.3	3.8	3.4*	3.9
No. of different isolates	2683	750	574	542*	526	538	513
Classified organisms Number	142	67	61	52	57*	54	62
No. $>$ 20,000 g^{-1} from $>$ 10 samples	21	20	19	19	20*	18	20
Biological activity. No. of classified organisms against:							
starch	29	13	12	10	12*	10	6
rose bengal	8	4	3	4*	3	2	3
fungi	19	1	0	2	3*	2	1
No. of unknown isolates active against:							
starch	114	36	26	28	25	26	21
cellulose	1	0	0	0	0	1	0
rose bengal	21	9	4	6	5	4	4
fungi	45	3	2	2	2	2	3

* Indicates score as discussed in the text.

of species active in the three biological tests to that of the total collection. The data given in Table IV for the collection expanded to 8047 are abnormal, as none of the species inhibited other fungi. The mean value for the ratio of starch hydrolysis to antifungal activity in 8 random samples was 11.5:1 and this is at greater variance with the ratio for the total collection than with that of the random samples (16) of the collection expanded to 14,950, which was 4.5:1. The reason for this odd result is not apparent to us, but it eased the choice of the latter expanded collection.

Choice of a Particular Random Sample for Study

The 3 sets of data given in Table IV for 3 random samples selected from the collection expanded to 14,950 entries show small differences. To enable a rational choice to be made between such samples a scoring system has been adopted where points are given to characters that are in closest accord with those of the total collection. The method is illustrated by reference to Table IV. One of the random samples contains isolates from one more collection date than the others and thus scores 1. The same sample also contains isolates from considerably more soil samples and thus scores another point. However, another sample has a linear least mean squares frequency-time fit that approximates more closely to that of the total collection and thus scores a point for this character, while the third random sample has a closer fit for the geographical distribution of its isolates for the different sectors. The scoring is indicated by asterisks in Table IV, and the sums of the scores for the 3 random samples are 2, 7 and 1. We have been surprised at the differences thus revealed and at how easily a choice could be made in this way. In addition to such objective criteria, it is desirable to look at random samples from the point of view of biological and/or chemical interest. For example, a choice between 2 random samples that scored very evenly by the method described might be made if one sample contained isolates having unusual properties e.g. the ability to hydrolyze cellulose, or unusual species, or species showing antifungal activity that have not been reported in the literature to produce such metabolites.

Quantitative Description of a Random Sample of 750 Fungal Isolates

The random sample of 750 isolates chosen for study, represented 182 soil samples collected on 96 collection dates. The gradient of the linear least mean squares fit of time against mean propagules $g^{-1}d^{-1}$ was 109, and the standard deviation of the percentage of isolates selected from each sector was 2.9. The number of different isolates selected was 543 and the ratios of the species that hydrolyzed starch, reduced rose bengal and inhibited other fungi were 9:3:2. One isolate of *Pachybasium niveum* inhibited the growth of *Zygorhynchus moelleri*. Of the isolates that have not so far been classified, 22 produced enzymes that hydrolyzed starch, 1 hydrolyzed cellulose, 3 reduced rose bengal, 1 isolate of *Penicillium* sp. 27 inhibited the growth of several fungal isolates and another unknown isolate inhibited the growth of a *Penicillium* sp. Sixty-one of the 750 samples (8%) were not viable at the time of assay (1978-1979); 40 of them (66%) were collected in 1969.

Sixty-seven species have been classified—the number is not comparable to those in Table IV because these isolates have been the object of closer scrutiny. This represents 49% of the total. They are given in Table V in the same format as Table II except that the number of isolates is the number in the random sample and therefore is greater than the number in the collection in a few cases. Of the 21 species in the total collection that have frequencies greater than 2×10^4 g^{-1} and occurred in more than 10 soil samples, 19 are found in this random sample. When the temperature range (4 degrees C) at which the greatest number of propagules were found was calculated for the species selected in the random sample it was found to

Table V. Fungi selected in the random sample used for growth inhibition studies, mean frequencies of selected species, number of soil samples from which they were isolated, and "optimum" temperatures for growth in soil.

