THE INTERACTIONS OF THE SOIL MICRO-ORGANISMS

by

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I. INTRODUCTION.

It has been known for some time that the soil harbours a rich population of Bacteria, Actinomycetes, Fungi, Algae, and Protozoa. Until the last decade or two, however, research was confined almost entirely to observations on the bacterial flora. Fungi were first isolated from the soil by Adametz (1886), in 1886, but it was not until 1902 (Oudemans et alii 1902) that any proper systematic account of the Soil Fungal Flora was published. Since then papers have appeared by many different authors and from some fifteen different countries.

This greater prominence accorded to the fungal part of the soil microflora is the result of several contributory factors. Fungi were found to play, in certain instances, an equal or greater part than bacteria in soil processes. As mycorrhiza they were found to have an important role in plant nutrition. The most urgent problem, however, arose from the rapid increase in root-invading soil fungi where methods of continuous cropping were in practice. In the wheat belts of Canada and Australia, and the cotton areas of Egypt and America, years of continuous cropping led to greatly increased numbers of fungal pathogens in the soil. There was a corresponding increase in the incidence of plant disease, and ultimately conditions

sometimes became so unsuitable that production of a particular crop was an economic impossibility.

It therefore became essential that methods for the control of these soil pathogens should be discovered, leading directly to increased study of plant pathogens in particular, and soil fungi in general. On the occurrence and behaviour of fungi in the soil much work has thus been done of late years. Most of this work has however been carried out over relatively large areas. By replication and randomisation over a large area it is possible to obtain much data as regards numbers of micro-organisms etc. On the other hand the behaviour of individual members of the population tends to be obscured. For this reason the present investigation was confined to detailed observation of a small area. In this way it was hoped to obtain information with regard to the soil micro-organisms, and particularly soil fungi, not forthcoming from previous investigations.

The work is divided into eight parts, the Introduction, an account of the Experimental Methods used, a Description of the Microflora, investigations on its Origin and Distribution, a Discussion of the worker's and other results, a Conclusion, Summary and Bibliography.

II. EXPERIMENTAL METHODS.

A plot of ground in the Royal Botanic Garden, Edinburgh, twelve yards by two, was used for all the soil samples. It had lain fallow for a year before use and was hand-weeded both before and during the investigation to prevent any weed growth. The plot was divided up into sub-plots as shown in the diagram (Fig.I). Sub-plot A remained fallow the whole time, and was used for the periodicity counts. B was allowed to become overgrown with weeds, mostly grass. C had an application of sterilised dung, which was buried about 3 ins. down. D received an application of sterilised filter paper, turned in to the same depth as C. E received three applications of a nutrient solution (Czapech's).

Soil samples for examination were taken from the surface 3 ins. by means of a cork borer. The top $\frac{1}{2}$ ins. of soil was always removed so that samples were actually taken from the first $\frac{1}{2}$ to $3\frac{1}{2}$ ins. If a particular soil sample was to be used for a quantitative investigation it was always composed of twenty of these small samples taken at random over the sub-plot.

For determination of fungal, bacterial, and actinomycete numbers the method of Brierley et alii (1927) was adhered to as closely as possible. The soil sample was intimately mixed and 10 grms. put aside

for estimation of moisture content, and total organic matter. 25 grms. of soil were then hand shaken in 250 ccs. of sterile water for 20 minutes. 10 ccs. of this suspension were transferred to 90 ccs. of sterile water, and mixed, and 10 ccs. of this suspension added to 190 ccs. of sterile water. This dilution, 1/2,000, was found sufficient for the fungal counts. For the bacteria and actinomyces a further dilution to 1/100,000 was necessary.

For plating out the fungi Conn's medium (Conn 1914) was found to be the most satisfactory. It was brought to a pH 4·0 by addition of N/10 sulphuric acid. Waksman and Fred's medium (Waksman & Fred 1922) was used for the bacteria and actinomyces. In this case a pH 7·5 was obtained, by addition of $^{N}/_{10}$ caustic soda. Care was taken that these two media were always made up in exactly the same manner.

The method of inoculation was the usual one, previously described by Brierley (1927). 10 ccs. of the medium was poured into a petri dish 10 cms. in diameter. When the medium had cooled to approximately 50°C. 1 cc. of the requisite soil suspension was added. The cultures were then incubated at 25°C. Fungal counts were taken after five days, bacterial and actinomycete counts also after five days. Individual fungal colonies could not as a rule be identified until

after a week or a fortnight's incubation. Plates I and II show typical fungal, and bacterial plates respectively.

The number of micro-organisms in any given sample is based on the average of at least eight plate counts. Ten replicates were normally plated from each sample but aggressive growth of one of the Trichoderma spp. frequently rendered the discarding of one or two plates from each set necessary. With each set of replicates two control plates were kept. In one sterile water instead of soil suspension was used as inoculum, in the other a pipette was introduced under the petri-dish lid but no inoculum added. Any contamination occurring during the plating out process could therefore be detected.

It has been shown by Thornton and Gray (1930) that, in arable soil, bacteria exhibit a diurnal periodicity. Moreover a maximum in numbers of bacteria was found round about 10 a.m. Soil samples were therefore always collected at this time and the suspensions and plates prepared immediately.

Simultaneously with the collection of soil samples records were taken of the soil temperature and soil pH at a depth of 3 ins.

For a given soil sample the following data were thus recorded; total numbers of fungi, and bacteria and actinomycetes, numbers of certain

individual species of fungi, soil temperature, soil moisture content, soil organic matter content, and soil pH.

In the case of sub-plot A samples were taken in duplicate and as far as possible at fortnightly. intervals.

Owing, as previously stated, to the fact that the experiments were carried out on a small area without replication, full statistical analysis of the results was not possible. Tests for comparison of two means and for homogeneity of samples could be applied however, and were generally used. Table I shows the result of an experiment in which four soil samples were plated out simultaneously for fungal counts. Fisher's (1936) Table VI, for use when the numbers of degrees of freedom are small and unequal, gives for the 5% point Z = 0·5403. The differences shown are therefore insignificant and the dilution and plating method can be taken in this investigation to give a reliable estimate of the soil population, or rather, that section of the soil population which is capable of developing under these particular experimental conditions.

For qualitative work two supplementary methods for identification of soil micro-organisms were used, Conn's direct method (Conn 1922) and hanging drop cultures. The former was used primarily for detection

of basidiomycetes, and the latter of yeasts, both of which of course failed to develop on the media used for soil counts. Figs. II and III, and Plates III and IV illustrate basidiomycetes and yeasts distinguished by these two methods.

A number of media were actually tried for fungal counts before Conn's was selected, and it did appear that, apart from the two groups mentioned above, the majority of the soil fungi grew reasonably well on Conn's medium. Provided only fluctuation in numbers, and not numbers of fungi themselves were compared, no error could be introduced owing to the fact that any given medium must be more favourable to the growth of a given species than to others.

In the quantitative investigations each colony developing on a plate was taken to represent one unit of that particular species. An experiment is described later where a distinction between colonies developing from fungal spares, and colonies developing from fragments of hyphae, was attempted.

III. DESCRIPTION OF THE MICROFLORA.

In sub-plot A the fungal counts were found to give numbers varying between 122,000 per gram dry soil in October to 54,000 in February. Numbers of bacteria and actinomycetes varied between 9½ millions per gram dry soil in May and 2½ millions in February. Table II records the actual numbers obtained from plot A during the course of a year. These are also expressed in graph form (Graph I) where they are plotted along with the soil moisture and soil temperature.

A comparitively small number of fungi were isolated from the plot, small, that is, as regards number of species. The following were identified:-

Trichoderma alba.
Trichoderma koningi.
Trichoderma lignorum.
Mucor hiemalis (plus and minus)
Mucor Ramanianus.
Zygorhynchus vuilleminii.
Mortierella sp.
Pestalozzia Hartigii.
Coniothyrium fuckelii.
Dematium sp.
Fusarium spp.
Penicillium spp.
Eurotium spp.
Cephalosperium sp.

75% of the fungal flora developing on the plates belonged to these species or genera. The identification of the other dozen or so odd species also occurring was not attempted as it was considered that a record of the individual behaviour of this 75% would be almost as valuable as a record of the whole. There would of course have been some difficulty, and a considerable amount of time would have been spent, in the identification of these other species. For the purposes of the experiment the ten or twelve species of Penicillium occurring were recorded as one, similarly the two or three species of Eurotium and Fusarium isolated.

Fluctuation in the numbers of these individual soil species from sub-plot A was found to be considerable, not always coinciding with fluctuations in numbers of the population as a whole. Graph II shows numbers of individual species plotted with numbers of the total fungal population over the course of a year.

Sub-plots B. C. D. and E. were sampled approximately 3 months after receiving their respective treatments. Table III shows the number of fungi per gram dry soil for each treatment and its control plot. There is a slight, though significant, depression in fungal numbers, as shown by the plate method, in treatments B, C, and E. Table III also shows the

numbers of individual species for each treatment.

Various species are apparently quite markedly affected by the different treatments. Trichoderma lignorum shows a very large response to increase in nutrients, as can be seen from the two photographs illustrating platings from sub-plot A and C (Plates I and V).

The Dematium sp. on the other hand, although stimulated by the addition of filter paper is suppressed by the addition of nutrients. Penicillium species apparently increase most under a weed growth or on addition of filter paper. It may be significant that the least, or no, depression in total numbers is effected by filter paper, and the greatest by dung.

The various findings here recorded are discussed at some length later.

IV. ORIGIN AND DISTRIBUTION OF THE FUNGAL MICROFLORA.

During the last twenty years an ever-increasing number of fungi has been recorded from the soil. The exact meaning of the term "soil fungus" is still however a little vague. Despite the fact that many species probably occur in the soil only as wind-borne or animal distributed spores, all species isolated from the soil, and not actually growing visibly on organic matter, are classified together as soil fungi.

