SEASONAL FLUCTUATIONS OF THE FUNGUSFLORA IN MULL AND MOR OF AN OAK FOREST

Dit proefschrift met stellingen van

MARTINUS WITKAMP,

landbouwkundig ingenieur,
geboren te Wijk aan Zee en -Duin, 23 juni 1922,
is goedgekeurd door de promotor

DR. A. J. P. OORT,

hoogleraar in de planteziektenkunde

De Wnd. Rector Magnificus
der Landbouwhogeschool,

W. F. EIJSVOOGEL

Wageningen, 5 december 1959

STELLINGEN

1. In het algemeen moet de saprophytische mycoflora van de grond betrokken worden bij de bestudering van phytopathogene bodemschimmels.

2. De term 'systemica' voor het collectief van systemische phytochemotherapeutica is een nomen confusum.

3. Bepaling van de absolute activiteit van de microflora van de bodem moet bij voorkeur aan ongestoorde grond plaats vinden.

4. De produktie van antibiotica in bosgrond is een feit en heeft oecologische gevolgen.

5. Bij het waarderen van paddestoelsoorten als oecologische indicatoren moet rekening worden gehouden met hun substraat.

6. Voor de verklaring van veranderingen in een vegetatie is kennis van de autoecologie van de vegetatie-elementen vereist.

7. Bij de groeiplaatswaardering aan ongestoorde podzolprofielen, ten behoeve van de bosbouw, is een chemische analyse veelal onontbeerlijk.

8. Het humustype wordt op zandgrond onder natuurlijke omstandigheden in eerste instantie door de waterhuishouding bepaald.

9. De betekenis van de saprophage fauna van de bosgrond voor de humusvorming ligt in de eerste plaats in zijn mechanische werking.

10. Het populatiedynamisch onderzoek aan insekten kan belangrijk bijdragen tot de insektenbestrijding in land- en tuinbouw.

11. Het is in veel gevallen mogelijk en economisch verantwoord om door milieu-aanpassing wildschade in de bosbouw te vermijden.

12. Het is in verband met de bevolkingsdichtheid en de geestelijke volkshygiëne gewenst, dat de financiële verantwoordelijkheid voor de kinderen meer bij de ouders komt te liggen.

Diss. M. Witkamp
22 januari 1960

SEASONAL FLUCTUATIONS OF THE FUNGUSFLORA IN MULL AND MOR OF AN OAK FOREST

SEIZOENSCHOMMELINGEN VAN DE SCHIMMELFLORA
IN MULL EN MOR VAN EEN EIKENBOS

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR. W. DE JONG,
HOOGLERAAR IN DE VEETEELTWETENSCHAP,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 22 JANUARI 1960 TE 16 UUR

DOOR

MARTINUS WITKAMP

G. W. VAN DER WIEL & CO. — ARNHEM

Dit proefschrift verschijnt tevens als mededeling nr. 46/1960 van het Instituut voor toegepast biologisch onderzoek in de natuur (ITBON) te Arnhem

Aan mijn ouders,
die dit mogelijk maakten

Aan mijn vrouw en kinderen,
die dit noodzakelijk maken

DANK ben ik verschuldigd aan

Professor Dr. A. J. P. Oort voor zijn bereidheid mijn onderzoek als basis voor een proefschrift te aanvaarden en voor de aandachtige en prettige medewerking bij de totstandkoming ervan;

Dr. A. D. Voûte, die als directeur van het Itbon heeft gestimuleerd dit werk naar eigen inzicht uit te voeren;

Dr. J. C. Went, Dr. J. van der Drift en G. Minderman, mijn collegae, met wie opzet, uitvoering en resultaten van het onderzoek zijn uitgewerkt;

Professor Dr. H. M. Quanjer, Professor Dr. L. C. P. Kerling en Professor Dr. R. L. Starkey, die allen aan mijn vorming tot microbioloog hebben bijgedragen;

Professor Dr. Ir. C. H. Edelman, Professor Dr. W. Roepke en Professor Dr. H. J. Venema, voor hun aandeel in mijn oecologische scholing;

Professor Ir. J. H. Jager Gerlings, Professor Dr. J. H. Becking en Professor Dr. Ir. J. F. Kools, mijn leermeesters op het gebied van de bosbouw;

Dr. D. Pramer voor de snelle en gedegen bijstand bij de vormgeving van dit proefschrift;

Mej. J. Bierling voor de bekwame en stimulerende hulp bij het verrichten en uitwerken van dit onderzoek;

het bestuur van het Itbon voor zijn toestemming de resultaten van dit onderzoek tot een proefschrift te verwerken.

CONTENTS

SAMENVATTING

Dit werk was een onderdeel van een onderzoek over het mechanisme van de afbraak van bosstrooisel. Enkele kwantitatieve aspecten van de mycoflora van bosgrond werden bepaald. De verkregen gegevens werden besproken in samenhang met het milieu.

1. Pasgevallen blad van verschillende boomsoorten bezat een kwantitatief en kwalitatief beperkte flora van schimmels die zich op agarvoedingsbodems ontwikkelden. Deze flora bestond voornamelijk uit Sphaeropsidales, *Pullularia* en *Cladosporium*. Tijdens de kolonisatie van het blad nam zij zowel in aantal als soortenrijkdom toe. Binnen een bepaald type bosgrond werd deze flora kwalitatief eenvormiger. Gedurende de eerste winter nam het aantal kolonies afkomstig van eikeblad af. In de tweede herfst werd een maximum bereikt. Dit viel samen met maxima van schimmels die zich op lignine- en celluloseagar ontwikkelden.In het tweede jaar was het aantal kolonies dat zich uit eikeblad ontwikkelde hoger dan in het eerste jaar. De geslachten *Penicillium* en *Alternaria* werden gedeeltelijk vervangen door *Trichoderma* en *Mortierella*. Uit eikeblad van de mull kwamen meer kolonies en soorten tot ontwikkeling dan uit eikeblad van de mor.

2. In de minerale ondergrond van de kalkrijke mull waren plaattellingen van fungi en myceliumgroei en -dichtheid lager dan in de mor. Plaattellingen van bacteriën en actinomyceten waren het hoogst in de kalkrijke mull. De waarden die voor deze factoren in zure mull werden verkregen, waren gewoonlijk intermediair. De kenmerken van de schimmelflora's in de verschillende typen bosgrond leken in de eerste plaats door waterhuishouding en kalkgehalte te zijn bepaald. Deze factoren bepaalden de begroeiing, die op haar beurt de chemische samenstelling van het strooisel beïnvloedde. Het pasgevallen strooisel van de kalkrijke mull bevatte meer eiwit en minder cellulose en lignine dan dat van de mor. Samenstelling en ontwikkeling van de schimmelflora van de minerale ondergrond werden onmiddellijk beïnvloed door het strooisel, uitdroging en pH van de grond, en door de activiteit van de overige micro-organismen en de strooiseletende bodemdieren.

3. Schommelingen van de aantallen micro-organismen en de myceliumgroei in de minerale ondergrond werden voornamelijk veroorzaakt door schommelingen in temperatuur en vochtgehalte, en de toevoeging van vers strooisel. Deze schommelingen waren synchroon in de verschillende typen bosgrond. Schommelingen in de groeisnelheid van mycelium en in het aantal bacteriën vertoonden grote overeenkomst.

4. De myceliumdichtheid in eikenbos was het hoogst in herfst of winter, en het laagst in de zomer. Deze jaarlijkse schommeling werd niet waargenomen in dennenbos. De snelheid waarmee mycelium werd afgebroken was in de kalkrijke mull en mor vrijwel gelijk. In de kalkrijke mull waren 4 tot 10 × zoveel chitineafbrekende en mycolytische micro-organismen dan in de mor. In de mor overheersten myceliumetende hoornmijten. Hun vraat was in de zomer per dier 3 × zo groot als in de winter.

5. De myceliumdichtheid in de minerale ondergrond binnen één type bosgrond, bleek positief gecorreleerd te zijn met het humus- en vochtgehalte en negatief met de diepte. De myceliumdichtheid in de minerale ondergrond van de mor was gewoonlijk hoger dan in die van de mull.

6. Het overheersen van dikke bruine hyphen op de grondsuspensieplaatjes en hun gering percentage op de Cholodnyplaatjes duidde op tragere groei en afbraak van dit mycelium dan van het dunne witte mycelium.

7. Het hoogste aantal soorten paddestoelen verscheen ongeveer drie maanden vóór de hoogste myceliumdichtheid. Hun aantal en gewicht hield geen verband met de myceliumdichtheid in de verschillende typen bosgrond. Hun verschijnen werd gedeeltelijk bepaald door het vochtgehalte van de ondergrond. Droogte beperkte het aantal soorten paddestoelen. Grotere organische strooiseldelen beschermden het mycelium tegen uitdroging. Er waren kenmerkende paddestoelen voor elk type bosgrond. In de kalkrijke mull waren dit voornamelijk humus- en strooiselzwammen. Mycorrhiza-zwammen overheersten in de zure mull en de mor. Strooiselzwammen kwamen in alle typen voor.

8. Opeenhopingen van schimmeldraad, gevormd onder natuurlijke omstandigheden, waren in staat de ontwikkeling van micro-organismen te belemmeren.

9. De verkleining van strooisel door strooiseletende dieren verhoogde de schimmeltellingen, vooral van arme grond. Bacterietellingen werden meer verhoogd dan schimmeltellingen.

INTRODUCTION

This paper describes the fungusflora of three types of forest litter. The distribution of the fungi in space and time and their interrelationships with various aspects of the soil environment are considered. The work was part of a study of the mechanism of transformation of organic matter into soil humus. The information obtained is of importance in the Netherlands for two main reasons:

1. As a result of the rapidly increasing population, and the shift of the rural population to urban areas, valuable horticultural land, surrounding towns and cities, is constantly lost. Horticultural crops require soil with a good humus status. As this becomes unavailable, it must be created by proper soil supplementation and management. To this end, knowledge of the mechanism of the decomposition of organic material and the formation of good humus is desired.

2. As a result of the intensive use of soil for more profitable agricultural pursuits, only the poorer soils are available for forestry. This, combined with the long rotation period required in forestry, has decreased revenues, and it is clear that costs must be kept at a minimum. Fertilizers become uneconomical even on these poor soils. Consequently, more efficient use of the minerals present in the soil is a necessity. Minerals, accumulated in undecomposed litter, must be made available, and their loss by leaching must be prevented. Knowledge of the mechanism of the decomposition of organic materials in soil is fundamental to attainment of these objectives.

To achieve information concerning the transformation of organic matter to minerals and soil humus, a research group was formed at the Institute for Biological Field Research, to which the author of this paper partakes as a mycologist. At present this group has four members, studying respectively the fauna, the microfauna, the bacteria and the fungi involved in the decomposition of organic matter on and in the soil. Characterization of the environment is a joint task of the team.

The forest soil used in this work was undisturbed and the litter at various locations showed different types of decomposition, and transformation to humus. The site offered an opportunity to investigate the dynamics of humus formation in a natural environment under known climatological conditions and sylvicultural methods.

Four methods were used to determine the nature of the fungus flora:

1. The numbers of fungi in soil and litter were determined by the agar plate method. This method does not supply information concerning the activity of fungi and, due to its selectivity, excludes the important litter decomposing Basidiomycetes.

2. Information about the Basidiomycetes was obtained by periodically collecting the fruiting bodies that appeared at the experimental site. The organisms were identified and their dry weight was measured.

3. To determine the amount of mycelium in the soil, direct measurements were made, but the procedure did not distinguish between living and dead mycelium.

4. An estimation of the extent to which fungus mycelium grew in soil was obtained by measuring hyphae that developed on glass slides buried in the soil.

In addition the influence of certain biotic factors on the fungus flora was determined experimentally. In some cases studies made at other locations are mentioned to illustrate methods, trends or processes. Ecological data, collected by others, are used. Data concerning the soil fauna and litter were collected by Dr. J. van der Drift. The carbon contents of litter and humus and some of the edaphic factors were determined by G. Minderman. The air temperature and precipitation 6 km north of the experimental area were kindly furnished by the Royal Dutch Metereological Institute (K.N.M.I.). A number of related papers by other members of the group are in press or being prepared for publication.

SITE CHARACTERISTICS

General description

The site of the present work is a 20 years old stand mainly of slashoak, on 1.3 ha of low sandy soil, 6 km southeast of Zutphen. This stand formerly belonged to the Estate of Hackfort and will be referred to as the Hackfort oak forest. The whole area slopes slightly toward the northwest, the maximum difference in heigth being 65 cm.

At the lowest part free calcium is present. Here, decomposition of the litter is completed within a year and the breakdown products are well mixed with the mineral soil. This area is referred to as calcareous mull. An intermediate area, in which decomposition is completed in approximately a year, is hereafter referred to as acid mull.

At the highest section, decomposition of the litter is slow and consequently at the end of the season, there is an accumulation of organic material on the surface of the mineral soil. This area is designated as mor.

The original distinction and description of mull and mor are from the studies of Müller (1887) on forest and heath in Denmark. These biologically distinct types were defined at the Third International Congress of Soil Science (1935) as follows: 'mull: mixture of organic matter and mineral soil, of crumbly or compact structure, with the transition to lower layers not sharp', and: 'mor: organic matter practically unmixed with mineral soil, usually more or less matted or compacted. Transition to mineral soil always distinct. Often composed of two layers named F and H (after Hesselman)'.

In this paper mull and mor will be referred to as forest floor types The F- and H-layers will be termed fermentation layer and humus layer respectively. The fermentation layer is characterized by comminution of the organic material, whereas in the humus layer humification prevails and the debris is not recognizable as plant material. The combined organic matter above the mineral soil will be referred to as litter, and the soil directly under the litter will be referred to as mineral soil.

Environmental factors

Edaphic factors

The soil of the calcareous mull type is a sand containing from 0.5 to 10% free calcium carbonate in the form of calcite (table 1). It has an extremely loose structure due to the burrowing action of a great many soil animals. The pH varies from 5.5 to 7.5. The humus content of the top 2 cm of the mineral soil is about 10%, and the wilting point (pF 4.2) is reached when the moisture content of the soil drops below 6.9% w/w. The depth of the water table varies according to the season from 25 to over 200 cm under the surface.

The soil of the mor type is also a sand, but contains no free calcium. The structure of this soil is usually compact with only local activity of burrowing animals. The pH of the mor varies from 3.0 to 3.7. The humus content of the top 2 cm of the mineral soil is about $2\frac{1}{2}$ × as high as in the calcareous mull and consequently the wilting point is

Table 1 - SITE CHARACTERISTICS OF THE OAK STAND AT HACKFORT, 27 KM E.N.E. OF ARNHEM

Soil

forest floor type	calcareous mull	acid mull	mor
soil texture	sand	loamy sand	sand
humus content in % w/w uppermost 2 cm	10	11	24
pH	7.2	3.6	3.3
moisture in % w/w at pF 4.2	6.9	8.2	13.0
minimum depth of water table in cm (1958)	25	interm.	90
maximum depth of water table in cm (1958)	120	interm.	190
$CaCO_3$ in % w/w	7.2	<0.1	<0.1
C/N of humus in mineral soil	<20	<20	>20
humus of mineral soil, 0–90 cm, in kg/m²	9.6	12.2	18.4

Vegetation

heighth of tree canopy in m	10.5–8.5	10.5–8.5	7.5–6.5
cover of tree canopy in %	100	100	95
number of species in ground cover	30–35	10–15	7–10
mass of ground cover in g oven dry/m²	75	50	12
C/N ground cover	20.4	22.7	34.1
cover of groundcover in %	100–70	50–30	15–5

Litter

tree litter in g air dry/m² year	560	580	490
tree leaves in g air dry/m² year	390	400	350
% oak leaves	28	66	92
% birch leaves	36	34	8
% poplar	17	0	0
% alder leaves	19	0	0
C/N of all fresh tree leaves	29.2	33.1	33.2
% lignin tree leaves	17.5	17.2	19.1
% cellulose tree leaves	8.7	10.9	12.3
C/N all fresh litter	27.8	31.9	33.2

reached at a higher moisture content. As the average soil surface of this type is about 60 cm higher than that of the calcareous mull type, there is a similar difference in the depths of the corresponding water tables.

The acid mull type occupies in many instances an intermediate position between the calcareous mull and the mor. Altitude and depth of the water table in the soil show such intermediate values. The calcium content of this soil is low, as in the mor type. Consequently there is little difference in acidity. Even though the humus content of the acid mull is only slightly higher than that of the calcareous mull, the wilting point of the former is reached at a higher moisture content than of the latter. This effect is

caused by the presence of loam in the area of the acid mull. The moisture content of the mineral soil was measured from June 1958 until November 1959 (FIG. 1).

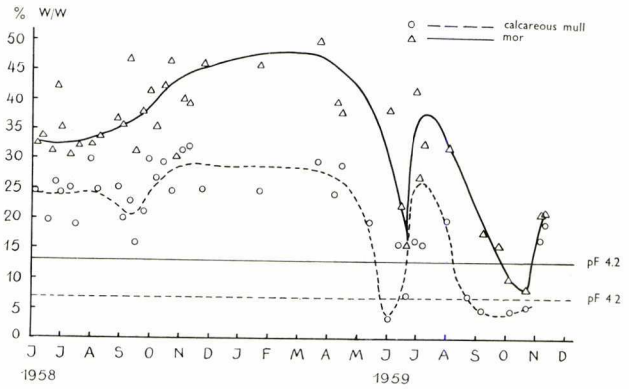

Fig. 1. Moisture content and wilting point (pF 4.2) of the top 2 cm of mineral soil in the Hackfort oak forest.

