# FUNGI IN FENCE POSTS - ISOLATION, DISTRIBUTION AND INTERACTION OF FUNGAL SPECIES PRESENT AND THEIR EFFECT

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by

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## ABSTRACT

The ecology of fungi on birch (Betula sp.) and Scots pine (Pinus sylvestris L.) fence posts was studied. Techniques for obtaining isolates in the field from the surface and known depths of the posts were developed. Fungal colonization on the surface and at 5, 25 and 45 mm depths of the posts in relation to time of exposure was described and a number of factors relating to the establishment of Basidiomycetes were discussed. The pattern of fungal succession was postulated for three zones, (a) at ground line, (b) 18 inches above ground line, and (c) six inches below the top of the post.

Interactions between certain fungi isolated during this study were tested, by inoculating the test fungi (Trichoderma viride, Botryodiplodia sp. and Polystictus versicolor) in birch and Scots pine blocks in succession in different sequences. It was found that the sapstain fungus Botryodiplodia sp. was able to overcome the inhibitory effect of Trichoderma viride in the establishment of Polystictus versicolor. Interactions between several other fungi were tested in malt agar medium and their possible role in fungal succession was discussed.

In the soft-rot ability test certain fungi isolated from the posts were found to produce soft-rot cavities in hardwood but did not produce cavities in soft-wood. Chaetomium globosum a soft-rot fungus when subcultured several times in Abrams medium with Whatmans cellulose powder before testing for soft-rot ability was found to produce different types of degrade of cell wall in Scots pine and several types of cavities in birch.

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## SECTION I

## Seasonal distribution and Succession of fungi on birch and Scots pine fence posts

### Introduction

The project was undertaken to study the distribution and variation of fungi colonizing birch and Scots pine posts at different regions of the post, and to investigate the pattern of succession leading to the establishment of decay fungi inside the wood. Various interactions between fungi were encountered during their isolation, and a few of these were examined in the laboratory. An attempt has been made to correlate their effect on the general pattern of distribution and succession. In the course of these studies observations were made on the possible impact of the seasonal variation of air borne spores on the wood surface flora.

Information available on the distribution and succession of fungi on debarked wood especially from the surface to the inside is severely lacking. The little that is known on the subject is based on isolations from the sub-surface layer, and this may include only those fungi which have survived the first phase of succession, namely active competition in the surface flora and have then entered the second phase by penetration inside the wood. The present work suggests that the surface colonizers may have an important role in wood decay. It may be that the surface colonizers have a significance and that the sequence of succession and the colonization of decay fungi inside the wood is determined by the competition, interaction and synergism between the fungi colonizing the surface of the wood.

Several schemes of fungal succession on different substrates have been postulated in the last thirty years and some of these are discussed in the following pages. The colonization and succession will no doubt differ in different wood substrates and in different environments, yet it would be worthwhile comparing the present results with the existing knowledge on the subject.

Several early workers have isolated fungi from the wood by removing periodically a number of stakes or posts to the laboratory, assuming that the fungal succession followed the same pattern in subsequent samples. In this experiment this has been avoided as far as possible and the series of periodic isolations have been made from a number of posts with the minimum disturbance of the environment and the process of succession in progress. The sampling technique developed was only partially destructive, and made it possible to keep the posts undisturbed in the ground and yet enabled samples to be taken periodically from both the surface layers and in depth.

As will be discussed later there are clearly a number of problems to be overcome, such as air-borne contamination at the time of making the inoculations and the opening up of the inside of the post to chance infection in the bore hole which could seriously affect the results of subsequent samplings. Evidence will be presented to show that neither of these theoretical sources of error are at all serious if proper precautions are taken.

Literature Survey

The ecology of fungi has received considerable attention in the last few decades. Some of the early workers laid stress on the study of distribution of fungi related to the habitat, season or plant communities. The subject has been reviewed by Cooke (1948 & 1953). The published literature on the colonization and succession of fungi mainly associated with wood as a substrate is briefly discussed here.

In a survey of interaction between fungi D'Aeth (1939) has pointed out that "successional diseases may occur on the living host through the action of one fungus in developing a substrate which is more favourable for a second fungus, a process comparable to the successive metabolic stages of decay under natural conditions". A year later Findlay (1940) recorded a sequence of species at various stages in decay in logs after felling. In birch logs he cited that the non-decay fungi such as Bispora sp. and Lasiosphaera sp. and the Hymenomycete Stereum purpureum were the pioneer invaders and these were followed by other wood-rotting Hymenomycetes. In coniferous wood sap-staining fungi appeared first and these were followed by wood rotting fungi. He concluded that the first organisms to appear were those which feed on sugar and starch, and were followed by true wood-rotting fungi. If the log has been attacked by white-rot a single species could cause complete decomposition but if a brown-rot fungus had attacked the log then the thirty per cent of the material which was left after decay would be decomposed by soil micro-organisms. In his paper he put forward some interesting questions as a guide to future workers on the subject, "How far succession is accidental and dependent merely on the intensity of spore infection of the different species and the rapidity with which they develop, and how far it is true succession conditioned by slowly changing chemical composition of the substratum are matters of interest".

The succession of fungi on wood remained a neglected subject for a long period. The more intensive study on fungal succession started after the remark Chesters (1949) made "Have we given enough thought to what goes on above the soil in relation to what we are striving to examine within the soil".

Chesters (1950) reported that Ascomycetes were the main primary and secondary colonizers on fallen logs and branches of birch and beech, but many Basidiomycetes especially Polystictus versicolor and Stereum hirsutum were also primary colonizers. The primary colonizers attacked the bark and surface layer of the wood. Once the primary phase of colonization was well advanced, succession depended on internal and external factors. He pointed out that after decortication the exposed wood could then be colonized by the original primary colonists of the bark which could continue to grow with secondary colonists of the bark along with a few other fungi which colonised the debarked wood directly. He noticed some peculiar associations between the colonizers in the secondary stages of colonization, and mentioned that a number of species of wood inhabiting fungi were associated with the succession of fungi on birch and beech logs.

The most detailed study of succession of fungi on lignicolous substrates was made by Mangenot (1952). He studied the decomposition of trunks of some hard woods and suggested the following stages of decomposition:

1. Phellinus stage: which included a list of Basidiomycetes most of which were parasitic and same bark-inhabiting Ascomycetes.

2. Phialophora fastigiata stage: in this stage the surface of the wood was invaded by "sugar fungi" which did not penetrate deeply into the wood. Cladosporium herbarum was one of the first fungi to appear on the surface.

3. Melanomma stage: this included several Ascomycetes and Fungi Imperfecti which colonized the wood where bark has been removed.

4. Mortierella ramanniana stage: This was the first stage of decomposition of wood which included Chaetomium, Fusarium, Cylindrocarpon spp. and two species of Mortierella.

5. Leptoporus stage: This included some species of Basidiomycete, which were responsible for the decay.

6. Mollisia stage: This phase included those fungi which colonized the trunk in advance stage of decay.

7. Bisporomyces stage: This was the final phase of colonization and included the species of fungi which often developed on the remains of other fungi.

The sequence of these stages depended on the presence and absence of stage 1., and the superficial humidity of the wood.

Another succession pattern with similar trends was given by Meredith (1959, 1960) on freshly cut pine stumps. He found that several Basidiomycetes and Ceratocystis spp., were the primary colonizers and these were followed by several other agarics and polypores. Käärik and Rennerfelt (1957) also claimed that the primary colonizers were Basidiomycetes along with several species of Fungi Imperfecti. They mentioned that the number of non-Basidiomycetes increased with the age of spruce and pine stumps.

A similar but more extended sequence of succession has been pointed out by Findlay (1966). The sap-staining moulds and Basidiomycetes are the pioneer colonizers and these are followed by cellulose and lignin decomposing fungi and finally succeeded by Lycoperdon pyreforme and Myxomycetes capable of living on partially decomposed cell wall and on residues of earlier colonizers. He has also mentioned that "it is often a matter of chance which fungus succeeded in establishing itself first in a stump and this may determine the subsequent succession of fungi that colonise it. The survival of the infection would depend on its tolerance to the seasonal variation of moisture content".

Basidiomycetes have not been found as the primary colonizer on logs and branches in many of the recent works. Etheridge (1961) found working with dead aspen branches that the primary invaders were bacteria followed by non-decay fungi and then by decay fungi. In the succession of fungi on felled beech logs Ueyama (1961) reported that the sap-stain fungus Ceratocystis moniliforme was the early colonizer. This was followed by Aspergillus spp., Penicillium spp., and Trichoderma spp. and then the logs were colonized by wood-rotting fungi. A similar finding has been

recorded by Shigo (1962) on cut ends of hard wood bolts but the species of fungi involved were different. On unpeeled pulpwood logs of birch and aspen Henningson (1967b, 1967c) found that the fungi with low wood-destroying activity were the early invaders and severe wood-destroyers appeared much later. A number of non-Basidiomycetes and bacteria were found to have invaded the stored logs in addition to decay fungi. He also mentioned that almost all decay fungi isolated were white rot fungi.

More recently Maloy and Robinson (1968) suggested that the bacteria were the initial colonizers followed by Fungi Imperfecti which probably invade in some order of succession. Basidiomycetes either followed Fungi Imperfecti and bacteria or appeared concurrently in fir. Good, Basham and Kadzielawa (1968) from their study of the decay process in maple also reported that the initial invasion was by Fungi Imperfecti and bacteria and not Basidiomycetes.

Several interesting papers have been published by Garretton the sequence of micro-organisms in plant root diseases and in the utilization of plant debris. Garrett(1950) pointed out that the root parasites were the primary colonizers and with the progressive lowering of host resistance the active colonisation of the parasite in the host tissues might be shortened by the extensive invasion of secondary micro-organisms. A similar pattern of succession has been recorded by Rishbeth(1950) in pine roots in which Fomes annosus is replaced by Trichoderma viride and Peniophora gigantea.

On injured, moribund and dead tissues Garrett(1951 & 1956) suggested that the saprophytic "sugar fungi" representing some members of Phycomycetes were the pioneer colonizers but were the first to disappear during decomposition. Rapid germination and growth were the reasons for their success as pioneer colonizers. However, the sequence of colonization by micro-organisms on a substrate must be determined by the nature of the substrate and by the micro-habitat (Garrett 1955). The question Garrett (1955) raised that "the colonies of fungi developing on the isolation

plate may have come either from the currently active mycelium or from resting bodies produced by fungi active during an earlier stage of this succession" poses a complicated problem. This can probably be evaded if the emphasis is mainly laid on the total number of fungi involved in the succession and the sequence with which they appear on the substrate. However, the period for which the primary colonizers persist in an active state may have some significance in determining the succession and therefore should not be neglected though it may not be possible to trace this period experimentally.

A very generalized pattern of fungal succession on dead plant tissue within or upon the soil was proposed by Garrett(1963). He postulated that the primary saprophytic "sugar fungi" appear in the first stage of decay followed by the cellulose decomposers and associated secondary saprophytic "sugar fungi". Finally the lignin decomposers and associated fungi colonise the substrate. The present results presented on the succession of fungi on fence posts will be discussed later on the lines of Garrett's proposed scheme.

It would be worthwhile to mention some of the investigations of the succession of fungi in discoloration and decay in wood of living trees and trees killed by various agents. Good & Nelson (1962) found a large number of fungi associated with Fomes igniarius in decay of living poplar. They concluded that wood in which F. igniarius grows has in most cases, already been colonized by other organisms. These primary colonizers included a number of moulds, sap-stain and soft-rot fungi which were thought of as saprophytes rather than as initial invaders of living woody tissues. Shigo (1965) made similar observations in several living hardwood trees. He found that several bacteria, Ascomycetes and Fungi Imperfecti which were associated with discoloration were the first to invade the wood of living trees. "Hymenomycetes did not always follow these organisms, but these organisms always preceded the invasion by Hymenomycetes". Shigo (1969) recently pointed out that the bacteria and non-decay fungi are the first organisms to inhabit the injured tissues of the tree, which are followed by

decay fungi in the formation of cankers. That sap stain fungi preceded invasion by Basidiomycetes was also reported by Basham (1957, 1958, 1959) in trees killed by fire or spruce bud worms. In jack pine Basham (1966) found that non-decay fungi preceded Peniophora pseudo-pini and Fomes pini, and with increasing host age P. pseudo-pini was progressively replaced by other fungi notably Fomes pini.

Some interesting work has been done on the succession of fungi on herbaceous stems. Webster (1956, 1957) and Hudson and Webster (1958) found different courses of succession on the three upper and the three lower internodes of grasses. In dicotyledonous herbaceous shootsYadav (1960) recorded that the upper internodes of the shootswere colonized first and the lower internodes were colonized subsequently.

The distribution and succession of fungi in debarked timber has received far less attention especially for timber in contact with soil. The fungal succession on wood blocks submerged in the sea has been discussed by Jones (1963). He found that Fungi Imperfecti were the early colonizers in submerged wood and these were followed by Ascomycetes. He and others have published several papers on the distribution of marine fungi in wood.

Corbett and Levy (1963) were probably the first to study in detail the distribution and succession of fungi on untreated and debarked wood in contact with soil. This work was briefly mentioned earlier by Levy (1962) and he suggested that the moulds and other fungi growing on the surface are important precursors in the initiation of decay. They based their results on the periodic isolation of fungi from fence posts of birch and Scots pine at a depth of 5 mm. from the surface. The distribution of fungi on the posts situated in three different environments was compared and a pattern of succession was postulated. They recorded that moulds such as Trichoderma viride, Penicillium spp. and Botrytis sp., were the early colonizers which were followed by several soft-rot fungi belonging to the Sphaeropsidales. Some of the species of Moniliales such as Fusarium spp., Gliocladiopsis sp., Cylindrocarpon sp., Memnoniella sp. and Streptomyces sp., appeared later in the succession following the soft-rot fungi. The decay-

fungi such as Coprinus and unidentified species of Basidiomycetes were late colonizers and were isolated from the centre of birch posts after about 18 months exposure.

A similar pattern of succession of moulds - soft-rot - decay fungi has been described on pine stakes by Merrill and French (1966). Fusarium solani, T. viride, Aspergillus ustus and Penicillium spp., which accounted for 92 per cent of all isolates were the early colonizers. They recorded that soft-rot was visible in sections of the wood with polarized light after 6 weeks exposure and after 10 weeks small pockets of brown-rot were visible.

The distribution of fungi throughout the sub-surface zones of birch and Scots pine was described by Greaves (1966). He divided the post into nine zones (three above the ground line and six below the ground line), and isolated fungi from 5 mm. below the surface from each of the nine zones. Some of these fungi were tested for their cellulolytic and lignolytic activity and also for soft-rot ability.

The succession of fungi on pine and spruce poles in contact with soil was studied by Käärik (1967, 1968). He found that in six months the posts were colonised by a large number of Ascomycetes and Fungi Imperfecti and attacked by different Basidiomycetes in localised patches. There was no change in the next six winter months. After 18 months the non-basidiomycetes fungi on the posts were different from those which had colonized earlier and Basidiomycetes e.g. Peniophora gigantea, Polyporus borealis and Stereum sanguinolentum occurred in high frequency in some poles, and was distributed over large areas of the pole.

An investigation in the succession of fungi on untreated stakes of Pinus radiata was carried out by Butcher (1968a, 1968b). He isolated fungi from just beneath the surface of the wood from several zones at regular intervals. In above-ground zones the succession did not advance further than the mould stage. At the ground line Fusarium spp. and Epicoccum nigrum were the primary colonizers followed by T. viride and a few other moulds.

Basidiomycetes appeared after the stakes had been exposed for 3 months but were not encountered after nine months of exposure. He also mentioned that white-rot fungi may precede brown-rot fungi. More recently Levy (1968) raised a new aspect of the problem in his statement that "clearly there is a complex of organisms present, and the indications are that the succession or the association of certain organisms may either inhibit or stimulate decay".

The ecology of fungi on treated wood has also received little attention. A number of fungi associated with treated timber were isolated by Greaves & Savory (1965) and the pattern of succession of fungi on treated wood was investigated by Butcher (1968a, 1968b). [redacted] Fuller (1969) isolated and identified several species of moulds, sap-stain and soft-rot fungi from treated stakes from the Ivory Coast. The breakdown of preservatives by fungi has been investigated by several workers and relevant literature has been cited elsewhere, along with the literature on the interaction and antagonism between the fungi. (c.f. Sections II and III)

Recently the subject has been reviewed by Shigo (1967) and Hudson (1968). Shigo discussed the succession of organisms in discoloration and decay of wood, in living trees, trees killed by various agents, in pulp wood, and in wood products. He suggested that bacteria and non-hymenocycetes are the early colonizers and Basidiomycetes usually follow these organisms in decay. In conclusion he stated that "in nature many organisms are involved in the processes that begin with infection and end in total decomposition. Through an understanding of succession, the process perhaps can be regulated to suit our needs".

Hudson (1968) published a review on succession of micro-fungi on plant remains above the soil surface. He compared the schemes postulated by Chesters, Garrett, Mangenot and others on succession of micro-organisms on lignicolous substrates. He suggested that the effect of competition and synergism must be considered in the further development of the ecology of fungi on plant remains above the soil.

A few of those fungi isolated from the fence post were tested in the laboratory for their soft-rot ability. They were grouped into

soft-rot fungi on the basis of available lists of those organisms from the literature (Savory, 1954; Duncan, 1960; Duncan and Eslyn, 1966; Greaves, 1966; Gambetta & Orlandi, 1967; and Rösch & Liese, 1968.)

From the authors cited there would appear to be conflict between those who claim that wood-rotting Basidiomycetes are primary colonisers and those who say that these organisms only occur after a succession of other fungi. If, however, one thinks in terms of the condition of the woody substrate at the time of sampling this conflict can be resolved. Rishbeth & Garrettwere working on tree stumps and roots, Chesters and others on felled logs and branches complete with bark, in the forest floor in an environment of active Basidiomycete soil flora. Shigo and others worked on living trees which had been infected from above ground. Corbett, and others were using clear, sound timbers erected in an environment which did not necessarily contain a large flora of active wood-decaying organisms, since the amount of woody material in the litter and upper layers of the soil was very much less than that likely to be found in a forest or woodland. As will be discussed later the fungi likely to be found on a wood substrate in each of these environments is likely to depend initially on either the local soil flora or on the organisms already present in the wood.

Material and Methods

Initial tests were made to establish the isolation techniques to be used. Untreated Birch (Betula sp.) and Scots pine (Pinus sylvestris L.) posts erected on the site in 1961 were used and a series of isolations were made over a period of five months to determine the effectiveness of the existing procedures, and make modifications where necessary; to decide the most useful isolation media; and to see how far it was possible to carry out this work on site so that the posts could be sampled without removing them from the field to the laboratory. This would have meant that the successive isolations would have had to be carried out on different posts rather than taking a series of isolations successively from the same post. It was also necessary to determine the maximum number of isolates that could be handled and processed at any one time and the minimum time that would be required between each sampling date.