Reinking (1934 and 1935) and Reinking and Manns (1934) recognised the need for a distinction of types of soil fungi. They concluded certain Fusarium spp. isolated from the soil could be divided into true soil inhabitants and soil invaders.

It is clear that the air must contain a vast reservoir of fungal forms which are all potentially soil invaders.

Stakman et alii (1923) investigating the occurrence of spores in the upper air, found spores of Puccinia, Alternaria, Helminthosporium, Cladosporium, Cephalothecium, Ustilago, Tilletia, and Scolecotrichum.

Dillon Weston (1929) in a similar investigation, showed the ubiquitous nature of fungal spores and bacteria in the upper air, a large number being viable even from an altitude of two miles above the

earth.

In an investigation of the fungi present in the air over apple orchards Carter (1935 a.) found Stycanus stemonitis, Alternaria humicola, Trichothecium roseum, Pestalozzia Hartigii, Cladosporium herbarum, and other fungi isolated from soil.

Aerial distribution of soil-invading pathogens has frequently been held responsible for serious epidemics of plant disease.

Samuel and Garrett (1933) have suggested that aerial dispersal of ascospores in showery weather is responsible for the widespread occurrence of the "take-all" disease in South Australian wheat crops in certain seasons.

Petch (1928), Gadd (1936), Bryce (1922), and others, consider air-borne spores to take a significant part in the distribution of the root-rots of tea, cacao, rubber, and other tropical crops. Criticisms have however been raised by Briton-Jones (1934) and Napper (1932) and it would appear that air-borne infections, in some cases, are not so important as was originally supposed.

But it is still apparent that many fungi isolated from the soil can also be isolated from the air.

The return of these air-borne fungi to the

soil can be facilitated in a number of ways. They can be carried down in rain or washed down from the surface of the soil by rain. They can also be carried down from the soil surface by the numerous micro-fauna of the soil and the smaller soil fauna. Several workers have demonstrated that earthworms are capable of dispersing soil fungi. Drainage and irrigation water too, apparently assist in the return or spread of soil fungi (Wardlaw (1935) King et alii (1934b) Thung (1932)).

In consideration of these various facts an attempt was made in the present investigation to determine which part of the fungal microflora was actually confined to the soil, and the origin and means of invasion of that section which was not.

The numbers of fungi in the air above the experimental plot were determined by exposing agar plates for a given time. In February half-an-hour's exposure was necessary to give a reasonable number of colonies per plate. In June 5 mins. sufficed. The average of colonies developing on ten plates of Conn's medium was always taken, and the photograph (Plate VI) shows colonies developing typically on a plate after 5 days incubation at 25°C. Table IV gives the numbers of colonies developing on the agar plates over a period of 12 months. In graph III these numbers are plotted against numbers of fungi in the soil, and in graph IV

numbers of individual fungi are compared with regard to their fluctuation in numbers. The actual numbers in graph III are not comparable, one being in thousands per gram of soil and the other in colonies developing per plate. Table V shows a list of (a) fungi isolated from both soil and air, (b) fungi isolated from air only, (c) fungi isolated from the soil only.

The numbers of fungi developing from rain water were estimated. It was calculated that during a medium shower of rain three times as many fungi were deposited on the surface of the soil in a given time as would be deposited from the air.

As previously stated several workers have considered earthworms capable of distributing various fungi through the soil. Their methods have, however, been open to criticism as in the majority of cases the finding of viable spores or mycelium in the intestine of the worm was considered sufficient evidence for assuming dispersal. In this investigation worm casts were actually examined for their fungal content. The following method was used to obtain 'worm' casts free from external contamination.

Worms were dug from the experimental plot and washed. They were then dipped in absolute alcohol for a second, then washed in several changes of sterile water. The mortality rate from the alcohol

was fairly high. Those worms surviving were placed in a sterile container, while the dead ones were plated out on agar to determine whether all surface infection had been removed. No immediate fungal growth was observed in the latter case, indicating that the external disinfection of the worms had been complete.

Worm casts deposited in the sterile receptacle were collected after 24 hours and treated in exactly the same way as a soil sample, except that only 5 grms. instead of 25 grms. of soil were available.

The results of this experiment are shown in Table VI. Numbers of individual species as well as numbers of total fungi for the worm casts, and the surface 3 ins. of soil from under which the earthworms were removed, are compared.

The movement of large quantities of soil by earthworms is a well known fact, first described by Darwin (1881). If earthworms are capable of carrying intact the majority of the soil fungous species, and continually moving both horizontally and vertically through the soil they must effect a very considerable mixing of the microflora.

An experiment was designed to test the rate of dispersal of a fungus through the soil by earthworms as compared with its own progress by growth. Two bell jars were fitted up as in the diagram Fig IV.

At the bottom was a layer of agar medium, separated from the surface of the soil by an air space about 1 in. deep. The soil rested on wire gauze and was itself some 9 ins. in depth. The bell-jar was closed by a glass plate resting on a ring of cotton wool. The whole apparatus was sterilised in an autoclave after fitting up.

Sterile earthworms were now prepared, or rather Penicillium free earthworms, by feeding on successive lots of sterile soil and periodic external sterilisation. After a fortnight it was found that the earthworms no longer gave colonies of Penicillium on plating out on agar. Some odd dozen were therefore introduced into one of the bell jars. When these had disappeared into the soil spores of a Penicillium sp. were dusted on the surface of the soil in both bell-jars. The time of appearance of colonies of Penicillium on the agar at the bottom of the bell-jars was now recorded. Where the earthworms had been introduced this took seven days, in the control a month. It is extremely likely this difference would be much greater in unsterilised soil, where growth of the fungus would be more restricted.

V. DISCUSSION.

By analogy with the Phanerogamic flora it might be expected that the soil microflora should show variations in its component species in regard to soil type, soil treatment, season, etc. Admittedly there is a much smaller range in such factors as temperature and moisture under the soil, as opposed to above it. On the other hand the effects of cultivation, different crops, different manurial treatments etc., must produce a wide range of physical and chemical characteristics in the soil. Possibly methods for the determination of the soil microflora are somewhat crude as yet, but certainly, so far, no worker has brought forward definite evidence of the delimitation of soil fungal floras by environmental conditions to anything like the extent distinct communities are encountered in the ecology of Higher Plants.

Hagem (1908) showed that cultivated soils have a distinctly different population of Mucorales from pine forest soils. Ling-Young (1930) states Mucor Mucedo, Thamnidium elegans, Rhizopus nigricans, Chaetocladium Jonesii, and other Mucors, are probably indicators of the intensity of human or animal influence.

On the other hand Mucor hiemalis, M. griseo-cyanus, Absidia glauca, A. cylindrospora, mentioned by Pispek (1925) as occurring only as alpine species, are

commonly found in low-lying districts. Campbell (1938) states that "soil conditions do not effect the distribution of some of the species of the Mucorales to the extent that might be expected."

Such data concerning the specific occurrence of given species in certain soils is very misleading unless accompanied by statements of frequency as well as occurrence. Records of occurrence alone fail to take into account the fact that a given isolation may be from an "alien" spore accidentally deposited in that particular soil, and quite incapable of germinating and establishing itself therein.

The individual soil factors which may influence the microflora are numerous. The more obvious are moisture content, temperature, organic matter content, amount of available mineral salts, reaction, aeration, and interaction between higher plants or other members of the microflora.

On the relative importance of these various factors there is a considerable divergence of opinion.

Coleman (1916) records that variations in moisture content vary the group relations of soil micro-organisms. Conn (1912) found a correlation between total numbers of bacteria and actinomycetes and soil moisture, and Waksman (1922a) a relationship between bacterial numbers and soil moisture. Jensen

(1934) also found a strong positive correlation between moisture content and the numbers of bacteria. Soil fungi showed a less pronounced correlation, and actinomyces were apparently unaffected by variations in moisture content. On the other hand Smith and Worden (1925) could reach no definite conclusion as regards the influence of moisture content on bacterial numbers. Brown and Halverston (1919) go so far as to state "... the numbers of moulds present in the soil fluctuated from one sampling to the next but were apparently unaffected by moisture, temperature, or soil treatment."

Soil temperature as a controlling factor has approximately as much evidence for, as against. Brown and Halverston (1919), as stated above, consider soil fungal numbers are not affected by soil temperature. Engberding (1909) concluded soil temperature had very little, if any effect on bacterial numbers. Smith and Worden (1925) could reach no definite conclusion as to the effect of temperature, as well as soil moisture on bacterial numbers. Eggleton (1934) stated that soil temperature and moisture could influence numbers of microflora, although apparently subsequently changing his opinion (1938).

There is general agreement that organic matter content of the soil influences the microflora.

Even here however opinion is not unanimous. Erdman (1928) states " ..... in the great majority of cases the soil from the manure and lime-treated plots showed practically the same number of fungi as the soil from the check plots."

According to Jensen (1931) the actual abundance of fungi depends on many factors, including food supply, but the ratio of fungi to bacteria and actinomycetes seems to depend on little more than pH.

Addition of various chemical substances to the soil has a marked influence on the microflora. Waksman (1922a) found potassium and phosphates stimulated the development of micro-organisms, particularly in the presence of lime. Lime itself decreased the numbers of fungi and increased those of bacteria and actinomycetes - presumably by influencing the soil reaction. Sodium nitrate stimulated the development of bacteria and actinomycetes, but not fungi. Ammonium sulphate, making the soil distinctly acid, stimulated the fungi. Carbon di-sulphide is frequently used as a soil disinfectant. Fleming (1929) describes how treatment of soil with carbon di-sulphide increases threefold the total number of fungi present, while reducing the number of species represented.

The influence of higher plants on the soil microflora has been demonstrated by several workers.