Organism (Code No.)		Mean frequency (propagules g-1)	No. of soil samples	No. of isolates selected	Observed optimum temperature range (°C)	Calculated optimum temperature (°C)
Absidia glauca		40,000	2	3	17-20	
Alternaria alternata	(462)	14,000	1	1	17-20	
Cladosporium cladosporioides		230,000	2	3	21-24	
Cladosporium herbarum		29,000	4	4	21-24	
Cunninghamella elegans		71,000	1	1	25-28	
Cylindrocarpon destructans		16,000	1	1	17-20	
Fusarium	(270)	32,000	2	2	13-16	
Fusarium avenaceum		31,000	1	1	17-20	
Fusarium solani		29,000	1	1	17-20	
Gliocladium atrum		44,000	1	1	13-16	
Gliocladium deliquescens		24,000	1	1	29-32	
Gliocladium roseum		116,000	17	25	13-16	18
Gongronella butleri		239,000	15	24	13-16	18
Metarrhizium anisopliae		6,017,000	2	3	25-28	
Oidiodendron tenuissimum		86,000	10	12	9-12	
Pachybasium niveum		152,000	10	14	9-12	18
Paecilomyces carneus		90,000	12	16	21-24	20
Paecilomyces marquandi		43,000	9	11	21-24	20
Penicillium	(11)	127,000	6	9	21-24	
Penicillium	(12)	135,000	2	2	25-28	
Penicillium	(13)	128,000	5	7	17-20	
Penicillium	(14)	170,000	1	1	21-24	
Penicillium	(16)	130,000	1	1	13-16	
Penicillium	(17)	180,000	1	1	17-20	
Penicillium	(18)	110,000	1	1	21-24	
Penicillium	(19)	74,000	4	6	9-12	
Penicillium	(20)	19,000	1	1	9-12	
Penicillium	(22)	164,000	2	4	17-20	
Penicillium	(24)	51,000	3	5	13-16	
Penicillium	(26)	483,000	3	7	13-16	
Penicillium	(27)	36,000	2	2	13-16	

Penicillium	(28)	100,000	1	3	9-12	
Penicillium	(33)	730,000	2	4	9-12	
Penicillium	(34)	79,000	1	1	21-24	
Penicillium	(36)	20,000	1	1	17-20	
Penicillium	(39)	110,000	4	5	9-12	
Penicillium	(41)	230,000	1	1	13-16	
Penicillium	(42)	21,000	1	1	9-12	
Penicillium	(46)	63,000	2	2	21-24	
Penicillium	(52)	38,000	1	2	9-12	
Penicillium	(56)	81,000	1	1	9-12	
Penicillium	(64)	43,000	1	1	25-28	
Penicillium	(66)	10,000	1	1	21-24	
Penicillium	(68)	71,000	1	1	13-16	
Penicillium	(75)	32,000	1	1	25-28	
Penicillium	(76)	680,000	1	3	25-38	
Penicillium	(94)	290,000	1	1	21-24	
Penicillium	(96)	114,000	2	3	21-24	
Penicillium	(711)	200,000	1	2	13-16	
Penicillium	(720)	11,000	1	1	21-24	
Penicillium	(726)	51,000	1	1	21-24	
Penicillium	(820)	165,000	2	5	21-24	
Penicillium	(825)	40,000	1	2	17-20	
Penicillium steckii		20,000	1	1	21-24	
Phoma	(532)	1,000,000	1	5	21-24	
Phoma	(533)	20,000	1	1	13-16	
Pseudeurotium zonatum		7,000	1	1	17-20	
Pyrenochaeta	(540)	34,000	1	1	9-12	
Trichoderma	(170)	32,000	3	3	17-20	
Trichoderma	(192)	76,000	1	1	21-24	
Trichoderma hamatum	(178)	84,000	26	38	13-16	18
Trichoderma harzianum	(174)	71,000	2	2	21-24	
Trichoderma koningii		169,000	25	46	21-24	19
Volutella	(701)	19,000	1	1	25-28	
Zygorhynchus moelleri		40,000	10	11	9-12	14
Unknown	(571)	03,000	19	38	17-20	20

be the same in 76% of the species as was calculated for the collection as a whole. Only two species—*Pachybasium niveum* [22°C (collection), 18°C (random sample)] and *Zygorhynchus moellerii* (21°C, 14°C)—differed greatly in the calculated optimum growth temperatures for the collection as a whole and for the random sample. The biological activity of the different species in this random sample is given in Table VI. Of the 750 isolates selected, 182 inhibited the growth of *S. lutea*, 24 in the growth of *C. utilis* and 22 the growth of both test organisms. Thus

Table VI. Metabolic and growth-inhibiting activity of fungal species randomly selected from an expanded culture collection of 14,950 isolates cultivated from soil of permanent pasture.