As a result of the thin litter layer in spring and summer, the high porosity and the relatively low humus content of the calcareous mull, the percentage of moisture was found to be lower than that in the mor type with its thick fermentation and humus layer on top of the relatively compact soil. The presence of some loam and a rather permanent litter layer in the acid mull type caused its moisture content to be close to that of the mor, notwithstanding its rather low humus content.

As a result of texture, capillary ascent and the depth of the water table, the subsoil water had no direct influence on the moisture content of the upper 2 cm of the mineral soil in the three forest floor types.

Climatic factors

As the various forest floor types in the Hackfort forest were less than 200 m apart, no differences in macroclimate were expected or observed. In the litter layer there were, however, differences in temperature. The average weekly maximum temperature in the litter layer of the mor type was in spring 3–5° C, and in summer 1–2° C higher than in the mull types. The average annual temperature was about 9.5° C. Minimum temperatures were recorded in January and maximum temperatures in July and August. The temperature, measured between the litter and the mineral soil in the acid mull type, was on average about 1° C lower than the temperature of the air outside the forest. This difference was maximal in summer and minimal in autumn (FIG. 2).

Partly as a result of these differences in temperature, from July until October the evaporation measured at 5 cm over the surface of the litter was found to be twice as high in the mor type as in the mull types. In spring this difference in evaporation was undoubtedly even larger.

During the three years of observation the precipitation was measured over an open field, 6 km north of the forest, and showed maxima in August–September and in January–February (FIG. 2). The average annual precipitation varied greatly.

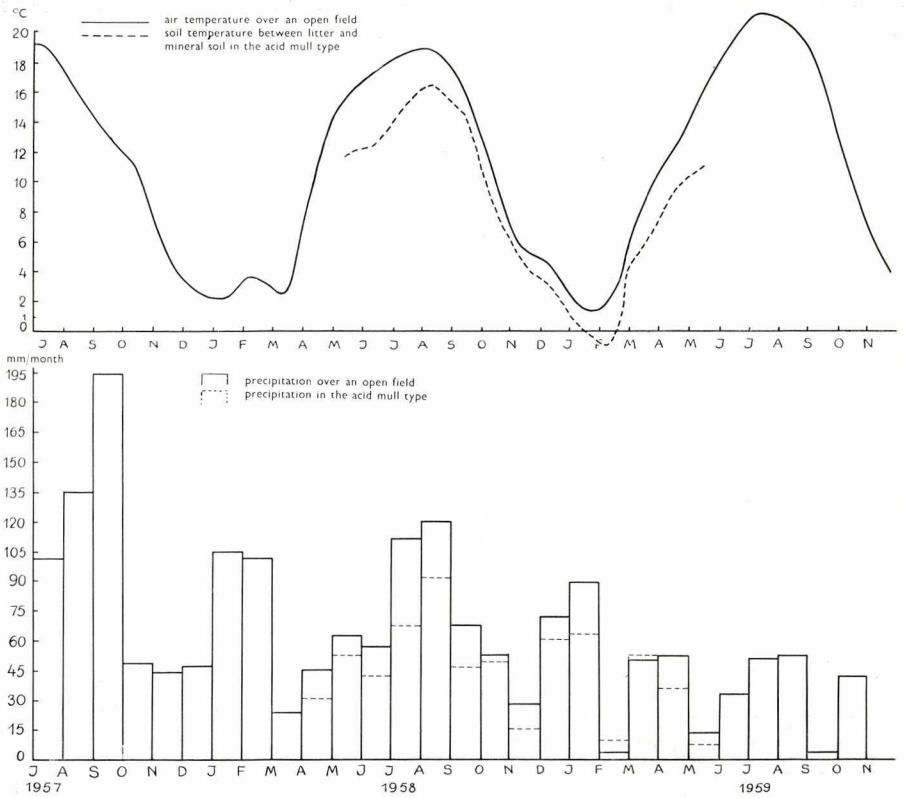

Fig. 2. Temperatures and precipitation at Hackfort.

Biotic factors

Vegetation

The vegetation on the calcareous mull consisted of a dense stand of oak *(Quercus robur* L.), birch *(Betula verrucosa* Ehrh.), alder *(Alnus glutinosa* (L.) Gaertn.) and poplar *(Populus tremula* L.), 8.5 to 10.5 m high. The lush undergrowth was composed of some 35 species of shrubs and herbs, covering in summer 60 to 95% of the area. The vegetation belonged to the Pruneto–Fraxinetum OBERDORFER 1953, but is now, due to increased drainage, turning into a Violeto-Quercetum OBERDORFER 1937 type of vegetation. The local vegetation of the calcareous mull was characterized by the presence of *Alnus glutinosa* L., *Listera ovata* (L.) R. Br., *Symphytum officinale* L. and *Daphne mezereum* L.

On the mor there was an equally dense stand of oak and a few birches, 6.5 to 7.5. m high and with about the same mass as the stand on the calcareous mull. The very sparse undergrowth covered about 10% of the area and consisted of only 10 species. This vegetation belonged to the Querceto-Betuletum Tx. 1930 and was here characterized by the presence of *Molinia caerulea* (L.) Moench, *Carex pilulifera* L. and *Melampyrum pratense* L.

The stand on the acid mull was composed of oak, birch and a few poplars, and just

as high as the stand on the calcareous mull. Its vegetation characteristics were intermediate between those of the two other forest floor types.

Litter

The amount of litter, shed annually from the trees, was collected in permanently exposed bags of cheese cloth, having round openings of 0.5 m^2, and determined by periodic weighing. Six bags were placed at each experimental site. The air dry weight of the litter falling in the period from September 1957 until September 1958 was for calcareous mull, acid mull and mor 560, 580 and 490 g/m^2 respectively. Botanical analysis of freshly fallen litter indicated an increasing percentage of oak leaves in forest floor types with a decreasing rate of decomposition. These percentages were for calcareous mull, acid mull and mor 28, 66 and 92 respectively. In all types about 70% of the tree litter consisted of leaves, 16 to 20% of twigs and the remainder of catkins (up to 7%), caterpillar excrements (up to 5%) etc.

The weight of herbs and grasses, annually returned to the soil, was estimated by mapping 6 density classes of the vegetation of the whole area in August and determining the weight after harvesting 3 × 1 m^2 of each class. *Anemone nemorosa* L., which disappeared before August, had been mapped and weighed in May.

The main period of shedding for birch was from August to October, for alder and poplar October and for oak November. This means that in the mull types fresh litter was deposited earlier than in the mor type. Of the total amount of tree litter, 70 to 75% fell during the last 4 months and 5 to 10% during the first 4 months of the year; 20 to 25% fell from May until September.

In order to evaluate the amounts of various groups of nutrients annually available for the microbial flora, an approximate chemical analyses of the litter was made. Easily decomposable carbohydrates were determined by the anthrone method (Morris, 1948) after hydrolizing the pulverized material with 1N. H_2SO_4. Cellulose was determined as the difference between total carbohydrates, after treatment with 75% H_2SO_4, and easily decomposable carbohydrates. Lignin was determined according to the glycocollic acid method (Holmberg, 1936) and nitrogen by the macro Kjeldahl method as described by Conway (1950).

The results of analyses of fresh litter and the calculated amounts of various constituents indicated that the percentages of lignin and cellulose in the tree litter of the mull types were lower than in the mor type. The nitrogen content of the total amount of litter fallen, was higher in the mull than in the mor.

There were also differences between the leaves of one species, collected from the litter of different forest floor types. The pH-values, measured in January on the wet surface of oak leaves, were for leaves from the calcareous mull, acid mull and mor 4.9, 3.9 and 3.7 respectively, whereas the corresponding pH-values for pulverized oak leaves in water were 5.2, 4.2 and 3.8 respectively. Another difference between oak litter from mull and mor was, that in the mull more shadow leaves were found than in the mor. This was caused by the position of the late budding and slowly growing oak trees under a canopy of early budding, fast growing birches, poplars or alders on the mull. In the almost pure stand of oak on the mor, sun leaves were consequently most numerous. These sun leaves were smaller and twice as thick as the shadow leaves and the amount of palisade parenchyma of sun leaves was greatly increased. Consequently the ligning content of sun leaves was higher than that of the shadow leaves, and the oak litter of the mor contained a higher percentage of lignin than that of the mull types.

The moisture content of the litter was determined from November 1957 until October

1958. Except for the dry period in April, May and June, there was always a higher percentage of moisture in the litter of the mor than in that of the calcareous mull. Notwithstanding the higher rate of evaporation, the thick litter of the mor was able to retain in general more moisture than the well drained thin litter layer of the calcareous mull. On prolonged drying, however, the thick sun leaves of the oak litter prevailing in the mor type curled strongly and permitted desiccation of the lower layers of the litter. The litter of the acid mull type constantly contained more moisture than that of the mor, even though the thickness of the respective layers suggests the opposite. Here the lesser curling of the leaves, the relatively low rate of evaporation and presence of loam in the sub-soil seemed responsible for the relatively high moisture content.

In order to study the loss of moisture of the top 6 cm of the whole profile, in which almost all of the biological activity was concentrated, undisturbed soil samples of 1 dm^2 surface area and 6 cm depth were stored in glass jars in an unheated room. The samples were taken after heavy rain and were well saturated with moisture. Samples of the calcareous mull, acid mull and mor retained 6, 15 and 45% respectively of their original moisture after 50 days storage.

The losses of moisture were in accordance with the observed moisture percentages of the litter and mineral soil in the forest. The same trend was observed in the dry spring and summer of 1959. The moisture contents of the mineral soil of the calcareous mull, acid mull and mor were 3.5, 26 and 37% respectively in June. After rain and renewed and prolonged drought in September and October the corresponding moisture contents were 6, 11 and 8%. This indicates that the wilting point in the mineral soil was first reached in the calcareous mull, next in the mor and not in the loamy soil of the acid mull.

The soil fauna

The soil fauna of the upper 5 cm of the mineral soil of the three forest floor types was analysed in the various seasons. Of the eleven groups of saprophageous animals, only the Acarina prevailed in the mor type. This group consists mainly of litter and fungus feeding oribatid mites. Springtails, many of which are fungus eaters, were about equally numerous in the acid mull and the mor, and half as numerous in the calcareous mull. All other groups of saprophageous animals prevailed in the mull types. Many of these are burrowing animals and they are in general considerably larger than springtails and mites. Most of these larger burrowing animals, like earthworms and dipterous larvae, live below the litter. They were most active in late fall, winter and early spring, when the litter was rather fresh, and the litter layer protected the soil against freezing and desiccation.

FUNGI DEVELOPING ON AGAR MEDIA

Colonization of newly shed litter

In 1886, Adametz isolated the first microfungi from soil on solid media. In 1904, Koning put this method on a more or less quantitative basis by mixing known dilutions of forest soil or litter fragments with gelatin and agar plates. The serial dilution technique has been used widely to enumerate the number of fungi in litter and soil.

From the studies of Warcup (1957), it appeared that on dilution plates the majority of colonies developing from soil of a wheat field arose from spores. Consequently heavily sporulating species were represented beyond proportion. Fast growing species were found to outnumber the slower growing fungi. Many species, present in the soil, were not found on dilution plates. Viable hyphae, belonging mostly to Basidiomycetes and Mycelia Sterila, were never observed to give rise to fungal colonies on dilution plates. These observations indicate that the dilution plate method is not a valuable tool to estimate the activity of fungi or to determine the mycoflora in soil. It may, however, be used to compare certain aspects of the soil fungi at different times and habitats, as appears from the work of Guillemat & Montégut (1956, 1957).

In the present investigation the dilution plate method was used to study the colonization of freshly fallen leaves.

In the fall of 1959, the microbial population present in fresh litter was determined for 7 weeks after shedding. To this end, leaves of the main tree species were collected in the calcareous mull type, cut in strips of $\frac{1}{2}$ mm width, and ground in a rotor type coffee mill for 30 seconds. Suspensions of 1 g of the resulting powder were plated with peptone-dextrose agar, containing 33 p.p.m. of rose bengal and 30 p.p.m. of streptomycin, in order to suppress bacterial growth (Martin, 1950), and nutrient agar (8 g Difco dehydrated nutrientbroth/l). After incubation at 25 and 30° C for 2, 4 and 7 days the number of bacterial and fungal colonies and the abundance of the dominant genera of the fungi were determined. The data obtained are recorded in table 2. From the results it appeared that the numbers of bacteria and fungi increased rapidly during the first weeks. The dominant flora of Sphaeropsidales disappeared, to be replaced by *Cladosporium herbarum*. The freshly fallen leaves of the various tree species were characterized by a specific combination of dominant fungi. Colonies of *Mucor*, *Trichoderma* and *Alternaria* were rare. The presence of fungi belonging to the genera *Cladosporium* and *Pullularia*, and the absence of *Mucor* on freshly fallen leaves has also been observed by Meyer (1959) and Smit & Wieringa (1953). Differences in the mycoflora of litter of different tree species were noted by Koning (1904). In December 1958, the microbial population of $1\frac{1}{2}$ months old oak leaves contained fungi and bacteria. The respective counts in 10^3/g were in the calcareous mull 500 and 6100, in the acid mull 400 and 1000, and in the mor 50 and 40. Most likely the fungal and bacterial flora on the surface of the leaves was greatly influenced by direct contact with the environment.

Microfungi from one and two years old oak leaves

During preliminary studies on the microflora of oak leaves, an attempt was made to isolate only those organisms actively growing on and in the material. This was done by

Table 2 — THE NUMBER OF FUNGI, BACTERIA + ACTINYMOCETES *(a)* AND THE PERCENTAGES OF SOME TAXONOMIC GROUPS OF FUNGI *(b)* DEVELOPING FROM 1 ML OF A 10^{-4} DILUTION OF GROUND LEAVES COLLECTED FROM CALCAREOUS MULL AT DIFFERENT TIMES AFTER SHEDDING (averages of duplicate series, — = < 10%)

	leaves of	alder					poplar					birch					oak				
	weeks after shedding	½	1	3	5	7	½	1	3	5	7	½	1	3	5	7	½	1	3	5	7
a	fungi	8	59	150	47	272	8	26	153	74	508	40	93	556	156	176	54	187	424	57	129
a	bacteria + actinomycetes	7	23	59	151	310	51	333	337	310	341	22	112	66	135	198	96	399	788	95	182
b	Sphaeropsidales	58	—	18	—	—	50	—	18	—	—	—	—	—	—	—	70	—	18	27	—
b	Pullularia	21	11	23	—	17	—	16	—	—	10	—	—	10	—	—	—	31	—	18	—
b	Cladosporium	—	18	22	14	48	—	28	—	21	57	—	38	12	18	72	—	41	21	24	22
b	Penicillium	—	—	—	18	—	—	—	—	31	—	22	—	—	—	—	—	—	—	—	—
b	submersed sterile mycelium	—	—	—	—	—	48	35	44	14	—	—	—	—	—	—	—	—	—	—	—
b	aerial sterile mycelium	—	—	—	—	—	—	—	—	—	—	32	—	20	—	—	—	—	—	—	24

creating a separation between the leaf material and the agar substrate, to prevent activation by nutrients of non-proliferating organisms. Moreover the technique limited the number of bacterial colonies that developed. Similar techniques involving soil or organic matter and agar media have been used previously (CHESTERS, 1940; LA TOUCHE, 1948; THORNTON 1952 and 1958).

The method employed discs of oak leaves (diameter 5.2 mm) punched with an aseptic cork borer. These were placed on sterile coverslips (18 × 18 mm) on the surface of peptone-dextrose agar pH 5.6 (TABLE 3). The leaves were collected monthly from a mor type oak stand at Doorwerth (TABLE 4) from December 1952 through August 1953. Both, fresh leaves of 1952 (F_0) and leaves shed in 1951 (F_1) were used. Each month, 8 discs of each material were placed on cover slips (4 slides on each plate). For purposes of comparison, another 12 discs from the same leaves were put directly on the peptone-dextrose agar surface. The plates were incubated at 25° C, and examined periodically until no more new colonies developed. To prevent desiccation of the discs on the slides, a drop of sterile water was administered to each disc every other day. The colonies that developed were counted and identified to genera.

Table 3 — COMPOSITION OF VARIOUS MEDIA (g/l)

constituents of medium	peptone-dextrose agar pH 5.6 [1]	cellulose agar	lignin agar	pectin agar
dextrose	20			
cellulose [2]		20		
meadol [3]			20	
pectin [4]				2% aq.
peptone	10			
$(NH_4)_2SO_4$		1	1	
$(NH_4)Cl$				0.5
asparagin				0.5
$MgSO_4$	0.25	0.5	0.5	0.5
$CaCO_3$		1	1	
$CaCl_2$				1
NaCl		0.5	0.5	
Na_2CO_3				0.5
K_2HPO_4	0.25	0.5	0.5	1
agar	20	15	15	15

[1]) For peptone-dextrose agar pH 4.5 add lactic acid.
[2]) Cellulose powder from Whatman I filter paper.
[3]) Meadol powder, supplied by The Mead Corporation, Chillicothe, Ohio. Contains, according to thioglycollic acid method, 72.5% of lignin.
[4]) Pectin powder 'Rotband Unipectin, Zürich, Switzerland'. 5 ml 2% aqueous solution on top of cooled plates.