Starkey (1929) investigated the effect of the stage of development of the plant on bacterial numbers for a large variety of plants in greenhouse and field. Bacterial numbers were found to increase with the development of the plants to have a maximum at the period of maximum growth, and to show a rapid decline on the death of the plants. Subsequent work by Starkey (1929, 1938 etc.) fully confirms his findings. Eggleton (1938) states "It is suggested that the seasonal changes in moisture and temperature are not the direct causes of the seasonal changes in numbers of bacteria, but that in controlling the growth of the herbage, these climatic factors control the amount of energy material reaching the micro-organisms in the form of root excretions or sloughed-off root material. Thus, in grassland soils the long-term changes in the numbers of micro-organisms are closely associated with the amount and growth activity of the surface vegetation." Wilson and Lyon (1926) find that numbers of various species of bacteria grown in sterilized soil are much higher in the presence of maize or timothy plants than in their absence, due they concluded, to the excretion of nutrient substances from the plant roots.

There is also the factor of biological competition between the various component species of the microflora. The significance of antagonistic

relations between micro-organisms was first recorded by De Bary (1879). The literature on this subject is considerable, but mostly concerns experiments on artificial substrates. Work of this nature has been published by Vasudekva (1930) Endo (1933, 1935 etc.) Rosen and Shaw (1929) Carter (1935b) and others. Porter (1924) reviewed the work done up to 1924, and Waksman (1937) also gives a historical review of Antagonistic Relationships.

The suppression of a particular soil pathogen by the sapraphytic population of the soil is recorded in a number of papers. Millard (1927) suggested the beneficial effect of green manuring on potato scab, under field conditions, was possibly due to the competitive action of bacteria and Actinomycetes. Fellows (1929) records that several kinds of organic matter added to infected greenhouse or field soil, greatly reduced the severity of Take-all. Similarly Garrett (1934) states that the addition of fowl manure was found greatly to retard the progress of infection by Take-all. He suggested the difference in rate of growth in Take-all in light sandy sails and heavier clay-loam was due to biological antagonism of the micro-organisms. King, Hope, and Eaton (1934b) also found "The rapid and prolonged reduction of root-rot activity on the manured plots suggests that the

dense population of organisms engaged in breaking down the organic materials developed a soil condition temporarily unfavourable for the growth and activity of the root-rot fungus."

Inhibition of soil pathogens by specific fungi has also been described. Allen and Haenseler (1935) found that seed decay and damping off of cucumbers induced by Rhizoctonia and Pythium is appreciably reduced by inoculating the soil heavily with Trichoderma sp. Muller (1935) records that Trichoderma sp. when added to soil infested with Sclerotium Rolfsii and planted with Mimosa gave complete control of the disease. Weindling and Fawcett (1936) obtained control of damping off of citrus seedlings, caused by Rhizoctonia solani, by the application of aluminium sulphate or acid peat moss, which produced an initial soil reaction of about pH 4.0. The decisive factor is apparently a change in the microflora of the soil, favouring organisms such as Trichoderma, which may be antagonistic or parasitic towards Rhizoctonia solani. Weindling (1934, 1937, etc.) has also described the parasitic action of Trichoderma lignorum on Rhizoctonia solani and some of the properties of a lethal principle that is instrumental in this action. Van Luijk (1938) suggests the isolation of inhibiting substances from fungi and their use as soil disinfectants.

The effect of these various chemical, physical, and biological properties described, as determined in the present investigation, is in general agreement with the results of the workers quoted above.

The most important result is the recording of a pronounced and definite fluctuation in numbers of soil fungi practically parallel with the now generally accepted long-term fluctuation in numbers of soil bacteria.

Of the various factors which might bring about this periodicity in fungal numbers soil organic matter content and soil reaction may be disregarded as these remained constant during the period of the investigation. Neither, from examination of the two curves in Graph I would it appear that the fungal periodicity is imposed by the antagonistic influences of the soil bacteria and actinomyces. On the other hand it is apparent that changes in fungal numbers are coincident with changes in soil temperature and moisture content. Most British soil fungi have an optimum temperature for growth between 20°C. and 25°C. For the soil fungi of this particular plot the soil temperature must therefore have been always below the optimum. But the fungal curve does not follow the temperature curve except at its two ends. Moreover the points where the fungal curve drops away from the

temperature curve coincide with decreasing soil moisture content.

So a similar hypothesis may be put forward to explain periodicity in soil fungi as is advanced, though by no means universally accepted, for bacteria. The low winter temperatures reduce fungal activity despite high soil moisture contents. With more favourable temperatures in April and May numbers rise, but activity is curtailed by a drop in soil moisture content. In June and July soil moistures are low and correspondingly so are bacterial numbers. At the end of July soil moisture starts to rise again and another peak of activity occurs in October when numbers again drop owing to a fall in soil temperature.

Extending this hypothesis to other soils it would be expected in an Arctic soil where temperature was a limiting factor, that soil fungi would show only one peak of activity and that in summer. In a tropical soil, where temperature was never a limiting factor there would be again one peak of activity, but this time in the rainy season.

No information on soil fungi of Arctic or Antarctic soils is available, but Corbet (1934) finds, in Malayan forest sails, bacterial and fungal numbers are constant, within narrow limits, all the year. Soil moistures are also almost constant, and soil temperature

varied from 78°-80°F. This fact is consistent with the hypothesis presented that seasonal periodicity in soil fungi is a function of soil moisture and soil temperature. It is also consistent, however, with the hypothesis, previously mentioned, of Eggleton (1938) that periodicity in the microflora merely reflects the periodicity in growth of the higher plants. That is assuming that growth of higher plants in a Malayan forest shows no seasonal activity. On Eggleton's hypothesis there should appear no seasonal variation in numbers of bacteria under a fallow soil. This is contrary to the present findings where a similar biennial maximum was found in the numbers of both bacteria and actinomycetes, and fungi.

Ward Cutler and Crump (1935) hold that the numbers of soil fungi fluctuate at short intervals of time, and exhibit some seasonal variation. The explanation they give for this behaviour merely restates the problem. They say "It appears there is in protoplasm an innate capacity to behave rhythmically, and the majority of soil organisms, no less than man himself, observe the fundamental law that vitality shall be at its maximum in spring and autumn." The work of Corbet (1934) previously quoted, shows this "fundamental law does not hold at any rate for Malaya."

In a recent work Singh (1938) finds, either that periodicity in soil fungi is not a definite

phenomenon, or the methods he employed obscured any such fluctuation. It would appear from the work here described that the second alternative is the correct one.

Another new fact was brought to light by the various treatments of sub-plots B. C. D. and E. That is, unless organic matter is added to soil with the microflora essential to its decomposition already present, there will be at first no increase in numbers of oil fungi as recorded by the plate method. The addition of unsterilised dung to the soil may bring about an increase in numbers of the microflora, not by virtue of stimulating the activity of those micro-organisms already present, but rather by addition of an alien microflora.

Addition of cellulose (sub-plot D) in the absence of minerals produced no significant change in the numbers of fungi. Apparently the soil did contain some "available" cellulose because the addition of a nutrient solution (sub-plot E) produced a large increase in numbers of Trichoderma lignorum.

The apparent depression in numbers on these three sub-plots can be also interpreted as a change in the activity of the fungi from a sporing phase to a vegetative one. In this case there would be a time lag before the increased activity was detected by the

dilution plating method, exceeding the period of the experiment. The obvious drawback to the dilute plating method is that it assumes that the frequency of the spores of micro-organisms are a measure of the activity.

Several workers have therefore proposed alternative methods whereby a distinction can be made between fungal spores and fungal mycelium.

Waksman (1922b) evolved a method of plating out soil crumbs, by isolating, after 24 hours incubation at 25°C, the growth which had appeared on the plates. The majority of organisms isolated from soil in this way belonged to the Mucorales. Penicillia, Aspergilli and Cladosporia, were hardly obtained at all. The reverse holds true when the dilution plating method is used. Waksman was thus led to conclude Mucorales and Trichodermae are always present in the soil in the form of spores and vegetative mycelium, and the Penicillia, Aspergilli and Cladosporia in the form of spores, which may germinate when soil conditions become favourable. The photograph (Plate VII) shows a growth of Mucor hiemalis obtained by Waksman's method. A similar growth was obtained under the same condition from spores of Mucor hiemalis so the method is apparently not infallible.

A direct method for demonstrating fungi and

actinomycetes in soil has been described by Conn (1922). The drawback here obviously lies in the difficulty of identifying fragments of hyphae. Photographs (Plates III, VIII, and IX) and Figs II, III, and V-IX show fungi detected in soil by this method.

The same criticism applies to the method of Cholodny (1930) where hyphae in the soil are allowed to grow out and adhere by a water film to the surface of sterile glass plates. Further, the fact that varying amounts of the fungal growth are left behind on removal of the glass plates, prohibits the use of this method in quantitative investigations.

McLennan (1928) perfected a much improved technique. Differential dehydration was employed to kill out the fungous mycelium while leaving intact the fungal spores. The difficulty here seems to lie in subjecting a given soil sample to a uniform dehydration over its whole area.

A technique based on similar lines was designed for use in connection with the present work, but its use had to be discontinued as it constituted an investigation in itself. It was simple, and made use of a differential power of resistance to moist heat which apparently exists between fungal spores and mycelium.

Suspensions of spores of various soil fungi

were heated for varying times at varying temperatures. The maximum period of heating, at a convenient temperature, which fungal spores could withstand without affecting their percentage germination, was found to be 2 mins. at 47·5°C. Table VII shows the results of a typical test of a spore suspension. It was found rather difficult to discover whether all soil fungous mycelium was destroyed by heating at this temperature for 2 mins. A sterile mycelium isolated from soil failed to survive no matter what the age of the culture. So also did mycelium of a Penicillium sp. which was tested before it had commenced spore-production.

The great advantage of this method lies in the fact that it can be applied to ultimate dilutions of soil suspensions prepared for plating. Comparison of plate counts inoculated with heated and non-heated suspension should therefore give accurate estimates of the total of viable fungal spores.