Organism (Code no.)		Metabolic activity (propagules g^{-1} soil)									
		Starch hydrolysis		Rose bengal reduction		Growth inhibition					
						Antifungal activity		*S. lutea*		*C. utilis*	
			No.*		No.*		No.*		No.*		No.*
Fusarium	(270)	0	0	0	0	0	0	32,000	2	22,000	1
Fusarium solani		0	0	0	0	0	0	29,000	1	0	0
Gliocladium atrum		0	0	0	0	0	0	44,000	1	0	0
Gliocladium roseum		0	0	0	0	0	0	112,940	15	36,000	1
Gongronella butleri		0	0	0	0	0	0	0	0	14,000	1
Metarrhizium anisopliae		0	0	0	0	0	0	6,017,000	2	0	0
Oidiodendron tenuissimum		95,500	2	0	0	0	0	0	0	29,000	1
Pachybasium niveum		0	0	0	0	130,000	1	124,750	4	200,000	1
Paecilomyces marquandi		0	0	0	0	0	0	18,600	2	0	0
Paecilomyces carneus		0	0	0	0	0	0	30,400	2	60,200	5
Penicillium	(11)	63,000	1	0	0	0	0	12,000	1	0	0
Penicillium	(12)	0	0	0	0	0	0	110,000	1	0	0
Penicillium	(13)	0	0	0	0	0	0	83,600	5	0	0
Penicillium	(19)	130,000	1	130,000	1	0	0	130,000	1	0	0
Penicillium	(20)	0	0	0	0	0	0	19,000	1	0	0
Penicillium	(24)	0	0	0	0	0	0	40,000	1	0	0
Penicillium	(26)	730,000	1	0	0	0	0	483,000	3	0	0
Penicillium	(27)	0	0	0	0	11,000	1	36,000	2	0	0
Penicillium	(33)	930,000	1	0	0	0	0	730,000	2	0	0
Penicillium	(34)	79,000	1	0	0	0	0	79,000	1	79,000	1
Penicillium	(39)	0	0	0	0	0	0	40,000	2	0	0
Penicillium	(41)	0	0	0	0	0	0	230,000	1	0	0
Penicillium	(46)	0	0	0	0	0	0	100,000	1	0	0
Penicillium	(56)	0	0	0	0	0	0	81,000	1	0	0
Penicillium	(76)	0	0	0	0	0	0	680,000	1	0	0
Penicillium	(720)	11,000	1	1	0	0	0	0	0	0	0
Penicillium	(825)	0	0	0	0	0	0	40,000	1	0	0
Phoma	(533)	20,000	1	0	0	0	0	0	0	0	0
Pseudeurotium zonatum		0	0	0	0	0	0	7,000	1	0	0
Pyrenochaeta	(540)	0	0	0	0	0	0	34,000	1	0	0
Trichoderma	(170)	0	0	0	0	0	0	46,000	1	0	0
Trichoderma hamatum	(178)	0	0	3,400	1	0	0	44,200	15	50,800	3
Trichoderma koningii		0	0	171,000	9	0	0	252,000	10	0	0
Volutella	(701)	0	0	0	0	0	0	19,000	1	0	0
Unknown	(571)	1,660,000	4	0	0	0	0	0	0	0	0

*Number of isolates in random sample.

growth-inhibitory activity was found in about 30% of the cultures selected for the random sample. The proportion is probably somewhat bigger if the fact that 8% of the selected cultures were not viable and could not be tested, is taken into account.