Table 4 – SITE CHARACTERISTICS OF VARIOUS SAMPLING AREAS

locality	Doorwerth	Middachten	National Park 'de Hoge Veluwe'		
forest floor type	light mor	acid mull	6 cm of litter	3 cm of litter	0– 0.5 cm of litter
soil texture	86% sand 7% loess	18% sand 60% clay	fine sand	sand	coarse sand
organic matter content of the mineral soil	7%	22%	10%	5%	4%
pH	3.5	3.7	4.6	4.7	4.9
depth of water table in m	>10	0–0.5	>10	>10	>10
main tree species	Q. Robur	Q. Robur	P. sylvestris	P. sylvestris	P. sylvestris
heighth of trees in m	12	20	10	8	6
cover of tree canopy in %	75	95	100	95	40
vegetation type	Querceto-Betuletum Tx. 1930	Pruneto-Fraxinetum Oberdorfer 1953	Deschampsia flexuosa + Pleurozium Schreberi	Deschampsia flexuosa + Hypnum cupressiforme	Calluna vulgaris + Cladonia sspp

Table 5 — PRESENCE OF SOME GENERA OF FUNGI AS PERCENTAGE OF THE TOTAL NUMBER OF COLONIES THAT DEVELOPED FROM DISCS OF OAK LEAVES ON VARIOUS MEDIA

locality	Doorwerth				Middachten	
forest floor type	light mor				acid mull	
period	December 1952 – September 1953				November 1953 – November 1954	
method and medium	discs on coverslip on peptone-dextrose agar pH 5.6		discs directly on peptone-dextrose agar pH 5.6		discs directly on peptone-dextrose agar pH 4.5	discs directly on 4 media
litter layer	F_0	F_1	F_0	F_1	F_0	F_0
Mucor	12	4	18	24		
Mortierella	5	8	24	27	41	32
Trichoderma	10	29	6	23	16	18
Aspergillus	46	39	27	7	0	2
Penicillium	7	2	4	2	14	15
Alternaria	7	0	8	2	5	7
miscellaneous	12	18	13	15	24	26
total number of colonies	41	49	90	113	349	1464
number of discs	72	72	108	108	192	768
colonies per disc	0.6	0.7	0.8	1.0	1.8	1.9

The results are listed in TABLE 5. The number of colonies formed from discs placed directly on the agar surface, was about 35% higher than that obtained from discs placed on coverslips. This may have resulted from either the absence of direct contact between fungus cells and the medium, or from desiccation of mycelium as it grew across the slides.

TABLE 5 shows that fewer colonies of *Mucor* and *Mortierella* developed from leaf discs on cover slips than from discs placed in direct contact with the agar. As mucoraceous fungi prefer moist conditions (GUILLEMAT & MONTÉGUT, 1956; ORPURT & CURTIS, 1957), desiccation may be responsible for their reduction on the slides. SAÎTO (1955a) suggests that Mucorales are present in the soil as spores which develop in dilution plates from soils lacking typical broad non-septate mycelium. If this is valid, the low numbers of these fungi from discs on cover slips may be due to failure of resting spores to develop. *Aspergillus*, which prefers dry areas (ORPURT & CURTIS, 1957), showed an effect opposite to that of the Mucorales.

The species isolated by both methods belonged mainly to the same genera. The numerical relation of these genera, however, depended on the method used. The species classified as miscellaneous belonged to the genera *Rhizopus*, *Phycomyces*, *Gymnoascus*, *Chaetomium*, *Lachnum*, *Geotrichum*, *Verticillium*, *Cladosporium*, *Tetracoccosporium* and *Fusarium*.

As to differences in the fungus flora of the two litter layers, both methods yielded about 20% more colonies from F_1- than from F_0-leaves. This may reflect an increased colonization of the oak leaves with increasing age as observed by TWINN and WAID (WAID, 1960), and the more favorable moisture conditions in the F_1-layer as compared to the exposed F_0-litter. The relative abundance of *Aspergillus* in the F_0-layer may have been due to the rather low moisture requirements of many species of this genus. *Alternaria* also seemed to prefer F_0-litter. On the other hand, *Trichoderma* appeared to prefer the deeper and older litter (F_1). SAÎTO (1956) also observed the disappearance of *Alternaria* with the aging of litter, and greatest counts of *Trichoderma* were obtained in the oldest litter layer.

PERIODICITY AND SUBSTRATE PREFERENCE OF MICROFUNGI FROM OAK LEAVES

As the methods used for the determination of microfungi in oak leaves failed to show seasonal fluctuations and yielded rather low numbers of colonies, a more extensive study was made.

Monthly, from November 1952 until November 1953, 16 discs (diameter 4.3 mm) were punched out of oak leaves collected from the F_0-layer of a mull type oak forest at Middachten (TABLE 4,) and placed on 4 different agar media. On each of these media 4 discs were placed in direct contact with the agar. The media contained different carbohydrates and were chosen to obtain a wide variety of physiological groups of fungi, and to indicate a possible substrate preference of the various microfungi from oak leaves. The media will be referred to as peptone-dextrose agar, cellulose agar, lignin agar and pectin agar; their composition is given in TABLE 3.

In total, 1464 colonies were counted and their genera were determined. The presence of over 25 different genera was established. The number of colonies expressed in the percentage of all colonies on all media and on peptone-dextrose agar pH 4.5 is given in TABLE 5.

A comparison between the relative abundance of the various genera in the mor stand

at Doorwerth and the mull forest at Middachten must be made with caution as the year and period of collecting, number and size of discs and the media used were different. Nevertheless these data show that Mucorales and *Alternaria* were present in comparable proportions. The percentage of *Aspergillus* colonies in the litter at Middachten was very low. *Aspergillus* colonies were replaced by colonies of *Penicillium*, *Trichoderma* and/or colonies classified as miscellaneous. The almost complete absence of *Aspergillus* is likely to be the result of the high water table at Middachten which kept the litter moist throughout the year. The strong admixture of decaying material from the rich ground cover may be responsible for the increased number of miscellaneous colonies. From discs of the moist and relatively rich litter at Middachten, twice as many colonies developed as from discs collected at Doorwerth, even though the latter were larger.

The total number of colonies on all media as determined monthly, showed a definite periodicity. During colonization of the fresh substrate in 1953 there was an indication of an increasing number of colonies growing from the discs. During the winter the numbers decreased. With rising temperatures in the spring there was a small increase but the number of colonies remained constant during late spring and early summer, and increased again with the summer rains in August and September. In October there was a rapid decrease, possibly due to competition from Basidiomycetes that colonized the now one year old leaves, as obeserved by TWINN and WAID (WAID, 1960). These fluctuations were less pronounced than those of counts on dilution plates. This may be related to the circumference of the discs limiting the number of fungi that can colonize the agar medium.

The counts of most genera fluctuated erratically. *Alternaria* gradually disappeared as the leaves aged, which is in accordance with the observations on this genus on the F_1- and F_0-leaves at Doorwerth. Some taxonomic groups classified as miscellaneous were observed for rather short periods only, like the Sphaeriales in April and May, *Helicosporium* in May and *Spicaria* in July and August.

Fluctuations in the number of colonies developing on the various media were limited. Maxima on the cellulose and lignin media were reached in August and September, when the content of easily decomposable substances in the oak leaves was low (FIG. 3).

Of the total number of colonies observed on all media throughout the year, 30% developed on the lignin medium, 29% on the cellulose agar, 24% on the peptone-dextrose

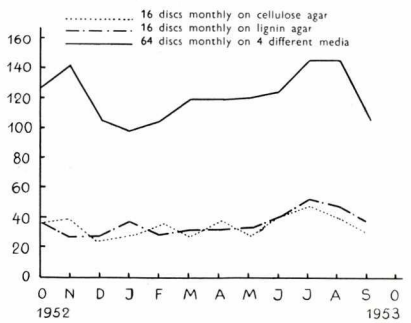

Fig. 3. Number of fungus colonies growing monthly from discs of oak leaves placed on agar plates.

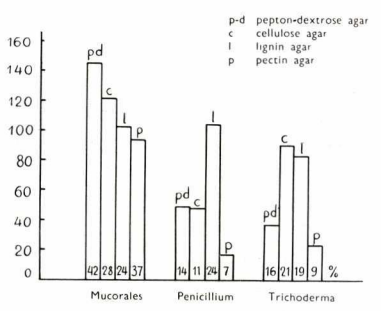

Fig. 4. Number of fungus colonies and the percentage of some taxonomic groups of the total number of colonies developing from discs of oak leaves on various media.

substrate and 19% on the pectin agar. As Fungi Imperfecti are in general not known to decompose lignin, and Mucorales do not seem to utilize cellulose, these results are rather surprising. The Meadol from the lignin medium contained some 27.5% of non-ligninous material, and this may have been used for the development of some fungal colonies. The cellulose used, however, did not contain any appreciable amount of extraneous carbohydrates, but a great number of Mucorales were able to sporulate on the cellulose agar. The reason was probably that the fungi growing from the discs, used the agar media as a substrate for sporulation and possibly to supply moisture and nitrogen, whereas the discs served as the main source of carbohydrates. Still there was in certain groups of fungi a substrate preference that seemed definite and reasonable. The distribution of colonies belonging to the Mucorales, *Penicillium* and *Trichoderma* among the various media is respresented in FIG. 4. Also shown are the number of colonies of these groups in percentages of the total number of fungi that developed on each medium. From these data it appeared that the Mucorales preferred the easily decomposable nutrients of the peptone-dextrose medium and that 42% of all colonies developing on these plates belonged to the Mucorales. On the pectin agar Mucorales constituted 37% of the total number of colonies developing. *Penicillium* showed a definite preference for the lignin medium and *Trichoderma*, of which many strains are known to decompose cellulose, preferred the cellulose agar.

In general, the method of placing discs punched out of leaves either on coverslips, or directly on agar media, did not seem to yield many advantages over other techniques designed to isolate microfungi from leaves. Quantitatively, the possibilities of this technique are limited. Qualitatively, the results were uncertain. When investigating substrate preference of microfungi from leaf material in this way, the relatively large substrate provided by the discs masked the ability of the fungi to utilize the various nutrients offered in the agar.

PLATE COUNTS OF FUNGI FROM THE MINERAL SOIL

In order to determine the density of fungi in the mineral soil of the three forest floor types at Hackfort, the dilution plate method was used.

Preliminary counts and respiration measurements on undisturbed soil in a Warburg apparatus indicated that most microorganisms and greatest respiratory activity occurred in the uppermost 2 cm of the mineral soil. Therefore samples were taken from this layer.

In each type 10 samples of 2 ml were collected with a sampling tube, (diameter 16 mm), and placed in a sterile Petri dish. These were kept overnight in the refrigerator and the next morning, after thorough mixing, 1 g of the wet soil was added to 1 l of sterile water. The soil suspension was then stirred for 20 minutes on a magnetic stirrer. Plates were prepared bij mixing 1 ml of a 10^{-4} dilution soil suspension, with 8 ml of rose bengal-streptomycin agar in each of duplicate series. The plates were incubated at 25° C and the number of fungal colonies that developed was counted under the low power of a dissection microscope after 2, 4 and 7 days.

During the period from September 1958 until December 1959, the soil of the forest floor types was sampled 9 times. The concentration of fungi in the soil was calculated on a dry weight basis. Simultaneously with the fungal study, the numbers of bacteria and actinomycetes in the same samples were determined by plating 1 ml of a 10^{-5} and a 10^{-4} soil suspension in nutrient agar. Bacterial counts were made after 2, 4 and 7 days incubation at 30° C.

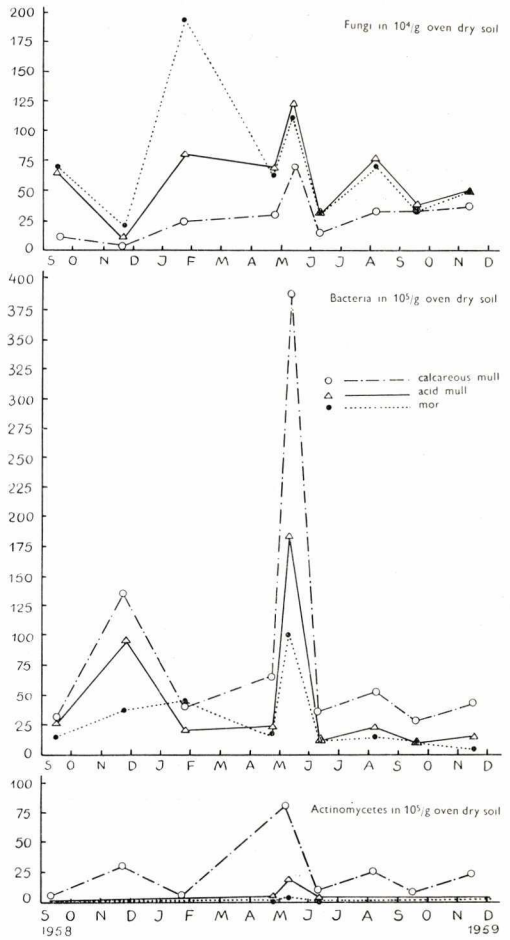

Fig. 5. Seasonal fluctuations in the counts of microorganisms from the mineral soil of three forest floor types of the Hackfort oak forest.

The results are listed in FIG. 5. The data obtained indicate that:

1. the fungal counts in the calcareous mull were lower than in the acid mull, and counts of bacteria and actinomycetes were highest in the calcareous mull type;
2. the annual fluctuations of the numbers of fungi as well as these of bacteria and actinomycetes were well synchronized in the three forest floor types;
3. the fungal counts in general showed maxima in January and May, whereas the bacterial and actinomycete counts were greatest in November and May.

In general, the ratio $\frac{\text{bacteria} + \text{actinomycetes}}{\text{fungi}}$ decreased in the sequence calcareous mull, acid mull and mor, the largest difference being between the first two humus

types. A similar trend for this ratio was reported by DUCHAUFOUR & POCHON (1955). Their values for the ratio $\frac{\text{bacteria}}{\text{fungi}}$ are for the same forest floor types 400, 75 and 50 respectively. In view of the large annual variations in this ratio its value seems of little importance.

The repeatedly observed correlation between the number of fungus colonies developing from a soil and its humus content (ORPURT & CURTIS, 1957; SAKSENA, 1955; TRESNER et al, 1954; WARCUP, 1951a; WEBLEY et al., 1952; WELVAERT, 1950) is not in accordance with the results obtained with the acid mull and mor samples, which had a considerable difference in humus content. A better basis for the understanding of the difference in numbers of fungi in the various forest floor types is the difference in pH between the calcareous mull on one hand, and the acid mull and mor on the other. WAKSMAN (1924) and JENSEN (1931) demonstrated that there was a definite correlation between the ratio $\frac{\text{fungi}}{\text{bacteria}+\text{actinomycetes}}$ obtained and the H-ion concentration of the soil, especially for pH-values below 5.5.

In November, under the influence of freshly fallen leaves, the soil of the mull types supported a relatively large number of bacteria, but few microfungi developed from these soils.

In January, however, fungal counts were high, especially in the mor soil. Possibly as a result of low temperatures and exhaustion of easily decomposible nutrients, bacterial numbers decreased and sugar and cellulose fungi took over. Only in the mor, bacterial counts did not decrease in January. This was possibly due to the late shedding and the dominance of oak in this area.

In April, both, bacterial and fungal counts were lower in the mor and higher in the calcareous mull than in January. The acid mull occupied an intermediate position. This difference in trend of the population densities between mull and mor was possibly caused by the difference in soil fauna of the humus types concerned. In the mull there were numerous soil burrowing and litter feeding animals. Their activity, which was most pronounced during winter and early spring, increased bacterial and fungal counts. In the acid mull, their activity was moderate, and in the mor almost absent.

The common maximum for fungi and bacteria in May was thought to be the result of favorable temperature and moisture conditions. Possibly, there was a release of easily decomposable nutrients after freezing and subsequent thawing in winter and spring. The maximum in May was more pronounced for bacteria than for fungi, and in the calcareous mull it was more pronounced than in the mor. A partial explanation may be found in the rhizosphere effect. This is rather strong in spring and results in a greater increase in bacteria than in fungi. It would also be more obvious in the mull type, with a higher vegetation mass and cover, than in the mor type. Many roots in the mor type were located in the fermentation and humus layer, and the rootmass in the mineral soil of the mor type was very low. The actinomycetes were most numerous in the calcareous mull and undoubtedly the high pH-values and the generally lower moisture content of the material were favorable to these organisms. Their most pronounced increase took place from January to April and counts were highest in May.

In June the low moisture content of the soil reduced all counts. Incidental rain showers in July and August, and subsequent remoistening of the soil caused a temporary recuperation of the microflora. Renewed drought in September and October reduced all counts anew. In November the moisture content of the soil and all counts increased.

FRUITING BODIES OF MACROFUNGI

MACROFUNGI OF MULL AND MOR

As plate counts did not supply any information as to the amount and species of macrofungi, an attempt was made to determine their presence at Hackfort.

During the fall of 1957 and 1958, and until December 1959, the number of mushroom species growing in all three forest floor types of the Hackfort oak forest was determined. The experimental area of the three forest floor types was searched and the fruiting bodies of the various species were taken to the laboratory for indentification. The total number of species collected in the three types is represented in FIG. 6. The species found more than once, and the number of times they were encountered during 13 searches in the period from August 1 until December 15, are listed in TABLE 6. Excluded from this table were 9 species growing on decaying tree stumps, twigs, branches and roots.