In Table VI is shown a comparison of the numbers of fungi at the actual surface of the soil with those of the first $\frac{1}{2}$ in. to $3\frac{1}{2}$ ins. This supports the results of calculations of the number of fungi deposited on the soil from the air. Compared with numbers of fungi in the soil those deposited on the surface are insignificant. Supposing however that a particular fungus dies out in the soil owing

to the persistence of disadvantageous conditions, if suitable conditions were to occur once again the existence of a supply of inoculum in the air and a rapid means of distribution by earthworms, becomes vital to the re-establishment of that fungus.

A soil-inhabiting fungus is here taken to denote a fungus which is not capable of establishing itself, growing, and reproducing outside the soil. A soil invader is taken as capable of existing in this manner independently of the soil. Some soil-inhabiting fungi may be isolated from the air owing to their habit of fruiting at the soil surface or being distributed with dust particles. From Table V it can be seen however that Mucor hiemalis is the only one of the generally accepted soil-inhabiting fungi which was isolated from the air.

Little is known of the persistence of soil-invading species in soil. Fungal material has been known to remain viable for a very considerable number of years in a dry condition (Collett (1921)). In soil however it is apparently rapidly destroyed by bacterial action, or the action of parasitic fungi (Drechsler (1938)). For soil-invaders therefore this reservoir of inoculum in the air must be essential to their rapid spread where suitable conditions occur.

During the course of this investigation a

number of minor problems arose, of which time did not usually permit a full investigation.

Despite the fact that both plus and minus forms of Mucor hiemalis were isolated from sub-plot A no zygospores were ever detected by Conn's direct method. A small experiment was carried out to determine whether or not Mucor hiemalis could form zygospores in soil. A number of cultures of plus and minus forms of Mucor hiemalis were prepared on a little agar in bottles. When the cultures were growing strongly plus and minus cultures were put into the same bottles but separated by a layer of sterile or unsterilised soil. The photograph (Plate X) shows the arrangement. After 3 weeks the soil separating the cultures was examined for zygospores. It was found that where sterile soil was used zygospores had formed, but only where the soil was in contact with the sides of the glass tube. In the inner part of the soil layer there was no zygospore formation. Nor was there any zygospore formation in unsterilised soil. So it would appear there is possibly some connection between zygospore formation in Mucor hiemalis, soil aeration, and inhibitory action of other micro-organisms, which may explain the absence of zygospores from the test plot.

The relative abundance of the plus and minus forms of Mucor hiemalis was also rather interesting.

Campbell (1938) records the ratio of plus to minus forms as 25/4, Hagem (1908) 5/21, Linneman (1936) minus as being more common than plus. In this investigation the ratio of the two forms was found to vary over the year. In July the numbers of each form were approximately equal, previously there had been a preponderance of the minus type and later there tended to be greater numbers of the plus form. Sufficient numbers of samples were not taken for a definite statement to be made, but a seasonal variation in the ratio of production of plus and minus forms by Mucor hiemalis would reconcile the divergent views of the various authorities.

Two fungi were absent from the soil isolations, Botrytis cinerea, and Rhizopus nigricans, which are usually recorded from arable soils. Rhizopus nigricans was not even found in the air above the soil plot, and its behaviour was consistent with the belief that it is only present where human influence is at work, i.e. in and round houses etc. In the case of Botrytis cinerea comparitively large numbers were found in air samplings. It can only be concluded either that conditions in the soil led to rapid destruction of the spores, or the mycelium very soon after germination of the spore. No species of Pythium or Phytophthora also, were isolated from the plot by any method.

Several types of mycelial fragments and spores were detected in the soil by Conn's method (Plates III, VIII and IX, Figs. II, III, and V-IX) Some, such as spores of Pestalozzia Hartigii, were easily identifiable but one large multicellular spore (Plate IX, Figs. V and VI) was never identified. In fact it could not actually be referred with certainty even to a fungal origin. Possibly it was a soil alga.

An interesting point came out in the experiments with hanging drop cultures of soil crumbs. Young, actively growing hyphae, by excretion of some antagonistic substance, using up all available oxygen, or some other method kept bacteria repelled to a certain distance (Plate XI). As they grew older, however, they did not retain this power and bacteria were not repelled from the vicinity of the hyphae (Plate XII).

VI. CONCLUSIONS.

It was apparent that soil fungi in the top layers of an arable soil can exhibit a seasonal periodicity very similar to that described for soil bacteria. Peaks of activity occur in April and October with a tendency for the latter to be the greater. This periodicity occurred, and a periodicity in bacterial numbers also, despite the fact that the experimental plot was maintained in a strict fallow before and during the experiment, and the amount of soil organic matter remained to all intents and purposes constant. The possible influence of higher plants on periodicity of the micro-organisms was therefore eliminated.

By postulating the limitation of fungal activity, in winter by low soil temperatures, and in summer by low soil moisture contents, it is possible to arrive at a hypothesis which will explain the seasonal periodicity of soil fungi in Britain. When information from countries where either soil temperature or soil moisture content is never a limiting factor in soil fungal growth, it should be possible to verify this hypothesis.

The peaks of activity as shown by total numbers of soil fungi are only a mean. The individual behaviour of a few species may depart considerably from

this mean.

The fungal flora was found to fall quite definitely into two groups for which the terms "soil inhabitants" and "soil invaders" have been adopted from Reinking and Manns (Reinking (1934, 1935) Reinking and Manns (1934)). The "soil invaders" were invariably present in the air above the soil, and when deposited on the surface could rapidly be distributed through the soil by earthworms, or washed down by rain. This reservoir of fungal forms in the air was insignificant in numbers compared with the soil flora, but important if a period of unsuitable conditions should eliminate any particular species of soil invader from the soil. This had apparently happened in the case of Botrytis cinerea which was not present in the plot under investigation. When conditions became suitable for its growth as a soil invader the presence of this fungus in the air above the plot would permit of it rapidly establishing itself in the soil.

It is possible then to conceive of two fungal populations in the soil. One relatively static and confined to the soil, though occasionally distributed by air. The other more dynamic, frequently dying out but re-establishing itself quickly on the return of suitable conditions by virtue of its possession of the power of existence independent of the soil. In the soil these

populations are equally influenced by the various soil factors and conform to the same seasonal periodicity.

Total numbers of soil fungi, and the ratio of the component species of the population to one another, can be influenced by various soil treatments. Sterilised dung after 3 months produced decreased numbers of soil fungi, due, it is claimed, to the fact that sterilization removes from the dung the flora normally responsible for its decomposition. Addition of nutrients salts enables Trichoderma lignorum to carry out cellulose decomposition. The depression in numbers of other fungi after 3 months in this treatment is most likely due to the antagonistic action of Trichoderma lignorum. The absence of any significant effect of added cellulose after 3 months can then be taken as due to the lack of suitable nutrients for the growth of cellulose - decomposing fungi. The fact that numbers of soil fungi under a 3 months' growth of weeds were lower than the control cannot at present be explained.

VII. SUMMARY.

(1) A record of numbers of soil fungi, and bacteria and actinomycetes, in a fallow plot in the Royal Botanic Garden, Edinburgh, was kept over the course of one year, 1937-1938, together with variations in soil moisture, soil temperature, soil pH, and soil organic matter content.

(2) The numbers of soil fungi exhibited a marked seasonal periodicity with a biennial maximum in April and October.

(3) The numbers of soil bacteria and actinomycetes exhibited similar fluctuations, but with maximums in May and September.

(4) Records of numbers of certain individual species were also taken. Fluctuations in numbers of one or two of the species departed considerably from the mean as indicated by the fluctuations in total numbers of soil fungi.

(5) Numbers of fungi developing on agar plates exposed to the air over the soil plot were recorded over the year.

(6) The effect of four different soil treatments on soil fungi was investigated. Sterilised dung, nutrient /

nutrient solution, and weak growth caused a significant depression in numbers after 3 months. Sterilised filter-paper had no effect.

(7) A new method of distinguishing between fungal spores and fungal mycelium is outlined.

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LIST OF ILLUSTRATIONS.

Plate. 1.

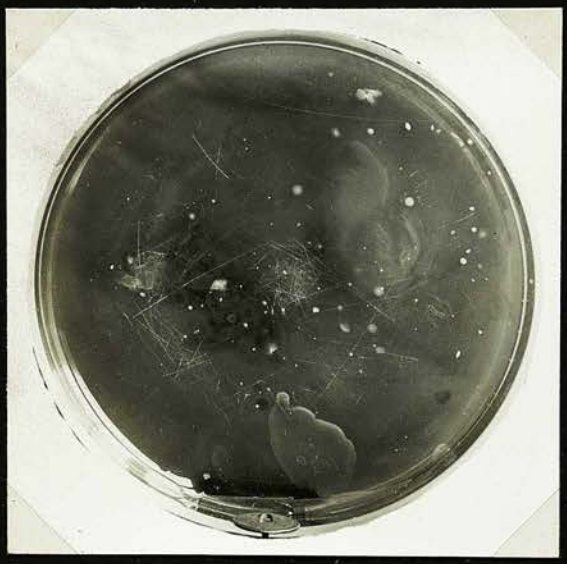
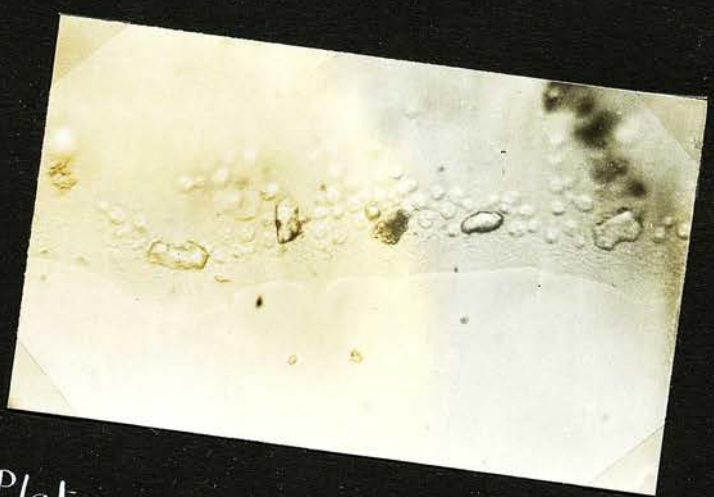


Plate. 2.