An examination of Tables V and VI reveals that 91% of isolates of *Gliocladium roseum*, 40% of isolates of *Trichoderma koningii*, and 58% of isolates of *T. hamatum* produce growth-inhibiting metabolites in culture. Isolates having this ability were found in 16%, 11%, and 16%, respectively, of all the soil samples collected. In Table II it is shown that the 3 species rank sixth (6.6×10^6), fifth (6.7×10^6) and ninth (4.7×10^6) in the numbers of fungal propagules of particular species collected in the experimental period 1967 to 1975. The species that is next in ability to produce growth-inhibiting metabolites is *Paecilomyces carneus*, found in 7% of the soil samples collected, ranked eleventh (3.3×10^6) in Table II, and 58% of whose isolates (7 out of 12) were active. Similar calculations in the cases of other species reveal a large decrease in the probability that a soil sample will provide an isolate in culture that will produce a growth-inhibiting metabolite.

Of the 383 (278 different) isolates in the random sample of 750 that are unclassified at present, 86 (60 different) inhibited the growth of *S. lutea* and 26 (22) the growth of *C. utilis*. The growth of both test organisms was inhibited by 13 isolates (10 different).

Discussion

In Figure 2, 763 determinations of the number of propagules g^{-1} are recorded. Some of the data are incorporated into both histograms, so the histograms cannot simply be added. Nevertheless, this large body of data seems to support the argument that the number of propagules g^{-1} follows a probability distribution with a maximum. It is noteworthy that this maximum remains the same (Fig 2) when the mean number of propagules g^{-1} collected on a particular day is used as the measure of soil fungal density even though the mean number of propagules g^{-1} in the latter case is 15% greater. The stability of the flora over the period of 9 years is remarkable and is in accord with the conclusions of Brewer et al. (1971) concerning the stability of fungal floras in forest-derived and marshland-derived soils. Much work remains to be done on the classification of isolates, especially the *Penicillia* isolated in 1974 and 1975—many of those classified as unknown in the random sample belong to this genus. Despite this ignorance we have the impression that certain species, e.g. *Chaetomium cochliodes*, *Fusarium poae*, and *F. avenaceum*, are less common among the 1975 isolates than they were among those collected in 1967. It is clear that much information concerning the behaviour of individual species during the period of our studies will come to light when the detailed taxonomic work is completed. We hope to report work on individual species when it is to hand.

The results in Table II are an attempt to assess the flora of these pasture soils quantitatively. Thus 2 species, *Gongronella butleri* and the unknown fungus 571, account for 8% of all the fungal propagules isolated. This assessment of the importance of different species could be mechanically applied to all those in the list, but is of doubtful value in the case of those species that have been found in less than 10 to 20 soil samples. However, it is clear that relatively few species constitute a major portion of the soil fungal flora of these pastures, and since the majority (90%) of these predominant species were found in the random sample used to determine the ability of the flora to produce growth inhibiting metabolites, the results might be justifiably extrapolated to the flora as a whole.

We consider the results given in Tables V and VI remarkable. The 3 species—*Gliocladium roseum*, *Trichoderma koningii*, and *T. hamatum* collectively

constitute about 6% of all the fungal propagules isolated from 437 g soil and from the biological assay results it can be calculated that about 4% of the propagules isolated consist of these 3 species that have the ability to hinder the growth of other organisms. On the basis of our earlier qualitative work (Brewer et al. 1972), we concluded that *Trichoderma* spp. were an important part of the soil fungal flora, capable of producing metabolites that were inhibitory to growth. This work is therefore in agreement and, further, places the evidence on a more quantitative basis. It has been shown that some of the toxic metabolites of *Trichoderma hamatum* affect rumen fermentation (Brewer et al. 1979; Jones and Jen unpubl.) thus augmenting the circumstantial evidence that links this genus with the ill-thrift of young sheep and cattle that graze these pastures (Brewer et al. 1971).

On a wider scene it seems possible that the techniques we have used in our attempt to get quantitative information about the fungal flora of pasture soils, will have applicability to other ecological niches. One immediately thinks of the aerial fungal flora which changes more rapidly than its soil counterpart, but other situations such as distributions of microscopic aquatic plants and animals or even lichens in a prescribed area, may well benefit from similar analysis.

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References

Ainsworth, G.C. 1968. The number of fungi. In *The Fungi. An Advanced Treatise.* Vol. III. (ed. G.C. Ainsworth and A.S. Sussman), Academic Press, New York, pp. 505-514.