Asmah (1967) had shown that it was possible to make isolations from both the surface of a standing post and at various depths beneath the surface, but it was not known whether the chance contamination likely to occur as a result of opening petri dishes in the open air would be so great as to mask the fungal species likely to be isolated from the wood, nor was it known whether infection in the sampling bore holes would readily take place, and if this occurred whether the resulting fungal growth would seriously affect the results in succession from subsequent samplings.

As a result of these initial tests a technique was evolved which made it possible to check the contamination from air-borne spores and at the same time keep the petri dishes used for isolating the fungi from the posts free from chance contamination to a remarkably high degree, certainly greater than with the other sampling techniques.

New untreated half round stobs of birch and Scots pine with a top diameter of 5 inches, a length of about 5 feet 6 inches and pointed at one end were driven vertically into the soil to a depth of approximately 18 inches. These were erected in the field at the farm site (described later) and set up in a row some six inches apart parallel to the original

row of posts and some four feet from them. They were sampled at monthly intervals for over ten months.

Field Site: The posts were set up at the farm site of the Imperial College Field Station, (Silwood Park, Sunninghill). The posts of birch and Scots pine were set up randomly in the row instead of placing the posts of the same species of wood together or arranging them alternatively. The soil in the farm site is usually wet. Grass surrounds the base of the posts to a depth varying from 3 inches to 2 feet (according to the season of the year) above ground level. These are exposed to the South-east, but are sheltered on the opposite side by rising ground covered with bracken and scrub which provides a wind break and some degree of shelter (Levy 1965, Corbett and Levy 1963).

Sampling procedure:

Each post was divided into three zones and samples were taken at four depths in each zone (Fig. 1.).

Each zone represents a particular environment in the post, based chiefly on the difference in the moisture content of the post at different points along its length (Corbett and Levy 1963, Levy 1965).

Zone 1 comprised the top six inches of the post. Here the moisture content varied both from one season of the year to another and during a particular day. Rain or dew or frost would be deposited on the cut end surface and water would soak into the wood along the grain. In sunny weather this end grain would dry out rapidly and after repeated wettings and dryings checks and splits were observed - in this zone. Spores deposited either from the air-borne flora or by insects or birds undoubtedly penetrated these checks and splits, drawn in with the deposited water. It was therefore decided that a fair sample of this flora could be obtained by making isolations at about six inches from the top of the post.

Zone II comprised the region between zone I and a point near the ground line where the surrounding vegetation might bring about an environment of high local humidity. Penetration of water across the grain

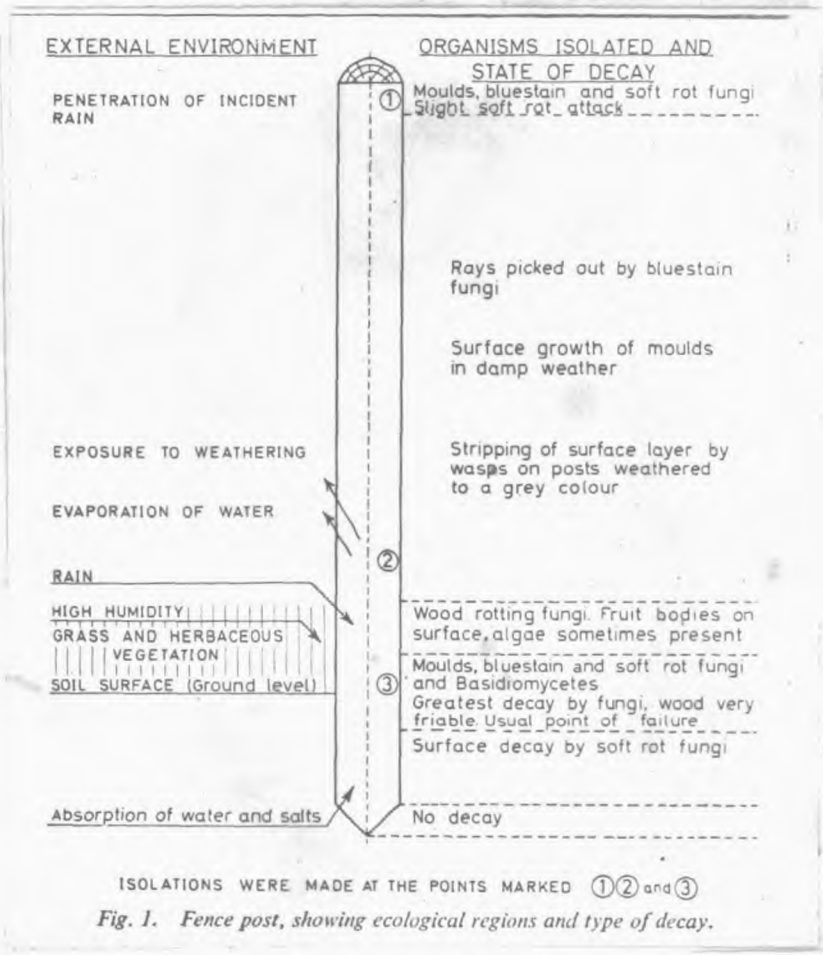



*Fig. 1. Fence post, showing ecological regions and type of decay.*

**(Levy - 1968)**

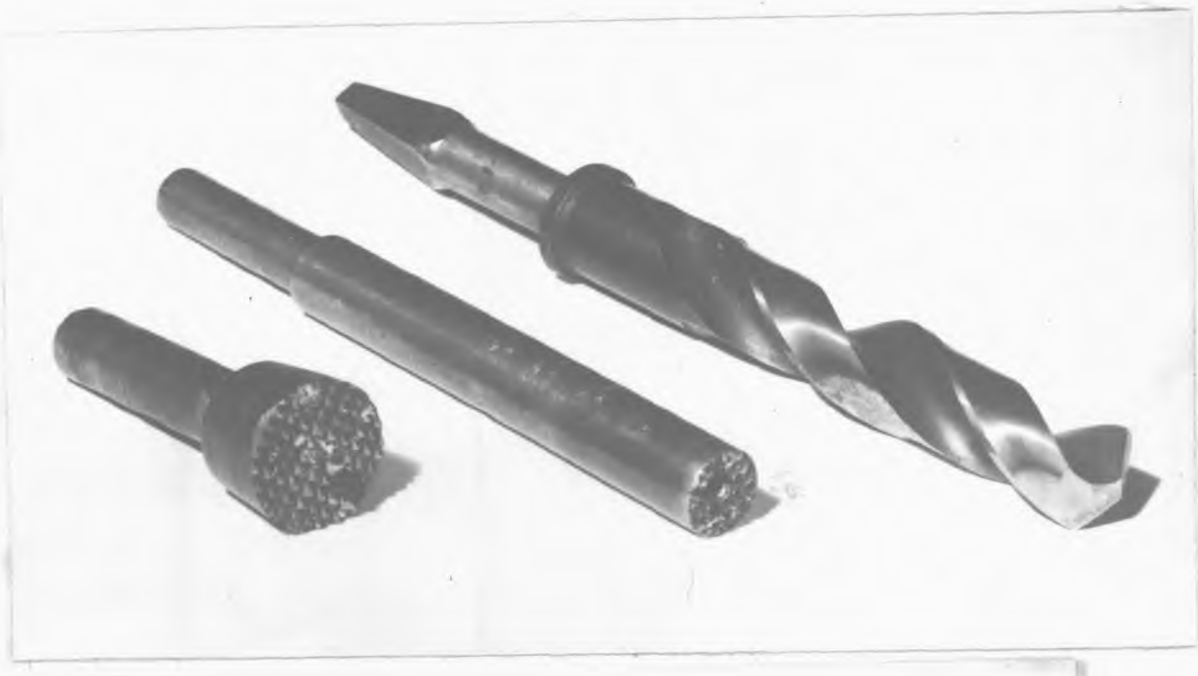


**Fig. 1B - From left to right, (a) Surface grinder (b) Calibrated grinder bit for sampling base of hole, (c) Calibrated twist drill for boring hole.**

is known to be much less than that along the grain and since vertical surfaces only were present, there was a much smaller chance of lodgement of air-borne spores. This was borne out by the subsequent isolations which showed a markedly smaller fungal flora present in this region, particularly inside the post. The moisture content of this region was low due to the poor penetration of water across the grain of the wood and the rapid drying effect of the wind. Samples were taken at 18 inches above the soil level.

Zone III comprised the region at the ground line and just below the surface of the soil. Here the moisture content was always high - the soil acting as a constant reservoir of water which was readily picked up by the wood. In addition the surface of the post was in direct contact with the soil flora which consisted not only of spores, but of actively growing mycelium. It is in this region of the post that the most active decay usually occurs - which will ultimately cause the post to fail by breaking off at the ground line or just below.

Two newly installed posts, one each of birch and Scots pine were sampled each month for a period of ten months. The samples of wood particles were taken from the surface and at 5 mm, 25 mm and 45 mm depth at each zone and were inoculated in the field to four or five nutrient media in petri dishes. (Details of these techniques are given later).

It was not possible to sample a post for more than three or four consecutive months (depending on the diameter of the post), therefore, when one post was fully sampled the subsequent samples were taken from another post of the same species of wood (which was selected at random). Each zone was sampled successively at the same horizontal line leaving approximately $\frac{3}{4}$ inch gap between the sampling areas, in order to avoid sampling the area where the fungal flora might have been disturbed during the previous operation. (Care was taken not to touch the sampling area which could very much alter the results). It was decided not to sample from above or below the previously sampled area especially at ground line because the

fungal flora might significantly differ at slightly above or below the ground line. Greaves (1966) and Butcher (1968a, 1968b, 1969) have shown that the fungal flora on the post or stake may be significantly different, a few inches above the ground line, at the ground line and a few inches below the ground line. c.f. Fig.2a which shows the infection of the post and the vertical and horizontal spread of that infection after 18 months.

Three posts of each wood species were sampled during the experiment to study the succession of fungi and a fourth post was sampled every three months to study the variation in the fungal flora in two different posts of the same wood species, sampled at the same time. In the results and discussion all the posts have been considered as a part of a unit, assuming that the succession of fungi have been initiated simultaneously and proceeded in an identical pattern in each species of wood. This is the assumption that most workers in this type of problem have made. (See Tables 11, 12 pages 74, 75)

During the time of sampling the open petri dishes with different media (the types used for inoculation) were placed on the top of other posts near the one which was being sampled, as freely exposed traps for collecting the air-borne spores. These petri dishes are referred to in the text as control plates. All the control plates were changed every 15 minutes. A very large number of fungi, mostly belonging to Fungi Imperfecti and several Phycomycetes were trapped, which helped in finding out some of the possible contamination by air-borne spores in the petri dishes inoculated with the wood particles. One species of Basidiomycetes was once isolated from the control plates. Hirst (1953) has also reported that in freely exposed traps for collecting air-borne spores the "hyaline basidiomycetes" are rarely isolated. This method for trapping the air-borne spores is not as efficient as the modern apparatus for collecting spores. Gregory (1950) pointed out "the trap efficiency varies with conditions, sometimes widely enough to make a direct interpretation misleading". But perhaps for checking the contamination of air-borne spores during inoculation of the samples in the field this simple method for spore trapping could serve a

Fig. 2B - Birch post cut vertically to show plugged hole after boring. Note no fungal stain from hole indicating no contamination due to sampling

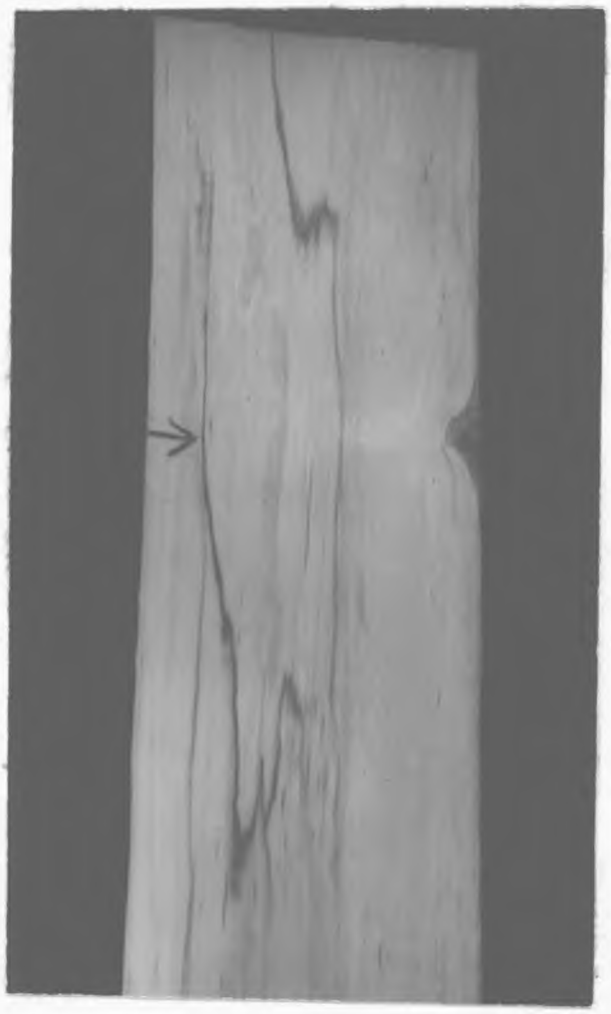


Fig. 2A - Birch post cut vertically to show discolouration and zonation by fungi inside the post. Arrow indicates the direction of sampling

useful purpose since the main point for contamination was the open petri dishes at the time the inoculations were made. Considering the volume of work involved in this study an elaborate method for spore analysis was barely possible.

After each sampling the hole made for collecting samples from different depths was plugged by a sterilized wooden peg or a sterilized cotton wool plug and sealed with molten wax to prevent any contamination entering the wood. Special precautions were taken as far as possible not to interfere with the area around the hole, and have proved effective (Fig 2b).

Technique for extracting samples: Several techniques have been used for extracting small size samples from the decayed wood to be used as inocula for isolating fungi. The different techniques used have been described by Levy (1962, 1967, 1968), Corbett and Levy (1963), Greaves and Savory (1965), Shigo (1965), Greaves (1966a), Okigbo (1966), Asmah (1967), Käärik (1967), Butcher (1968a, 1968b, 1969), and Grant and Savory (1968). The techniques are briefly described below:

1. The Pressler Increment Borer: With the borer a plug is removed from the wood core and all or part of this is planted in the medium as inoculum (Corbett and Levy 1963, Butcher 1969).
2. The Two Chisel Technique: With one sterilized chisel the superficial layer of decayed wood is removed and with the other sterilized chisel the samples are taken from the underlying wood and they may be split into small segments before planting on the medium. (Greaves & Savory, 1965; Greaves, 1966a; Butcher, 1968a, 1968b).
3. The Cut-Block System: A small block is removed from the wood which is then split into smaller blocks for planting in the medium (Corbett & Levy, 1963). Käärik (1967) has sawn out a disc from the pole, split it into small blocks and planted them on the medium.
4. The Split-Billet Technique: Samples are cut from the freshly split surface of decayed wood and are planted on the medium (Shigo, 1965, Basham, 1958).
5. The Saw-Cut Technique: The material being sampled is cut by a

hack-saw blade and the saw-dust is allowed to fall on the petri dishes with media (Greaves & Savory, 1965; Greaves, 1966a).

6. The Drill Technique: This technique was earlier developed in this laboratory (Okigbo 1966, Asmah 1967, Levy 1967, 1968) and it has been used in the present investigation with certain modifications.

"The drill technique involved the preparation of fine particles of wood produced at known depths and at particular places on the material to be sampled" (Levy 1967). Okigbo (1966) used a metal drill, $\frac{5}{8}$ inch diameter, fitted to the standard carpenter's brace for boring through wood. The size of the particles produced depended on the speed of cut and the pressure applied to the brace, slow drilling with light pressure produced very small particles and fast drilling with heavy pressure produced large chips. To drill at known depth a rubber band was slipped on the neck of the drill as marker. The drill was sterilized and a bore hole of required depth was made. The bore-hole was then flamed, and small particles of wood were produced by inserting the flame sterilized drill and giving a few slow turns. The wood particles were then plated out with a pair of sterile forceps. Asmah (1967) modified the technique and used grinding bits of $\frac{1}{2}$ inch and $\frac{3}{8}$ inch diameter, with a serrated end surface for extracting the wood particles from the hole. Okigbo and Asmah had shown that this method could be used for extracting the wood particles in the field for isolation of fungi from the decayed post.

In the present study, a surface grinder of $\frac{7}{8}$ inch diameter with a serrated surface has been used for extracting surface samples. The subsequent depths below the surface have been obtained using a $\frac{5}{8}$ inch diameter twist drill, calibrated to show the required depths, and the samples taken with a $\frac{1}{2}$ inch grinding tool with a serrated end surface (See Figure 1b). A detailed description of the technique used in the present experiment is given in the following paragraphs.

Small particles of decayed wood samples were extracted first from the surface of the post and subsequently from 5, 25 and 45 mm depths. These were aseptically planted as inocula on different media in plates in the field.

The surface grinder inserted in a carpenter's brace was sterilized by dipping in ethanol and flaming lightly. It was then softly pressed against the surface and rotated slowly, the samples collected on the grinder head were evenly spread on the media with a sterilized needle or a pair of forceps. The surface was then cleaned with sterilized cotton wool swab and at the same place a 5 mm deep hole was bored with the sterilized $\frac{5}{8}$ inch twist drill fitted in the brace. The hole was cleaned thoroughly with sterilized cotton wool swabs and the samples were extracted with the sterilized grinding tool and were evenly planted on different media with a sterilized needle. The same procedure was followed for extracting samples from 25 and 45 mm depth. Dry swabbingwith sterile cotton wool was carried out with several changed of swab in an attempt to remove any air-borne fungal spores or loose wood particles from the bored hole. Samples were collected three or four times from each depth to inoculate the full series of petri dishes with different media. The chances of isolating all the fungi from a sampling area would probably be greater if the samples are collected several times for the inoculation, instead of inoculating several petri dishes from one sample.

The inocula were plated out in petri dishes. To reduce contamination a portable formica covered picnic table was used as a bench and swabbed down regularly with ethanol. The table was set up close to the line of posts and up-wind to avoid, as far as possible, small particles of wood being blown onto it during the drilling operation. This was thought to be the weakest part of the technique from the point of view of chance contamination, but, as will be seen from the results, it proved to be highly successful in as much as the petri dishes containing inocula from the inner regions of the posts showed a complete absence of fungal growth from any source in the great majority of the plates in the first few samplings. Even in the later samplings in both wood species there were no fungi present in zone II at 25 and 45 mm depth.