Plate. III.



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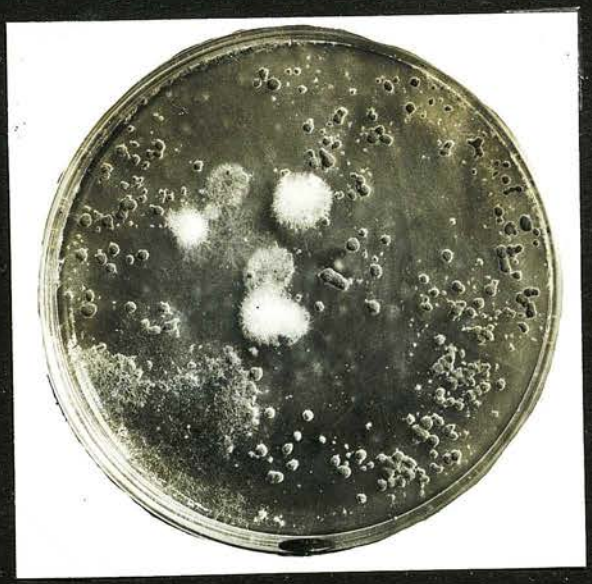

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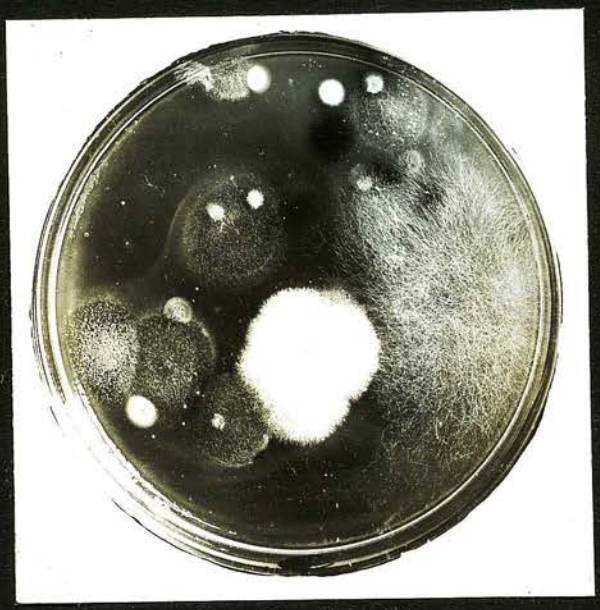


Plate VI.

Plate. VII

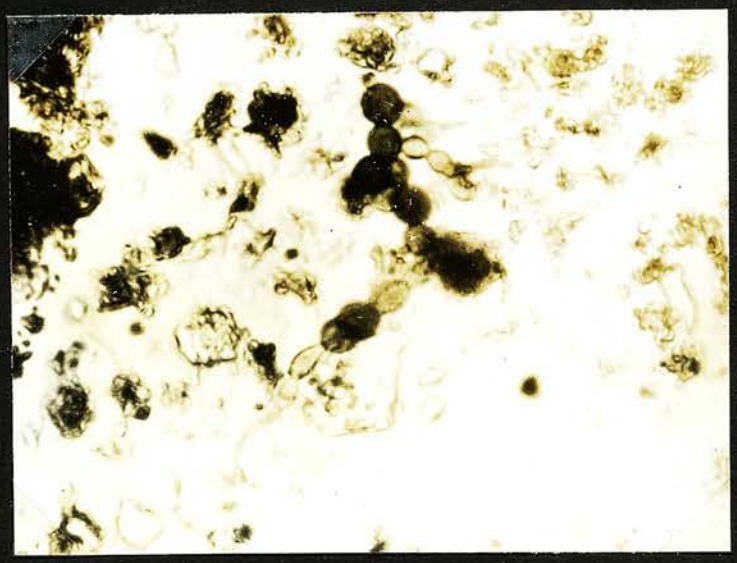


Plate. VIII.

Plate. IX.

Plate X

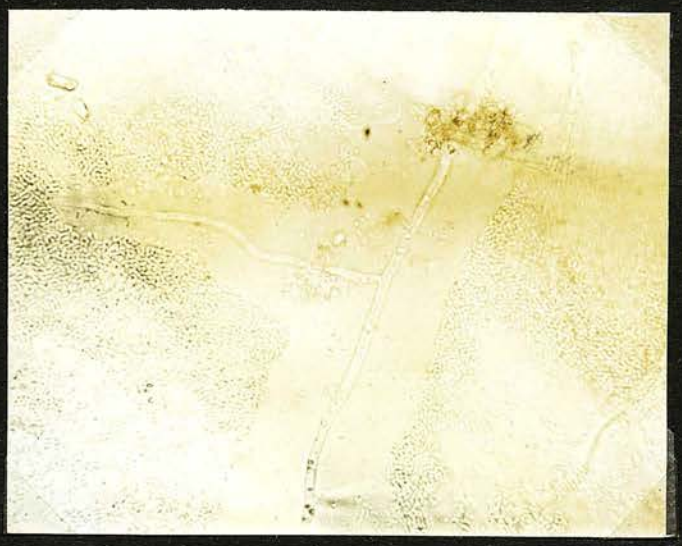


Plate. XI.

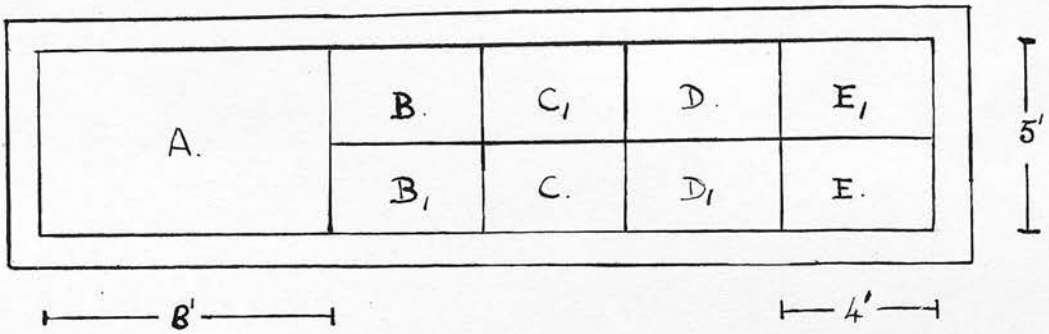
Plate. XII.

LIST OF FIGURES.

Figs. II, III, and V-VIII are of fungi detected by the use of Conn's method.

FIG. I

PLOT LAY-OUT.



A. Fallow.

B. Weed growth permitted

D. Filter paper added.

C. Dung added.

E. Nutrient solution added.

B, C, D, E, Controls of B.C.D.E.

Fig. 1

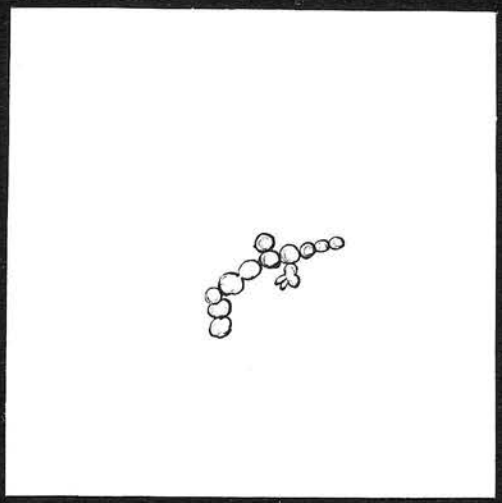


Fig. 2.

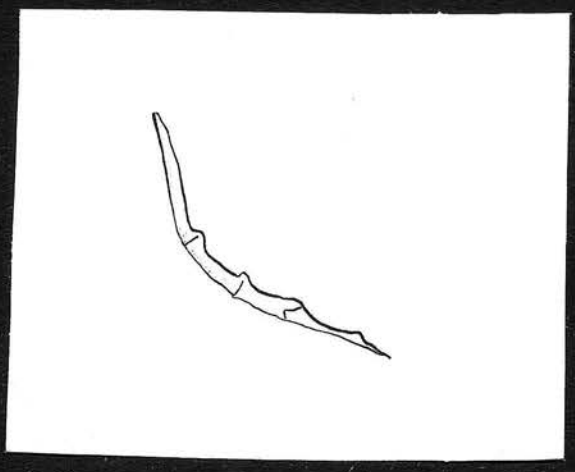


Fig. 3.