Brewer, D., Calder, F.W., MacIntyre, T.M. and **Taylor, A.** 1971. Ovine ill-thrift in Nova Scotia. 1. The possible regulation of the rumen flora in sheep by the fungal flora of permanent pasture. *J. Agric. Sci.* 76: 465-477.

Brewer, D., Hanson, A.W., Shaw, I.M., Taylor, A. and **Jones, G.A.** 1979. A crystalline toxic peptide metabolite of *Trichoderma* spp. isolated from soil. *Experientia* 35: 294-295.

Brewer, D., Taylor, A. and **Evans, A.** 1978. A mechanical search and retrieval system for mycotoxins. *Proc. N.S. Inst. Sci.* 28: 163-169.

Brewer, D., Taylor, A. and **Hoehn, M.M.** 1972. Ovine ill-thrift in Nova Scotia. 2. The production of antibiotics by fungi isolated from forest and marshland soil. *J. Agric. Sci.* 78: 257-264.

Brewer, D., Taylor, A. and **Hoehn, M.M.** 1974. The antibiotic activity of cultures from fungal spores collected by a spore trap in permanent pasture. *Can. J. Microbiol.* 20: 721-729.

Brierley, W.B., Jewson, S.T., and **Brierley, M.** 1927. A quantitative study of soil fungi. *Proc. Pap. 1st Int. Congr. Soil Sci. 1927,* 3: 1-24.

Jones, G.A. and **Jen, W-C.** Unpublished results.

Kliejunas, J.T. and **Nagata, J.T.** 1979. *Phytophthora cinnamomi* in Hawaiian forest soils: seasonal variation in population levels. *Phytopathology* 69: 1268-1272.

Montégut, J. 1960. Value of the dilution method. In *The Ecology of the Soil Fungi. An International Symposium.* (ed. D. Parkinson and J.S. Waid), Liverpool Univ. Press, Liverpool, pp. 43-52.

Pontecorvo, G., Roper, J.A. and **Forbes, E.** 1953. Genetic recombination without sexual reproduction in *Aspergillus nidulans*. *J. gen. Microbiol.* 8: 198-210.
Rifai, M.A. 1969. A revision of the genus *Trichoderma*. *Mycol. Pap.* 116: 1-56.

Appendices

A. Program used to write files of data describing fungal isolates from soil.

```
01000 FILE#1="A1",#2="OLDFIL",
01005#3 = "NEWFIL"
01010 ON ERROR THEN 9040
01020 RESTORE #2
01030 FOR X=1 TO 2000
01040 READ D,M,Y,P,S,I,T,T1,N,D1,S1
01050 READ T2,S2,S3,A,C,R1,R
01060 R=R*25.4
01070 R=INT(R+.5)
01080 S2=(S2-S3)/2
01090 S2=INT(S2+.5)
01100 IF S2<10 THEN 1120
01110 S2=9
01120 N=(N*10**D1)/S1
01130 FOR Z=0 TO 9
01140 IF INT(N/10**Z)>10 THEN 1160
01150 GOTO 1170
01160 NEXT Z
01170 N=(N/(10**(Z-1)))+.5
01180 N=INT(N)
01190 N=(N*10)+Z
01200 T=INT(T+.5)
01210 T1=INT(T1+.5)
01220 D=(D*10**4)+(M*1000)+(Y*100)+P
01230 S=(S*10**5)+(I*1000)+N
01240 A=(A*10**7)+(C*10**6)+(R1*10**5)
01250 A=A+(S2*10**4)+T2
01260 T=(T*10**4)+(T1*100)+R
01270 WRITE #1,D,S,A,T
```

Each time the program is run the data are written on the temporary file "A1" in correct format. This file is then added to existing information residing on "Oldfil" to create "Newfil". Each experimental result is read from right to left as it appears in the field book (lines 1040 and 1050).

Lines 1060 to 1210 calculate values that appear on the file from those read on lines 1040 and 1050; e.g. rainfall is converted from inches to an integral number of millimeters by lines 1060 and 1070. The frequency (propagules g^{-1}) is calculated on line 1120 and its value converted to the format described in the text by lines 1130 to 1190.

Lines 1220 to 1260 put the data into the correct format.