The semicircular shape of the post made it necessary to drill

where there was less possibility of penetrating beyond the middle of the post, in order to avoid sampling from an area colonized by fungi which might have entered from the other side of the post. The maximum width of the post was about $4\frac{1}{2}$ inches therefore the first hole at the centre of the post was drilled straight but the subsequent holes were made at an angle. At the ground line the drill was inserted at approximately $30^{\circ}$ angle pointing downwards, to sample the area which would possibly cover the ground line and a few millimeters below the ground line. The drill was inserted for the sampling from sapwood to heartwood along the rays in a radial direction.

In the course of study it was found that the drill technique for extracting samples from the desirable depth could be gainfully used where a series of samples were to be taken periodically from the same post without disturbing the existing ecological conditions to which the post had been subjected. The sample extraction and inoculation in the field could be done with this technique, in much less time than by those used earlier. The other advantage was, that samples of various particle sizes could be obtained easily by controlling the pressure and speed of rotation of the drill. Small size wood particles were used in this experiment in the hope (Greaves 1966) that the smaller the particle the greater the chance of there being only one fungal species present. This was found especially necessary for the isolation from surface where several fungi might be present in a very small area.

The other techniques for extracting samples, which had been mentioned before, were not used in the present work. With the saw-cut technique and the method Kääarik (1967) used, it might be possible to obtain samples from a known depth but the post had to be removed for sampling and could not be used for subsequent samplings. With two-chisel and "cut-block" techniques sampling at four different known depths from the same point was not practicable. The Pressler borer can be used for extracting a long plug from the wood then cutting out small discs from the desired depths, the discs are then split into small particles and planted on the medium (Butcher, 1969). This is

too long a procedure with little advantage over the drill technique which is perhaps the most convenient method for sampling from several known depths wothout removing the post from the ground.

The sampling and inoculation were done very carefully to minimize the chance contamination of petri dishes by air-borne spores. However, the results have shown that the contamination was too insignificant to pose any serious problem in the experiment. From a survey of the literature this appears to be the first time in the study of ecology of fungi on wood that a successful attempt has been made to extract and inoculate the samples in the field.

Media used:

Four or five different media were used during each sampling in order to isolate as many of the fungi as possible which have inhabited the sampled area. Eight different media were tried out during the experiment. They are as follows:

1. Malt agar medium: 2.5 % malt extract and 1.5 % of agar in distilled water was autoclaved at about 10 p.s.i., for 30 minutes, before pouring in the petri dishes. This was the most suitable medium. At 3.5 or 4 pH the medium was found to eliminate some of the bacteria without effecting the fungal growth. The bacterial growth often created problemsin the isolation of fungi.

2. Malt agar, rose bengal and streptomycin: In 2.5 % malt agar medium, 1:100 dilution of rose bengal was added at the rate of 1 ml/ 100 ml of medium. The mixture was autoclaved at 10 p.s.i., for 30 minutes. In the cooled liquid medium streptomycin was added in a proportion of 30 µg/ml., before pouring the plates. (Martin - 1950 used rose bengal and streptomycin in a medium with peptone, dextrose, agar and mineral salts to exclude most bacteria).

   This medium was used especially for isolating fungi from the zone III surface which was often found to be heavily infested with bacteria. The bacterial growth was suppressed and the fungal growth was also retarded on this medium. It could be used as an additional

medium for isolation from zone III.

3. Czapek-Dox Agar medium: (Johnson et al 1959)
(Agar - 15.0 g., $NaNO_3$ - 2.0 gm, $K_2HPO_4$ - 1.0 gm, $MgSO_4.7H_2O$ - 0.5 gm, KCl - 0.5 gm, $FeSO_4.7H_2O$ - 0.01 gm, Sucrose - 30 gm and distilled water 1000 ml. The mixture was sterilized at 10 p.s.i. for 30 minutes).

In this medium bacterial growth was retarded and the fungi sporulated early which helped in quick identification. The fungal colonies were conveniently picked up for subculturing due to the slow mycelial growth in this medium, but it appeared to be more selective. This could be used as an additional medium.

* 4. Abrams medium (Abrams 1948) (with cellulose powder)
($NH_4NO_3$ - 3.0 gm, $K_2HPO_4$ - 2.0 gm, $KH_2PO_4$ - 2.5 gm, $MgSO_4.7H_2O$ - 2.0 gm, 10 gm cellulose powder, Agar - 15 gm, tap water 1 litre; the mixture was autoclaved at 15 p.s.i. for 15 minutes).
The medium was not found very suitable for this work. The growth of fungi was very slow and often it was found difficult to group them on culture characters and identify before the next sampling date. This medium was found very suitable for testing the soft-rot ability of certain fungi.

5. Whatman cellulose medium: (Greaves 1966a):
(12 gm cellulose powder, 4 gm. yeast extract, 20 gm agar and 1 litre distilled water; autoclaved at 15 p.s.i. for 15 minutes).
This medium was not found suitable for the work.

6. Malt agar and Diphenyl (Russell 1956): (0.05 gm O-diphenyl phenol was added to 2.5 % malt agar medium and autoclaved). This is a suitable medium for isolating Basidiomycetes, but the growth of most fungi was suppressed. A lower strength of Diphenyl might be more suitable for the isolation of fungi.

7. Samples were collected in empty petri dishes and after about 18 hours 2.5 % cooled liquid malt agar was poured into the plates. This method prevented bacterial growth but the samples formed

* (With Whatman's cellulose powder added)

lumps when the medium was poured and the isolation of fungi was unsatisfactory.

8. Eggins & Pugh medium (1962) - (modified by Bravery 1968) was used for testing cellulolytic activity and soft-rot ability of certain fungi isolated from the fence posts.

It appeared that malt agar medium is by far the best for isolating the majority of fungi from the decayed wood, but it may be necessary to use in addition a few different media to be sure of isolating all the organisms present. Some of the fungi grow very fast in malt and are often found to suppress the slow growing ones. The malt agar medium of lower strength than 2.5 % may give better results in certain cases (Hudson 1969, Butcher 1969).

Isolation of fungi:

The petri dishes with inoculated samples from the field were incubated at 25°C. After about 48 hours the plates were examined and the young colonies were punched out with specially designed cutters of various diameters (this can also be done with a cork borer). The colony was lifted with a sharp arrow-head needle and transferred to another plate containing a similar medium from which it was taken. The fungi were isolated from the sample plates for about 15 days. The subcultured fungi were then grouped according to their culture characters and the characters visible within 40 times magnification under a stereoscopic microscope. A few cultures representing each group were finally sub-cultured in 2.5 % malt agar medium, and were identified from their microscopic characters. (The cutter and needle were flame sterilized each time before using).

Identification:

In the ecological study of fungi the number of isolates reaches such large figures that the identification of each with microscopic characters becomes almost impossible. It was observed that in most cases several of the culture characteristics of a fungus were constant and did not vary with the source of isolate or time of sampling. With the help of such

characters of a fungus which has been already identified, it is generally possible to reach generic identification of subsequent colonies of the same fungus without going into microscopic details. The identification of wood inhabiting fungi (belonging to Fungi Imperfecti) with their culture characters and some of the easily discernible microscopic characters, has been attempted.

The identification of wood-rotting Basidiomycetes based on the culture characters, temperature and oxidase reactions and reactions to certain chemical tests, and the microscopic characters has received considerable attention (Cartwright & Findlay 1958; Nobles 1948, 1958a, 1958b, 1965). The identification of the members of Fungi Imperfecti by culture characters has received far less attention although perhaps more than 90 % of the fungi which colonize exposed wood belong to this group. The 'Imperfects' have been identified with the help of published works of Clements & Shear (1931), Ainsworth & Bisby (1954), Gilman (1959), Barnett (1960), Bessey (1950), Grove (1935, 1937) and several other publications on taxonomy. Before being able to consult this literature profitably, a good working knowledge of taxonomy has to be acquired and a lot more time is needed to make the suitable preparations for the microscopic studies. Even after making the necessary mounts some of the more important diagnostic features are often difficult to see under the microscope; such as attachment of conidia inside the pycnidium, the endoconidia of Chalara, paired conidia at the end of the conidiophore in Bisporomyces, the attachment of conidia to the phialides or conidiophores of many fungi and several other diagnostic characters.

Levy (1967, 1968) has pointed out the difficulties involved in the identification and classification of these organisms. Levy (1967) also stressed the need for developing a method of spot identification of fungi encountered during ecological studies, based on the characters exhibited by the fungal culture, otherwise the collection of isolates in periodic sampling could reach such an astronomical number that it would be impossible to deal with them in the end. Asmah (1967) described in detail

the culture characters he used in developing the punch card technique for identification of fungi. He has shown the possibility of using the punch card system for identification. In the present work many of the characters included are taken from those described by Asmah, and Cartwright & Findlay (1958). The generic classification of fungi is based on Barnett's (1960) Illustrated Genera of Imperfect Fungi.

It is necessary to mention that to give a fool-proof system for identification of Fungi Imperfecti by this method is extremely difficult. The 'Imperfects' which include asexual stages of many fungi known to produce perfect stages are a complicated group. Many minor or variable characters are arbitrarily used to separate some of the genera. It is important to select the characters for identification very carefully and their variations within a genus should be thoroughly studied before a technique for quick identification of wood inhabiting "Imperfects" can be developed, (Findlay 1968)

The fungi isolated from treated and untreated exposed timbers, dead trees and fallen branches, by Corbett & Levy (1963), Greaves & Savory (1965), Butcher (1968a, 1968b), Basham (1958), Chesters (1950), Duncan & Eslyn (1966), Gambetta & Orlandi (1967), Greaves (1966a) and the present work, showed that they belong to about 50 genera of Fungi Imperfecti. Perhaps it is possible to make a key for the identification of the common wood-inhabiting Fungi Imperfecti with a more intensive study on the subject and thus attract many students to the study of fungal ecology who otherwise might not be prepared to go through the ordeal of acquiring a good working knowledge of taxonomy before studying any ecological problem.

The characters exhibited by fungi growing on 2.5 % malt agar plates incubated at 25°C were used as diagnostic features for identification. The characters are grouped as follows :

A. Culture Characters:

Colony colour (in one week old culture): (1) Hyaline, (2) Milk white, (3) White, (4) Yellow/orange/cream, (5) Fawn, (6) Black/very dark, (7) Green/yellow-green/dark green/blue-green, (8) Red/dark brown, (9) Pink/light brown, (10) Grey.

Change in colour of the colony with aging (in 2 - 3 week old colony): (11) Hyaline, (12) Milk white, (13) White, (14) Yellow/orange/ cream, (15) Fawn, (16) Black/very dark, (17) Green/yellow-green/dark green/ blue-green, (18) Red/dark brown, (19) Pink/light brown, (20) Grey, (21) No change in colour.

Uniformity in colony colour (in one to two week old culture): (22) Uniform, (23) Varied (mixed colour or significantly different shades of the same colour)

The colour of the colony can be a distinguishing feature for grouping the fungi, if an easily available standard colour chart is used for the comparison, otherwise it may lead to serious errors. If the colour scheme is narrowed down to a few broad based colour groups, this will perhaps eliminate to a considerable extent the chance of error, especially when the standard colour chart is not available. However, if the colour is used as the first character to group the fungi then it may be advisable not to lay much stress on the intermediate shades.

Outline of the colony (shape or form): (24) circular/nearly circular, (25) Irregular.

The outline was observed from the third day of inoculation for one week. It was found difficult to study the outline of a fast growing fungus.

Elevation: (26) Submerged/appressed, (27) Flat (slightly above the surface of the medium), (28) Raised.

Texture: (29) Powdery/granular, (30) cottony/woolly, (31) Felty/ velvety, (32) Smooth (to describe the texture of an appressed colony).

Margins (in three days culture): (33) Even/entire, (34) Filamentous, (35) Wavy, (36) Irregular.

Rate of growth (mean diameter of the colony from four measurements taken from the reverse side of petri dish): (37) Fast growing $>$ 7.5 cm, (38) Moderate - 4.5 to 7.5 cm, (39) Slow - 2 to 4.5 cm, (40) Very slow $<$ 2 cm.

Change in colour of the medium: (41) No change, (42) Discolouration, (43) Black/dark, (44) Pink/red/brown, (45) Yellow/yellowish white.

The colour of the medium may often be due to the growth and colour of the mycelium.

The 'odour' as an identifying character for lower fungi was not included because it was not distinctive.

B. Microscopic characters:

Conidia and conidiophore: (46) Absent, (47) Visible, (48) Inside pycnidium, (49) Inside acervulus.

Pycnidium: (50) ostiolate, (51) without ostiole, (52) separate, (53) clustered, (54) Erupment, (55) Superficial.

Hyphae: (56) Hyaline, (57) Dark,

Spores: (59) Hyaline, (60) Dark.

Number of cells in the spores: (61) One, (62) Two, (63) Many.

For detailed microscopic examination hyphae with conidia and conidiophores were removed by placing a small strip of adhesive sellotape over the fungus and mounting in lactophenol or aniline blue in lactophenol. Fruiting bodies were also mounted by removing them with pointed glass needles (made by drawing the thin glass rod on the flame). Pycnidia were dissected under a stereoscopic binocular microscope to examine the type of attachment of conidia and conidiophores.

The shape and size of the spores can also be included in the key for the identification. From the observations it appears that if these two characters are divided into broad sub-groups, they can be used as diagnostic characters for generic identification.

This punch card system for the description of cultures was an attempt to find out a suitable means for quick generic identification of the wood-inhabiting Fungi Imperfecti. The result is based on 116 cultures grown on 2.5 % malt agar and incubated at 25°C. (The results have been given in Appendix I). This number is not adequate enough to know the range of variations of these characters within a genus. However, from the

information available from the trial run it appears that this system is workable. The order in which the characters have been described earlier may need some alteration, and needs further investigation.

The system described here is basically meant for the identification of wood inhabiting Fungi Imperfecti and therefore several diagnostic characters for the identification of Basidiomycetes included by Cartwright and Findlay (1958) were omitted here. It is suggested that a detailed work with a larger number of cultures could produce a more acurate system with wider application.

It has to be borne in mind that if sampling, isolation, sub-culturing, classification and identifications have to be done in a month before proceeding to the next series of sampling, it is very necessary to develop and use more convenient techniques to cope with the work.

It would be clearly valuable to have some idea of the numbers of organisms involved and discussions were held with a view to planning the experiments so that they could be analysed statistically and possibly even written up as a Computers programme. The advice received from the Computer Centre was that this could be done provided a suitably large number of samples were taken each time. With one person involved in the work this was simply not possible. Each sampling involved the isolation of fungi on to some 130 petri dishes initially. The subsequent processing, screening and subculturing of the isolations involved on the average another large number of petri dishes each month, all of which had to be examined and some attempt made at identification of the fungi present. The tenfold increase required by the statisticians made this beyond the capabilities of one man.

Recently an attempt has been made to tackle this problem in a manner similar to that used byhigher-plant ecologists (Butcher 1969) and the results of this are awaited with interest.

## Results

### Seasonal distribution of fungi on birch and Scots pine posts

The seasonal distribution of fungi in the posts at different zones and depths is described in this chapter. The sequence of colonization by fungi has been critically analysed with respect to the factors which could affect the distribution. The effects of some of these factors have not been examined experimentally; The conclusions arrived at being based on keen observations and information available from published work. Finally, a comparison has been made on the distribution of fungi between the zones in each post and between the posts of different wood species.

The distribution of fungi is based on their presence and absence. It has been mentioned earlier that the number of isolates obtained from about 130 plates at each sampling in each series, was so high that the frequency count of the fungal colonies became physically impossible to cope with and was therefore abandoned later. In the trial run on fence posts erected in 1961, an attempt was made to find the possibility of studying the distribution of fungi in the light of frequency counts of fungal colonies.

The questions can be raised that the fungi represented in the present distribution may be from spores which are just chance contaminants on the wood and not actual colonizers, or they may be the resting spores or dormant mycelia of some of the early colonizers. Some logical arguments based on the data of distribution of fungi have been given in answer to this question . It has been shown that there is a progressive increase and subsequent decline in the number of different species of fungi which colonized the post from the time it was first exposed to the end of nine months exposure. The composition of the flora thus showed a definite pattern . Had these fungi been just chance contaminants it is likely that they would have shown a random curve in the graph instead of the present definite pattern ( Fig. 3 2.). For example, in zone III of birch, when the

post was later colonized by the cellulose decomposers no new mould was found to colonize, although several mould fungi were abundant in the aerospora throughout the year. It was also evident from the distribution tables that several fungi persisted for a very long period and others for short duration. It is difficult to accept that the chance contaminants appeared persistently in such a way to give the present distribution pattern. It has been assumed therefore that the species of fungi isolated were those which colonized the post.

The factors which may affect the colonization of fungi on the surface and inside the wood are the moisture content of the post, the rate of sporulation, spore germination and growth, the interaction and competition initially between fungi and bacteria and subsequently between fungus and fungus, the secretion of antibiotics, toxic and stimulatory substances, space available in which to grow and suitable nutritional requirements. The three zones of the post selected for study represent three different environments. This has been discussed earlier. The moisture content on the surface and below varies considerably between the zones (Corbett & Levy 1963). The post above the ground is air dry timber with a normal moisture content of 14 - 20 % which is generally not very suitable for fungal growth but under certain seasonal conditions the moisture content may reach an acceptable level for a sufficient period to support the life of some fungi. In zone I the moisture content may be higher than zone II especially below the surface due to the penetration of water from the top of the post along the grain. Therefore it may be expected that a few fungi would be able to penetrate into the wood especially the spores of those which could be washed in from the top along with the rain water or dew when this soaks into the end grain of the wood. In zone II the moisture content below the surface remains too low throughout the year to support the life of any fungus. In the surface layers of zone I and II there may be much fluctuation in the moisture content depending on the dry and wet spells of the weather.

Therefore the fungi which are more tolerant to low moisture content or to fluctuations in moisture content may have some advantage over others in colonization and survival in these zones. In zone III the moisture content remains near optimum through the year and in consequence the fungal flora may be different from the other two zones. The aeration which is inversely proportional to the moisture gradiant may also be an important factor affecting the distribution of fungi. Temperature may be a major factor especially in the colonization of surface layers of the post. The influence of this factor has not been examined, and it needs further investigation.

The high rate of spore germination of some fungi may give them a better opportunity to colonize and become established earlier than those fungi which have a slower rate of germination. This factor may play an important role in the success of establishment of early colonizers. The growth rate of these fungi may effect the distribution in that certain species may overgrow the slow growing species or, by occupying space, prevent several other fungi colonizing.

The interaction between fungi may affect the fungal flora both on the surface and below. One fungus may suppress another and may consequently pave the way for a more virulent species. The effect of antagonism on the distribution of fungi based on the experimental evidence has been discussed in Sections II and III.

Similarly, the antibiotic and toxic substances secreted by one fungus may prevent a second fungus from colonizing, but later it may be suppressed by a third fungus which is more tolerant to the particular substances present. The early colonizers may leave behind some toxins or stimulatery substances which may either inhibit or accelerate the growth of the other fungi which follow, or the early colonizers may have no effect on subsequent colonizers.