FIG IV

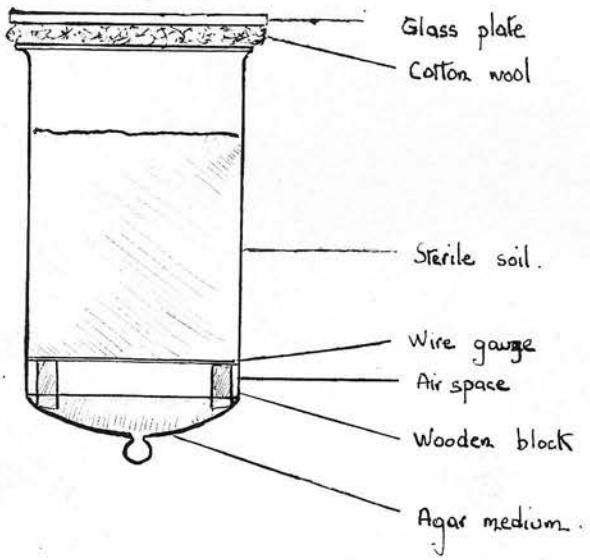
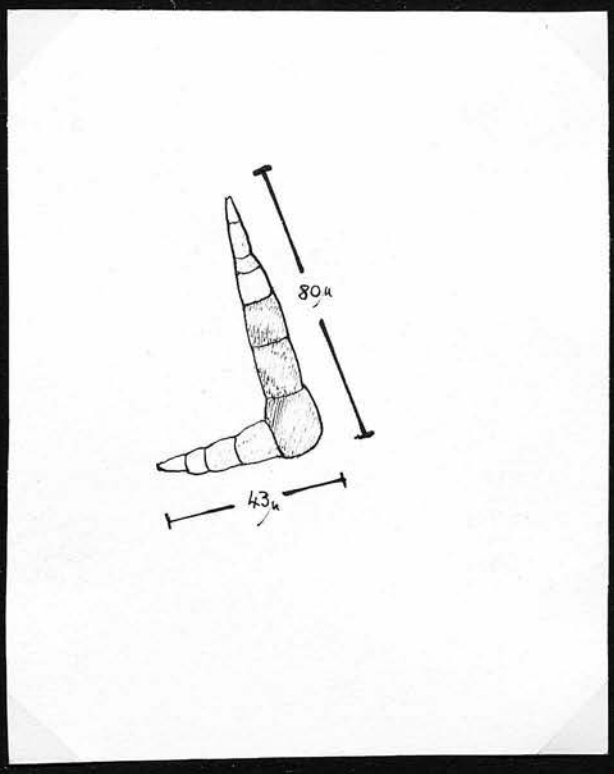

Glass plate
Cotton wool
Sterile soil.
Wire gauze
Air space
Wooden block
Agar medium.

Fig. 4



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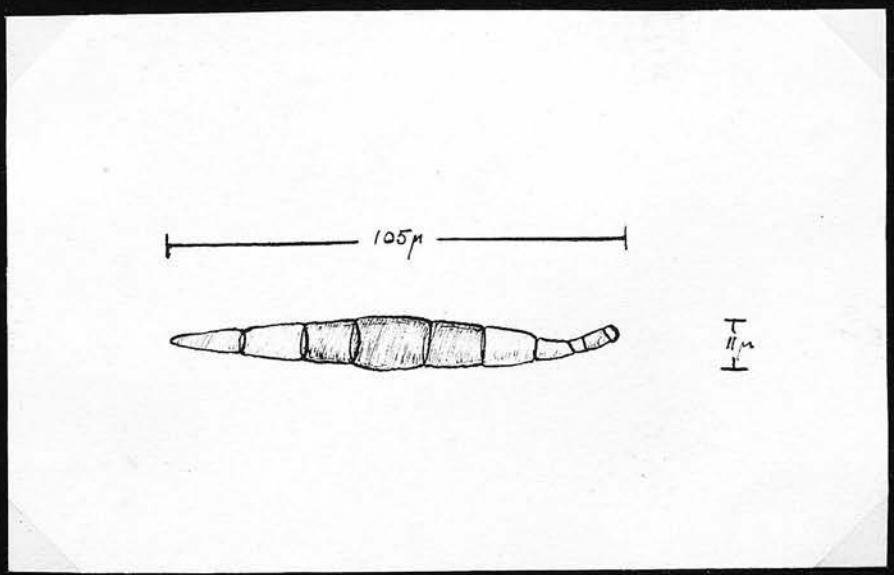

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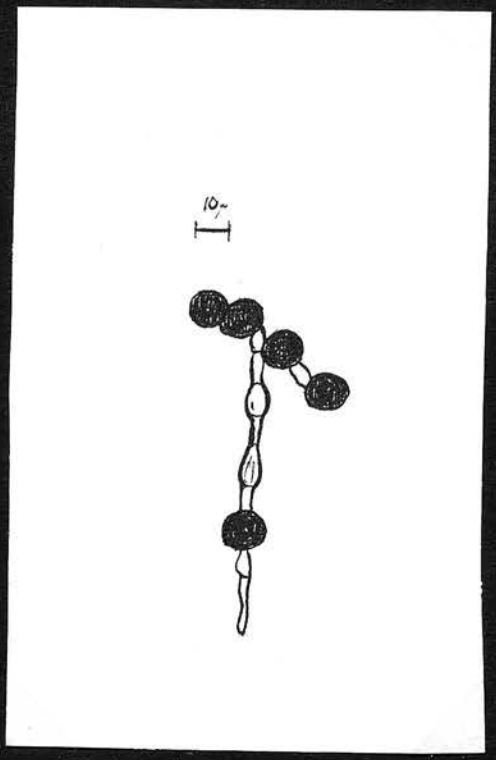


Fig. 7.

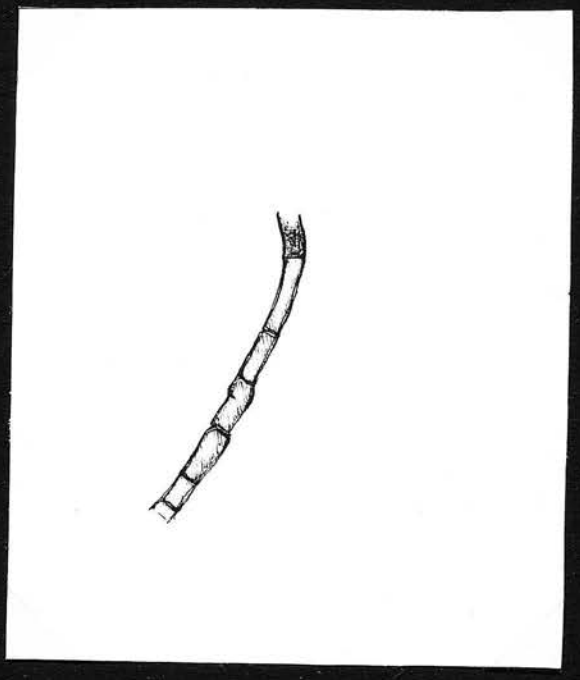


Fig. 8

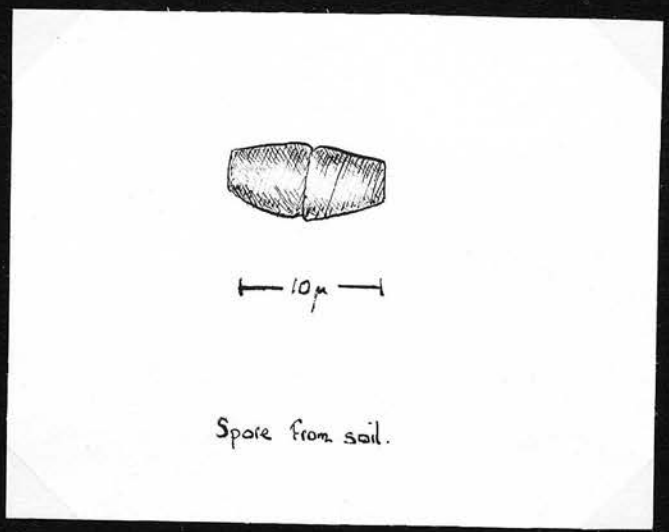


Fig. 9(a).

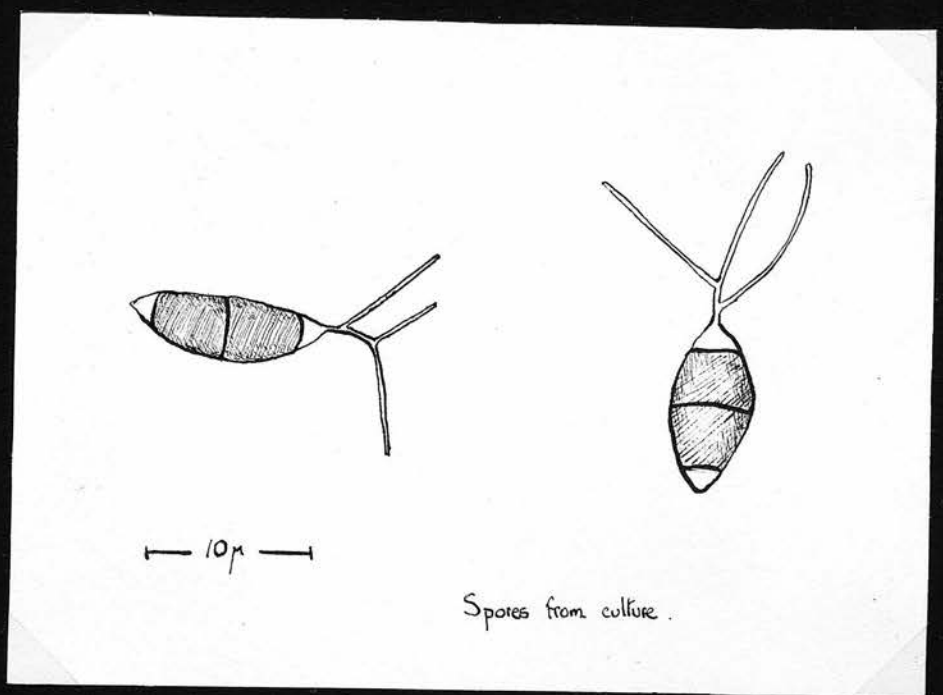


Fig. 9.(b)

TABLE I

Fungal Counts from 4 different soil samples of sub-plot A taken and plated simultaneously.

PLATE NUMBER	SERIES A.	SERIES B.	SERIES C.	SERIES D.
1.	42	29	34	33
2.	24	37	27	25
3.	31	32	26	40
4.	27	37	30	39
5.	26	34	32	37
6.	36	36	29	27
7.	32	26	31	26.
8.	36	35	28	30
AVERAGE	31·8	33·3	29·4	34·6

TABLE II

Monthly Average of Numbers of Fungi and Numbers of Bacteria and Actinomycetes per gram oven-dry soil

Sub-plot A.