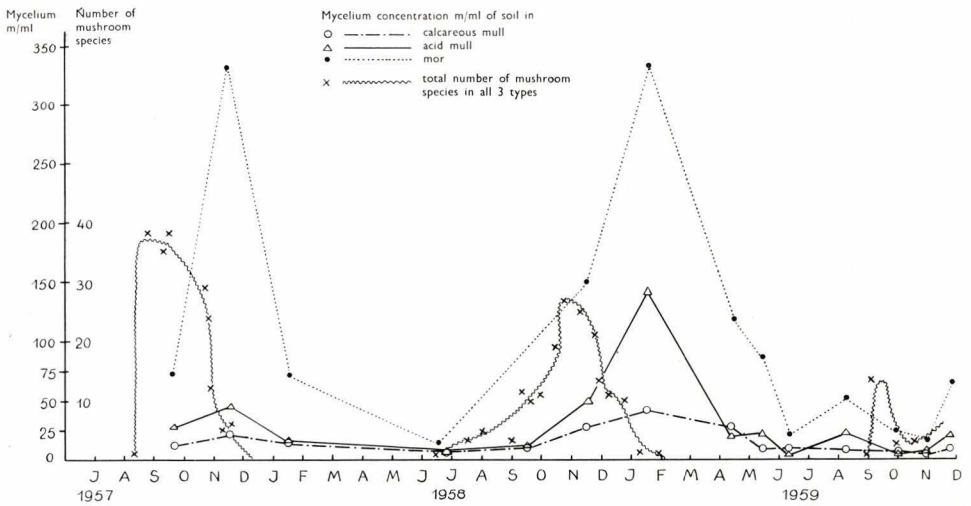

Fig. 6. Seasonal aspects of fruiting bodies of macrofungi and mycelium of the Hackfort oak forest.

The species belonging to the genera *Russula*, *Boletus* and *Lactarius* were identified and named according to PEARSON (1950). The remaining Basidiomycetes were named according to MOSER (1955), and the Ascomycetes according to the system of LINDAU (1922).

The total number of species found in 1957 and 1958 in the calcareous mull type was 35 and 32, in the acid mull type 44 and 33 and in the mor 46 and 31 respectively, indicating that from one year to another the absolute and relative number of species varied within and between the three forest floor types. The distribution of the species over the three forest floor types indicated a definite difference between the mushroom flora of

Table 6 — NUMBER OF FINDS OF SPECIES OF MACROFUNGI FOUND MORE THAN ONCE DURING 13 SEARCHES BETWEEN AUGUST 1 AND DECEMBER 15 IN THREE FOREST FLOOR TYPES AT HACKFORT

forest floor type	calcareous mull		acid mull		mor	
year	1957	1958	1957	1958	1957	1958
Lachnea hemisphaerica (Wigg.)	1	1				
Plicaria badia (Pers.)	2					
Macropodia macropus (Pers.)	2					
Humaria rutilans (Fr.)		2				
Clavulina rugosa (Bull. ex Fr.) Schroeter	1	1				
Typhula juncea (A. et S. ex Fr.) Karst	2	2				
Inocybe asterospora Quél.	1	2				
Inocybe geophylla (Sow. ex Fr.) Quél.	4	1				
Scleroderma verrucosum Vaill. ex Pers.		2				
Clavaria fistulosa Holmskj. ex Fr.	2	1		2		
Amanita muscaria (L. ex Fr.) Hooker		1	1	1		
Lycoperdon perlatum Pers.	3	1	1			
Lactarius necator (Pers. ex Fr.) Karst.			4	2		
Lactarius blennius Fr.			2			
Lactarius vietus Fr.			2			
Boletus subtomentosus (L.) Fr.			2	2		2
Boletus badius Fr.			1		1	1
Amanita vaginata (Bull. ex Fr.) Quél.			2	1	4	2
Psathyrella appendiculata Fr.			1	1	1	
Russula delica Fr.				2		1
Russula nigricans (Bull.) Fr.			1		3	
Lactarius vellereus Fr.			2	2	4	3
Lactarius subdulcis (Bull.) Fr.			2		3	
Hygrophoropsis aurantiaca (Wulf. ex Fr.) R.Mre					1	*
Hydrocybe hemitricha (Fr.)					1	2
Dermocybe cinnabarina (Fr.)					3	4
Russula emetica Fr.					3	
Russula cyanoxantha Schff. ex Fr.					1	3
Russula fragilis Fr.					4	4
Lactarius quietus Fr.					1	5
Paxillus involutus (Batsch) Fr.	1	5	3	2		1
Calocybe ionides (Bull. ex Fr.) Kühn	1	1	3		1	1
Clitocybe brumalis (Fr.) Quél.	1		4	3	1	2
Laccaria laccata (Scop. en Fr.) Bk. & Br.	5	4	5	1	5	3
Laccaria amethystina (Bolt. ex Fr.) Bk. & Br.	4	3	1	2	6	2
Collybia confluens (Pers. ex Fr.) Quél.	2	2	4	1	4	1
Collybia dryophila (Bull. ex Fr.) Quél.	2		3	2	5	3
Collybia butyracea (Bull. ex Fr.) Quél.	2	1	3	*	3	2
Oudemansiella platyphylla (Pers. ex Fr.) Mos.n.c.		1	2		1	1
Oudemansiella radicata (Relh. ex. Fr.) Bours.		1	2	1	2	
Mycena galopoda (Pers. ex Fr.) Quél.	5	3	6	4	5	4
Mycena sanguinolenta (A. & S. ex Fr.) Quél.	1		4	*	4	
Mycena epipterygia (Scop. ec Fr.) Gray	1		2	1	2	
Amanita phalloides (Vaill. ex Fr.) Secr.	1	1	2	1	2	7
Amanita mappa Batsch	2	1	1	2	3	5
Hydrocybe paleacea (Fr.)		1	1	2	1	
Stropharia aeruginosa (Curt. ex Fr.) Quél.	7	5	3	2	4	3
Coprinus disseminatus (Pers.) Fr.		*	1		1	
Lactarius chrysorrheus Fr.		1	3		2	
Scleroderma aurantium Vaill. ex Pers.	6	1	5	2	6	6

* = encountered outside the observation period.

the calcareous mull and mor. Since the forest floor types were characterized by combinations of phanerogams, a correlation between the herbaceous vegetation and macrofungi was expected. This type of correlation has been observed repeatedly (HAAS, 1933; PARKER–RHODES, 1951) and was discussed by COOKE (1948, 1953). HAAS considered mushrooms as valuable ecological indicators in areas where there is no vegetation of phanerogams. Whether the composition of the mushroom fiora is influenced directly by the soil or by the vegetation and forest floor type, will depend on the substrate of the various species.

The humus fungi, *Inocybe*, *Lycoperdon* and the Ascomycetes preferred the mull, with its exposed mineral soil, into which rather fresh litter material had been incorporated by the active burrowing saprophageous soil and litter fauna.

The mycorrhizal fungi were rare in the calcareous mull. Of the 3 *Lactarius* species, that appeared to be restricted to the acid mull type, 2 viz. *L. necator* and *L. vietus* Fr. are indicated in the literature to be associated with birch, as is *Amanita muscaria* (L. ex. Fr.) Hooker. As birch attained maximum growth on acid mull and was almost absent on mor, the occurrence of these fungi appeared to depend on the presence of birch. No such relation between oak and *Russula* species was established for the mor but *Lactarius quietus* Fr., also an exclusive mor species, seemed to be associated with oak.

Most litter fungi were present in all three forest floor types. The Clavariaceae were invariably found on poplar leaves. None of the fungi that were limited to the mor is known to have a preference for oak leaves. The most constant litter species on the mor, *Dermocybe cinnabarina* (Fr.), was recognized as a common inhabitant of beech litter. Only *Collybia dryophila* appeared almost exclusively on oak leaves.

The relatively high frequency of most species common to all types, indicated that their edaphic and climatic requirements were factors, neither limited nor critical.

Apart from the results with *Collybia dryophila* and the Clavariaceae, differences in the mycofloras of the various forest floor types appeared to be quantitative rather than qualitative and not suitable for characterization of the experimental areas.

PERIODICITY AND ABUNDANCE OF THE MACROFUNGI

The results shown in FIG. 6 indicate that a mushroom flora had developed by August 1957, but in 1958 it was October before a reasonable mushroom flora developed, having less species than in 1957. In 1959 the number of species was less than in 1958 and the time of their appearance was later. This was understandable since the amount of precipitation during August and September was 330 mm in 1957, 188 mm in 1958 and 52 mm in 1959.

The delay in appearance of mushrooms in 1958 as compared to 1957 amounted to 20 to 40 days, as listed below for some conspicuous species:

Paxillus involutus	20 days
Laccaria laccata	40 days
Lactarius necator	40 days
Stropharia aeruginosa	27 days
Clavaria fistulosa	20 days
Typhula juncea	20 days

The delay in appearance of Clavariaceae was caused by the late shedding of the poplar leaves in 1958. A similar shift in the time of appearance of mushrooms in relation to precipitation has been described by WARCUP (1951b).

An influence of precipitation on the associations of fungi was observed during the summer of 1958, when ephemeral species such as *Coprinus disseminatus*, *Mycena sanguinolenta* and *M. galopoda*, living on the surface layers of the litter, occurred together with mycorrhizal fungi like *Russula*, *Lactarius*, *Amanita* and *Boletus* species. The larger litter fungi were absent. The appearance of short lived species after light rain has also been observed by FRIEDRICH (1940) en WILKINS & HARRIS (1946).

The number of the species of macrofungi per 100 m² was in 1957 and 1958 for calcareous mull 4.4 and 4.0, for acid mull 0.7 and 0.5 and for mor 2.9 and 2.0. The areas considered were about 800, 6600 and 1600 m² respectively. The abundance of species indicated that the mushroom flora of these various forest floor types occupied a minimal area of approximately 800 m². This does not imply that these areas were populated by all species adapted to their ecological condition. PARKER-RHODES (1956) showed that as a result of the slow spread of the macrofungi, many species are lacking in suitable environments. Consequently the absence of a species in one or more forest floor types may create a false impression of specificity of the species.

The number and dry weight of fruiting bodies per 100 m², collected during the dry period of 1959 on 6 trips between September 1 and October 15, are shown in TABLE 7. Also presented are the average mycelium concentration and moisture content in the respective profiles, determined at 4 stations in each type at the beginning and the end of September.

Table 7 — ASPECTS OF FUNGAL GROWTH AND SOIL MOISTURE IN FOREST FLOOR TYPES AT HACKFORT DURING THE DRY PERIOD BETWEEN SEPTEMBER 1 AND OCTOBER 15, 1959

forest floor type	mushrooms		mycelium	moisture
	number of fruiting bodies per 100 m²	dry weight of fruiting bodies g/100 m²	average concentration m/ml 0–40 cm depth	average percentage w/w 0–40 cm depth
calcareous mull	8.3	17	1.7	5.2
acid mull	9.6	50	7.9	10.0
mor	2.5	13	7.6	4.1

These results indicate that the number of mushrooms and their dry weight per unit of surface area were highest in the acid mull type where, as a result of the prolonged drought, the moisture content of the loamy soil was less reduced than in the sandy soils of the other types. The influence of moisture was also expressed in the mycelium concentration in the profile.

During this period, the flora of macrofungi consisted almost entirely of a limited number of woodrotting and mycorrhizal fungi. SHOPE (1936, 1937) observed that extreme drought may even cause the absence of the fruiting bodies of certain species for several years.

In 1957 and 1958, the first appearance of the mushrooms coincided more or less with the accumulation of mycelium in the soil. The disappearance of the last fruiting bodies in both years was obviously the result of early night frosts. Before that time, however, the number of species decreased while the mycelium concentration increased.

MYCELIUM IN SOIL

MYCELIUM CONCENTRATION IN SOIL

A method to determine the mycelium concentration in soil

In 1916, CONN, discussing the relative importance of fungi and bacteria in soil, asserted that fungi were not active in the soil, but occurred merely as resting spores. In that same year WAKSMAN demonstrated indirectly, that there was mycelial growth in the soil. Later, CONN (1922) observed directly the presence of fungal hyphae in dried and stained soil smears on microscope slides. This method was put on a quantitative basis by JONES & MOLLISON (1948), by counting the number of fragments of fungal hyphae in a certain area of an agar film containing a soil suspension of known dilution. The method used in this work is based on that of JONES & MOLLISON, and will be referred to as the soil suspension slide method.

The following procedure was used to prepare the slides: One gram portions of a soil sample were weighed into each of 10 test tubes. To each tube 10 ml of a 5% aqueous solution of a mounting medium (Celodal I, Merck) was pipetted. The tubes were then shaken 20 times end over end, and permitted to stand undisturbed for 15 seconds, to allow for setting of the heavier particles. A 0.5 ml aliquot was withdrawn 2 cm below the surface of the suspension and spread over the surface of a slide (4 × 5 cm), warmed on a hot water bath at 90° C. During evaporation, it was necessary to stir the suspension with a needle in order to prevent flocculation of small mineral or humus particles. After drying, 25 microscope fields were examined under low power (300–400 ×) on each of the 10 slides. The mycelium observed in these fields was traced on paper using a camera lucida. The total length of the mycelium traced from the area examined, was measured with a map runner and the total length of mycelium per g or per ml of soil was calculated.

At the magnifications used, it was not necessary to stain the slides. Soil suspension slides can be filed as a permanent record and are practically indestructible. The accuracy of the method was ± 10.5 %. The method can be simplified by determining the number of intersections of the observed hyphae and the lines in an integration eyepiece (HENNING, 1958), rather than using a mirror and map runner.

Quantitative aspects of the mycelium concentration in mull and mor

From September 1957 until December 1959 the uppermost 2 cm of the mineral soil of the various types of the Hackfort forest were periodically sampled and the mycelium concentration was determined. The results are illustrated in FIG. 6.

It appeared that there was constantly more mycelium present in mor than in mull soil. Practically all this mycelium was thick and brown, and belonged possibly to Basidiomycetes. In November 1959 the share of this type mycelium on the soil suspension slides was 91, 98 and 100 % for calcareous mull, acid mull and mor respectively.

The difference in the amount of fungus mycelium found in mull and mor, was one of the factors used by MÜLLER (1887) to describe mull and mor. His observation has been confirmed repeatedly (WAKSMAN 1932, WILDE, 1954).

The rather constant difference of the mycelium concentration in mull and mor indicated that the causative factor acts continuously, albeit with variable effect. A constant

difference between mull and mor soil was found in their respective humus contents. Since organic matter is the source of energy for the soil fungi, it provides a possible explanation for the higher mycelium concentration in mor than in mull. As well as a difference in humus content, there was a difference in moisture content which may also contribute to the differences in the amounts of fungus mycelium. The loam in the acid mull type improved moisture conditions and favored fungus development. Other differences, apparent in the C/N ratio and the cellulose and lignin content of the litter, tended to favor growth of fungi, especially cellulose or lignin decomposing Basidiomycetes, in the mor soil.

Seasonal aspects of the mycelium concentrations in soil

FIG. 6 shows that the mycelium concentration in the various types fluctuated, due to an imbalance between growth and break down rates of mycelium. This fluctuation indicates that the high concentration of fungus mycelium in the soil was not an artifact resulting from accumulation of only dead mycelium.

The fluctuations observed in the mor were greater than in the calcareous mull, but were synchronized in all types and had a frequency of one cycle annually. The causative factor should thus have an annual rhythm, and should act in all types simultaneously. Climatic factors have the required characteristiscs. However, of these only the low moisture content of the soil in spring may be correlated with the already low and decreasing mycelium concentrations at that time. Indirectly, however, climatic factors may change the environment of the fungi and influence the amount of nutrients and growth factors in the soil by leaching.

The increase of the mycelium concentration after the new litter had been shed, appeared to be a biotic effect occurring after the number of bacteria had reached its fall maximum, and simultaneous with the increase of fungus counts in the same soil. The decline in mycelium concentrations after January 1959 coincided with a pronounced increase in actinomycetes, followed by an increase in bacteria.

The annual fluctuation of the mycelium concentration was similar in all soil types, but differed each year. The maxima in 1957–1958 and 1958–1959, differed by 14 instead of 12 months, and so there was a phase shift of about 2 months. As climatic factors were thought to be primarily responsible for the annual fluctuations, they are also apt to have caused the observed shift. In the previous chapter it was shown that climatic factors retarded the development of mushrooms for one to two months. It seems possible that both phenomena were governed by the same factor, in this case drought.

Shortly after the initiation of mycelium growth, mushrooms appeared and the mycelium concentration in the soil increased. This increase of mycelium continued, even when the number of species of mushrooms decreased, partly as a result of early night frost. In both years, $2\frac{1}{2}$ months after the number of mushroom species had reached a maximum, the rate of decomposition of mycelium appeared to surpass its rate of growth. This suggests that the mechanism of mycelium decomposition, may depend on some biotic factor rather than directly on the climatic factors.

Mycelium concentration in relation to soil conditions

In the previous section of this chapter the influence of season on the mycelium concentration of mull and mor was discussed. To study the influence of various soil factors, it was necessary to eliminate the influence of differing forest floors, soil types and seasons.

This was achieved by collecting mor samples in a Scotch pine wood at the National Park 'de Hoge Veluwe', and obtaining the average annual data of some soil factors and the corresponding mycelium concentrations. In this area on the slope of a fixed inland dune, there was a continuum which ranged from a relatively high dense stand with a thick litter layer on top of a deep layer of fine sand, having a water holding capacity of 32% w/w at the top of the slope, to an open and low stand, with little litter above shallow coarse sand, having a low waterholding capacity (18% w/w) at the foot of the slope. Concurrent with the decrease of productivity and surface litter there was a decrease of organic matter in the mineral soil.