The lack of space for germination of spores in an area where earlier fungi are growing luxuriantly may prevent the germination of certain fungi

with greater decay capability.

Availability of food is also one of the important factors to affect distribution. In zone I and II the surface colonizers are entirely dependent on the food available on the surface of the post, therefore when the nutrients are depleted the fungal flora on the post is likely to change. Some members of the existing flora which are capable of adapting to the change may continue to survive, but most of the existing flora is likely to be replaced by other species. The colonies formed from the spores on the surface of zone III may depend primarily on the food available on the post, but mycelia of the other fungi which colonized the post from the soil have an alternative source of nutrients in the soil substrate and may not be entirely dependent on the food from surface of the post. Therefore, the depletion of nutrients from the surface may have a more severe effect on the distribution of air borne fungi which colonized the post rather than on the fungi which colonize by hyphal contact.

The fungi which have penetrated the wood are probably not affected by the depletion of nutrients from the surface because of their alternative source of energy from the cell wall components or from the cell contents of the parenchyma.

The other factors which can also affect the distribution of fungi are the pH, the anatomical features of the wood and the increase in permeability due to the colonization by a certain fungal type. The pH which is about the same throughout the wood may not be a critical factor but the anatomical features may be of greater importance in the distribution of fungi. Several saprophytic fungi are selective to the wood species and consequently the difference between the soft wood and hard wood may affect the fungal flora on Scots pine and birch. The change in the physical characteristics by the colonization of a fungus type may also affect the distribution. A sap-stain fungus by penetrating into the wood may increase the moisture content by bringing about a change in the permeability of the timber (Findlay 1959) and consequently may allow other fungi to penetrate into the wood. Greaves (1966) has shown an increase in the permeability of the wood by the

presence of bacteria and certain fungi.

The ecological studies involve many complexities. Several variable factors can affect the distribution of fungi and therefore it may be difficult to draw any definite conclusion. However, an attempt has been made to interpret the sequence in which the fungi have appeared on the post and the significance of various fungi leading to the establishment of decay fungi in the wood.

Relationship between the flora of the post surface and the aerospora (Table 7 & 8)

The study of fungal colonization on the surface with non-destructive type of samplings, poses a much greater problem than in a similar study of colonization below the surface. It has been mentioned before that open plate spore traps were set up during each sampling to evaluate the relationship of the aerospora to the surface colonization. The possible drawbacks of this spore trapping method have already been discussed. In the following paragraphs the correlation between surface colonization in three zones of birch and Scots pine and the aerospora is briefly discussed.

Several common moulds, e.g. Mucor sp., Penicillium spp., Fusarium spp., Cladosporium herbarum, and Botrytis sp. were isolated throughout the year from the control plates which were set up as spore traps. P. varioti, Cephalosporium sp., Epicoccum sp., Aureobasidium pullulans, Verticillium spp., Aspergillus sp., Gliocladium sp., and Geotrichum sp. were isolated very frequently from the air. Several other species of identified fungi and about 7 unidentified fungi were isolated very rarely. The data on fungi in the air is insufficient to draw any definite conclusion on the seasonal variations in aerospora. However it appears that the number of species of fungi present in the air increases during September and October and remains fairly constant throughout the rest of the year.

About 50% of the fungi from zone I and II, and 35% from zone III appeared at the same time, both in air and on the surface of the posts. Several other fungi were either isolated first from the air and later from the posts, or were isolated from the post first and later picked up from air.

A number of fungi present in the air did not colonize the post and a few fungi from the posts were not picked up from air. Details are given in Tables 1 - 12, and Figure 3.

The distribution of fungi in the air poses a great problem to interpret the results of surface isolations e.g. Fusarium spp., C. herbarum and Penicillium spp. were isolated each month from the air, but these fungi also appear to have dominated zones I and II for a considerable length of time. Whether dominance of these fungi is due to their adaptability to the conditions existing in the post or due to the repeated recolonization or due to both raises an important question. It will be worthwhile discussing this question by taking the example of Fusarium spp. which dominated zone I and II for a very long period but were replaced very early from zone III. During the period Fusarium spp. were dominant on the posts, several other mould fungi (which were constantly isolated from the air) might have also been deposited in the same place. The successful recolonization of Fusarium spp. in competition with other fungi may be due to their adaptibility to live in a condition not suitable for the development of other fungi. In zone III Fusarium spp. were replaced because the condition existing in this zone was favourable for the growth of other more competitive fungi which resulted in their replacement. This suggests that the successful colonization by Fusarium spp. in above ground line zones is probably due more to their adaptibility to the conditions than due to recolonization.

Contrary to the example cited above where Fusarium spp. were dominant in both air and on the posts, C. piceae, Phialophora spp., Stysanus sp. and T. viride the dominant fungi in zone III of birch or Scots pine were very rarely isolated from air. From a comparative study on the surface flora and aerospora, it appears that the colonization may be affected by the aerospora but the establishment of fungi would mainly depend on several other factors.

The seasonal distribution of fungi on birch posts in zone I (Table 1)

Surface

The early colonizers on the surface of the post were Paecilomyces varioti, Penicillium spp., and Cladosporium herbarum. These fungi were followed by Fusarium spp., and Cephalosporium sp. P. varioti was isolated intermittently but C. herbarum and Fusarium spp. remained as dominant members of the flora throughout the year. Penicillium spp. persisted on the surface of the wood for about six months after exposure and were later replaced by other fungi. The main soft-rot fungi which remained for a long period as surface colonists were Cephalosporium sp., and Dactylosporium sp. These two soft-rot fungi were present on the post at different times of the year and Dactylosporium sp. colonized the post shortly after Cephalosporium sp. disappeared. A few other moulds, sap-stain and soft-rot fungi, including a few pycnidial fungi were isolated from the post from the third month. These fungi stayed for a very short period and did not appear to have affected the dominance of the early colonizers.

5 mm depth

The samples from below the surface were observed (by the reaction of the drill) to be very dry. Three fungi P. varioti, C. herbarum and Cephalosporium sp. were isolated from 5 mm depth but no fungus was isolated from 25 mm and 45 mm depth. The first fungus to penetrate into the wood was C. herbarum which was followed by Cephalosporium sp. and P. varioti. The presence of C. herbarum was recorded but it was later replaced by P. varioti. Cephalosporium sp., persisted for a short period but was not isolated after P. varioti was well established. in the wood. P. varioti was the only fungus which remained in the wood for the later part of the year.

The low moisture content of the wood below the surface layer might be the main factor inhibiting the growth of the other fungi into the wood.

Table 1

Sequence of colonization of fungal species in zone I of birch posts in relation to exposure months

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| On the surface layer | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
| Paecilomyces varioti | X | | | | X | | | | | |
| Penicillium spp. | X | X | X | X | X | X | | | | |
| Cladosporium herbarum | X | X | X | X | X | X | | X | | X |
| Fusarium spp. | | X | X | X | X | X | X | X | | X |
| Cephalosporium sp. | | X | X | X | | | | | | |
| Phoma sp. | | | | X | | | | | | |
| Trichoderma viride | | | | | X | | | | | |
| Verticillium sp. | | | | | X | | | | | |
| Dactylosporium sp. | | | | | X | X | X | X | | |
| Alternaria sp. | | | | | X | | | | | |
| Aposphaeria | | | | | | | | | | X |
| | | | | | | | | | | |
| At 5 mm depth | | | | | | | | | | |
| C. herbarum | | X | X | X | X | X | | | | |
| Cephalosporium sp. | | | X | X | | | | | | |
| Paecilomyces varioti | | | | | X | X | X | X | | |

Although the top of the post may be susceptible to wetting by the soaking of rain along the grain of the wood, it is equally susceptible to drying out. End splitting of the posts would increase the rapidity of wetting and drying. P. varioti may therefore survive by being able to cope with this changeable environment.

Analysis of the results:

It is difficult to distinguish a definite pattern of succession of fungi but it may be possible to discuss the sequence in which the fungi were isolated from the post. On the surface of the post the moulds were the early colonizers which were shortly followed by sap-stain and soft-rot fungi. These fungi were then succeeded by several other moulds, a pycnidial fungus and another soft-rot fungus and then again by new sap-stain, soft-rot and pycnidial fungi.

The moulds and sap-stain fungus which colonized the post earlier remained as dominant members of the flora throughout the year. This may be due to their ability to stand the seasonal variation in moisture content and also to their high rate of sporulation and spore germination. That the soft-rot fungi did not have much success in the build up might be due to the low moisture content. The inability of the subsequent fungi to persist might be due to depletion of nutrients, lack of space to develop, suppression by already existing fungi, or to the inability to adapt to the seasonal changes. These factors might be operating individually or in combination.

The trend of fungal succession on the surface can be summarized as follows : Moulds (Penicillium spp and Fusarium spp.) ⟶ Sap-stain fungus (C. herbarum ⟶ Soft-rot fungi (Cephalosporium sp. and Dactylosporium sp.) The fungi included in the scheme are only those which were isolated repeatedly for a long time. The soft-rot fungi were later completely replaced by moulds, therefore it has been suggested that the succession did not reach beyond the mould stage.

At 5 mm depth the sequence in which C. herbarum, Cephalosporium sp. and P. varioti appeared on the post can be explained in the following way. C. herbarum living on cell content established first. This was followed by soft-rot fungus Cephalosporium sp. which could not successfully develop and grow due to inadequate moisture content. P. varioti which colonized the post later, eliminated the other two fungi. The reasona for this could not be determined but would be most interesting to know. The penetration of C. herbarum with the consequent increase of the permeability of the wood (Findlay, 1959) might well have brought about a change in the moisture content of the area which helped the penetration of both the later colonizers.

Dactylosporium sp. which persisted on the surface for long period, and other cellulose decomposers which appeared later on the surface, were unable to penetrate into the wood possibly due to the presence of P. varioti. However, P. varioti by its ability to utilize cellulose from the cell wall and presumably to live at a low moisture content level might have had the advantage over the other cellulose decomposers and therefore remained the dominant fungus.

The competition and interaction between the fungi on the surface and the ecological conditions may have an affect in determining the species of fungi to colonize below the surface.

The succession of fungi in this zone did not reach beyond the second mould stage, and could be summarized as the primary saprophytic "sugar fungi" being followed by the cellulose decomposers, this may be compared with Garrett's (1963) stage I and II in the general trend of fungal succession.

The succession of fungi below the surface can be summarized as follows: Sap-stain fungus (C. herbarum) ⟶ Moulds (P. varioti) The scheme includes only those fungi which have been isolated repeatedly for long time.

Seasonal distribution of fungi on birch post in zone II (Table 2)

The early colonizers on the surface were Penicillium spp. and Cladosporium herbarum and these were shortly followed by Fusarium spp., Cephalosporium sp. and Dactylosporium sp. None of these early colonizers persisted for more than two months. C. herbarum and Penicillium re-appeared for a short period after a gap of a few months but was not encountered in the later months

On second and third month Botrytis sp., Alternaria sp. Aposphaeria sp. and P. varioti were isolated from the post and it appeared that these fungi had almost eliminated the primary colonizers from the surface. A few of the secondary colonizers persisted for a longer period and Alternaria sp.and Aposphaeria re-appeared later.

Several other moulds, sap-stain, soft-rot and pycnidial fungi were isolated from the third month onwards but none of these fungi remained long except Chaetomium dolichotrichum which was present for about two months Fusarium spp., . which re-appeared in the fifth month remained as the dominant colonizer for the rest of the year. No fungus was isolated from below the surface layer (Table 2. ) probably the wood below the surface was too dry to support the life of any of these surface colonizers.

Analysis of the results

The moulds and sap-stain fungus were the first colonizers and these were followed by soft-rot fungi. Then several other moulds sap-stain, soft-rot and pycnidial fungi appeared on the surface. There was no definite pattern with which these later fungi appeared. A few of these fungi remained longer and the others were present for a shorter period. The soft-rot fungi and other cellulose decomposers could not establish, possibly because the very low moisture content below the surface of the wood prevented their penetration and/or survival.

Several moulds persisted for a longer period than the other groups of fungi. Fusarium spp. were among the most successful colonizers and remained throughout the later part of the year, this might possibly be because of their tolerance to the low moisture content and the seasonal

Table 2

Sequence of colonization of fungal species in zone II of birch posts in relation to exposure months

| On surface layer | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
|---|---|---|---|---|---|---|---|---|---|---|
| Penicillium spp. | X | | | X | X | | | | | |
| Cladosporium herbarum | X | X | X | | | X | X | | | |
| Fusarium spp. | | X | | | | X | X | X | | X |
| Cephalosporium sp. | | X | X | | | | | | | |
| Dactylosporium sp. | | X | | | | | | | | |
| Botrytis sp. | | | X | X | | X | | | | |
| Alternaria sp. | | | X | X | | | | | | X |
| Aposphaeria sp. | | | X | X | | | | | | X |
| Paecilomyces varioti | | | X | X | X | | | | | |
| Epicoccum sp. | | | | X | | | | X | | |
| Phoma sp. | | | | X | | | | | | |
| Trichoderma viride | | | | | X | | | | | |
| Aureobasidium pullulans | | | | | X | | | | | |
| Chaetomium dolichotrichum | | | | | | X | X | | | |
| Chaetophoma sp. | | | | | | | X | | | |

variation and/or the high rate of growth and germination. This genus formed encrustations on the surface of both Scots pine and birch posts.

In the early stage there is a trend in colonization from primary saprophytic "sugar fungi" to soft-rot fungi, but because this zone of the post was not suitable for the growth of most of the fungi the succession did not reach beyond mould stage.

Seasonal distribution of fungi on birch post in Zone III (Table 3)

Surface

Bacteria, Fusarium spp. and Botrytis sp. were isolated from the surface of the post one week after it had been exposed. In the first isolation the bacterial colonies were found to develop from most of the samples and only a few colonies of fungi were isolated. In the subsequent isolation the number of bacterial colonies decreased and after about two months bacteria were seldom encountered. It is therefore suggested that the bacteria were probably the early colonizers followed by moulds.

One month after exposure the post was colonized for a short period by Penicillium spp., C. herbarum and Aureobasidium pullulans. These fungi were replaced in the third month by Mucor and Cephalosporium sp., Geotrichum sp., Phialophora sp. and Ceratocystis piceae, From the third month onward the post was colonized by several other sap-stain and

Table 3

Sequence of colonization of fungal species in zone III of birch posts in relation to exposure months

| On surface layer | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
|---|---|---|---|---|---|---|---|---|---|---|
| Fusarium spp. | X | X | X | X | X | X | | | | |
| Botrytis sp. | X | X | | | | | | | | |
| Cladosporium herbarum | | X | X | | | | | | | |
| Aureobasidium pullulans | | X | | | | | | | | |
| Penicillium spp. | | X | X | | | | | | | |
| Mucor sp. | | | X | X | X | | | | | |
| Ceratocystis piceae | | | X | X | X | | X | X | | X |
| Cephalosporium sp. | | | X | | | | | | | |
| Phialophora spp. | | | X | X | X | | | X | | X |
| Geotrichum sp. | | | X | X | | | | | | |
| Alternaria sp. | | | | X | | | | | | |
| Dactylosporium sp. | | | | X | | | | | | |
| Aposphaeria sp. | | | | X | | | | | | |
| Discula pinicula | | | | X | X | | | | | |
| Stysanus sp. | | | | | X | X | X | | | |
| Chaetomium deolichotrichum | | | | | X | X | | | | |
| Chaetophoma sp. | | | | | | X | | | | |
| Pestalotia sp. | | | | | | | | X | | X |
| Trichoderma viride | | | | | | | | X | | X |
| Coniothyrium fuckelii | | | | | | | | X | | X |

contd.../

Table 3 (contd.)

Sequence of colonization of fungal species in zone III of birch posts in relation to exposure months

| At 5 mm depth | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
|---|---|---|---|---|---|---|---|---|---|---|
| Cladosporium herbarum | | X | X | | | | | | | |
| Aureobasidium pullulans | | X | X | | | | | | | |
| Sporotrichum sp. | | | X | X | | | | | | |
| Geotrichum sp. | | | X | X | | X | X | | | |
| Alternaria sp. | | | | X | X | | | | | |
| Ceratocystis piceae | | | | X | X | X | X | X | | X |
| Cephalosporium sp. | | | | X | X | X | | | | |
| Phialophora sp. | | | | X | X | X | X | X | | X |
| Polystictus sp. | | | | | X | | | | | X |
| Unidentified Basidiomycetes species | | | | | | | X | | | |
| Pestalotia sp. | | | | | | | | X | | |
| Coniotherium sp. | | | | | | | | X | | |
| **At 25 mm depth** | | | | | | | | | | |
| Geotrichum sp. | | | | X | | | | | | |
| Phialophora sp. | | | | X | X | X | X | X | | X |
| Ceratocystis piceae | | | | | X | X | X | | | |
| Polystictus sp. | | | | | X | X | | X | | X |
| Unidentified Basidiomycetes species | | | | | | | X | | | X |
| **At 45 mm depth** | | | | | | | | | | |
| Phialophora sp. | | | | X | X | | | | | X |
| Ceratocystis piceae | | | | | X | X | X | | | |
| Polystictus sp. | | | | | X | X | X | X | | X |
| Unidentified Basidiomycetes species | | | | | | | X | X | | |

soft-rot fungi from Moniliaceae, Dematiaceae and Sphaeropsidales of Fungi Imperfecti and a member of Ascomycetes Chaetomium dolichotrichum sp. However, C. piceae, Phialophora spp. remained dominant throughout the period of investigation. Stysanus sp. was the only fungi among the late colonizers which persisted for a few months.

5 mm depth.

C. herbarum and A. pullulans penetrated into the wood first but were not isolated from beyond 5 mm depth. These fungi were present in the wood for a short period (not more than 3 months) and were replaced by Sporotrichum sp. and Geotrichum sp. Three months after the exposure C. piceae and Phialophora sp. penetrated the wood followed by Basidiomycetes spp. The last three fungi mentioned remained as dominant members of the flora for the rest of the year. A few other fungi e.g. Alternaria sp., Cephalosporium sp., Pestalotia sp. and Coniothyrium fuckelii were isolated from the wood at this depth but did not become established. Basidiomycetes spp. which were isolated after four months of exposure were successively isolated from the seventh month,

25 mm depth.

A very few fungi were able to penetrate to 25 mm depth. Phialophora sp. which penetrated first along with Geotrichum sp. remained for four months but Phialophora sp. reappeared later. C. piceae and Basidiomycetes spp. appeared shortly after Phialophora sp. had established itself. Basidiomycetes spp. were not isolated for a short period but later became dominant. C. piceae grew for a short period but later completely disappeared once the Basidiomycetes were well established.

45 mm depth.

Phialophora sp. was the first fungus to penetrate to 45 mm. depth and was followed by C. piceae and Basidiomycetes spp. Phialophora sp.

remained for a very short period but was again isolated during the last sampling. C. piceae also colonized the wood for a short period and the Basidiomycetes spp. remained the only dominant group for the rest of the year. The Basidiomycete species isolated were Polystictus sp. and an unidentified species.