Each line of code is written onto the scratch file "A1".

```
01280 NODATA 1300
01290 NEXT X
01300 PRINT "NUMBER OF CULTURES";
01305 PRINT "WRITTEN";
01310 PRINT " ON SCRATCH FILE ";X
01320 PRINT " "
01330 NODATA #2,1390
01340 FOR X1=1 TO 20000
01350 READ #2,D,S,A,T
01360 WRITE #3,D,S,A,T
01370 NODATA #2,1390
01380 NEXT X1
01390 RESTORE #1
01400 FOR X2=1 TO X
01410 READ #1,D,S,A,T
01420 WRITE #3,D,S,A,T
01430 NEXT X2
01440 RESTORE #3
01450 PRINT "FIRST 3 LINES ON NEW FILE"
01460 PRINT " "
01470 FOR X3=1 TO 30000
01480 READ #3,D,S,A,T
01490 IF X3>3 THEN 1560
01500 PRINT D,S,A,T,
01510 IF X3 <> 3 THEN 1580
01520 PRINT " "
01530 PRINT "LAST 3 LINES ON NEW FILE"
01540 PRINT " "
01550 GOTO 1580
01560 IF X3<X+X1-2 THEN 1580
01570 PRINT D,S,A,T
01580 NODATA # 3,1600
01590 NEXT X3
01600 PRINT " "
01610 PRINT "NUMBER OF CULTURES";
```

The x loop continues to cycle (lines 1030 to 1290) until all the field book data are read.

The x1 loop starts to write the updated file "Newfil", beginning with the data already in the computer system. Line 1330 serves to bypass this section if such data do not exist.

The new data are added to the old data by the loop x2.

The loop X3 is provided so that the mycologist knows that:

(a) The correct number of cultures has been written on the new file.
(b) The total number of isolates on the new file is correct.
(c) The first 3 lines and the last 3 lines on the new file are given so that these can be checked with the record in the field book.

```
01620 PRINT " ON NEW FILE ";X3
01630 STOP
09040 RESTORE #1
09050 PRINT "NUMBER OF CULTURES";
09055 PRINT "WRITTEN";
09060 PRINT " ON FILE BEFORE CRASH";X
09070 PRINT " "
09080 FOR X4=1 TO X
09090 READ#1,D,S,A,T
09100 IF X4>3 THEN 9180
09110 PRINT D,S,A,T
09120 IF X4<>3 THEN 9200
09130 PRINT " "
09140 PRINT " "
09150 PRINT "LAST 3 LINES WRITTEN"
09160 PRINT " "
09170 GOTO 9200
09180 IF X4<X-2 THEN 9200
09190 PRINT D,S,A,T
09200 NODATA #1,9220
09210 NEXT X4
09220 END
```

The lines 9040 to 9220 give information if an error has occurred in the calculations. The following facts are given:

(a) The number of cultures written on the file before the error occurred.
(b) The first 3 lines on the file of new data (this indicates if the data were entered correctly).
(c) The last lines of data read. This is useful to trace the error.

Notes.
Before running the program, data are typed into it from the field book or transmitted from a tape cassette. Data should be in the format: 2000 data 23,6,4,3, . . etc. 18 entries on each line, each entry separated by a comma. Zeros are used when no observations exist. The program is then 'run'; the exact command differs slightly with different systems.

Finally the new file 'Newfil' must be renamed 'Oldfil', the old file discarded and the 'Newfil' saved.

B. Program for expanding fungal data files with respect to frequency, and selecting random samples therefrom.

```
01000 DATA 90,4,120,5,151,6,181,7,212,8
01010 DATA 243,9,273,0,304,1
01020 FILE #1="CULTFIL",#2="EXPCOL"
01030 FILE #3="RANDSEL",#4="ISOLATS"
01040 RESTORE #1
01050 DIM N(750), M(10,2),S(100,6)
```