Three areas along the continuum were selected as sampling sites. The characteristics of these sites are listed in TABLE 4. In each of the areas the soil was sampled with an auger at monthly intervals from December 1951 through October 1952. From the soil obtained at 5, 10, 25, 50, 75 and 100 cm beneath the surface, 6 ml samples were taken using a sampling tube of 16 mm diameter. One soil suspension slide was prepared from each sample. The mycelium concentration was determined by counting 45 fields per slide and the moisture and humus content of the samples were determined. Thus 3 areas were sampled for 11 months at 6 levels. By taking the average annual values for each sampling point, seasonal influences were eliminated. The data, thus obtained, are shown in FIG. 7.

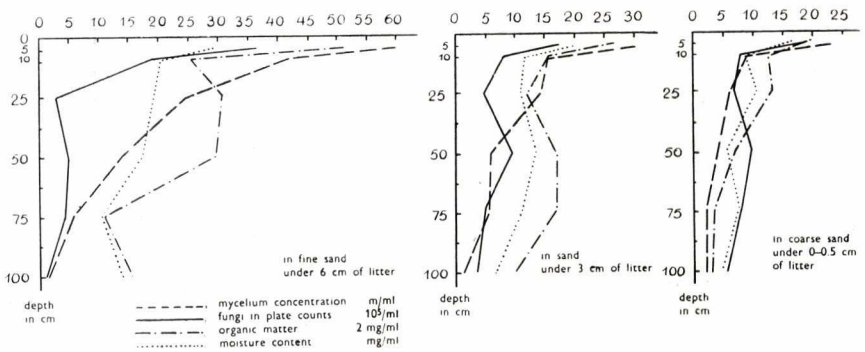

Fig. 7. Mycelium, fungal counts, organic matter and moisture in three areas of a Scotch pine continuum at the National Park „de Hoge Veluwe".

There appeared to be a positive correlation between mycelium concentration, humus content, and water content of the soil. The correlation coefficients calculated from the data were $r_{mh} = + 0.90$ (standard deviation mr = 0.05) for mycelium and humus, and $r_{mw} = + 0.92$ (mr = 0.04) for mycelium and water. A better correlation existed between the average annual mycelium concentration and the combined quantities of humus and water: $r_{m.hw} = + 0.94$ (mr = 0.03). The correlations were highly significant. The respective regression formulae were $m = 0.61\ h - 6.6$ and $m = 0.2\ w - 11.88$, in which m represents the mycelium concentration in m/ml, and h and w represent the quantities of humus and water respectively in mg/ml of soil. From the regression formulae it appeared that, as an average, 11 mg of humus and 59 mg of water should be present per ml, to yield a measurable amount of mycelium in this kind of soil. All calculations were according to the equations for correlation coefficients and linear regression functions as given by PATERSON (1939).

As in all the experimental areas the mineral soil was poor and as the main substrate

for fungus growth was Scotch pine litter, the correlation between mycelium concentration and humus content of the mineral soil is quite understandable. SAÎTO (1955b) did direct microscopy of mycelium in dunes and noted an increasing amount of mycelium with increasing humus content.

Humus increases the waterholding capacity of mineral soil, and in the present study there was a high correlation between water and humus content ($r_{wh} = + 0.91$).

FIG. 7 shows that with increasing depth, there was a greater decrease of mycelium concentration than of water and humus content. This decrease of the microbial population of the soil with increasing depth has also been observed in homogenous peat profiles (TIMONIN, 1935). BROWN (1958) mentioned that the quantity of mycelial fragments in dune sand was correlated with humus content and depth. RICHTER, as cited by WAKSMAN (1932) found a decreasing number of fragments of hyphae with increasing depth, and observed more mycelium in forest soil than in sand.

In FIG. 8, the mycelium concentration in samples taken from a depth of 5 cm below the litter layer in the three sampling areas is plotted against the month of the year. Each result was obtained from only one sample, but there was a general decrease in mycelium concentration after March, and rather high values were obtained in June and September for all areas.

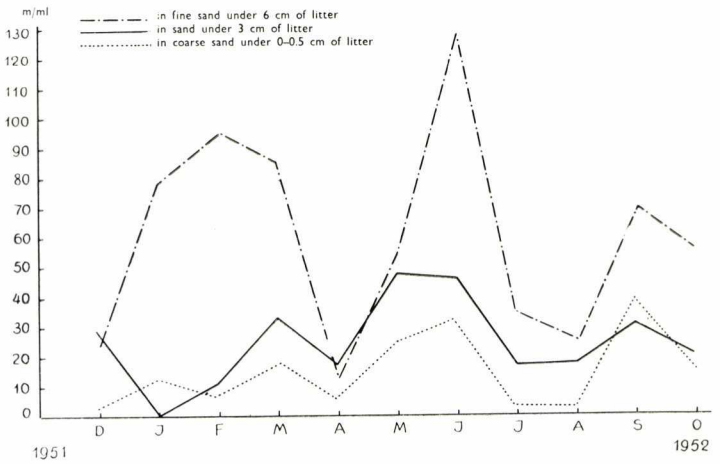

Fig. 8. Monthly variations in the mycelium concentration in m/ml at 5 cm depth in the mineral soil of three areas of a Scotch pine forest.

These results indicate that in 1952 there was no seasonal fluctuation in the pine stand as was observed in the oak stand at Hackfort. This difference suggests that the fluctuations were determined by the periodicity of litter fall, viz. the litter in the pine stand falls almost continuously throughout the year, whereas oak litter falls primarily in autumn.

Mycelium concentration in soil of various forest types

In previous sections of this chapter the mycelium content of a number of forest stands was described. For purposes of comparison, the annual average values obtained are listed in TABLE 8.

It is apparent that there was, in general, a high mycelium concentration in mor and a

low one in mull. In the soil of a poor mor with a thin litter layer, as in the pine stand, there was less mycelium than in a mor with a well developed litter layer. From TABLE 8 it appears that tree species were not primarily responsible for the mycelium concentrations or the type of humus.

Table 8 — ANNUAL AVERAGE MYCELIUM CONCENTRATION IN SOIL OF VARIOUS FOREST TYPES

year	locality	stand	forest floor type	mycelium m/ml
1959	Hackfort	oak	mor	108
1952	Hoge Veluwe	Scotch pine	mor	60
1952	Hoge Veluwe	Scotch pine	mor	31
1952	Hoge Veluwe	Scotch pine	mor, impoverished	27
1959	Hackfort	oak and birch	mull, acid	31
1959	Hackfort	oak, birch, alder and poplar	mull, calcareous	16

Mycelium concentration in relation to plate counts of fungi

The data obtained from the three forest floor types at Hackfort showed that the mycelium content and the number of fungal propagules fluctuated independently. This may be due to the fact that the soil suspension slides mostly detect hyphae of Basidiomycetes, whereas dilution plates favor the development of microfungi. Both groups of fungi have different ecological requirements.

However, in the previous chapter a high correlation between mycelium concentration and the amount of organic matter was established in three areas within a pine stand, having a mor forest floor type. Here, the waterholding capacity and consequently the organic matter and moisture content of the mineral soil controlled the mycelium concentration. Since organic matter is the source of energy for all saprophytic fungi, a correlation between numbers of microfungi and Basidiomycetes was expected.

This was tested by making counts of microfungi by the serial dilution technique on peptone-dextrose agar, pH 4.5, using soil collected at all sample points in the three areas of the continuum in January. The data thus obtained are shown in FIG. 7. A positive correlation between the mycelium concentration and the number of fungal propagules at the various sample points is obvious. For the 18 paired data the correlation coefficient for mycelium concentration and colonies developed (r_{mc}) was + 0.88 (mr = 0.05).

Similar tests were made using samples of soil profiles from an oak forest near Arnhem (Mariëndaal) and a heath at the National Park 'de Hoge Veluwe', both on mor. The results listed in FIG. 9 show a definite positive correlation between mycelium concentration and plate counts of fungi.

In these examples (FIG. 7, FIG. 9) there was a tendency for the fungal counts to decrease with depth at a slower rate than the corresponding mycelium concentrations. This may have been due to the tendency of microfungi to produce more spores under poor substrate conditions. The addition of fresh organic material to the upper layers of the profile may have caused lysis of spores or germtubes (CHINN, 1953). The washing down of spores (BURGES, 1950) may also have caused this effect.

To determine the extent to which mycelium on the soil suspension slides could develop into fungus colonies on rose bengal – streptomycin agar, the following procedure was used. An 1 g portion of the same soil samples taken from and used for the preparation of suspension slides and agar plates of the Hackfort forest in September 1958, was suspended in 10 ml of rose bengal – streptomycin agar. A 0.5 ml aliquot of this suspension was spread over an area of 18 × 32 mm on a microscope slide and allowed to solidify. The slides were incubated in a sterile moist chamber for 40 hours at 20° C, then covered with a coverslip and examined under the microscope. A similar method has been described by ROBERTS & BOLLEN (1955).

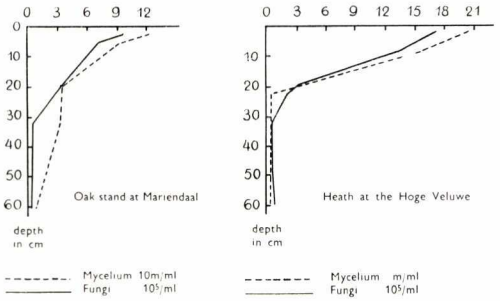

Fig. 9. Mycelium concentration and plate counts of fungi at various depths in the profiles of an oak stand and a heath.

In no case a fungus colony was observed to develop from hyphae, such as measured on soil suspension slides. This may be due to the short incubation period, the high concentration of the suspension, the composition of the medium, or the length of the fragments (average 0.06 mm). During the mixing of the sample, the hyphae broke up and lost part of their contents. SAÎTO (1955a) suggested that many hyphal fragments in soil

Table 9 — MYCELIUM CONCENTRATION, PLATE COUNTS AND ORIGIN OF FUNGUS COLONIES FROM SOIL OF THE FOREST FLOOR TYPES AT HACKFORT, SEPTEMBER 1958

forest floor type	calcareous mull	acid mull	mor
organic matter in %	10	12	26.5
number of fungi in 10^4/g from plate counts	30	64	66
mycelium concentration m/ml of soil	12	26	70
percentage of hyphae developing into fungus colonies	0	0	0
percentage of fungus colonies developing from humus particles	65	71	84
percentage of humus particles from which fungus colonies grew	17.5	17	18

the calcareous mull than in the mor, and so were the corresponding percentages of microfungi. This may have resulted from the richer composition of the mull litter as compared to that of the mor. The decrease in growth rate of the mycelium on the contact slides from December to February, coincided with an increased rate of accumulation of mycelium in the soil. The former was mainly due to microfungi, and the latter to Basidiomycetes.

The results indicate a marked decrease in growth rate of microfungi, although counts of microfungi on agar plates indicate a large increase in the number of their propagules over the same period (FIG. 14). This apparent contradiction may be clarified by the fact that sporulation of microfungi occurs after the exploitation of freshly exposed substrate, that is, after the main period of mycelial growth. BRIERLEY, JEWSON & BRIERLEY (1927) emphasized this phenomenon as a reason to reject the dilution plate method as a mean of measuring fungal activity in soil.

STUDIES OF SOME BIOTIC FACTORS INFLUENCING THE FUNGUS FLORA

Antibiotic influences

The first three sections of this chapter deal with the decrease in the amount of mycelium in soil, observed during the last part of the winter of 1958 and the spring of 1959. This decrease may have been caused by a reduction in growth rate or by an increased decomposition of mycelium or by both. Antibiosis may have been responsible for a reduction in growth rate. Wide-spread antibiotic effects were observed to originate from vegetation (Hessayon, 1953), litter (Melin, 1946; Melin & Wikén, 1946; Winter & Bublitz, 1953) or soil (Dobbs & Bywater, 1959; Dobbs & Hinson, 1953; Jackson, 1958).

To demonstrate the presence of antibiotic activity, soil strips (Witkamp & Starkey, 1956) were prepared containing various materials. These strips were made by impregnating the test materials in a metal mold, 1 × 8 cm and 0.5 cm high, with cooled 2% water agar. When the agar solidified, the strips were placed on a cellulose film, partly covering the surface of an assay plate. The plates were stored at 2° C for 16 hours, to permit diffusion of antibiotics from the soil strips into the test agar. In some tests the agar was seeded with a test organism before the soil strip was applied, in other experiments the cellulose film and soil strip were removed and the surface of the plates was streaked with several test organisms. In all cases the plates were incubated for 30 hours and the inhibition zones were measured. The medium used for fungi was rose bengal – streptomycin agar, and for bacteria nutrient agar was employed. The incubation temperatures for fungi and bacteria were 25 and 30° C respectively. Table 10 lists the results obtained with various constituants of a forest floor.

The data show that mineral soil from the acid mull and mor type inhibited the growth of *B. subtilis*, possibly due to its low pH. The test fungi were not inhibited by soil or by fresh leaves. Fresh leaf material of birch inhibited *B. subtilis*, whereas the activity of similar material from oak and poplar leaves was limited to a zone located underneath the strip. These results were not reflected in the number of fungi and bacteria isolated from the same leaves (table 2). By October, the leaves had lost most of their inhibitive effect.

Dobbs & Bywater (1959) indicated that there was a wide-spread fungistasis in soil, fluctuating with season. Since this fungistasis was eliminated by the addition of glucose (Dobbs & Hinson, 1953), it was understandable that on the peptone – dextrose agar as used in the present studies, no such effect was demonstrated.

Examples of local fungistasis or antibiosis in soil have been described (Brian, 1957; Novogroudsky, 1948; Stevenson, 1956a). Wright (1956) demonstrated the production of gliotoxin in unsterilized soil, containing pieces of straw inoculated with *Trichoderma viride*. This prompted tests with dead twigs from oak that were covered with profusely sporulating mycelium of *T. viride*. In the fall of 1957, 1958 and 1959, 36 infected twigs were collected from litter, and all caused inhibition of *B. subtilis*, when tested by the soil strip method (fig. 11).

To determine if *T. viride* was the causative organism, 10 twigs with and 10 without visible presence of this fungus, all collected from the same area (acid mull type of the

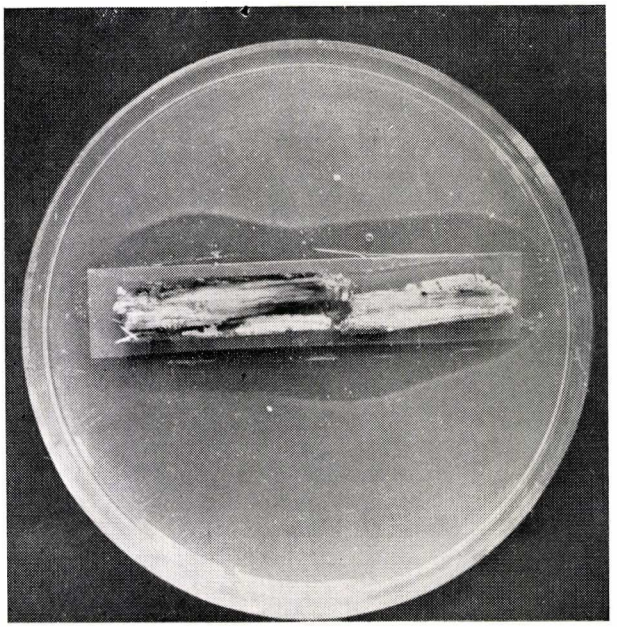

Fig. 11. Inhibition of *B. subtilis* by twig infested with *T. viride* (after incubation, agar strip with twig was put back on plate, to illustrate situation during diffusion).

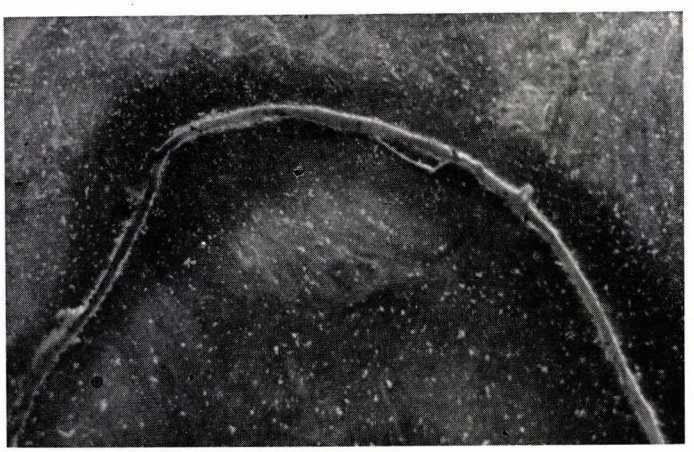

Fig. 12. Inhibition of *B. subtilis* by a mycelial strand of a Basidiomycete placed directly on agar surface.