Analysis of the results:

In zone III the conditions in the post were near optimum throughout the year for favourable growth of many saprophytic fungi. The competition was therefore between a large number of fungi both on the surface and below. Several fungi were eliminated in the competition at each depth and a limited number penetrated to 25 mm and 45 mm depth. The sequence in which the fungi colonized the post is discussed here.

The fungal flora in zone III comprises a large number of soil fungi. The initial colonization on the surface was by air-borne spores of moulds. The subsequent colonization was by soil fungi probably by direct contact with mycelium. The competition on the surface was between germinating air-borne spores of fungi living on the available nutrition on the surface and the mycelium of soil fungi deriving nutrition from the post as well as the soil substrate. With the gradual depletion of nutrients from the surface those fungi (including most of the initial colonists) which depended only on this source of supply of food were eliminated. The depletion of surface nutrients probably did not affect so severely those members of the fungal flora with an alternative source of nutrition in the soil substrate. This may be one of the reasons for the isolation of more soil fungi than air-borne species from the surface in zone III.

The aerospora may consist of a very large percentage of spores of moulds and C. herbarum, but (after 2 months of exposure) none of these fungi were found to re-colonize the surface after having been eliminated. The spores of these fungi gave rise to fungal colonies in the exposed "control" plates at all isolations, and were therefore probably deposited constantly on the surface but since they might not have got adequate

space for germination did not colonize. The depletion of nutrients from the surface layers may also inhibit the germination and growth of air-borne spores which are deposited at late stages of colonization. Therefore several fungi which colonize the substrate by air-borne spores may be eliminated from the succession, although they may have a potential decay ability.

The high moisture content in zone III enables many of the soft-rot fungi to become established on the surface. The cellulose decomposers which have become established below the surface, continued as dominant surface flora throughout the year. Even after the depletion of nutrients from the surface they probably survived due to the constant supply of energy from the breakdown of the cellulose by their hyphae below the surface. These fungi in turn may be able to eliminate those which are depending on the nutrition from soil substrate because the ageing of the mycelia of the latter may hamper the supply of nutrition, leading to their disappearance. The successful colonization of fungi on the surface may therefore also depend on their build up below the surface. This may be one of the reasons for the dominance of C. piceae and Phialophora sp. on the surface of the post.

It appears that the species of fungi involved in the succession may depend more on the soil micro-flora of the region than that of the air. The species of fungi which dominate the surface flora may eventually depend on their ability to become established below the surface and maintain a constant supply of nutrition to their growing mycelium on the surface.

The succesion of fungi on the surface of the post may be summarized as being from moulds to sap-stain and soft-rot (these also exhibiting characteristics of sapstain / fungi) to soft-rot fungi. The fungi involved in the succession were Moulds (Fusarium spp. and Mucor sp.) ——→ Sapstain and soft-rot fungi (C. piceae and Phialophora sp.) Soft-rot fungus (Chaetomium dolichotrichum).

At 5 mm depth only about half the number of species of fungi

which inhabited the surface were isolated and the rest of the fungi were either eliminated or remained confined to the surface. Penetration of fungi into the wood might depend on several factors including inoculum potential of the fungi on the surface and their ability to utilize the nutrients in the cell wall/contents below the surface. Several sap-stain, soft-rot and decay fungi colonized at 5 mm depth but only a few became dominant. The sequence in which the fungi appeared at this depth could be summarized as follows : Sap-stain to soft-rot fungi, followed by other soft-rot fungi ( with sapstain characteristics) and decay fungi.

C. piceae and Phialophora sp. were the only fungi except Basidiomycetes spp., which appeared to have dominated below the surface. It is suggested that the reasons for their success are : (a) these fungi developed sufficient inoculum potential due to their long stay on the surface, (b) their ~~high rate of~~ ability to cellulose decomposition and ~~their ability~~ to utilize the cell contents provided sufficient energy for rapid colonization (Findlay & Levy, 1969, Findlay 1969 have shown that Phialophora sp. formed soft-rot cavities in birch and beech within 2 weeks of exposure, (C. piceae and Phialophora also formed cavities in the tests)/(c) the growth rate of these fungi within the wood and (d) possible inhibitory effects of C. piceae (this fungus has shown mutual antagonism with Discula pinicola which has been found to be antagonistic to several fungi in the laboratory test (see p.144 and Fig.17 )

The further penetration of the fungi to 25 mm and 45 mm depth might depend on the build up at 5 mm depth, the ability to utilize the substrate, and the mechanism of penetration. C. piceae and Phialophora sp. penetrated to 25 mm and 45 mm depth probably due to their build up nearer the surface but Basidiomycetes spp. appeared to have penetrated solely due to their ability to bore through the cell wall. Basidiomycetes did not become dominant at the surface but dominated the flora at 25 mm and 45 mm depths earlier than at 5 mm even though they were first isolated from all three depths at the same time. The former two

fungi remained sub-dominant for a short period but were later eliminated by decay fungi.

In the distribution of Basidiomycetes certain interesting features were noticed which are discussed here. These fungi were never isolated from the surface, probably because their slow growth rate in culture prevented them competing against the existing fast growing species of Fungi Imperfecti in the plates. Also the earlier colonization by cellulose decomposers had left insufficient nutrients for the decay fungi to colonize the surface layers; thus inducing them to penetrate to deeper regions. The brown-rot fungi might possibly have very little chance to become established within one or two millimetres from the surface (probable depth for the surface samples) against several cellulose decomposers which had colonized earlier and utilized the cellulose. The white-rot fungi could possibly colonize depending on the available space and the ability to live in presence other fungi - though in this experiment none were isolated from the surface.

A question could be raised that Basidiomycetes were present on the surface but were not isolated from the plates with samples of several other fast grown species, as the former were overgrown by the latter group; This does not seem to be very probable because Basidiomycetes were isolated several times from the plates with mixed fungal cultures, and also special isolation media were used to inhibit the growth of moulds and other lower fungi. Basidiomycetes were first isolated from 5 mm to 45 mm depth, then for a certain period from 25 mm and 45 mm depth and later from all the three depths. This might be just a chance isolation with no significance or it might be the general trend and could be interpreted in the following way. The presence of several other fungi at 5 mm depth probably posed difficulties for the decay fungi and prevented their establishment in this over populated area. Because of their ability to penetrate further inside where competititors were far less in numbers than at 5 mm depth they were able to colonize better at greater depths. Later with sufficient build up they migrated again towards the surface.

(After about 18 months of exposure the surface in zone III of Scots pine

was covered by Peniophora sp. and in birch fruit bodies of Polystictus sp. were formed).

The Basidiomycetes spp. were found to follow closely the colonization of C. piceae from the surface of the post to 45 mm depth, but when Basidiomycetes spp. were established at 25 mm and 45 mm depth, C. piceae disappeared from the area. The disappearance of C. piceae might be due to the parasitism by Basidiomycetes spp. on this fungus. Shigo (1967) quoting Griffith (1964) has mentioned that "endoconidia of Ceratocystis spp. were highly susceptible to attack by certain Basidiomycetes". It is suggested that the initial growth of Basidiomycetes spp. may be accelerated due to their parasitism on certain early colonizers. This may also be the reason for the disappearance of other members of the fungal flora after the attack by certain decay fungi (see Figure II , and page 133 ).

The presence of a sap-stain fungus Discula penicala on the surface of the post before the attack of Basidiomycetes spp. might be of some significance. This fungus was found to be antagonistic to several other fungi but was suppressed by Polystictus versicolor in malt-agar plate. The presence of such antagonistic fungi on the surface of the post may pave the way for decay fungi by suppressing other fungi which could have prevented their entry. (Discussed in detail in Section III).

The species of Basidiomycetes isolated from the post were very often found to suppress the growth of other fungi including Penicillium spp., C. herbarum and P. varioti, in the plates during isolation (see page 142 ). It is therefore suggested that the first decay fungi to attack the post may be those which have the ability to compete successfully with other fungi and these may be followed by less aggressive species of Basidiomycetes, (a similar suggestion was made by D'Aeth 1939) for the entrance of more virulent parasite following the attack by another pathogen.

It would appear that the successful colonization of Basidiomycetes in exposed timber in ground contact is a more complex phenomenon involving a wider range of factors than has previously been imagined.

After about 16 months the fruit bodies of one species of Basidiomycetes appeared on birch posts. The species was Polystictus sp. An unidentified Basidiomycetes species (culture No. IC 101)was also isolated.

The succession of fungi below the surface nan be summarized as follows: Sapstain (A. pullulans) ⟶ Sapstain and soft-rot (C. piceae and Phialophora sp.) ⟶ Basidiomycetes (Polystictus sp. and an unidentified species)

A comparative account of the distribution of fungi in three zones of birch post (Table 7 )

Fungi belonging to 13 genera were isolated from zone I, 16 from zone II and 23 genera from zone III. The fungi which were isolated from all three zones were Penicillium spp., Fusarium spp., T. viride, C. herbarum Alternaria sp. Aposphaeria sp., Cephalosporium sp. and Dactylosporium sp. The other fungi common to different zones were, Phoma sp. and P. varioti in zones I and II; Botrytis sp., Chaetophoma sp. and Chaetomium sp. in zones II and III. Although several species of fungi were common to all the zones yet the difference between the fungal flora of each zone was quite clear. The fungi which colonized the post at the ground line and above ground line were very different, and this is probably due to the difference in the moisture content and the colonization of soil fungi at ground line.

In each zone the surface was heavily colonized by a number of fungi but below the surface the colonization was very variable, therefore, the comparison has been confined to the surface flora of the three zones. In all the zones the primary colonizers on the surface were moulds but the species involved were different. Penicillium spp. were among the first fungi to appear in zones I and II, but appeared with the second flush of colonizers in zone III. There were marked differences in the periods for which Penicillium persisted in different zones, in zone I they remained

as dominant fungi for a very long period, in zone II they stayed for a short period but in zone III these were only isolated ~~at~~ during early sampling operations. The distribution of C. herbarum was found to be similar to Penicillium with a slight difference in the period during which these fungi persisted on the post. Fusarium spp. on the other hand were the first fungi (along with Botrytis sp.) to colonize zone III but appeared with the second flush of fungi in zone I and II.

It would be interesting to discuss the question as to whether distribution is merely a reflection of the random deposition of spores or is related to the different conditions prevailing at each zone. Among the moulds Fusarium spp. and Botrytis sp. were often found as first colonizers in zone III and Penicillium spp. in zones I and II. Is this distribution due to random deposition of spores or has it another significance ? The spores of these fungi are always present in abundance in air, they might have been also present on all the zones of the posts when these were erected, but their germination and first appearance would depend on the suitability of the substrate.

In zone III which was heavily infected with bacteria, Penicillium spp. with closely appressed or submerged myc., probably were unable to grow in presence of bacteria, but Fusarium and Botrytis with their profuse aerial mycelium grew happily over the bacteria, eventually suppressing them, opening the way for the future colonizers. These two fungi were the first to colonize zone III, not only because the spores were deposited there by chance, but also because of their ability to grow on a substrate infested with bacteria.

In zone I and II Penicillium appeared first along with Paecilomyces and Cladosporium, probably because the rate of germination of their spores was higher than that of Fusarium, so that if spores of Fusarium and Penicillium were present in similar condition to zone I and II, Penicillium might colonize first but later the aerial mycelium of Fusarium would ~~later~~ suppress these fungi.

Penicillium and Cladosporium persisted for a long period in zone I and II, because the other fungi colonizing the area did not have normal vegetative growth due to the low moisture content. In zone III they were almost immediately suppressed by other fungi with rapidly growing aerial mycelium.

Several other fungi were also isolated from the three zones at different times of the year. Alternaria sp. and Aposphaeria sp. were isolated from zone II and III after a few months of exposure but they appeared during the seventh month in zone I. Dactylosporium sp. also colonized zone II and III earlier than zone I. The type of distribution where fungi first colonize the lower region on the post and later colonize the upper region (zone I) of the post may be due to the following reasons: Some of the soil fungi probably do not produce large numbers of spores when growing in the soil or heavy spores do not migrate long distance. The mycelium of such fungi may colonize the post near the soil and produce spores which migrate to zone II and later to zone I. It is also possible that insects such as Collembola which are known to feed on fungi carry spores on their bodies to different parts of the post or deposit both spores and mycelium in their faecal pellets (Goto and Levy (1955) unpublished data).

The distribution of T. viride and P. varioti was of a different type. T. viride appeared very early in zone I and II but appeared much later in zone III; P. varioti which was isolated from zone I and II several times was never isolated from zone III. These fungi probably could not colonize in zone III at the early stage due to the presence of bacteria (as described earlier for Penicillium) and later because of the competition for space and nutrients from the already established fungi.

A very striking difference between the fungal flora of the ground line and above ground line of the post was the presence of a larger number of soft-rot species in zone III than in other zones. Dominant fungi in zone III were soft-rot fungi with sapstain characteristics, whereas moulds were the dominant members of the flora in zones I and II.

On the surface of zones I and II the succession of fungi did not reach beyond mould stage but in zone III the succession reached to the soft-rot stage. The reasons for this have already been discussed.

At 5 mm depth eleven species of fungi were isolated from zone III, three species from zone I and none from zone II. C. herbarum persisted for a long period in zone I probably because of less severe competition but in zone III it was eliminated by soft-rot fungi within two months. P. varioti which was not isolated from any depth in zone III was the only fungus to persist through the latter half of the year in zone I. Several fungi colonized at 25 mm and 45 mm depths in zone III but none were isolated from other zones.

## Seasonal distribution of fungi on Scots pine post in zone I

## (Table 4)

### Surface

On the surface of the post Penicillium spp. and T. viride were the pioneer colonizers and a few bacterial colonies were also isolated. Both bacteria and Penicillium spp. did not persist for long but T. viride was more regularly isolated in the later series of isolations. In the second flush of invasion, Fusarium spp., Epicoccum sp., C. herbarum and Cephalosporium sp. appeared on the post. Fusarium spp. and C. herbarum were the only two fungi which dominated the flora throughout the year along with T. viride, the others were eliminated after a short period. After the post had been exposed for two months, Botrytis sp. and Botryodiplodia sp. were the only new isolates. Both Botryodiplodia and Botrytis sp. were regularly isolated for about five months from the time of their colonization. The last major invasion of fresh colonizers was after the posts were exposed for four months (which included Mucor sp., P. varioti, Alternaria sp. and Aposphaeria sp.) and there after Dactylosporium sp. was the only new inclusion in the flora. However all the fungi which appeared from the third month onwards colonized the post for a very short period and had no impact on the fungi which were already established.

At 5 mm depth: C. herbarum was the first fungus isolated from below the surface - and was soon followed by T. viride and later by Botryodiplodia sp. All these fungi were isolated regularly in subsequent isolations. P. varioti was the only other fungus which later penetrated into the post but could not become established.

At 25 mm depth: C. herbarum was the first fungus isolated from this depth. It was later followed by T. viride and Botryodiplodia. These fungi were not isolated in the last three or four series of isolations.

Table 4

Sequence of colonization of fungal species in zone I of Scots pine posts in relation to exposure months

| On surface layer | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
|---|---|---|---|---|---|---|---|---|---|---|
| Penicillium spp. | X | X | | | | | | | | |
| Trichoderma viride | X | X | | X | X | X | | X | | X |
| Fusarium spp. | | X | X | X | X | X | X | X | | X |
| Epicoccum sp. | | X | X | | | | | | | |
| Cladosporium herbarum | | X | X | X | X | X | X | X | | X |
| Cephalosporium sp. | | X | X | | | | | | | |
| Botrytis sp. | | | X | | | X | X | | | |
| Botryodiplodia sp. | | | X | X | X | X | | | | |
| Aspergillus sp. | | | | X | | | | | | |
| Mucor sp. | | | | | X | X | | | | |
| Paecilomyces varioti | | | | | X | | | | | |
| Alternaria sp. | | | | | X | X | | | | |
| Aposphaeria sp. | | | | | X | | | | | |
| Dactylosporium sp. | | | | | | | X | | | |
| | | | | | | | | | | |
| At 5 mm depth | | | | | | | | | | |
| C. herbarum | | X | X | X | | X | | | | |
| T. viride | | | X | X | X | X | X | X | | X |
| Botryodiplodia sp. | | | X | X | X | X | X | X | | X |
| P. varioti | | | | | X | X | | | | |
| | | | | | | | | | | |
| At 25 mm depth | | | | | | | | | | |
| C. herbarum | | | X | X | X | | | | | |
| T. viride | | | | X | X | X | X | | | |
| Botryodiplodia sp. | | | | | X | X | | | | |

At 45 mm depth: No fungus was isolated from this depth.

Analysis of the results:

Fourteen fungi were isolated from the surface but only three of these remained dominant throughout the year. It is difficult to say whether T. viride which colonized the post first inhibited the growth of other colonizers. However Fusarium spp, C. herbarum and Botryodiplodia sp. were not affected by the antagonistic action of T. viride and remained dominant. The colonization on the surface of the posts did not follow any definite sequence and the succession of fungi did not proceed here beyond the mould stage, although several soft rot fungi (Cephalosporium sp. Alternaria sp., Dactylosporium sp.) and a pycnidial fungus (Aposphaeria sp.) appeared later for a short period. The peculiar sequence of fungi could be due to season variation. That the soft-rot fungi were unable to establish on the post was perhaps due to the low moisture content and the competition from moulds. The dominance of T. viride, C. herbarum and Botryodiplodia sp. on the surface was probably because these fungi were able to draw sufficient nutrients from inside the post to support their mycelial growth on the surface. Other fungi were eliminated probably due to the depletion of nutrients on the surface. The presence of Fusarium spp. on the post for long period may be due to their low nutrition requirements and adaptability to dry and wet spells.

T. viride, Botryodiplodia sp. and C. herbarum were the only colonizers below the surface layers. The successful colonization of these fungi is probably due to their close mutual association. The increase in permeability of wood in the presence of sapstain fungi ( Findlay 1959), may increase the moisture content and thus the growth of T. viride.