The first 2 lines are a calendar, used to calculate the day number.
The file 'cultfil' is the file of fungal isolates from which the expanded file 'expcol' is created. The file 'randsel' is the random sample of 750 en-

```
01060 MAT M=ZER
01070 MAT S=ZER
01080 MAT N=ZER
01090 T=0
01100 FOR X=1 TO 26000
01110 READ#1,A,B,C,D
01120 Z=INT(A/100)*100
01130 Z1=INT(Z/10**4)
01140 Z2=INT(Z/1000)-(Z1*10)
01150 Z=INT(Z/100)-((Z1*100)+(Z2*10))
01160 IF Z=0 THEN 01420
01170 IF Z=7 THEN 01420
01180 IF Z=8 THEN 01420
01190 GOSUB 02510
01200 M(Z3,1)=M(Z3,1)+1
01210 RESTORE
01220 FOR X1=1 TO 8
01230 READ J,J1
01240 IF J1<>Z2 THEN 01270
01250 Z1=Z1+J
01260 GOTO 01280
01270 NEXT X1
01280 IF Z>6 THEN 01300
01290 Z=Z+10
01300 Z1 = (((Z-7)*365)+ Z1)-113
01310 B1=INT(B/10)-(INT(B/1000)*100)
01320 B9=B-(INT(B/10)*10)
01330 B1=B1*(10**(B9-1))
01340 IF B1>6*10**5 THEN 01370
01350 B2=1+INT(B1/10**4)
01360 GOTO 01380
01370 B2=55
01380 FOR X2=1 TO B2
01390 WRITE#2,A,Z1,B,B1,C,D
01400 T=T+1
```

tries from 'expcol' and 'isolats' is a file of all the different isolates that have been written on 'randsel'.

The x-loop (lines 1100-1430) does the following jobs:

(a) It calculates the day number the propagule was isolated (lines 1210-1300).

(b) It calculates the frequency (propagules g^{-1}) (lines 1310-1330).

(c) It finds the sector from which the soil was collected and records in matrix m that an isolate was collected therefrom. (lines 1190 and 1200).

(d) It writes the expanded file 'expcol'; the number of multiple entries is calculated by lines 1350-1410.

```
01410 NEXT X2
01420 NODATA#1, 01440
01430 NEXT X
01440 RESTORE #2
01450 Z=Z1=Z2=0
01460 FOR X3=1 TO 750
01470 Z=INT(1+T*RND(-1))
01480 FOR X4=1 TO X3-1
01490 IF Z=N(X4) THEN 01470
01500 NEXT X4
01510 N(X3)=Z
01520 NEXT X3
01530 FOR X5=1 TO 750
01540 Z=2*T
01550 FOR X6=X5 TO 750
01560 IF Z < N(X6) THEN 01590
01570 Z=N(X6)
01580 Z1=X6
01590 NEXT X6
01600 Z2=N(X5)
01610 N(X5)=Z
01620 N(Z1)=Z2
01630 NEXT X5
01640 Z=Q=Q1=K=1
01650 T1=0
01660 FOR X7=1 TO T
01670 READ#2,A,Z1,B,B1,C,D
01680 IF Z=751 THEN 01730
01690 IF X7<>N(Z) THEN 01720
01700 WRITE #3,A,Z1,B,B1,C,D
01710 Z=Z+1
01720 NEXT X7
01730 RESTORE #3
01740 FOR Y=1 TO 750
01750 READ #3,A,Z1,B,B1,C,D
```

Lines 1460-1520 generate 750 random numbers—all different—lying between the values 1 and the number of entries on the expanded file ('expcol').

Lines 1530-1630 sort the 750 random numbers into *ascending* order.

Lines 1660-1720 write the file of randomly selected isolates and in addition (for convenience) record the day number and frequency of each isolate. The selection is done by the value of the counter x7 which varies between 1 and the number of entries in the expanded collection ('expcol'). When the value of x7 = the value of a selected random number, the entry that has been read is written on the file 'randsel'.

```
01760 IF A<> Q THEN 01790
01770 IF B<> Q1 THEN 01790
01780 GOTO 01990
01790 GOSUB 02510
01800 M(Z3,2)= M(Z3,2)+1
01810 FOR X8=1 TO K
01820 IF S(X8,1)= Z1 THEN 01880
01830 IF S(X8,1)<> 0 THEN 01870
01840 S(X8,1)= Z1
01850 K=K+1
01860 GOTO 01880
01870 NEXT X8
01880 S(X8,2)= S(X8,2)+ B1
01890 FOR X9=3 TO 6
01900 IF S(X8,X9)= Z3 THEN 01950
01910 IF S(X8,X9)<> 0 THEN 01940
01920 S(X8,X9)= Z3
01930 GOTO 01950
01940 NEXT X9
01950 Q=A
01960 Q1=B
01970 WRITE #4,A,B,C,D
01980 T1=T1+1
01990 NEXT Y
```