Table 10 — INHIBITION OF VARIOUS ORGANISMS BY LITTER CONSTITUENTS AND SOIL FROM THE HACKFORT OAK FOREST, TESTED BY THE SOIL STRIP METHOD (width of strip = 10 mm)

type of material tested	pH	total width of zone of inhibition in mm		
		Bacillus subtilis	Aspergillus niger	Mucor silvaticus
Collected in May				
mineral soil from:				
calcareous mull	7.2	0	—	—
acid mull	3.6	11	—	—
mor	3.3	13	—	—
Collected in September				
mineral soil from:				
calcareous mull	—	0	0	2
acid mull	—	12	0	3
mor	—	14	0	5
oak leaves	4.8	9	0	0
alder leaves	4.9	0	0	0
poplar leaves	5.9	10	0	0
birch leaves	5.2	11	0	0
Collected in October				
oak leaves	—	6	—	—
alder leaves	—	6	—	—
poplar leaves	—	0	—	—
birch leaves	—	5	—	—
soil with mycelium, acid mull	5.8	12	—	—
alder leaf with *T. viride*	6.0	35	—	—
oak twig with *T. viride.*	6.4	30—35	—	—
bark of oak with *T. viride*	6.6	35—38	—	—
sapwood of oak with *T. viride*	6.5	32—35	—	—
heartwood of oak with *T. viride*	6.6	28—31	—	—
birch twig with *T. viride*	6.3	35	—	—
oak branch with *Stereum gausapatum*	5.0	15	—	—
mycelial strand from calcareous mull	7.0	12—17	—	—
mycelial strand from mor	—	10	—	—

Hackfort oak forest) and of the same age (less than one year on the forest floor) and dimension (8 × 1 cm), were tested on nutrient agar plates seeded with *B. subtilis*. All the twigs with *Trichoderma* caused inhibition of the test bacterium, whereas of those lacking the fungus, 7 showed no effect and 3 caused limited inhibition. The total area of inhibition produced by the first group was 16.3 × that of the latter. Ether extracts and juice pressed from the twigs were equally active. Antibiotic activity was detected after storage of the twigs for 3 weeks at 2° C. The appearance of sporulating mycelium of *T. viride* on oak twigs on the forest floor is very common in fall and winter. In some cases the fungus was observed on birch twigs and large branches of oak and once on an alder leaf. All these materials also inhibited growth of *B. subtilis* on agar plates, as shown in TABLE 10.

Soil covered with mycelium of an unidentified fungus, and oak wood with *Stereum gausapatum* also caused inhibition. Mycelial strands of saprophytic fungi that appeared to be lignin decomposing Basidiomycetes inhibited *B. subtilis*, when tested by the soil strip method. Pieces of mycelium, collected from a wooden pole in the mor of the Hackfort oak stand, were put directly on the surface of nutrient agar plates seeded with *B. subtilis*, and on a peptone-dextrose plate inoculated with *Botrytis allii*. After incubation, inhibition zones of 5 and 4 mm respectively were observed on both sides of the strand (FIG. 12). Hyphae bordering the inhibition zones showed typical curling effects (BRIAN & HEMMING, 1945).

These results show that the growth of bacteria on agar media may be inhibited by litter, litter fragments and soil. The effects may be local and due to fungal growth, or general and of unknown origin.

The ecological significance of antibiosis in the forest floor was investigated, using oak twigs infested with *T. viride*. Twigs with and without visible presence of the fungus, but otherwise identical, were ground and sieved to produce a homogeneous powder with a particle size of 0.5 mm. One g of the powder was stirred for 30 minutes in 1 l of water, and duplicate series of dilution plates containing nutrient agar or cellulose agar were prepared. Cellulose agar was included to determine the effects on bacteria that utilized both, simple and complex carbohydrates within the tissue of the twigs. To suppress the profuse development of *T. viride* on the plates, similar series were prepared to contain 50 p.p.m. of cycloheximide (= Acti-dione). The results obtained are shown in TABLE 11.

Table 11 — MICROORGANISMS DEVELOPING ON AGAR MEDIA FROM OAK TWIGS, WITH AND WITHOUT VISIBLE PRESENCE OF *Trichoderma viride* IN THOUSANDS

Agar medium	Incubation at 25° C in days	without *Trichoderma*		with *Trichoderma*	
		bacteria	fungi	bacteria	fungi
nutrient agar	11	10.0	7.0	0.2	4.7
nutrient agar + 50 p.p.m. Acti-dione	11	3.2	5.2	0.2	2.5
cellulose agar	9	22.5	7.0	0	13.0
cellulose agar + 50 p.p.m. Acti-dione	9	41.0	0	0	0.7

The data indicate that the numbers of bacteria, utilizing the constituents of nutrient broth or cellulose agar, were greatly reduced by the presence of *T. viride*. As this was the only fungal species observed on all plates containing the twig powder, it appeared

that the fungus flora was also significantly inhibited. KOÄRIK & RENNERFELT (1958) found that in fall it was difficult to isolate other fungi, from tree stumps infested with *T. viride*.

DECOMPOSITION OF MYCELIUM BY THE MICROFLORA

A rapid disappearance of fungus mycelium from the soil has been observed repeatedly (DOBBS & HINSON, 1953; PARK, 1956; STARKEY, 1938; TRIBE, 1957; WAID, 1960). To gain an insight in the contribution of some factors concerned with reducing the amount of mycelium in the calcareous mull and the mor, the number of mycolytic and chitin decomposing microorganisms in the mineral soil of these types was determined. Moreover the rate of disappearance of mycelium in the forest floor types was measured early in April 1959.

The number of mycolytic microorganisms was determined using the method of CARTER & LOCKWOOD (1957). Plates were poured to contain 10 ml of peptone-agar (0.5% peptone, 2% agar) seeded with 1 ml of a suspension of spores of *Glomerella cingulata* (20.000 spores/ml). After incubation for 2 days at 25° C the plates were sprayed with 0.5 ml of an appropriate soil suspension and reincubated for 2 or 3 weeks, when they were examined for clear zones, resulting from the activity of mycolytic organisms. The numbers of these organisms found were in the calcareous mull and in the mor 8.10^3 and 18.10^3 per gram of oven dry soil respectively.

The number of chitin decomposing organisms was determined according to the method of VELDKAMP (1955). Plates were poured with 8 ml of 2% water agar and, after solidifying, 4 ml of a 1% chitin agar was added.* After cooling, 1 ml aliquots of a series of soil suspensions were added to the surface of the medium and dispersed with a bend glass rod. After 2 weeks of incubation at 25° C, the number of clear zones indicated the abundance of chitin decomposing microorganisms. The numbers of these organisms in the calcareous mull and the mor in April 1959 were 89.10^5 and 9.10^5, and in July 21.10^5 and 8.10^5/g of oven dry soil respectively. In April the majority of chitin decomposing organisms from the calcareous mull were actinomycetes. The rest consisted mainly of bacteria and a few Phycomycetes. In the mor type, actinomycetes were a minority. During June, the percentage of the total number of organisms that were actinomycetes on the chitin plates, inoculated with soil from the calcareous mull, acid mull and mor, was 63, 37 and 33 respectively. Since it was difficult to distinguish actinomycetes on these plates, the foregoing values are minima. The data show that as the mycelium content decreased during April, when actinomycete counts on nutrient agar were highest, the number of chitin decomposing organisms and of chitin decomposing actinomycetes was higher than in July, when the decrease in mycelium came to a stop and actinomycete counts on nutrient agar were low.

A measure of the disappearance of fungus mycelium was obtained in April 1959. For this purpose 16 discs (3 mm thick, ⌀ 8.5 mm) were punched from stems of the cultivated mushroom *Agaricus campestris* (L.) Fr., and placed between the litter layer and the mineral soil of the forest floor types of the Hackfort oak forest. After 6 days, 4 discs were removed from each soil and cleaned and dried. The loss of weight of the discs in calcareous mull and mor was 62 and 67% respectively. After 15 days only one small portion of a disc was recovered from the mor.

In June 1959, the experiment was repeated. At that time the moisture content of the soil was low. After 3 days, the discs in the calcareous mull were lysed, but those in the

* The chitin agar was prepared and kindly supplied by Dr. H. Veldkamp.

appeared to spread an average of one fungus colony each. Large species produced more fungus colonies on the plates than small species. Some small animals did not spread any fungus colony but all spread bacterial colonies. The fungi disseminated, belonged predominantly to the genera *Penicillium*, *Mucor* and *Alternaria*.

Mites grown on a culture of *Aspergillus niger* and transferred to peptone-dextrose slants produced colonies of this fungus in 33 out of 36 cases.

Thirty slugs collected from the fruiting bodies of Basidiomycetes and placed on peptone-dextrose agar plates spread, without exception, propagulus of Fungi Imperfecti.

Direct observation showed the presence of fungus spores, adhering to the bristles of oribated mites.

Each of a number of droppings of mites and springtails, sown on peptone-dextrose agar, caused fungal colonies to develop. On examination under the microscope, these droppings always appeared to contain fragments of mycelium, and in many cases spores were also observed.

In order to investigate the possible importance of mites for the dissemination of fungi in sterile soil, the following experiment was conducted:

Six glass tubes (25 cm long, ⌀ 1 cm) were filled with forest soil, stoppered with cotton and sterilized (3 × ½ hr. at 115° C). After sterilization, 1 cm of the soil was removed from 2 tubes and replaced by unsterilized forest soil containing 5 oribatid mites. This procedure was repeated with 2 other tubes, but the mites were omitted. Two tubes were kept as control. After incubation for 8 days at 20° C, there was no growth of fungus mycelium in the control tubes. In the tubes containing only forest soil, mycelium had spread 1.5 cm along the tubes, whereas in the tubes containing forest soil and mites, mycelium was found growing at a point 15 cm from the inoculum. It spread from various centers along the sides of the tube, probably from the droppings of the mites. The mycelium observed, belonged to Fungi Imperfecti. When repeating this experiment with water saturated forest soil, Phycomycetes outgrew the mites in all inoculated tubes. Their mycelium extended for 20 cm along the side of the tubes.

DISCUSSION

From the determinations, counts and experiments described in the previous chapters, and with data on forest soil and its flora and fauna obtained from the literature, the fungus populations of mull and mor and their dynamics will be discussed.

The quantitative differences of the fungus flora in the mineral soils of Hackfort calcareous mull and mor may be characterized as follows: throughout the year mycelium concentration, mycelium growth, and numbers of fungi determined by plate counts, were lower in the calcareous mull than in the mor (FIG. 14).

In general the acid mull was intermediate to the other two types. A predominance of fungi in the mor has also been noticed by other authors (MÜLLER, 1887; WAKSMAN, 1932; WILDE, 1954).

The difference in the organic matter content of the two extreme forest floor types seemed to provide a basis for a difference in plate counts since organic matter is the source of energy for saprophageous fungi. Indeed, within one forest floor type there was a high positive correlation between plate counts and organic matter content (page 26). The similar counts in the mineral soil of the acid mull, which contained about 11% of organic matter, and the mor, which contained about 24% of organic matter, indicated that organic matter was not the only factor determining the number of propagules. Differences in pH may also have influenced the respective counts. The low acidity of the calcareous mull was not likely to cause a reduction of the concentration of fungal propagules nor of the growth rate of fungi. However, it enabled a great variety of bacteria and actinomycetes to develop normally, whereas the high acidity in the acid mull and the mor was likely to limit development of many species of these organisms. This view was supported by differences in plate counts of bacteria and actinomycetes in the mineral soil of the calcareous mull and the mor (FIG. 5).

The occasionally relatively high counts of these organisms obtained from the acid mull, as compared to those from the equally acid mor, however, suggested that there were other factors stimulating their development in the mull types, or suppressing their number in the mor type. Factors favoring bacteria in the mull were the botanical nature of the tree stand, and mass and botanical nature of the ground cover. As a result of its botanical composition, the litter in the mull contained more protein and easily decomposable carbohydrates, and less lignin and cellulose than in the mor (TABLE 1). This resulted in high bacterial counts on the components of the mull litter.

The botanical composition of the mull litter also attracted a great number of litter feeding and soil burrowing animals. In general, these animals preferred the litter of alder, poplar and birch to oak litter (VAN DER DRIFT, 1958). The comminution effect, as a result of which plate counts of bacteria increased more than fungal counts, caused an apparent stimulation of the bacterial flora (VAN DER DRIFT & WITKAMP, 1960). As there was more comminution of litter in the mull than in the mor, the bacterial flora was more stimulated in the former.

This reasoning also applies to the rhizosphere effect, which in general increases bacterial counts more than those of fungi. The higher mass of the ground cover and the consequently higher root mass in the mull types will be manifested as relatively high counts of bacteria in the mull. Even though the contribution of the herbaceous vegetation to the total annual litter fall was rather small, the rhizosphere effect of the roots of these

plants may have been of importance. The rhizosphere effect of herbs (STARKEY, 1938), is in general greater than that of trees with as well as without mycorrhiza (IVARSON & KATZNELSON, 1959; KÜRBIS, 1937).

Since the surface of the mineral soil of the calcareous mull, and to a lesser extent that of the acid mull, was subjected to desiccation, actinomycetes found a better competitive position in the mull than in the more easily desiccating mineral soil of the mor (KLAUSZ, 1940; MORROW, 1931; NIETHAMMER, 1937; ORPURT & CURTIS, 1957). On the other hand, both the mycelium and spores of macro- and microfungi are sensitive to desiccation (COOKE, 1955; KLAUSZ, 1940; MC. LENNAN, 1928; SHOPE, 1936, 1937; WARCUP, 1951b, 1957). This is especially true of lignin decomposing Basidiomycetes, which require a large food basis for succesfull infestation of new substrate, and suffer mycelial destruction from periodic desiccation. This was the situation in the mineral soil of the calcareous mull. There was greater continuity of the Basidiomycetes in the mor type, due to increased moisture and substrate conditions.

From the observation that bacteria and actinomycets found more suitable ecological conditions in the mull than in the mor, it followed that the fungi in the mull faced greater competition. Judging from the plate counts (TABLE 2) and experiments with both, micro- and macrofungi (MIKOLA, 1954, 1958), the mull litter itself offered favorable conditions for the development of fungi. In experiments with *Clavaria cristata*, it was observed that ethylene oxide-sterilized leaves of alder and poplar were more rapidly decomposed than those of birch and oak.

The low mycelium content of mull could be explained by the greater numbers of chitin decomposing and mycolytic microorganisms in this forest floor type than in the mor. According to this view, the low mycelium content of the alkaline mull soil would be the result of a rapid bacterial break down of senescent and dead hyphae (WARCUP, 1951a), and the high concentration in the mor would mainly be the result of accumulation of dead hyphae (BURGES, 1958). Even though fossilization of chitinous material was observed in deep, biologically inactive layers of the soil (BRADLEY, 1958; BURGES, 1958), the rapid increase and decrease of the mycelium concentration, and the low minimum mycelium content of the mor were not in accordance with this idea.

The relatively low mycelial growth rate and fungal plate counts in the mull as compared to these in the mor, seemed to indicate that the inhibition of fungal development was not the result of lysis and destruction of already formed senescent or dead hyphae, but that the inhibition took place at an earlier stage.

On the Cholodny slides, the hyphae were often found to be surrounded by bacteria (CONN, 1932). STARKEY (1938) found fungal mycelium very susceptible to bacterial attack and suggested that hyphae in soil do not persist for long periods. WAID (1960) observed that accumulation of fungal mycelium on nylon gauze in the soil ceased after 8 days at temperatures between 13 and 25° C, and suggested that this was due to increased breakdown of the mycelium.

These observations may be related to the observation of NOVOGROUDSKY (1948) concerning an interaction between bacteria and fungi. He noticed that bacteria developed in the thin membrane of water around hyphae in a manner similar to that in the rhizosphere of many phanerogams. As a result of their metabolic activity, the bacteria create anaerobic conditions around the hyphae, thus inhibiting the activities of the fungus, including the production of antibiotics. This finally results in lysis of the cells.

In this way the large bacterial population in the mull may have been able to keep growth and development of fungi at a low level.

The addition of fresh organic material to the soil stimulates the bacterial population

and has been observed to increase the rate of disappearance of fungal mycelium (VERPLANKE, 1932), fungal spores (CHINN, 1953; PARK, 1954, 1955, 1956) and sclerotia (MITCHELL et al., 1941) from the soil.

Fungi may produce antibiotics, active against bacteria. In one single biotope, WALLHÄUSSER (1951a) found 44% of 368 combinations of fungi and bacteria to result in an antibacterial effect caused by the fungi. In an acid soil, 50% of the fungi appeared to produce antibiotics (JEFFERYS et al., 1953). Antibiotic production as indicated in the case of twigs infested with *Trichoderma viride* may result in a localized decrease in the bacterial flora (TABLE 11). As soil fungi tend to populate micro habitats (GARRETT, 1956; KUBIENA & RENN, 1934; SCHMIDT, 1959; STANIER, 1953), they may exert effective antibiotic influences in these small areas. Antibiotics from mycelial strands of Basidiomycetes may possibly inhibit other microorganisms.

In general, no wide-spread antibiosis caused by fungi may be expected (BRIAN, 1957; THAYSEN, 1950; STEVENSON, 1956a). Adsorption of the antibiotics by soil constituents (DOMSCH, 1955; GOTTLIEB & SIMINOFF, 1950; WITKAMP & STARKEY, 1956), microbial degradation (JEFFERYS, 1952; PRAMER & STARKEY, 1951), dilution (WALLHÄUSSER, 1951b) and an unfavorable pH (JEFFERYS, 1952; WITKAMP & STARKEY, 1956), are some factors that may render antibiotics ineffective in soil.

The present differences between the fungus flora of the forest floor types at Hackfort seem to be caused and maintained by differences in the botanical composition of the vegetation, and subsequently by differences in the chemical composition of the litter and by the bacterial flora, the soil fauna and microclimatological conditions.