Seasonal distribution of fungi in zone II (Table 5 )

Surface: C. herbarum was the first fungus isolated from the surface layers. A few bacterial colonies were also found on the petri dishes

Table 5

Sequence of colonization of fungal species in zone II of Scots pine posts in relation to exposure months

| On surface layer | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
|---|---|---|---|---|---|---|---|---|---|---|
| Cladosporium herbarum | X | X | X | X | X | | | | | |
| Cephalosporium sp. | | X | X | | | | | | | |
| Fusarium spp. | | | X | X | X | X | | X | | X |
| Alternaria sp. | | | X | X | | | | | | X |
| Aspergillus sp. | | | | X | | X | | | | |
| Botryodiplodia sp. | | | | X | X | | X | | | |
| Aureobasidium pullulans | | | | X | X | | | | | |
| Phoma sp. | | | | | X | | | | | |
| Penicillium spp. | | | | | X | X | | | | |
| Trichoderma viride | | | | | X | X | | | | X |
| Aposphaeria sp. | | | | | | X | | | | |
| Dactylosporium sp. | | | | | | X | | | | |
| Discula pinicola | | | | | | | X | | | |
| Botrytis sp. | | | | | | | | X | | |

with wood samples. In each subsequent isolation a few fresh fungal species were added to the flora on the post almost throughout the period of investigation. A few of the important fungi which later colonized the post are mentioned here. The early colonizers were followed by Cephalosporium sp., Fusarium spp. and Alternaria sp. within two months of exposure. Bacteria and the fungi mentioned above, except Fusarium spp. were not isolated after the posts had been in the ground for four months. Fusarium spp. were the only fungi isolated regularly throughout the period of investigation. The posts were later colonized by several other moulds, sapstain, soft-rot and pycnidial fungi. None of these fungi stayed long on the post except Botryodiplodia sp. and T. viride which were isolated from several subsequent isolation series.

At 25 mm to 45 mm depth: From these depths no fungus was isolated during the investigation.

Analysis of the results.

The moisture content on the surface of the post probably varied very sharply during the year, remaining at a low level for most of the year. The fungi which colonized the post in zone II, did not persist for any length of time probably because of their inability to live under such sharp seasonal changes. Fusarium spp. were the fungi isolated most regularly and were often found to encrust the surface in this zone.

The post inside was very dry and none of the surface colonizers penetrated the wood below the surface layers. A few fungi, e.g. C. herbarum, Botryodiplodia sp. and T. viride, which persisted longer than their competitors were later eliminated from the surface layers or due to seasonal change or both. The number of species of fungi in the surface flora reached a maximum within about four or five months of exposure and then the numbers declined in the subsequent months. This reduction in number of species in the flora of the post suggests that the depletion of nutrients or the temperature variation may be the cause of elimination of most of the

existing fungi.

Colonization by fungi in zone II did not follow any definite pattern. Fresh colonizers were isolated contiuously from the posts for about 6 to 7 months of exposure. The succession of fungi did not proceed beyond mould stage.

Seasonal distribution of fungi on Scots pine post in zone III

(Table 6)

Surface:

The post was first colonized by bacteria and moulds (Botrytis sp., Fusarium sppp., and C. herbarum) . Both bacteria and moulds were isolated at the same time, but the presence of a very large number of bacterial colonies in the first isolation and decline in number in subsequent isolations after the establishment of the fungi suggests that the moulds may have followed bacteria in the succession. The primary colonizers were followed by a large number of other moulds, sapstain, soft-rot and pycnidial fungi within a period of four or five months. Most of these fungi remained on the post for a very short time. The important fungi which dominated the surface flora for a longer period were Fusarium spp., T. viride, C. piceae, A. pullulans, Botryodiplodia sp., Phialophora spp. and Geotrichum sp.. Fusarium spp. were the first members among the dominant groups of fungi to be eliminated from the flora and later T. viride was also eliminated. C. piceae and Phialophora spp. were isolated regularly in most subsequent series of isolations.

The last flush of invasion by other fungi was after nine months of exposure and these included Dactylosporium sp., Alternaria, Dendrophoma sp. and Aposphaeria sp. These fungi were very rarely isolated from the air in the control plates. It is therefore assumed that these soil microfungi probably colonized the post from the soil by hyphal contact.

At 5 mm depth: Bacteria were not isolated from below the surface layers. Twenty three fungi colonized the surface and out of those only eleven fungi penetrated below the surface. The first isolates were a mixed group of fungi belonging to moulds, pycnidial and soft-rot fungi. These early colonizers were very soon eliminated by the secondary colonizers which included C. piceae, Phialophora sp., Botryodiplodia sp., Sporotrichum sp., Geotrichum sp., T. viride , Chaetomium globosum and Gliomastix sp. Many of the secondary colonizers persisted for a few months but in the last few isolation series C. piceae and Phialophora sp. were the only fungi isolated along with Basidiomycetes (Peniophora sp.). A Basidiomycete species was first isolated from the posts after these had been in the ground for about seven months.

At 25 mm depth: C. piceae was the first fungus to penetrate to this depth. This fungus was followed by Phialophora sp. and Gliomastix sp., and later by Peniophora sp. Both C. piceae and Phialophora sp. were isolated along with Peniophora sp. until the end of the investigation. Gliomastix sp. was not isolated later when C. piceae and Phialophora sp. were well established.

At 45 mm depth: Fungi which colonized at 25 mm also penetrated to 45 mm depth of the posts but, there is some difference in the order of colonization. At 45 mm depth C. piceae colonized first, followed by Gliomastix sp. Both these fungi were replaced very early by Phialophora sp. and later by Basidiomycetes.

Table 6

Sequence of colonization of fungal species in zone III of Scots pine posts in relation to exposure months

| On surface layer | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
|---|---|---|---|---|---|---|---|---|---|---|
| Botrytis sp. | X | X | | | | | | | | |
| Fusarium spp. | X | X | X | X | | | | | | |
| Cladosporium sp. | X | X | | | | | | | | |
| Penicillium spp. | | X | | | | | | | | |
| Mucor sp. | | X | X | X | | | | | | |
| Cephalosporium sp. | | X | | | | | | | | |
| Chaetophoma sp. | | X | X | | | | | | | |
| Trichoderma viride | | | X | X | X | X | | | | |
| Ceratocystis piceae | | | X | X | X | | X | X | | |
| Cytospora sp. | | | X | X | | | | | | |
| Aureobasidium pullulans | | | | X | X | X | | | | |
| Verticillium spp. | | | | X | | | | | | |
| Botryodiplodia sp. | | | | | X | X | | | | |
| Phialophora spp. | | | | | X | | X | X | | X |
| Sporotrichum sp. | | | | | X | X | | | | |
| Paecilomyces varioti | | | | | X | | | | | |
| Gliomastix sp. | | | | | X | X | | | | |
| Geotrichum sp. | | | | | X | X | | | | |
| Chaetomium globosum | | | | | X | X | | | | |
| Chaetomium dolichotrichum | | | | | X | | | | | |
| Pestalotia sp. | | | | | | | X | X | | |
| Aspergillus sp. | | | | | | | | X | | |
| Dactylosporium sp. | | | | | | | | | | X |
| Alternaria sp. | | | | | | | | | | X |
| Dendrophoma sp. | | | | | | | | | | X |
| Aposphaeria sp. | | | | | | | | | | X |

Table 6 (contd.)

| At 5 mm depth | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
|---|---|---|---|---|---|---|---|---|---|---|
| Botrytis sp. | | X | X | X | | | | | | |
| Chaetophoma sp. | | X | X | | | | | | | |
| Cephalosporium sp. | | X | X | X | | | | | | |
| Cylindrophora sp. | | | | X | | | | | | |
| Ceratocystis piceae | | | | X | X | X | X | X | | X |
| Phialophora sp. | | | | | X | X | X | X | | X |
| Botryodiplodia sp. | | | | X | | | | | | |
| Sporotrichum sp. | | | | | X | | | | | |
| Geotrichum sp. | | | | | X | X | | X | | |
| Trichoderma viride | | | | | X | X | | | | |
| Chaetomium globosum | | | | | X | X | | | | |
| Gliomastix sp. | | | | | X | X | X | X | | |
| Peniophora sp. | | | | | | | X | | | X |
| **At 25 mm depth** | | | | | | | | | | |
| Ceratocystis piceae | | | | | X | X | X | X | | X |
| Phialophora sp. | | | | | | X | X | X | | X |
| Gliomastix sp. | | | | | | X | X | | | |
| Peniophora sp. | | | | | | | | X | | X |
| **At 45 mm depth** | | | | | | | | | | |
| Ceratocystis piceae | | | | | | X | X | | | |
| Gliomastix sp. | | | | | | | X | | | |
| Phialophora sp. | | | | | | | X | X | | X |
| Peniophora sp. | | | | | | | | X | | X |

Analysis:

This series of isolations revealed a pattern of succession of fungi. In suggesting the pattern of succession only the fungi which were isolated for several consecutive months were taken into account, leaving out several of those which were thought to be chance colonizers.

The succession on the surface and at different depths is dealt with separately. On the surface the succession of fungi begins with bacteria and ends with soft-rot fungi; following the sequence:→ Bacteria → Moulds → Sapstains → Sapstaining and soft-rot fungi.

The fungi representing the groups are: in Moulds - Botrytis sp., Fusarium spp., Mucor sp. and T. viride; and in sapstain and soft-rot fungi - C. piceae, [redacted] and Phialophora spp.

The fungi mentioned above remained on the surfaces as dominant colonizers for a considerable length of time. These were eight of a total of twenty three fungi which colonized the surface in zone III at some time or other. Dominance of these fungi on the surfacewas probably due to their growth below the surface of the post, from where the necessary nutrition came when the nutrients on the surface were depleted. The fungi which did not penetrate inside were eventually eliminated from the surface by depletion of nutrients. For example, C. piceae and Phialophora sp. remained on the surface as dominant colonizers due to their heavy mycelial growth below the surface.

The role of Fusarium spp. and Botrytis sp. on the surface of the post infested with bacteria is of great significance in succession of fungi. These fungi by overgrowing bacteria on the surface layers probably pave the way for several subsequent colonizers, which otherwise might not have grown in the presence of bacteria.

T. viride a dominant member of the surface flora for several months, did not affect the growth of several other fungi which later colonized and survived for a considerable length of time. This suggests that the interaction between T. viride and several fungi is not very effective

when a number of fungi grow together on the surface layers of fence posts.

The record of surface colonization shows that the fresh colonizers were added to the flora regularly for about five months and thereafter no significant addition took place to the list of species of fungi. It is therefore suggested that once the colonization reaches the climax, within a period of four or five months, the subsequent random deposition of air-borne spores does not result in colonization, due to the lack of space and nutrients. Even among the existing fungi the severe competition for food and space appeared to have resulted in the elimination of some of these fungi. The activity of fungi on surface is probably lower at this stage than it ever had been before. It is therefore thought that Basidiomycetes may have a better chance of penetrating the wood after the fungal activity on the surface has declined due to the depletion of nutrients than at any time before this stage. The isolation of Basidiomycetes from the posts in most cases after 4 or 5 months exposure is supporting evidence of this view.

The succession of fungi below the surface layers is very similar at each depth, when only dominant species of fungi are considered. Below the surface layers of the posts fungal succession was sapstain and soft-rot fungi → ~~Soft-rot fungi~~ → Basidiomycetes. The fungi included in the sequence were as follows: Sapstain and soft-rot group - C. piceae and Phialophora sp.; and in Basidiomycetes Peniophora sp. A fruit body of this fungus was formed on the surface of the post.

A comparative account of distribution of fungi (in three zones) in Scots pine Table 8

The fungal flora in zone I falls into about 14 genera, in zone II, 15 genera and in zone III, about 23 genera. Fungi belonging to 9 genera were common to all the three zones, which includes five common moulds, a pycnidial fungus and three soft-rot fungi. Isolation of a largernumber

of fungi from zone III than other two zones indicates that the conditions here are more favourable for fungal growth. This also indicates that the number of fungi involved in the colonization in zone II is more than in other zones because it is exposed to both air-borne spores and mycelium of fungi living in the soil substrate.

Certain striking variations in the time of colonization of fungi common to all the zones are briefly discussed here. T. viride was the first fungusto colonize the surface layers of posts in zone I, but it was a very late colonizer in other zones. Another example is Fusarium spp. which was among the first colonizers in zone III but was a secondary colonizer in other zones. However both these fungi were isolated very regularly throughout the period of investigation. In zone III they were replaced by other colonizers which were possibly more efficient cellulose decomposers. The survival of these two fungi for a greater length of time in zone I and II may be due to the lack of competition from any efficient cellulose decomposers which failed to grow at the low moisture content level of these zones.

The species of fungi from the first isolation were significantly different in three zones. Fusarium spp. and Botrytis sp. were the early colonizers in zone III which was heavily covered with bacteria, where other moulds had far less chance of growth. In zone I and II, bacterial infection was very slight and the early colonizers were Penicillium spp., T. viride or C. herbarum. This suggests that the distribution of fungi is not always due to the random deposition of spores but the conditions prevailing on the substrate may be an important factor which would determine the distribution of species of fungi on the fence posts.

Fresh colonizers were continuously isolated from the surface layers of zone I and III until about four months of exposure but were isolated continuously in zone II until about seven months from the date the posts were set up. The sharp change in moisture content in zone II may have so greatly reduced the activity of the existing fungi, that sufficient nutrients are left for a greater length of time for the growth of

fresh colonizers. In zone I and III the conditions on the surface of the posts being more favourable for the growth and activity of fungi, the surface nutrients are depleted very early, thus the chances for growth and development of freshly deposited spores are greatly reduced after about 4 to 5 months.

After the depletion of nutrients from the surface layers in zone III there are three alternative possibilities for colonization: (1) Myxomycetes and other fungi capable of living on partially decomposed cell wall and on residues of earlier colonizers (Findlay 1940, others) (2) Fungi which were now established below the surface may reappear, (3) soil-microfungi may colonize through hyphal contact.

This suggests that the active phase of succession of fungi on the surface layers, where both air-borne spores and mycelium of the soil fungi are involved, probably ends within a few months with the depletion of nutrients. After this stage the fungi involved in the succession may be those which are able to colonize the post through hyphal contact and their success may primarily depend on their inoculum potential and their mycelial growth in the soil. It has been noticed that in zone III the fungi which colonized the surface during the last few series of samplings might have colonized the post by hyphal contact. The spores of these fungi were rarely isolated from the air. The succession of fungi did not proceed beyond the mould stage in zone I and II, but it is more pronounced and showed a definite pattern in zone III.

A comparative study of fungal colonization in birch and Scots pine

(Tables 7, 8, 9, 10)

In the present investigation two different wood species, one hard wood (birch) and one soft wood (Scots pine), were selected to examine the possible effect of wood species on the fungal colonization.

It has been mentioned before that each zone of a post is exposed to different ecological conditions. It is assumed that the conditions prevailing in the same zone of birch and Scots pine posts would be very similar. The distribution of fungi on each zone is therefore compared

separately in the text, assuming that other external factors were similar, the difference in the distribution of fungi if any, would be due to the difference in wood species.

Species isolated:

In general fungal species isolated from both birch and Scots pine were similar. Most of the fungi were common to both wood species but certain fungi, e.g. Botryodiplodia sp., Cytospora sp., Chaetomium globosum., Gliomastix sp., and Peniophora sp. were isolated only from Scots pine, and Stysanus sp., Coniothyrium fuckelii, Polystictus sp. and an unidentified Basidiomycetes species were isolated only from birch. The fungal flora in a zone is very similar in two wood species but several fungi were isolated either from birch or Scots pine. There is a marked difference in the order of colonization of common fungi in both wood species. A very large number of common fungi in a zone were isolated at different times of the year from two wood species. The difference in the time of isolation of a fungus from different wood may be 1 to 5 months within a zone, but between the zone the time of isolation of a fungus may vary from 1 to 8 months. (Tables 1 to 6)

Colonization on the surface.

In zone I Fusarium spp. and C. herbarum were common dominant fungi both in birch and Scots pine. Other dominant or sub-dominant fungi in birch were Penicillium spp., Cephalosporium sp. and Dactylosporium sp. in Scots pine - T. viride and Botryodiplodia sp. In Scots pine T. viride was the first colonizer but this fungus appeared very late in birch. Most of the species of fungi isolated were common to both but a few fungi were present either in birch or Scots pine.

In zone II a few dominant and sub-dominant fungi were common to both wood species, but among the sub-dominant groups of fungi, birch was colonized by Penicillium spp., Botrytis sp. and P. varioti, and Scots pine was colonized by T. viride and Botryodiplodia.

In zone III the important dominant fungi were common to both but among the sub-dominant groups of fungi, birch was colonized by Stysanus sp. and Scots pine by T. viride, A. pullulans and Geotrichum.

Colonization in depth

In zone I - the fungal colonization in birch did not proceed beyond 5 mm., but in Scots pine a few fungi were isolated from 25 mm depth. T. viride and Botryodiplodia were the dominant fungi in Scots pine but they were not isolated from birch. P. varioti a dominant fungus in birch was was present only for a very short period in Scots pine.

In zone II - the fungi did not colonize the posts below the surface in both birch and Scots pine.

In zone III - the difference in the fungal flora below the surface is more pronounced than it was on the surface. The common dominant fungi in both birch and Scots pine were C. piceae and Phialophora sp. In birch the only other dominant fungi were Basidiomycetes - Polystictus sp. and an unidentified species, but in Scots pine Geotrichum and Gliomastix were sub-dominant fungi along with the Basidiomycete - Peniophora sp. The birch posts were attacked by Basidiomycetes much earlier than Scots pine.

The Succession of fungi:

The pattern of succession of fungi is very similar in both birch and Scots pine, but the fungi involved in the succession were different. For example T. viride, Botryodiplodia and A. pullulans were among the dominant fungi in the early stage of succession of organisms in Scots pine and Peniophora sp. was dominant in the later stage of succession, but in birch these fungi persisted for a very short period (Peniophora sp. was not isolated from birch posts). In zone I and II the succession did not proceed beyond mould stage but in zone III the succession of organisms was more complete.