	Fungi	Bacteria and Actinomycetes
January	65,000	3,750,000
February	54,000	2,500,000
March	67,000	2,900,000
April	92,000	3,500,000
May	75,000	9,700,000
June	65,000	4,370,000
July	82,000	3,000,000
August	90,000	4,120,000
September	110,000 100,000	9,100,000
October	122,000	7,100,000
November	75,000	5,400,000
December	62,000	4,750,000

TABLE III

Numbers of Soil Fungi developing in the different subplot Treatments.

	A	B	C	D	E
Total numbers of fungi: treated	—	24	20	29	22
Total numbers of fungi: control	—	31	28	30	27
Trichoderma lignorum	—	—	—	—	2
T. koningi	—	—	—	—	—
T. alba	0·5	—	—	—	—
Pestalozzia Hartigii	—	0·3	0·2	1	1
Mucor hiemalis	—	0·1	0·2	0·5	—
M. Rammannianus	0·5	—	—	1	1·5
Zygorhynchus vuilleminii	—	—	1	—	1
Coniothyrium fuckelii	5	3	3	4	—
Dematium sp.	4	3	3	7	—
Cephalosporium sp.	3	4	3	—	—
Fusarium spp.	1·5	1·5	0·8	0·3	0·5
Martierella sp.	—	—	—	—	—
Penicillium spp.	3	4	2	5	1
Eurotium spp.	1.	—	—	—	—
Total of identifiable fungi	18·5	15·9	13·2	18·8	7·0

A. — Fallow
B. — Weed growth permitted
D. — Filter paper added
C. — Dung added
E. — Nutrient solution added.

Figures represent average numbers of fungal colonies developing per plate.

TABLE IV

Numbers of fungal colonies developing on Conn's agar medium after exposure for 30 mins to the air above the soil plot.

Jan.	Feb.	Mar.	Apr.	May 1st	May 2/5th	June	July	Sept.	Oct.
26.	7.	30.	207.	111.	43.	24.	108.	426.	123.

TABLE V

Classification of Fungi into Soil Invaders and Soil Inhabitants.

Fungi in Soil only [Soil Inhabitants]	Fungi in both Soil and Air. [Soil Invaders]	Fungi present in Air only.
Mucor Rammanianus.	Trichoderma lignorum.	Botrytis cinerea
Zygorhynchus vuilleminii.	Pestalozzia Hartigii	Cladosporium herbarum. [possibly the Dematium sp.]
Trichoderma Koningi.	Coniothyrium fuckelii	
Trichoderma alba.	[Mucor hiemalis]	
	Mortierella sp.	
	Dematium sp.	
	Cephalosporium sp.	
	Fusarium spp.	
	Penicillium sp.p.	
	Eurotium spp.	

TABLE VI

NUMBERS OF FUNGI IN TOP 3" SOIL, TOP $\frac{1}{4}$" OF SOIL AND IN WORM CASTS, COMPARED

	TOP 3" SOIL	TOP $\frac{1}{4}$" SOIL	WORM CASTS.
TOTAL NUMBERS FUNGI	31	42	39
Penicillium spp.	3	10	9
Coniothyrium fuckelii	5	7	6
Dematium sp.	4	8	5
Pestalozzia Hartigii	—	—	1
Mucor rammanianus	0·4	0·5	0·1
Eurotium spp.	0·6	0·5	0·1
Mucor hiemalis	—	0·5	0·1
Trichoderma alba	0·6	0·5	0·1
Cephalosporium sp.	4	4	5
Fusarium sp.	—	0·6	—
Total of identifiable fungi.	17·6	31·6	26·4

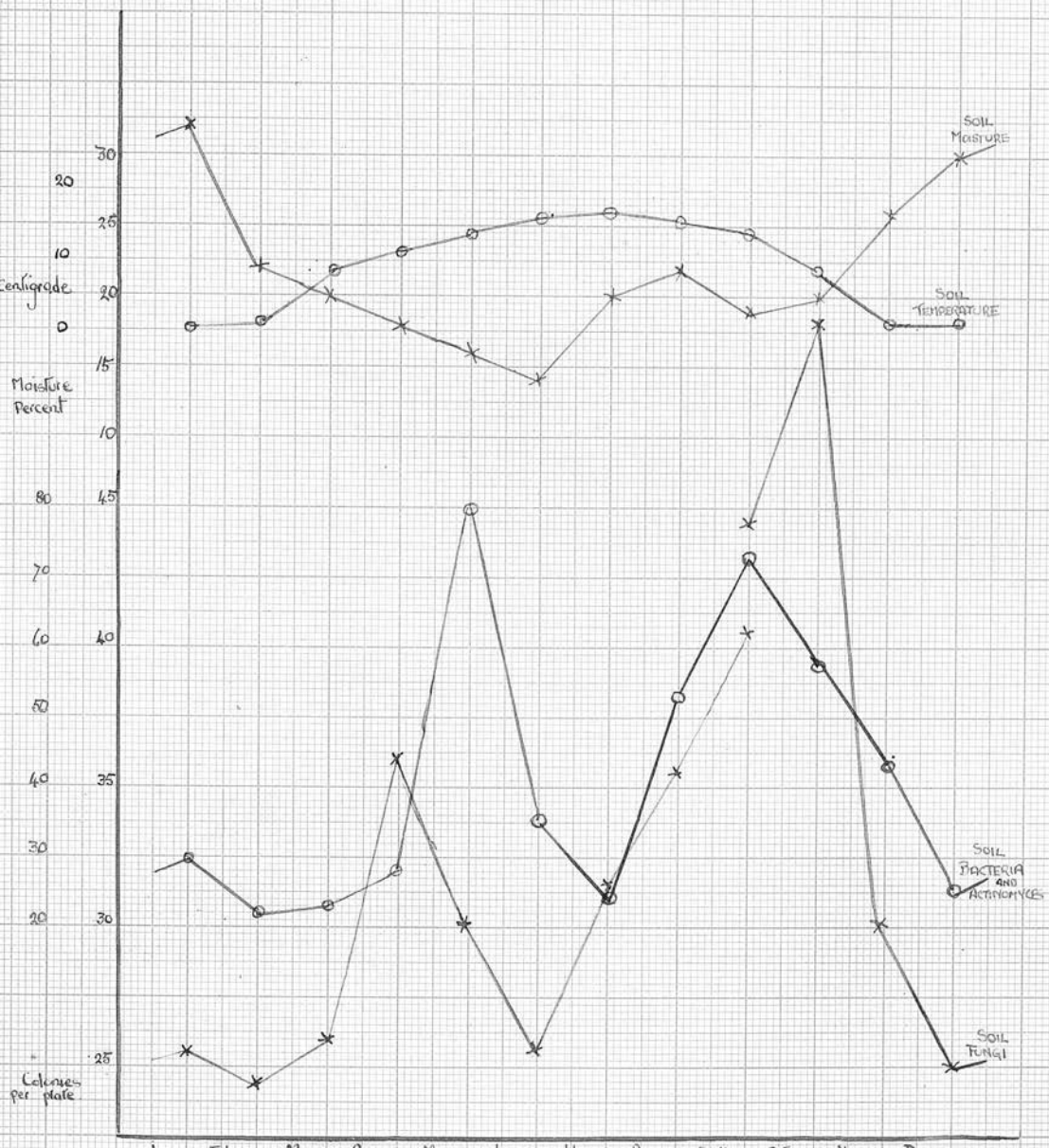
Figures represent average number of colonies developing on one plate.

TABLE VII

Effect of heating a suspension of spores of a Penicillium sp. for 2 mins. at 47·5 °C on the germination of the spores on an agar medium.

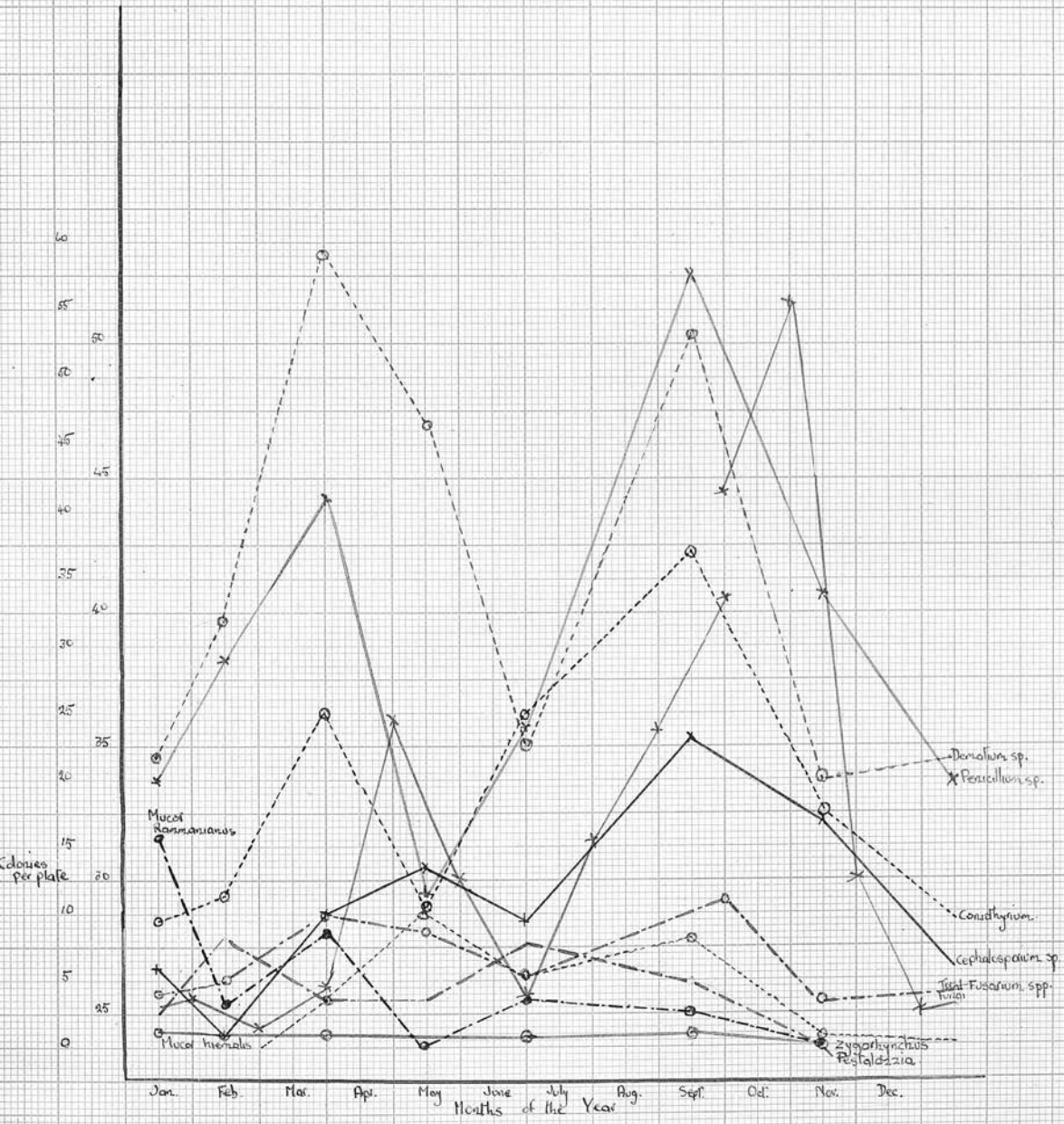
Number of plate	HEATED. Colonies developing per plate	CONTROL Colonies developing per plate
1.	44	36.
2.	40	43.
3.	38	43.
4.	40	37.
5.	37.	43.
6.	31.	48.
7.	42.	41.
8.	46.	32.
9.	45.	29.
10.	46.	42.
Average.	40·9	39·4