The factors responsible for differences in the vegetation and soil fauna, will be treated in subsequent publications.

The characteristics of each of the fungus floras in the forest floor types were found to fluctuate similarly throughout the year (FIG. 14). Changes in temperature and in moisture content of the soil were indicated as factors influencing changes in fungal and bacterial counts and the rate of mycelium growth. In addition to climatic factors, the time of shedding of the leaves was indicated as causing increased counts and maintenance of the mycelium growth rate, notwithstanding decreasing temperatures in the fall.

As a result of the combined action of these three main factors, plate counts showed two maxima. The first was in spring and was caused by increasing temperatures and possibly by the release of readily decomposable nutrients from litter that had fallen during winter. The second maximum was due to the readily decomposable content of the large litter fall in autumn. Between these two maxima, there were minimum counts or low growth rates, caused by low winter temperatures and summer desiccation of the soil. A similar trend was found in the number of colonies that developed from the leaves.

Variations in bacterial and fungal counts with season appeared to be caused by their modes of development. Bacterial counts reflected the numbers of individuals, able to develop on the substrate offered. Fungal counts were to a large extent a reflection of the number of spores in soil, formed by the mycelium as it aged.

In general, variations in the fungal population of the soil, as measured by plate counts, were small. (BROWN & HALVERSON, 1919; BRIERLY et al., 1927; TRESNER et al., 1954; WAKSMAN, 1932; WARCUP, 1951a). Maximum fungal counts in fall are most common (KLAUSZ, 1940; MILLER et al., 1957; SAÎTO, 1955a; STEVENSON & CHASE, 1957). Studies, using cultivated soil, confirm the observed influences of temperature, moisture and organic matter (GUILLEMAT & MONTÉGUT, 1956).

In contrast to the foregoing, the fluctuations of the mycelium concentration in the

mineral soil showed a different pattern (FIG. 14). Accumulation of mycelium starts before the falling of fresh litter, permitting moisture conditions. This becomes apparent with the appearance of tufts of mycelium, growing out from the larger organic debris, and of the fruiting bodies (FIG. 13).

Many suggestions as to the appearing of the fruiting bodies have been made. The influence of moisture on the time of their appearing has already been mentioned. According to GRAINGER (1946) and WILKINS & HARRIS (1946), the fall is the only season in which soil temperature, moisture conditions and nitrogen supply are simultaneously adequate to fulfil the requirements for the formation of the fruiting bodies. After the summer rains, the free amino acid content of the soil is relatively high (BIRCH, 1958; HAGENZIEKER, 1957; STEVENSON, 1956b), and numbers of microfungi and bacteria are still low. The fresh litter has not yet fallen, and last year's litter is devoid of most of

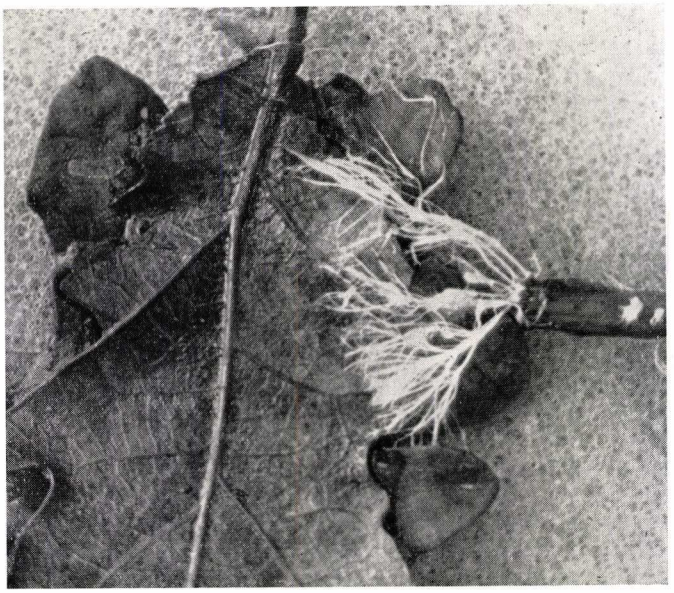

Fig. 13. After the dry summer, mycelium grows from pieces of wood onto freshly fallen leaves

its readily decomposable carbohydrates and proteins, and the percentage of cellulose and lignin in the litter is relatively high.

From this time on, the concentration of mycelium rises at an increasing rate. Growth rate increasingly exceeds death and disappearance of mycelium. During this period there is a decrease in temperature, which undoubtedly decreases the rate of mycelial growth (LINDEGREN, 1933). From this it seems plausible to assume that there is a decrease in some form of antibiosis, or an increase in some growth stimulating factor.

A general mycostasis in forest soil, inhibiting germination of spores of *Mucor rammannianus* until September was observed by DOBBS & BYWATER (1959). They also observed mineral soil to cause a reduction in the growth rate of mycelium of many Basidiomycetes. Whether this phenomenon showed a seasonal fluctuation similar to that observed for spores of *Mucor*, was not mentioned.

Addition of fresh organic matter may end such wide-spread fungistasis (CHINN & LEDINGHAM, 1957).

The numbers of bacteria and actinomycetes decreased in early winter and consequently the breakdown of mycelium by mycolytic and chitin decomposing bacteria and actinomycetes may also have decreased, resulting in accumulation of fungus mycelium.

Stimulation of the growth of Basidiomycete mycelium may be caused by the supply of vitamins (FRIES, 1943), resulting from bacterial growth on the fresh litter (SCHMIDT & STARKEY, 1951). It is also possible that the addition of readily decomposable substances increased the inoculum potential of the Basidiomycetes, resulting in an increased utilization of lignin and cellulose (NORKRANS, 1950).

The fluctuation of mycelium concentration from fall to spring was roughly inversely proportional to mycelium growth as measured on Cholodny slides and the soil temperature. Since the rate of mycelium growth of the Basidiomycetes was positively correlated with temperature, the decrease in mycelium concentrations in the spring may have been caused by factors decreasing mycelium growth, or by increased destruction of mycelium.

Adverse factors to mycelium development include:
substrate exhaustion and subsequent autolysis, (BRIAN, 1960);
inadequate replenishment of essential vitamins, resulting from low activity of yeasts and bacteria in winter;
cessation of mycelium growth, caused by staling products after complete colonization of the substrate (BARTON, 1960);
increased bacterial activity in spring and subsequent inhibition of mycelium development as described by NOVOGROUDSKY (1948).

In addition to autolysis, increased destruction of mycelium may be caused by increasing number or/and activity of chitin decomposing and mycolytic organisms, or by increased mycelium consumption by the soil fauna. In the calcareous mull type, where the first groups prevailed, there was actually an increase of actinomycetes from January until May. Both, the mycolytic bacteria and fungivorous fauna of soil will increase in numbers and activity during this period under the influence of rising temperatures. The total consumption by the fungivorous fauna at that time is adequate to account for a rate of decrease of mycelium from the mor soil of 2 m/cm^3 per day (page 37).

The $2\frac{1}{2}$ months between maximum development of the fruiting bodies and maximum mycelium concentration suggests the accumulation of a group of mycelium destroying organisms, that required $2\frac{1}{2}$ months to catch up with mycelium growth.

A possible explanation for the rapid increase in mycelium concentration during the winter is a different response of the fungi and their antagonists or predators to changes in temperature. If Basidiomycetes were more active at low temperatures than their microbial antagonists or animal predators, an inverse proportionality between mycelium concentration and soil temperature would be obtained.

It was found that various species of tree leaves have their own mycoflora (TABLE 2). KONING (1904) has already mentioned a difference in fungus species in the litter of the leaves of different tree species. SMIT & WIERINGA (1953) and CHESTERS (1950) found that tree leaves and branches were colonized by fungi, when still on the tree. After the leaves were on the soil surface for only a few days they were colonized by fungi, bacteria and actinomycetes. Incidentally, certain groups of fungi will be present on the leaves for only a short period of time, possibly because of climatic or substrate

conditions. The number of colonies growing from oak leaves of the mull was higher than that from oak leaves of the mor, possibly due to direct contact with the environment.

More than one year old oak leaves contained more microfungi than younger litter, and there was a qualitative difference in the respective mycofloras (TABLE 5). Similar differences in the fungus population of various layers of the forest floor have been described by KENDRIK (1958) and SAÎTO (1956). In general there was a sequence of bacteria, microfungi and finally Basidiomycetes on the decaying material, resulting in exhaustion of easily decomposable nutrients and subsequent attack on the more resistant residues. This succession is also known to occur during the preparation of compost for commercial growing of mushrooms (GARRETT, 1956). A similar sequence of bacteria and microfungi was found in the droppings of litter feeding soil animals (page 37).

Differentiation of the microflora during decay results in the characteristic mycofloras of the various litter layers and mineral soil of the mor.

The constant activity of the soil fauna, and the resulting lack of litter accumulation in the calcareous mull tend to make differentiation less pronounced in this forest floor type. Consequently the mycofloras of mull and mor are qualitatively different, especially in the later stages of decay, when Basidiomycetes prevail.

SUMMARY

This work was part of a study regarding the mechanism of the breakdown of forest litter. Some quantitative aspects of the mycoflora of the forest floor were determined. The results were discussed in view of environmental conditions:

1. Freshly shed leaves of various tree species had a qualitatively and quantitatively limited flora of fungi developing on agar plates, which consisted mainly of Sphearopsidales, *Pullularia* and *Cladosporium*. During colonization this flora increased in number and species (TABLE 2). Within one forest floor type it became qualitatively more similar for the leaves of different tree species. During the first winter the number of colonies developing from oak leaves decreased. In the second fall a maximum was reached. This coincided with an increase in fungi that developed on cellulose and lignin agar (FIG. 3). In the second year the number of colonies developing from oak leaves was higher than in the first year. The genera *Penicillium* and *Alternaria* were partly replaced by *Trichoderma* and *Mortierella*. From oak leaves from mull more colonies and species developed than from oak leaves from mor (TABLE 5, page 9).

2. In the mineral soil of the calcareous mull, fungal plate counts, mycelium growth and mycelium concentration were lower than in the mor. Plate counts of bacteria and actinomycetes were highest in the calcareous mull. The results obtained in the acid mull were usually intermediate (FIG. 5, 6, 10). The characteristics of the fungus flora of the various forest floor types seemed to be caused primarily by water supply and calcium content. These factors determined the vegetation, which influenced the chemical composition of the litter. The fresh litter in the calcareous mull contained more protein, and less cellulose and lignin than in the mor (TABLE 1). The composition and activity of the fungus flora of the mineral soil was influenced directly by the litter, the desiccation and pH of the soil, and the activity of the saprophageous soil fauna and the non-fungal microflora.

3. Fluctuations in the numbers of microorganisms and mycelium growth in the mineral soil were caused by fluctuations in temperature and moisture, and the addition of fresh litter. These fluctuations were similar in the various forest floor types. Fluctuations in the rate of growth of mycelium and numbers of bacteria were closely related (FIG. 10, FIG. 5).

4. The concentration of mycelium in an oak forest was highest in fall or winter, lowest in spring and summer (FIG. 6). This annual fluctuation was not found in a pine stand (FIG. 8). The rate of breakdown of mycelium was almost equal in calcareous mull and in mor. In the calcareous mull, there were 4 to 10 × as many chitin decomposing and mycolytic microorganisms as in the mor. In the mor, mycelium feeding oribatid mites prevailed. Their individual consumptive capacity was in summer 3 × as high as in winter.

5. The mycelium concentration in the mineral soil of one single forest floor type appeared to be positively correlated with humus and moisture content, and negatively with depth. The mycelium concentration in the mineral soil was in general higher in mor than in mull (TABLE 8).

6. The predominance of thick and brown hyphae on the soil suspension slides (page 22) and their low percentage on the Cholodny slides (page 28), indicated slower decomposition and growth of this mycelium than of the slender white mycelium.

7. Macrofungi appeared in highest numbers about 2½ months before the maximum myceli-

um concentration (FIG. 6). Their numbers and masses were not correlated with the mycelium concentrations in the various floor types (TABLE 7). Their appearance was partly determined by the moisture content of the substrate. Drought reduced the number of mushroom species (FIG. 6). Larger organic debris protected their mycelium against desiccation. There were characteristic macrofungi for each forest floor type. In the calcareous mull these were mostly humus and litter fungi. Mycorrhyzal fungi dominated in the acid mull and mor. Litter fungi appeared in all types (TABLE 6).

8. Concentrations of mycelium, formed under natural conditions, were able to inhibit the development of microorganisms (FIG. 11, 12; TABLE 11).

9. The comminution of litter by saprophageous animals stimulated fungal counts, especially in poor soil (page 37). Bacterial counts were more stimulated than fungal counts.

REFERENCES

ADAMETZ, I., 1886.Untersuchungen über die niederen Pilze der Ackerkrume. Inaug. Diss., Leipzig.

BARTON, R., 1960. Antagonism amongst some sugar fungi. *The ecology of soil fungi.* Univ. Press, Liverpool. (in the press)

BIRCH, H. F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil 10*, 9–31.

BRADLEY, P. L., 1958. An assemblage of arthropod remains from a Roman occupation site at St. Albans. *Nature 181*, 435–436.

BRIAN, P. W., 1957. The ecological significance of antibiotic production. *Microbial ecology*, 168–188, Univ. Press, Cambridge.

BRIAN, P. W., 1960. Antagonistic and competitive mechanisms limiting survival and activity of fungi in soil. *The ecology of soil fungi.* Univ. Press, Liverpool. (in the press)

BRIAN, P. W. & HEMMING, H. G., 1945. Gliotoxin, a fungistatic metabolic product of *Trichoderma viride. Ann. appl. Biol. 43*, 214–220.

BRIERLEY, W. B., JEWSON, S. T. & BRIERLEY, M., 1927. The quantitative study of soil fungi. *Proc. First Int. Congr. Soil Sci. 3*, 48–71.

BROWN, J. C., 1958. Fungal mycelium in dune soils estimated by a modified impression slide technique. *Trans. Brit. mycol. Soc. 41*, 81–88.

BROWN, P. E. & HALVORSEN, W. V., 1919. Effects of seasonal conditions and soil treatment on bacteria and molds in soil. *Iowa Agr. Exp. Stat. Res. Bull. 56*, 256.

BURGES, A., 1950. The downward movement of fungal spores in sandy soil. *Trans. Brit. mycol. Soc. 33*, 142–147.

BURGES, A., 1958. Micro-organisms in the soil. Hutchinson Univ. Library, London.

CARTER, H. P. & LOCKWOOD, J. L., 1957. Methods for estimating numbers of soil micro-organisms lytic to fungi. *Phytopathology 47*, 151–154.

CHESTERS, C. G. C., 1940. A method of isolating soil fungi. *Trans. Brit. mycol. Soc. 24*, 352–355.

CHESTERS, C. G. C., 1950. On the succession of microfungi associated with the decay of logs and branches. *Trans. Lincolnshire Naturalists Union*, 129–135.

CHINN, S. H. F., 1953. A slide technique for the study of fungi and actinomycetes in soil with special reference to *Helminthosporium sativum. Canad. J. Bot. 31*, 718–729.

CHINN, S. H. F. & LEDINGHAM, R. J., 1957. Studies on the influence of various substances on the germination of *Helminthosporium sativum* spores in soil. *Canad. J. Bot. 35*, 697–701.

CHOLODNY, N., 1930. Uber eine neue Methode zur Untersuchung der Bodenmikroflora. *Arch. f. Mikrobiol. 1*, 620–652.

CONN, H. J., 1916. Relative importance of fungi and bacteria in soil. *Science 44*, 857–858.

CONN, H. J., 1922. A microscopic method for demonstrating fungi and actinomycetes in soil. *Soil Sci. 14*, 149–152.

CONN, H. J., 1932. A microscopic study of certain changes in the microflora of soil. *N. Y. Agr. Exp. Stat., Techn. Bull. 204*, 1–21.

CONWAY, E. J., 1950. Microdiffusion and volumetric error, Crosby Lockwood, London.

COOKE, W. B., 1948. A survey of literature on fungus sociology and ecology. *Ecology 29*, 376–382.

COOKE, W. B., 1953. A survey of literature on fungus sociology and ecology-II. *Ecology 34*, 211–222.

COOKE, W. B., 1955. Subalpine fungi and snowbanks. *Ecology 36*, 124–130.

DOBBS, C. G. & BYWATER, J., 1959. Studies in soil mycology. *Rep. Forest Res. 1957/58, For. Comm.*, London, 98–104.

DOBBS, C. G. & HINSON, W. H., 1953. A widespread fungistasis in soils. *Nature 172*, 197.

DOMSCH, K. H., 1955. Die Kultivierung von Bodenpilzen auf bodenähnlichen Substraten. *Arch. f. Mikrobiol. 23*, 79–87.

DUCHAUFOUR, Ph. & POCHON, J., 1955. Note sur la biologie des humus forestiers. *Ann. Inst Pasteur 88*, 261–265.

FRIEDRICH, K., 1940. Untersuchungen zur Ökologie der höheren Pilze. Gustav Fisher, Jena.

FRIES, N., 1943. Untersuchungen über Sporenkeimung und Mycelentwicklung bodenbewohnender Hymenomycetes. *Symb. Bot. Upsal. 6*, 1–81.

GARRETT, S. D., 1956. Biology of root-infecting fungi. Univ. Press, Cambridge.

GOTTLIEB, D. & SIMINOFF, P., 1950. The activity of antibiotics in soil. *Proc. VII Int. Bot. Congr.*, 449–450.