# TABLE - 7

## A COMPARATIVE SEASONAL DISTRIBUTION OF FUNGI FROM THE AIR (C) AND ON THE SURFACE OF BIRCH FENCE POSTS IN ZONES I, II AND III

| | JUNE | | | | JULY | | | | AUGUST | | | | SEPTEMBER | | | | OCTOBER | | | | NOVEMBER | | | | DECEMBER | | | | JANUARY | | | | MARCH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | I | II | III | C | I | II | III | C | I | II | III | C | I | II | III | C | I | II | III | C | I | II | III | C | I | II | III | C | I | II | III | C | I | II | III |
| **PHYCOMYCETES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MUCOR sp. | X | | | | X | | | | X | | | X | X | | | X | X | | | X | X | | | | X | | | | X | | | | X | | | |
| **ASCOMYCETES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CERATOCYSTIS PICEAE | | | | | | | | | | | | X | | | | X | X | | | | | | | X | | | X | | | X | | | | | X |
| CHAETOMIUM DOLICHOTRICHUM | | | | | | | | | | | | | X | | | | X | | | | | X | X | | | X | | | | X | | | | | |
| **FUNGI IMPERFECTI** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **MONILIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ASPERGILLUS sp. | X | | | | | | | | | | | | X | | | | X | | | | | | | | | | | | | | | | | | | |
| BOTRYTIS sp. | X | | | X | X | | | X | X | | X | | X | | X | | X | | | | X | | X | | X | | | | X | | | | X | | |
| CEPHALOSPORIUM sp. | X | | | | X | X | X | | X | X | X | X | X | X | | | | | | | | | | | | X | | | | X | | | | X | | |
| GEOTRICHUM sp. | | | | | | | | | | | | X | | | | X | X | | | | | | | | X | | | | X | | | | X | | |
| GLIOCLADIUM sp. | | | | | | | | | | | | | | | | | | | | | | | | | | X | | | | X | | | | X | | |
| PAECILOMYCES VARIOTI | X | X | | | X | | | | | | X | | | | X | | X | X | X | | X | | | | X | | | | | | | | X | | |
| PENICILLIUM spp. | X | X | X | | X | X | | X | X | X | | X | X | X | X | | X | X | X | | X | X | | | X | | | | X | | | | X | | |
| TRICHODERMA VIRIDE | | | | | | | | | | | | | | | | | X | X | X | | | | | | | | | | X | | X | | | | X |
| VERTICILLIUM spp | | | | | X | | | | X | | | | X | | | | X | X | | | | | | | | | | | | | | | | | | |
| **DEMATIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ALTERNARIA sp. | | | | | | | | | | | X | | | | X | X | | X | | | | | | | X | | | | | | | | | X | | |
| AUREOBASIDIUM PULLULANS | | | | | | | | X | | | | | | | | | X | | X | | X | | | | | | | | X | | | | | | | |
| CLADOSPORIUM HERBARUM | X | X | X | | X | X | X | X | X | X | X | X | X | X | | | X | X | | | X | X | X | | X | | X | | X | X | | | X | X | | |
| DACTYLOSPORIUM sp. | | | | | | | X | | | | | | | | X | | X | X | | | X | X | | | | X | | | | X | | | | | | |
| GLIOMASTIX sp. | | | | | | | | | X | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PHIALOPHORA sp. | | | | | | | | | | | | X | | | | X | | | | X | | | | | | | | X | | | | | | | | X |
| **TUBERCULARIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| EPICOCCUM sp. | | | | | X | | | | | | | | | | X | | X | | | | X | | | | | | | | | X | | | X | | | |
| FUSARIUM spp. | X | | | X | X | X | X | X | X | X | | X | X | X | | X | X | X | | X | X | X | X | X | X | X | X | | X | X | X | | X | X | X | |
| **SPHAEROPSIDALES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| APOSPHAERIA sp. | | | | | | | | | | | X | | X | | X | X | | | | | | | | | | | | | | | | | | X | X | |
| CHAETOMELLA sp. | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | |
| CHAETOPHOMA sp. | | | | | X | | | | X | | | | | | | | X | | | | | | | X | | | X | | | | | | | | | |
| CONIOTHYRIUM FUCKELII | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| DENDROPHOMA sp. | | | | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | | | | | |
| PHOMA sp. | | | | | | | | | | | | | | X | X | | | | | | X | | | | | | | | | | | | | | | |
| PHOMOPSIS sp. | | | | | | | | | | | | | X | | | | | | | | X | | | | | | | | | | | | | | | |
| DISCULA PINICOLA | | | | | | | | | | | | X | | | | X | | | | | | | | | | | | | | | | | | | | |
| **MELANCONIALES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PESTALOTIA sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| **STILBACEAE** - STYSANUS sp. | | | | | | | | | | | | | | | | X | | | | X | | | X | | | | | | | | | | | | |

# TABLE-8

A COMPARATIVE SEASONAL DISTRIBUTION OF FUNGI FROM THE AIR (C) AND ON THE SURFACE OF SCOTS PINE POSTS - ZONE: I, II, III

| | JUNE | | | | JULY | | | | AUGUST | | | | SEPTEMBER | | | | OCTOBER | | | | NOVEMBER | | | | DECEMBER | | | | JANUARY | | | | MARCH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | I | II | III | C | I | II | III | C | I | II | III | C | I | II | III | C | I | II | III | C | I | II | III | C | I | II | III | C | I | II | III | C | I | II | III |
| **PHYCOMYCETES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MUCOR sp. | X | | | | X | | | X | X | | | X | X | | | X | X | X | | | X | X | | | X | | | | X | | | | X | | | |
| **ASCOMYCETES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CERATOCYSTIS PICEAE (MÜNCH) BAKSHI | | | | | | | | | | | | X | | | | X | | | | X | | | | X | | | X | | | | X | | | | |
| CHAETOMIUM GLOBOSUM KUNZE ex FR. | | | | | | | | | | | | | | | | X | | | | X | | | X | | | | | | X | | | | | | |
| CHAETOMIUM DOLICHOTRICHUM | | | | | | | | | | | | | X | | | X | | | | | | | | | | | | | | | | | | | | |
| **FUNGI IMPERFECTI - MONILIACEAE** | | | | | | | | | | | | | | | | | | | | | | X | | | | | | X | | | | X | | | | |
| ASPERGILLUS sp. | X | | | | | | | | | | | | X | X | X | | X | | | | | | | | X | X | | | X | | X | | X | | | |
| BOTRYTIS sp. | X | | | X | X | | | X | X | X | | | X | | | | X | | | | X | X | | | X | X | | | X | | | | X | | | |
| CEPHALOSPORIUM sp. | X | | | | X | X | X | X | X | X | X | | X | | | | | | | | | | | | X | | | | X | | | | X | | | |
| GEOTRICHUM sp. | | | | | | | | | | | | | | | | | X | | | X | | | | X | X | | X | | X | | | | X | | | |
| GLIOCLADIUM sp. | | | | | | | | | | | | | | | | | | | | | | | | | X | | | | X | | | | X | | | |
| PAECILOMYCES VARIOTI BAINIER | X | | | | X | | | | | | | | | | | | X | | | X | X | | | | X | | | | X | | | | X | | | |
| PENICILLIUM spp. | X | X | | | X | X | | X | X | | | | X | | | | X | | X | | X | | X | | X | | | | X | | | | X | | | |
| SPOROTRICHUM sp | X | | | | | | | | X | | | | | | | | | | | X | | | | X | | | | | | | | | | | | |
| TRICHODERMA VIRIDE Link ex Fr. | | X | | | | X | | | | | | X | | X | | X | X | X | X | | | X | X | X | | | | | X | X | | | | X | X | |
| VERTICILLIUM spp. | | | | | X | | | | X | | | | X | | | X | X | | | | | | | | | | | | | | | | X | | | |
| **DEMATIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ALTERNARIA sp. | | | | | | | | | | | | X | | | X | | X | | | | X | | | | X | | | | | | | | | | X | X | |
| AUREOBASIDIUM PULLULANS (DE BARY) ARN. | | | | | | | | | | | | | | | X | X | | X | X | | X | | | X | | | | | X | | | | X | | | |
| CLADOSPORIUM HERBARUM Link ex Fr. | X | | X | X | X | X | X | X | X | X | X | | X | X | X | | X | X | X | | X | X | | | X | X | | | X | X | | | X | X | | |
| DACTYLOSPORIUM sp. | | | | | | | | | | | | | | | | | X | X | | X | X | | X | | | X | | | | | | | | | | X |
| GLIOMASTIX sp. | | | | | | | | | X | | | | | | | | | | X | | | | | X | | | | | | | | | | | | |
| PHIALOPHORA sp. | | | | | | | | | | | | | | | | | | | X | | | | | | | | | X | | | | X | | | | X |
| **TUBERCULARIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| EPICOCCUM sp. | | | | | X | X | | | | X | | | | | | | X | | | | X | | | | | | | | | | | | X | | | |
| FUSARIUM spp. | X | | | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | X | | X | X | X | | X | X | | | X | X | X | | X | X | X | |
| **SPHAEROPSIDALES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| APOSPHAERIA sp. | | | | | | | | | | | | | X | | | | | X | | | | | X | | | | | | | | | | | | | X |
| BOTRYODIPLODIA sp. | | | | | | | | | X | X | | | X | X | X | | | X | X | X | | X | | X | | | X | | | | | | | | | |
| CHAETOMELLA sp. | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | |
| CHAETOPHOMA sp. | | | | | X | | X | | | | | X | | | | | X | | | | | | | | | | | | | | | | | | | |
| CONIOTHYRIUM FUCKELII Saccardo | | | | | | | | | | | | | | | | X | | | | | | | | | X | | | | | | | | | | | |
| CYTOSPORA sp. | | | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | | | | | | X |
| DENDROPHOMA sp. | | | | | | | | | | | | | X | | | | | | X | | | X | | | | | | | | | | | | | | |
| PHOMA sp. | | | | | | | | | | | | | X | | | | | | | | | X | | | | | | | | | | | | | | |
| PHOMOPSIS sp. | | | | | | | | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | |
| DISCULA PINICOLA (Naum.) Per | | | | | | | | | | | | | | | | | | | | | | | | | | | | X | | | | X | | | |
| **MELANCONIALES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PESTALOTIA sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

# TABLE - 9

SEASONAL DISTRIBUTION OF FUNGI IN BIRCH POST - ZONE III - SURFACE(S), 5, 25 AND 45mm. DEPTHS.

| | JUNE | | | | JULY | | | | AUGUST | | | | SEPTEMBER | | | | OCTOBER | | | | NOVEMBER | | | | DECEMBER | | | | JANUARY | | | | MARCH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 |
| **PHYCOMYCETES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MUCOR sp. | | | | | | | | | X | | | | X | | | | X | | | | | | | | | | | | | | | | | | | |
| **ASCOMYCETES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CERATOCYSTIS PICEAE | | | | | | | | | X | | | | X | X | | | X | X | X | X | | X | X | X | X | X | X | X | X | X | | | X | X | | |
| CHAETOMIUM GLOBOSUM | | | | | | | | | | | | | | | | | X | | | | X | | | | | | | | | | | | | | | |
| CHAETOMIUM DOLICHOTRICHUM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **BASIDOMYCETES** | | | | | | | | | | | | | | | | | | X | X | X | | | X | X | | | | X | | | X | X | | | X | X |
| POLYSTICTUS sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PENIPHORA sp. | | | | | | | | | | | | | | | | | | | | | | | | | | X | X | X | | | | X | | | | |
| Unidentified sp. C.No. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **FUNGI IMPERFECTI - MONILIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ASPERGILLUS sp. | X | | | | X | | | | | | | | | X | | | | | X | | | X | | | | | | | | | | | | | |
| BOTRYTIS sp. | | | | | | | | | X | | | | | | | | | X | | | | X | | | | | | | | | | | | | | |
| CEPHALOSPORIUM sp. | | | | | | | | | X | | | | | X | | | | | | | | | | | | | | | | | | | | | | |
| CYLINDROPHORA sp. | | | | | | | | | X | X | | | X | X | X | | | | | | | X | | | | X | | | | | | | | | | |
| GEOTRICHUM sp. | | | | | | | | | X | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PAECILOMYCES VARIOTI | | | | | X | | | | X | | | | | X | | | | X | | | | | | | | | | | | | | | | | | |
| PENICILLIUM spp. | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | X | | | | X | | | |
| SPOROTRICHUM sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TRICHODERMA VIRIDE | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| VERTICILLIUM spp. | | | | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | | | | | |
| **DEMATIACEAE** | | | | | | | | | | | | | | X | | | | X | | | | | | | | | | | | | | | | | | |
| ALTERNARIA sp. | | | | | X | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| AUREOBASIDIUM PULLULANS | | | | | | X | | | | X | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CLADOSPORIUM HERBARIUM | | | | | X | X | | | X | X | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DACTYLOSPORIUM sp. | | | | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | | | | | |
| GLIOMASTIX sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PHIALOPHORA sp. | | | | | | | | | X | | | | X | X | X | X | X | X | X | X | | X | X | | | X | X | | | X | X | X | | X | X | X |
| **STILBACEAE** - STYSANUS sp. | | | | | | | | | | | | | | | | | X | | | | X | | | | | X | | | | | | | | | |
| **TUBERCULARIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| EPICOCCUM sp. | | | | | | | | | | | | | X | | | | | | | | X | | | | | | | | | | | | | | | |
| FUSARIUM spp. | X | | | | X | | | | X | | | | | | | | X | | | | | | | | | | | | | | | | | | | |
| **SPHAEROPSIDALES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| APOSPHAERIA sp. | | | | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | | | | | |
| BOTRYDIPLODIA sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHAETOPHOMA sp. | | | | | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | | | | |
| CONIOTHYRIUM FUCKELII. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CYTOSPORA | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DISCULA PINICOLA | | | | | | | | | | | | | X | | | | | X | | | | | | | | | | | | | | | | | | |
| DENDROPHOMA sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **MELANCONIALES** - PESTALOTIA sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

# TABLE - 10

SEASONAL DISTRIBUTION OF FUNGI IN SCOTS PINE POSTS - ZONE III - SURFACE(S), 5, 25 and 45 mm DEPTHS

| | JUNE | | | | JULY | | | | AUGUST | | | | SEPTEMBER | | | | OCTOBER | | | | NOVEMBER | | | | DECEMBER | | | | JANUARY | | | | MARCH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 | S | 5 | 25 | 45 |
| **PHYCOMYCETES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MUCOR sp. | | | | | X | | | | X | | | | X | | | | | | | | | | | | | | | | | | | | | | | |
| **ASCOMYCETES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CERATOCYSTIS PICEAE | | | | | | | | | X | | | | X | X | | | X | X | X | | | X | X | X | X | X | X | X | X | X | X | | | X | X | |
| CHAETOMIUM GLOBOSUM | | | | | | | | | | | | | | | | | X | X | | | X | X | | | | | | | | | | | | | | |
| CHAETOMIUM DOLICHOTRICHUM | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | |
| **BASIDIOMYCETES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| POLYSTICTUS sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PENIOPHORA sp. | | | | | | | | | | | | | | | | | | | | | | | | | | X | | | | | X | X | | | X | X | X |
| Unidentified sp. C.No. IC 101 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **FUNGI IMPERFECTI - MONILIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ASPERGILLUS sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | X | | | | | | | |
| BOTRYTIS sp. | X | | | | X | X | | | | X | | | | X | | | | | | | | | | | | | | | | | | | | | | |
| CEPHALOSPORIUM sp. | | | | | X | X | | | | X | | | | X | | | | | | | | | | | | | | | | | | | | | | |
| CYLINDROPHORA sp. | | | | | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | | | | |
| GEOTRICHUM sp. | | | | | | | | | | | | | | | | | X | X | | | X | X | | | | | | | | X | | | | | | |
| PAECILOMYCES VARIOTI | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | |
| PENICILLIUM spp. | | | | | X | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SPOROTRICHUM sp. | | | | | | | | | | | | | | | | | X | X | | | X | | | | | | | | | | | | | | | |
| TRICHODERMA VIRIDE | | | | | | | | | X | | | | X | | | | X | X | | | X | X | | | | | | | | | | | | | | |
| VERTICILLIUM spp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | X | | | |
| **DEMATIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ALTERNARIA sp. | | | | | | | | | | | | | X | | | | | | | | | | | | | | | | | | | | | | | |
| AUREOBASIDIUM PULLULANS | | | | | | | | | | | | | | | | | X | | | | X | | | | | | | | | | | | | | | |
| CLADOSPORIUM HERBARUM | X | | | | X | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DACTYLOSPORIUM sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | X | | | |
| GLIOMASTIX sp. | | | | | | | | | | | | | | | | | X | X | | | X | X | X | | | X | X | X | | X | | | | | | |
| PHIALOPHORA sp. | | | | | | | | | | | | | | | | | X | X | | | | X | X | | X | X | X | X | X | X | X | X | X | X | X | X |
| **STILBACEAE** - STYSANUS sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **TUBERCULARIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| EPICOCCUM sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| FUSARIUM spp. | X | | | | X | | | | X | | | | X | | | | | | | | | | | | | | | | | | | | | | | |
| **SPHAEROPSIDALES** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| APOSPHAERIA sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| BOTRYODIPLODIA sp. | | | | | | | | | | | | | | | | X | X | | | | X | | | | | | | | X | | | | | | | |
| CHAETOPHOMA sp. | | | | | X | X | | | X | X | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CONIOTHYRIUM FUCKELII | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CYTOSPORA sp. | | | | | | | | | X | | | | X | | | | | | | | | | | | | | | | | | | | | | | |
| DISCULA PINICOLA | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DENDROPHOMA sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | X | | | |
| **MELANCONIALES** - PESTALOTIA sp. | | | | | | | | | | | | | | | | | | | | | | | | | X | | | | X | | | | | | | |

# TABLE - 11

## VARIATION IN FUNGAL FLORA IN REGULAR POST AND CONTROL POST OF BIRCH

| | JULY | | | | | | | | | DECEMBER | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ZONE I | | | | ZONE II | | | | ZONE III | ZONE I | | | | ZONE II | | | ZONE III | |
| | Su | 5 | 25 | 45 | Su | 5 | 25 | 45 | Su 5 25 45 | Su | 5 | 25 | 45 | Su | 5 | 25 | 45 | Su 5 25 45 |
| **PHYCOMYCETES** | | | | | | | | | | | | | | | | | | |
| MUCOR sp. | | | | | | | | | X | | | | | | | | | X |
| **ASCOMYCETES** | | | | | | | | | | | | | | | | | | |
| CERATOCYSTIS PICEAE | | | | | | | | | | | | | | | | | | O O |
| CHAETOMIUM DOLICHOTRICHUM | | | | | | | | | | | | | | O | | | | |
| **BASIDIOMYCETES** | | | | | | | | | | | | | | | | | | |
| POLYSTICTUS sp. | | | | | | | | | | | | | | | | | | O O |
| PENIOPHORA sp. | | | | | | | | | | | | | | | | | | O O O |
| Unidentified sp. 1. | | | | | | | | | | | | | | | | | | X X |
| Unidentified sp. 2. | | | | | | | | | | | | | | | | | | |
| Unidentified sp. 3. | | | | | | | | | | | | | | | | | | |
| **FUNGI IMPERFECTI** | | | | | | | | | | | | | | | | | | |
| **MONILIACEAE** | | | | | | | | | | | | | | | | | | |
| ASPERGILLUS sp. | | | | | | | | | | | | | | | | | | X |
| BOTRYTIS sp. | | | | | | | | | ⊗ | | | | | | | | | |
| CEPHALOSPORIUM sp. | ⊗ | | | | ⊗ | | | | | | | | | | | | | |
| GEOTRICHUM sp. | | | | | | | | | | | | | | | | | | O |
| PENICILLIUM spp. | O | | | | | | | | O | | | | | | | | | |
| TRICHODERMA VIRIDE | | | | | | | | | | X | | | | | | | | X X |
| **DEMATIACEAE** | | | | | | | | | | | | | | | | | | |
| ALTERNARIA sp. | | | | | | | | | X | X | | | | | | | | |
| AUREOBASIDIUM PULLULANS | | | | | | | | | O O | | | | | | | | | |
| CLADOSPORIUM HERBARUM | ⊗ | ⊗ | | | ⊗ | | | | ⊗ O | X | | | | ⊗ | | | | |
| DACTYLOSPORIUM sp. | X | | | | O | | | | | O | | | | X | | | | |
| GLIOMASTIX sp. | | | | | | | | | | | | | | | | | | |
| PHIALOPHORA sp | | | | | | | | | | | | | | | | | | O O |
| **TUBERCULARIACEAE** | | | | | | | | | | | | | | | | | | |
| FUSARIUM spp. | ⊗ | | | | ⊗ | | | | O | ⊗ | | | | ⊗ | | | | |
| **SPHAEROPSIDALES** | | | | | | | | | | | | | | | | | | |
| APOSPHAERIA sp. | | | | | | | | | X | | | | | | | | | |
| BOTRYODIPLODIA sp. | | | | | | | | | | | | | | | | | | |
| CYTOSPORA sp. | | | | | | | | | | X | | | | | | | | |
| DISCULA PINICOLA | | | | | | | | | | | | | | | | | | |
| PHOMA sp. | | | | | | | | | | X | | | | | | | | |
| **MELANCONIALES**-PESTALOTIA sp. | | | | | | | | | | | | | | | | | | X |

### NOTE

X = FUNGAL SPECIES ISOLATED FROM CONTROL POST.