GRAPH I.



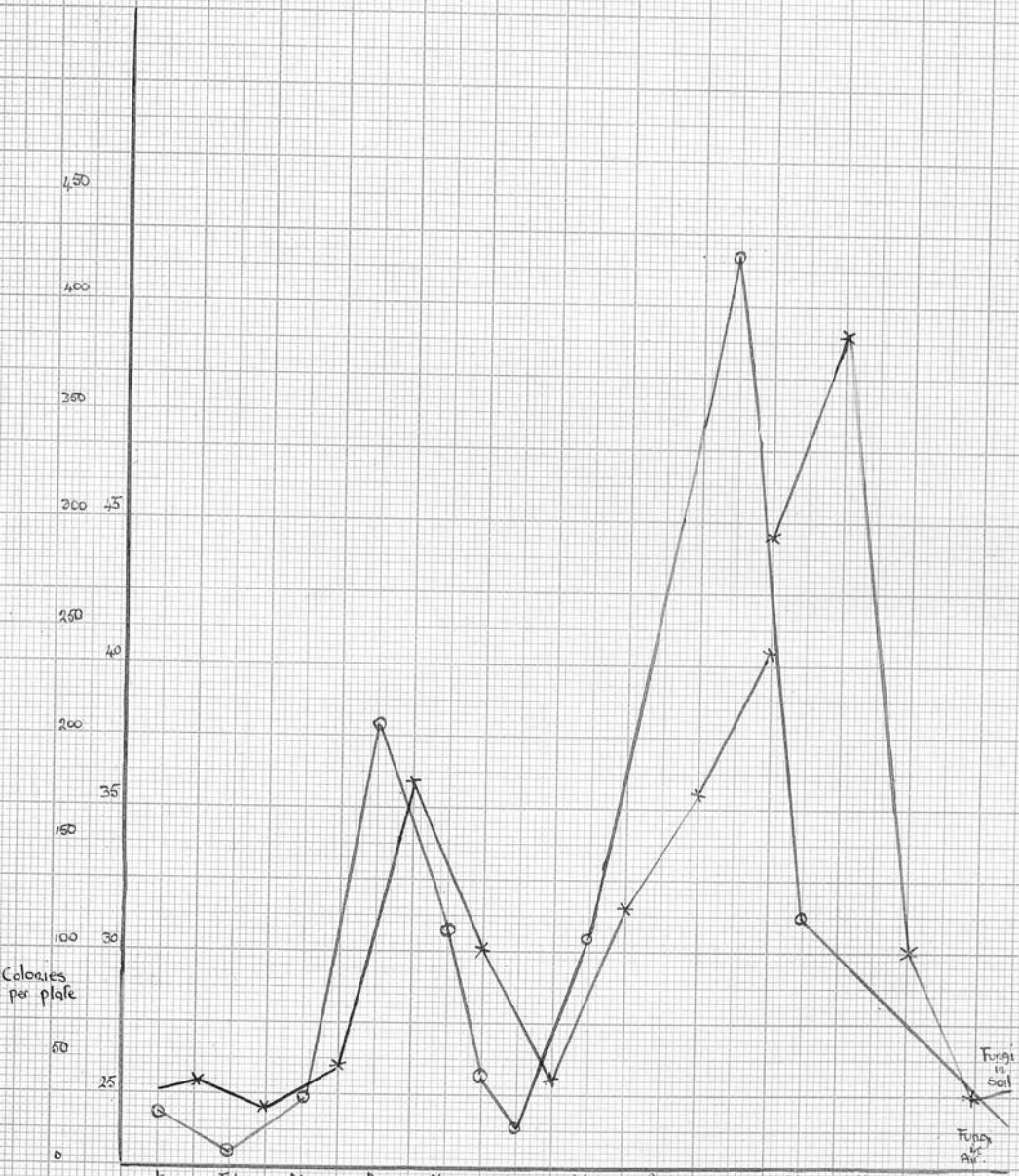
SEASONAL PERIODICITY of SOIL MICRO-ORGANISMS.

GRAPH II.

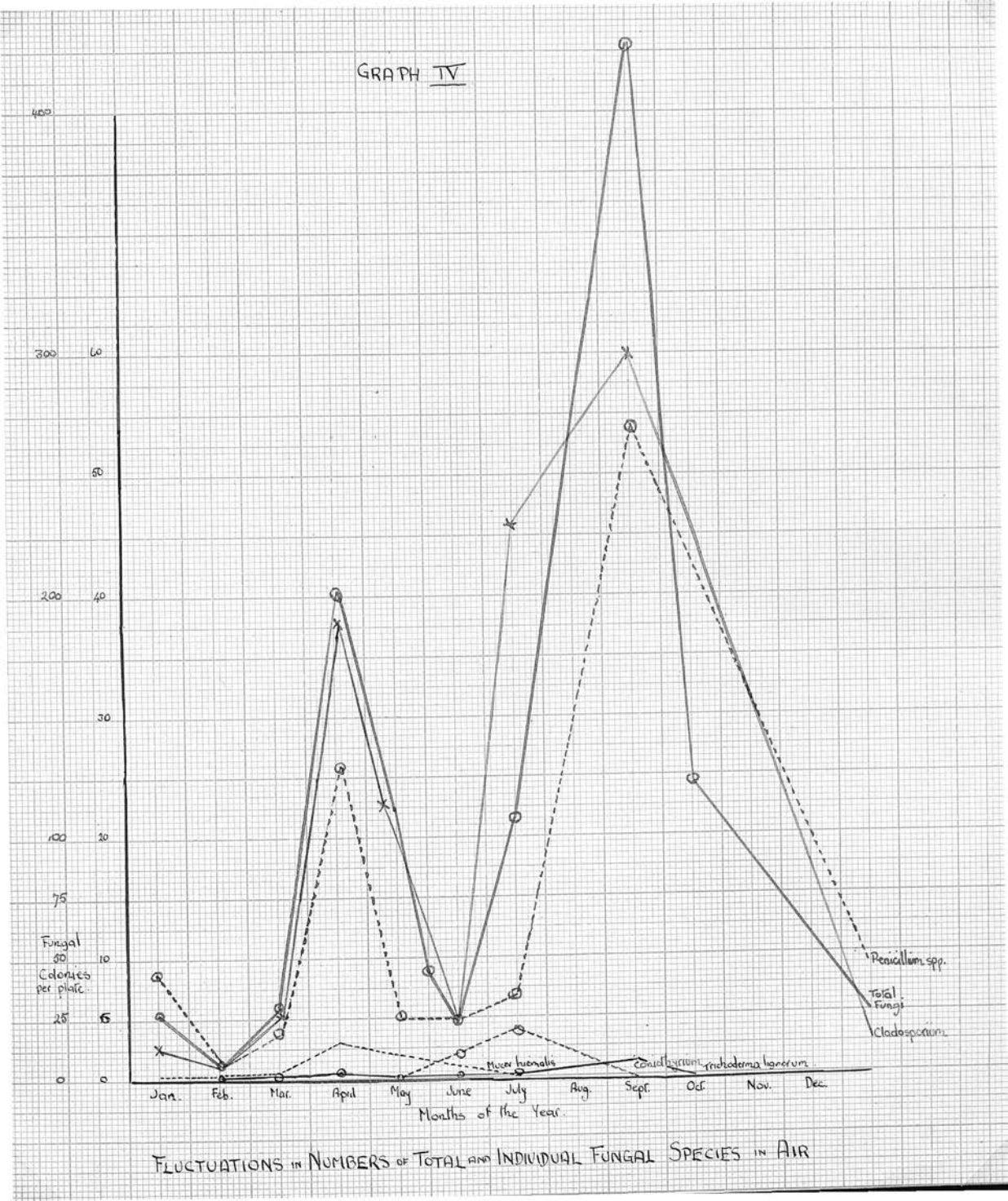


SEASONAL PERIODICITY IN INDIVIDUAL SPECIES OF SOIL FUNGI.

GRAPH III



SEASONAL PERIODICITY IN NUMBERS OF FUNGI FROM SOIL AND AIR.



FLUCTUATIONS IN NUMBERS OF TOTAL AND INDIVIDUAL FUNGAL SPECIES IN AIR