The y-loop—lines 1740-1990—does the following jobs:

(a) It writes a new file 'isolats' where all replicate entries on 'randsel' are rejected, i.e. all entries on 'isolats' are unique.
(b) It calculates the sector from which the soil was collected and records that an isolate from that sector was selected, accumulating the sum.
(c) It sums all the frequencies collected on each collection day and remembers how many soil samples were collected on that day.
(d) It sums all soil samples collected (k).
(e) It adds all the different isolates (t).

```
02000 FOR X0=1 TO K-1
02010 FOR X=1 TO 4
02020 IF S(XO,X+2)= 0 THEN 02040
02030 NEXT X
02040 S(XO,2)= S(XO,2)/(X-1)
02050 NEXT XO
02060 K=K-1
02070 Q=Q1=Q2=Z=0
02080 FOR X1=1 TO K
02090 Q=Q+S(X1,1)
```

The x0 loop calculates the mean propagules g^{-1} for each day of collection.

At this point in the program (after line 2030) the file 'isolats' can be printed out, by inserting a suitable loop. This is useful as a temporary notebook for recording further results acquired by subsequent study of the random sample.

```
02100 Q1 = Q1 + S(X1,2)
02110 Q2 = Q2 + (S(X1,1)**2
02120 Z = Z + (S(X1,1)*S(X1,2))
02130 NEXT X1
02140 Z7 = ((K*Z) - (D*Q1))/((K*Q2) - (Q*Q))
02150 Z8 = (Q1/K) - (Z7*(Q/K))
02160 PRINT "COEFFICIENTS OF LINEAR";
02170 PRINT "LEAST MEANS SQUARES FIT";
02180 PRINT Z8,Z7
02190 PRINT "NUMBER OF COLLECTION";
02195 PRINT "DATES";
02200 PRINT "SELECTED";K
02210 PRINT " "
02220 PRINT "NUMBER OF DIFFERENT"
02230 PRINT "ISOLATES SELECTED"; T1
02240 PRINT " "
02250 PRINT "GEOGRAPHICAL DISTRIBUTION";
02260 PRINT "OF ISOLATES"
02270 PRINT " "
02280 PRINT "SECTOR";TAB(12);"NO. OF";
02290 PRINT TAB(22); "PERCENT"
02300 PRINT TAB(12)"ISOLATES";TAB(22);
02310 PRINT "OF TOTAL"
02320 PRINT " "
02330 S = T = 0
02340 FOR X2 = 1 TO 10
02350 PRINT TAB(4);X2;TAB(11);M(X2,1);
02360 P = M(X2,2)*100/M(X2,1)
02370 PRINT TAB(16);M(X2,2);TAB(23);
02380 PRINT INT(P + .5)
02390 S = S + P
02400 NEXT X2
02410 S = S/10
02420 FOR X3 = 1 TO 10
02430 P = M(X3,2)*100/M(X3,1)
```

The remainder of the program is arithmtic. A linear least mean squares fit of the data (day number:mean frequency) is calculated in the usual way by the lines 2080-2150.

Lines 2420-2470 calculate the standard deviation of the % isolates of the total collected from each sector.

```
02440 T=T+((P-S)**2)
02450 NEXT X3
02460 T=T/9
02470 T=SQR(T)
02480 PRINT "STANDARD DEVIATION OF":
02490 PRINT"% DISTRIBUTION";T
02500 STOP
02510 Z3=INT(B/10**5)
02520 IF Z3=0 THEN 02550
02530 IF Z3>10 THEN 02570
02540 GOTO 02580
02550 Z3=10
02560 GOTO 02580
02570 Z3=INT(1+10*RND(-1))
02580 RETURN
02590 END
```

Lines 2510-2580 are a subroutine that calculates the sector from which the soil sample was collected. In the years 1967-1971 sectors in the experimental plots were different. In these cases a random integral number in the range 1-10 inclusive is generated (line 2570) and used thereafter.