GRAINGER, J., 1946. Ecology of the larger fungi. *Trans. Brit. mycol. Soc. 29*, 52–63.

GUILLEMAT, J. & MONTÉGUT, J., 1956. Contribution a l'étude de la microflora fongique des sols cultivés. *Ann. épiphyt. 7*, 472–540.

GUILLEMAT, J. & MONTÉGUT, J., 1957. Deuxième contribution a l'étude de la microflora fongique des sols cultivés. *Ann. épiphyt. 8*, 185–207.

HAAS, H., 1933. Die bodenbewohnenden Groszpilze in den Waldformationen einiger Gebiete von Württemberg. *Beih. Bot. Cbl. 50, 2e Abt.*, 35–134.

HAGENZIEKER, F., 1957. Soil-nitrogen studies at Urambo, Tanganyika Territory, East Africa. *Plant and Soil 9*, 97–113.

HENNING, A., 1958. Messen und Zählen in der Mikroskopie. *Zeiss-Werkz. 30*, 78–86.

HESSAYON, D. G., 1953. Fungitoxins in the soil: I Historical. *Soil Sci. 75*, 317–327.

HOLMBERG, B., 1936. Lignin-Untersuchungen, XI Mitt.: Fichtenholz und Mercapto-säuren. *Ber. dtsch. chem. Ges. 69*, 115–119.

IVARSON, K. C. & KATZNELSON, H., 1959. Studies on the rhizosphere microflora of yellow birch seedlings. *Bacteriol. Proc.*, Proc. 59th Gen. Meeting, 26.

JACKSON, R. M., 1958. An investigation of fungistasis in Nigerian soils. *J. gen. Microbiol. 18*, 248–258.

JEFFERYS, E. G., 1952. The stability of antibiotics in soils. *J. gen. Microbiol.* 7, 295–312.

JEFFERYS, E. G., BRIAN, P. W., HEMMING, H. G. & LOWE, D., 1953. Antibiotic production by the microfungi of acid heath soils. *J. gen. Microbiol. 9*, 314–341.

JENSEN, H. L., 1931. The fungus flora of the soil. *Soil Sci. 31*, 123–158.

JENSEN, H. L., 1934–36. Contributions to the microbiology of Australian soils I-IV. *Proc. Linn Soc. N.S.W. 59*, 101–117, 200–211; *60*, 145–154; *61*, 27–55.

JONES, P. C. T. & MOLLISON, J. E., 1948. A technique for the quantitative estimation of soil microorganisms. *J. gen. Microbiol. 2*, 54–69.

KENDRICK, W. B., 1958. Micro-fungi in pine litter. *Nature 181*, 432.

KLAUSZ, D., 1940. Zur Kenntnis der Bodenmikroorganismen und ihrer Tätigkeit zu verschiedener Jahreszeit und bei verschiedener Bodenreaktion. *Bodenk. und Pflanzenern. 66*, 365–407.

KOÄRIK, A. & RENNERFELT, E., 1958. Investigations on the fungal flora of spruce and pine stumps. *Medd. Stat. Skogsforskn. Inst. 47*, 7.

KONING, C. J., 1904. Contributions à la connaissance de la vie des champignons humicoles et des phénomènes chimiques qui constituent l'humification. *Arch. Neerl. Sci. Ext. Nat. 9*, 35–107.

KUBIENA, W. & RENN, C. E., 1934. Micropedological studies of the influence of different

organic compounds upon the microflora of the soil. *Zbl. f. Bakteriol. Parasitenk. u. Infektionskr. 2e Abt. 91*, 267–292.

KÜRBIS, P., 1937. Mykologische Untersuchungen über den Wurzelbereich der Esche *(Fraxinus excelsior* L.). *Flora 131*, 129–175.

LA TOUCHE, C. J., 1948. Slide traps for soil fungi. *Trans. Brit. mycol. Soc. 31*, 281–284.

LINDAU, G., 1922. Die mikroskopischen Pilze. Julius Springer, Berlin.

LINDEGREN, R. M., 1933. Decay of wood and growth of some Hymenomycetes as effected by temperature. *Phytopathology 23*, 73–81.

MARTIN, J. P., 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Sci. 69*, 215–232.

MC. LENNAN, E. I., 1928. The growth of fungi in soil. *Ann. appl. Biol. 15*, 95–109.

MELIN, E., 1946. Der Einflusz von Waldstreuextrakten auf das Wachstum von Bodenpilzen, mit besonderer Berücksichtigung der Wurzelpilze van Bäumen. *Symb. Bot. Ups. 8*, 116.

MELIN, E. & WIKÉN, T., 1946. Antibacterial substances in water extracts of pure forest litter. *Nature 158*, 200.

MEYER, F. H., 1959. Untersuchungen über die Aktivität der Mikroorganismen in Mull, Moder und Rohhumus. *Arch. f. Mikrobiol. 33*, 149–169.

MIKOLA, P., 1954. Experiments on the ability of forest soil Basidiomycetes to decompose litter material. *Commun. Inst. Forestry Fenn. 42*, 7.

MIKOLA, P., 1958. Studies on the decomposition of forest litter by Basidomycetes. *Commun. Inst. Forestry Fenn. 48*, 2.

MILLER, J. H., GIDDENS, J. E. & FOSTER, A. A., 1957. A survey of the fungi of forest and cultivated soils of Georgia. *Mycologia 49*, 779–808.

MITCHELL, R. B., Adams, J. E. & THOM, C., 1941. Microbial responses to organic amendments in Houston black clay. *J. agr. Res. 63*, 527–534.

MORRIS, D. L., 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. *Science 107*, 254–255.

MORROW, M. B., 1931, Correlation between plant communities and the reaction and microflora of the soil in South Central Texas. *Ecology 12*, 497–507.

MOSER, M., 1955. Agaricales und Gastromycetales. Kleine Kryptogamenflora von Mitteleuropa II b. Gustav Fisher, Stuttgart.

MÜLLER, P. E., 1887. Studien über die natürlichen Humusformen und deren Einwirkung auf Vegetation und Boden. Julius Springer, Berlin.

NIETHAMMER, A., 1937. Die mikroskopischen Boden Pilze. W. Junk, Den Haag.

NOORDAM, D. & VAN DER VAART-DE VLIEGER, S. H., 1943. Een onderzoek naar de samenstelling en betekenis van de fauna van eikenstrooisel. *Ned. Boschb. T. 16*, 470–492.

NORKRANS, B., 1950. Studies in growth and cellulolytic enzymes of *Tricholoma. Symb. Bot. Upsal. 11*, 1.

NOVOGROUDSKY, D. M., 1948. The colonisation of soil bacteria on fungal hyphae. *Microbiologiya 17*, 28–35.

ORPURT, P. A. & CURTIS, J. T., 1957. Soil microfungi in relation to the prairie continuum in Wisconsin. *Ecology 38*, 628–637.

PARK, D., 1954. An indirect method for the study of fungi in the soil. *Trans. Brit. mycol. Soc. 37*, 405–411.

PARK, D., 1955. Experimental studies on the ecology of fungi in soil. *Trans. Brit. mycol. Soc. 38*, 130–142.

PARK, D., 1956. Effect of substrate on a microbial antagonism with reference to soilconditions. *Trans. Brit. mycol. Soc. 39*, 239–259.

PARKER–RHODES, A. F., 1951. The Basidiomycetes of Skokholm Island XII. Correlation with the chief plant associations. *The New Phytologist 50*, 227–243.

PARKER–RHODES, A. F., 1956. Distribution of fungi in a small wood. *Ann. Bot 20*, 251–264.

PATERSON, D. D., 1939. Statistical technique in agricultural research. Mc. Graw–Hill, New York, London.

PEARSON, A. A., 1950. The genus *Lactarius*; British Boleti; The genus *Russula*. *The Naturalist*, The University, Leeds.

PRAMER, D. & STARKEY, R. L., 1951. Decomposition of streptomycin. *Science 113*, 127.

ROBERTS, J. E. & BOLLEN, W. B., 1955. A microplating method for soil molds and its use to detect some effects of certain insecticides and herbicides. *Appl. Microbiol. 3*, 190–194.

SAÎTO, T., 1955*a*. The significance of plate counts of soil fungi and the detection of their mycelia. *Ecol. Rev. 14*, 69–74.

SAÎTO, T., 1955*b*. Soil microflora of a coastal dune. *Sci. Rep. Tôhoku Univ.*, Ser. 4, *22*, 145–151.

SAÎTO, T., 1956. Microbiological decomposition of beech litter. *Ecol. Rev. 14*, 141–147.

SAKSENA, S. B., 1955. Ecological factors governing the distribution of soil microfungi in some forest soils of Sagar. *J. Indian bot. Soc. 34*, 262–298.

SCHMIDT, H. L., 1959. Beitrag zur Ermittelung der Pilzbesiedlung bei natürlichen Böden und ihrer Kennzeichnung durch ein besonderes Isolierungsverfahren. *Arch. f. Mikrobiol. 32*, 224–233.

SCHMIDT, E. L. & STARKEY, R. L., 1951. Soil organisms and plant growth substances. II Transformations of certain B-vitamins in soil. *Soil Sci. 71*, 221–231.

SHOPE, P. F., 1936. Drought and the fungus flora of Colorado. *Science 84*, 155.

SHOPE, P. F., 1937. Drought and the fungus flora of Colorado. *Science 86*, 177.

SMIT, J. & WIERINGA, K. T., 1953. Microbiological decomposition of litter. *Nature 171*, 794.

STANIER, R. Y., 1953. Adaptation, evolutionary and physiological: or Darwinism among the microorganisms. *Adaptation in Microorganisms*, 1–20. Univ. Press, Cambridge.

STARKEY, R. L., 1938. Some influences of the development of higher plants upon the micro-organisms in the soil. VI Microscopic examination of the rhizosphere. *Soil Sci. 45*, 207–228.

STEVENSON, I. L., 1956*a*. Antibiotic activity of actinomycetes in soil as demonstrated by direct observation techniques. *J. gen. Microbiol. 15*, 372–380.

STEVENSON, I. L., 1956*b*. Some observations on the microbial activity in remoistened air-dried soils. *Plant and Soil 8*, 170–182.

STEVENSON, I. L. & CHASE, F. E., 1957. Microbial studies on an orchard under three cultural practices. *Canad. J. Microbiol. 3*, 351–358.

THAYSEN, A. C., 1950. Antibiotics in the soil. *Nature 166*, 93–94.

THORNTON, R. H., 1952. The screened immersion plate: a method of isolating soil micro-organisms. *Research 5*, 190–191.

THORNTON, R. H., 1956. Fungi occurring in mixed oakwood and heath soil profiles. *Trans. Brit. mycol. Soc. 39*, 485–494.

THORNTON, R. H., 1958. A soil fungus trap. *Science 182*, 1690.

TIMONIN, M. I., 1935. The microorganisms in profiles of certain virgin soils in Manitoba. *Canad. J. Res. C 13*, 32–46.

TRESNER, H. D., BACKUS, M. P. & CURTIS, J. T., 1954. Soil microfungi in relation to the hardwood forest continuum in southern Wisconsin. *Mycologia 46*, 314–333.

TRIBE, H. T., 1957. Ecology of microorganisms in soils as observed during their development upon buried cellulose film. *Microbial ecology*, 287–298. Univ. Press. Cambridge.

VAN DER DRIFT, J., 1951. Analysis of the animal community in a beech forest floor. *Tijdschr. Entom. 94*, 1–168.

VAN DER DRIFT, J., 1958. The role of the soil fauna in the decomposition of forest litter. *15th Int. Congr. Zool. Proc.*, 357–360.

VAN DER DRIFT, J. & WITKAMP, M., 1960. The significance of the breakdown of oak litter by *Enoicyla pusilla*. Burm. *Arch. Neerl. de Zool.* 13 (in the press).

VELDKAMP, H., 1955. A study of the aerobic decomposition of chitin by microorganisms. *Meded. Landbouwhogesch. Wageningen 55*, 127–174.

VERPLANKE, G., 1932. l'Examen microbiologique du sol. *Bull Inst. Agron. Stat. Rech. Gembloux 1*, 35–45.

WAID, J. S., 1960. The growth of fungi in soil. *The ecology of soil fungi.* Univ. Press, Liverpool. (in the press).

WAID, J. S. & WOODMAN, M. J., 1957. A method of estimating hyphal activity in soil. *Pedologie 7, no. spec. (Symp. Méth. Microbiol. Sol)*, 155–158.

WAKSMAN, S. A., 1916. Do fungi actually live in soil and produce mycelium. *Science N.S. 44*, 320–322.

WAKSMAN, S. A., 1924. Influence of soil reaction upon the distribution of filamentous fungi in the soil. *Ecology 5*, 54–59.

WAKSMAN, S. A., 1932. Principles of soil microbiology. Baillière, Tindall & Cox, London.

WALLHÄUSSER, K. H., 1951*a*. Die antibiotischen Beziehungen einer natürlichen Mikroflora. *Arch. f. Mikrobiol. 16*, 201–236.

WALLHÄUSSER, K. H., 1951*b*. Untersuchungen über das antagonistische Verhalten von Mikroorganismen am natürlichen Standort. *Arch. f. Mikrobiol. 16*, 237–251

WARCUP, J. H., 1951*a*. The ecology of soil fungi. *Trans. Brit. mycol. Soc. 34*, 378–399.

WARCUP, J. H., 1951*b*. Studies on the growth of Basidiomycetes in soil. *Ann. Bot. 15*, 305–317.

WARCUP, J. H., 1955. On the origin of colonies of fungi developing on soil dilution plates. *Trans. Brit. mycol. Soc. 38*, 298–301.

WARCUP, J. H., 1957. Studies on the occurrence and activity of fungi in a wheat-field soil. *Trans. Brit. mycol. Soc. 40*, 237–262.

WEBLEY, D. M., EASTWOOD, D. J. & GIMINGHAM, C. H., 1952. Development of soil microflora in relation to plant succession and sand dunes including the ‚rhizosphere' flora associated with colonising species. *J. Ecol. 40*, 168–178.

WELVAERT, W., 1950. Enkele schimmels geïsoleerd uit duinzand. *Meded. Landbouwhogesch. en opzoek stat. Gent 15*, 686–689.

WILDE, S. A., 1954. Forest humus, its genetic classification. *Trans. Wisconsin Acad. Sci., Arts a. Letters 43*, 137–163.

WILKINS, W. H. & HARRIS, G. C., 1946. The ecology of the larger fungi V. *Ann. appl. Biol. 33*, 179–190.

WINTER, A. G. & BUBLITZ, W., 1953. Untersuchungen über antibakterielle Wirkungen im Bodenwasser der Fichtenstreu. *Naturwissenschaften 40*, 345–346.

WITKAMP, M. & STARKEY, R. L., 1956. Tests of some methods for detecting antibiotics in soil. *Soil Sci. Soc. Am. Proc. 20*, 500–504.

WRIGHT, J. M., 1956. The production of antibiotics in soil III. Production of gliotoxin in wheatstraw buried in soil. *Ann. appl. Biol. 44*, 461–466.

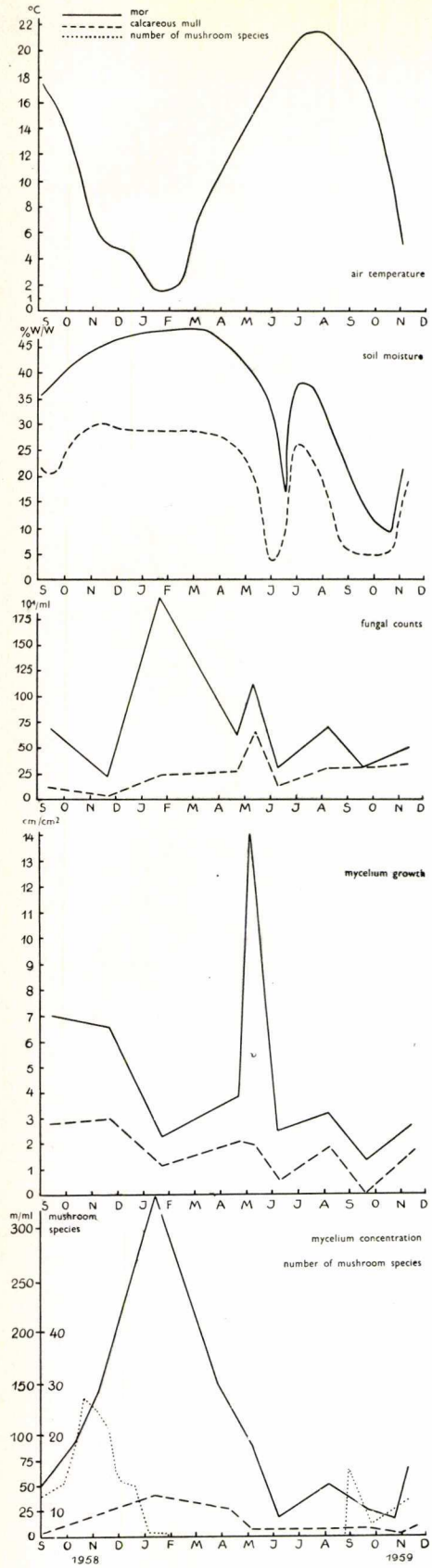

Fig. 14. Seasonal fluctuations of air temperature, soil moisture, fungal plate counts, mycelium growth, and mycelium concentration in calcareous mull and mor, and the total number of mushroom species in mull and mor of the Hackfort oak forest.