O = FUNGAL SPECIES ISOLATED FROM REGULAR POST

⊗ = FUNGAL SPECIES ISOLATED FROM BOTH REGULAR AND CONTROL POSTS.

# TABLE - 12

## VARIATION IN FUNGAL FLORA IN REGULAR POST AND CONTROL POST OF SCOTS PINE

| | JULY | | | | | | | | | | | | DECEMBER | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ZONE I | | | | ZONE II | | | | ZONE III | | | | ZONE I | | | | ZONE II | | | | ZONE III | | | |
| | Su | 5 | 25 | 45 | Su | 5 | 25 | 45 | Su | 5 | 25 | 45 | Su | 5 | 25 | 45 | Su | 5 | 25 | 45 | Su | 5 | 25 | 45 |
| **PHYCOMYCETES** | | | | | | | | | | | | | | | | | | | | | | | | |
| MUCOR sp. | | | | | | | | | ⊗ | | | | | O | | | | | | | | X | | |
| **ASCOMYCETES** | | | | | | | | | | | | | | | | | | | | | | | | |
| CERATOCYSTIS PICEAE | | | | | | | | | | | | | | | | | | | | | | O | ⊗ | O O |
| CHAETOMIUM DOLICHOTRICHUM | | | | | | | | | | | | | | | | | | | | | | | O | |
| **BASIDIOMYCETES** | | | | | | | | | | | | | | | | | | | | | | | X | X |
| POLYSTICTUS sp. | | | | | | | | | | | | | | | | | | | | | | | | |
| PENIOPHORA sp. | | | | | | | | | | | | | | | | | | | | | | | | |
| Unidentified sp. 3. | | | | | | | | | | | | | | | | | | | | | | | | |
| **FUNGI IMPERFECTI** | | | | | | | | | | | | | | | | | | | | | | | | |
| **MONILIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | |
| ASPERGILLUS sp. | | | | | | | | | ⊗ | O | | | | | | | | | | | | | | |
| BOTRYTIS sp. | ⊗ | | | | ⊗ | | | | O | O | | | | ⊗ | | | ⊗ | | | | | | X | |
| CEPHALOSPORIUM sp. | O | | | | | | | | O | | | | | | | | | | | | | | | |
| GEOTRICHUM sp. | O | X | | | | | | | O | | | | | ⊗ | ⊗ | | | | | | | | | |
| PENICILLIUM sp. | | | | | | | | | | | | | | | | | | | | | | | | |
| TRICHODERMA VIRIDE | | | | | | | | | | | | | | | | | | | | | | | | |
| **DEMATIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | |
| ALTERNARIA sp. | ⊗ | ⊗ | | | ⊗ | | | | O | | | | ⊗ | X | X | | X | | | | X | | | |
| AUREOBASIDIUM PULLULANS | | | | | | | | | | | | | | O | X | | | | | | | | | |
| CLADOSPORIUM HERBARUM | | | | | | | | | | | | | | | | | | | | | | X | ⊗ | ⊗ ⊗ |
| DACTYLOSPORIUM sp. | | | | | | | | | | | | | | | | | | | | | | O | O | ⊗ O |
| GLIOMASTIX sp. | | | | | | | | | | | | | | | | | | | | | | | | |
| PHIALOPHORA sp. | | | | | | | | | | | | | | | | | | | | | | | | |
| **TUBERCULARIACEAE** | | | | | | | | | | | | | | | | | | | | | | | | |
| FUSARIUM sp. | ⊗ | | | | | | | | ⊗ | | | | | ⊗ | X | | X | | | | X | | | |
| **SPHAEROPSIDALES** | | | | | | | | | | | | | | | | | | | | | | | | |
| APOSPHAERIA sp. | | | | | | | | | | | | | | X | X | | | | | | | | | |
| BOTRYODIPLODIA sp. | | | | | | | | | | | | | | | | | O | | | | | | | |
| CHAETOPHOMA sp. | | | | | O | | | | | | | | | | | | | | | | | | | |
| DISCULA PINICOLA | | | | | | | | | | | | | | | | | O | | | | | | | |
| PHOMA sp. | | | | | | | | | | | | | | | | | | | | | | | | |
| **MELANCONIALES** | | | | | | | | | | | | | | | | | | | | | | | | |
| PESTALOTIA sp. | | | | | | | | | | | | | | | | | X | | | | O | | | |

NOTE: X = FUNGAL SPECIES ISOLATED FROM CONTROL POST
O = FUNGAL SPECIES ISOLATED FROM REGULAR POST
⊗ = FUNGAL SPECIES ISOLATED FROM REGULAR AND CONTROL POSTS.

## Influence of Seasonal factors on the fungal colonization on birch and Scots pine posts.

It is very difficult to correlate the influence of 'aerospora' and temperature or any other physical factors with fungal colonization on the posts. The 'aerospora' mentioned in the text represent the fungi obtained by open plate spore trap method. It has been mentioned earlier about the drawbacks of this spore trap method. Spore trapping for a few hours during each isolation may not give a correct picture of the actual seasonal variation in 'aerospora'. It is therefore not possible to genralize the influence of 'aerospora' on fungal colonization in the posts. It is also very difficult to correlate the influence of temperature on fungal colonization on fence posts. This involves soil temperature, atmospheric temperature, adequate knowledge on adaptibility of the fungi isolated from the posts to the sharp variation in temperature, and several other allied problems. It is therefore not possible to give any conclusive suggestion on the influence of seasonal factors. However a short discussion on the subject based on the results is given here.

Total number of fungal species isolated from the posts, 'aerospora', and temperature in centegrade is plotted in the figure against time of exposure (Fig. 3 ). (Although several species of Penicillium, Fusarium and Verticilllium and Phialophora were isolated, in the figure each of these genera represent one unit.) In describing fungal distribution only the identified species have been included in the text. Fungal species isolated only from the air are shown in table 13. Seven species of unidentified fungi which produced sterile mycelium with considerable variation in colour and form have been omitted from the text. The results showed a rise in the number of fungal species on the posts between June and August when both temperature and number of species in the air were nearly constant. A further rise in number of fungi on the post was observed between August and September or October which corresponded with the rise in number of species in air, but during the same period a fall in temperature was observed. The maximum

number of fungi on the posts was during September and October. Wilkins and Harris (1946) also observed similar trend in the distribution of higher fungi in two different localities. A sharp fall in the number of species isolated from the posts was observed from October to December which corresponded favourably with the fall in temperature, but during the same period the number of species in air did not fall so sharply. Again between December and January when there was a rise in temperature the number of fungal species in Scots pine post continued to fall whereas in birch there was a rise which shows that temperature may have certain influence in this case. However, the data are not sufficient to draw any inference from these results.

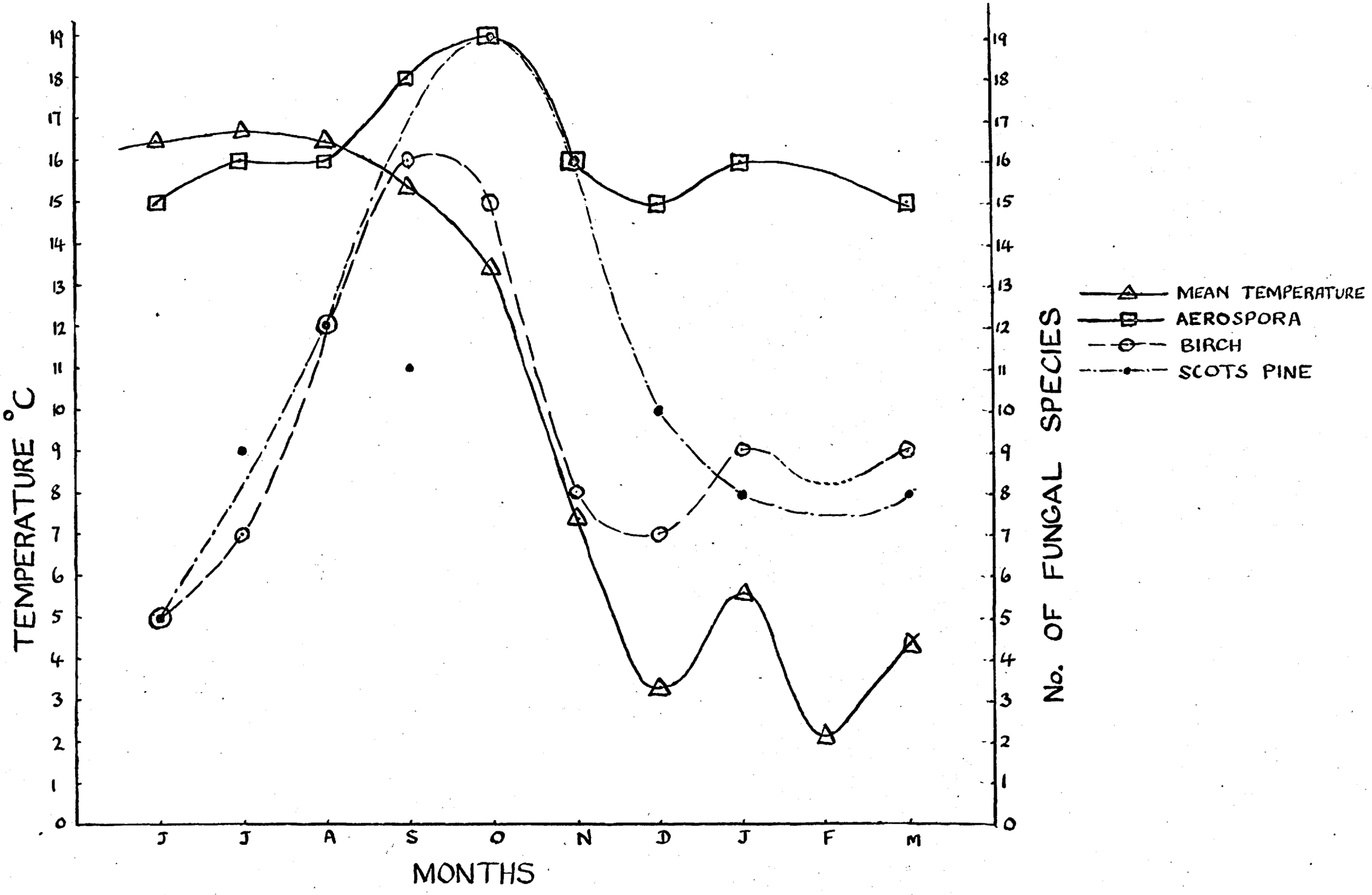
It would be interesting to investigate the influence, that the time of erection of the posts may have on the fungal colonization. It is therefore suggested that in further investigation if posts are erected in September when temperature begins to fall and 'aerospora' nearly reaches the maximum number, then it may be possible to know more about their influence on fungal colonization. If the number of fungi on the posts shows a rising trend between September and December when both temperature and 'aerospora' fall from their maximum value to minimum, then probably the influence of nutrients can be established. A further critical investigation on fungal colonisation from March to July may more clearly establish which of these factors have greater influence.

Table 13

Fungal species isolated from the air which did not colonize the posts

| | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of Fungi isolated from the posts and air (included in the tables (1 - 10) | 9 | 10 | 10 | 13 | 15 | 11 | 12 | 11 | | 11 |
| Fungi isolated only from the air | | | | | | | | | | |
| Phycomycetes | | | | | | | | | | |
| Cunninghamella sp. | X | | | | | | | | | |
| Moniliaceae | | | | | | | | | | |
| Camarosporium sp. | | | X | | X | X | | | | |
| Candida sp. | X | X | | | X | | | X | | |
| Cylindrophora sp. | | | X | | | X | | | | X |
| Rhinotrichum | | | | X | | | | | | |
| Spedonium sp. | | X | | | | | X | | | |
| Spicaria sp. | | X | X | | X | | | X | | |
| Streptomyces sp. | | X | | | | X | | | | X |
| Demateaceae | | | | | | | | | | |
| Bispora sp. | X | | | X | | | | | | |
| Bisporomyces sp. | | | X | | | | | | | X |
| Catenularia sp. | | | | | | | | | | X |
| Piricauda/coniothecium sp. | X | | | | | | X | X | | |
| Stachybotrys sp. | X | | X | | | X | | | | |
| Stachylidium sp. | | | | | | | X | | | |
| Streptothrix sp. | | | | X | | X | | | | |
| Torula sp. | | X | | | | | | X | | |
| Sphaeropsidales | | | | | | | | | | |
| Coreothyriella sp. | | | | X | | | | | | |
| Diplodia sp. | | | | X | | | | | | |
| Ephelis (mexicana) | X | | | | X | | | | | |
| Pyrenochaeta sp. | | X | | | | | | X | | |
| Basidiomycetes (unidientified) | | | X | | | | | | | |
| Total number of fungi isolated from the air | 15 | 16 | 16 | 18 | 19 | 16 | 15 | 16 | | 15 |

FIG. 3 THE NUMBER OF FUNGAL SPECIES COLONISING THE SURFACE LAYERS OF BIRCH AND PINE FENCE POSTS SHOWN IN RELATION TO THE NUMBER OF SPECIES IN THE AEROSPORA AND THE MEAN MONTHLY TEMPERATURE.



## Discussion

The seasonal distribution and fungal succession already described have been based on the results of periodic isolation of fungi from birch (Betula sp.) and Scots pine (Pinus sylvestris) fence posts. The 'drill technique' (Asmah 1965) was used to obtain samples of infected wood particles from the surface layers and at three different depths (5 mm., 25 mm., 45 mm.) from a standing fence post. The wood particles were obtained from about 6 inches below the top of the posts. (zone I), from 18 inches above ground line (zone II), and at the ground line (zone III), to investigate the distribution and succession of fungi in three ecological zones with respect to environmental conditions prevailing at different zones of the posts (see p. 13 ). The isolation techniques used by the previous workers have been described by Greaves and Savory (1965) and Grant and Savory (1968) (see p. 18 ). To isolate fungi from the infected wood particles obtained from the posts several different media were used. The influence that culture media have on the number and type of fungi isolated has been discussed by Warcup (1960) Greaves and Savory (1965). Further findings on the suitability of media for similar ecological study have been discussed earlier (see p. 22 ).

In discussing the results of the investigation it should be mentioned that it cannot be assumed that all the fungi which colonized the posts were isolated. It is also possible that some of the isolates may have been obtained from spores or resting bodies produced by fungi which were active during the early stage of succession (Garrett 1955), and in particular some of the isolates obtained from the surface zones may in fact have arisen from spores. The fungal colonies isolated from the wood were not counted so no figures were produced on their relative frequency of occurrence. The 'drill technique' used in this investigation for obtaining inocula from the posts is not very suitable for studying frequency of occurrence. With this technique a very large number of isolates are

obtained in each petri dish which make a colony count very difficult. However, it is a very suitable technique for a qualitative study of the ecology of fungi. Several other advantages of this technique are discussed later in the text. In the study of the succession of fungi the importance and feasibility of using relative frequencies of occurrence of fungi as a criterion of studying the quantitative aspect of fungal colonization is under investigation at the present time (Butcher 1969).

The early workers concerned with the succession of fungi have generally removed the post to the laboratory for sampling. In the present investigation 3 to 4 periodic isolations were carried out on a post without disturbing its existing environment. In the non-destructive type of sampling (where posts are not removed from the ground), the 'drill technique' is a very satisfactory method for obtaining isolates, both from the surface layers and at different depths. Sampling a post for several consecutive months has given some information on the progressive colonization by fungi from the time of their appearance on the surface to their establishment into the post. It had been thought that in the case of isolations carried out in the open air, a large number of contaminants from air-borne spores might render difficulties. In fact these were surprisingly few in number and the complete absence of fungal colonies at 25 mm and 45 mm in the early stages of those studies clearly shows that it is a negligible factor. The fungi which colonized the post at 5 mm depth were often isolated one or two months earlier from the surface. The colonization of fungi beneath 5 mm depth may also depend on their ability to penetrate through the wood tissues. For example Phialophora sp., Geotrichum sp., Gliomastix sp. and the species of Basidiomycetes were isolated from 25 mm and 45 mm depth during the same period that they first appeared at 5 mm depth, but C. piceae was isolated from 45 mm depth on the third month after it had appeared at 5 mm depth. The results have also shown that the initial hyphal penetration is mostly in the radial direction from sapwood to heartwood. This was confirmed when after 18 months exposure the posts cut into several vertical sections showed a shallower penetration from heartwood

side and major white-rot pockets in the centre which spread towards the surface in zone III.

It has been observed that the fungi isolated from greater than 25 mm depths have invariably colonized the intervening area between that point and the surface layers. This shows that the fungal colonization is unidirectional and progresses from the surface layers to the centre of the post. It also indicates that even if there were vertical migration of fungi from below ground line to ground line zone no new fungus was added to the existing flora at 25 mm and 45 mm depth. Butcher (1968) has suggested that there may be a migration of Basidiomycetes from the ground line to areas below the ground line, No isolations were made below the ground line in these investigations.

The isolation of fungi from surface to subsequent known depths has opened new concepts in the field of study of fungal ecology. It is now possible to examine the following aspects of fungal colonization on a fence post: (1) at what stages and depths certain fungi are eliminated, (2) the fungal flora at different depths, (3) the fungi which commonly do not penetrate below a certain depth but may have a high intensity of fungal growth nearest to the surface and are likely to be included among the dominant fungi in a grouped sampling, (4) the fungi which are associated with the colonization by Basidiomycetes and other fungi at different stages, (5) those fungi capable of penetrating beneath the surface and the possible associations of the surface flora which make this possible. The gradual decrease in the number of fungal species from surface to subsequent depths suggests that competition, interaction and association may be involved in this process. As the number of fungal species in a post forms the basis for interpreting succession of fungi, therefore it appears that the fungal succession is also influenced by competition, interaction and association. A similar suggestion was also given by D'Aeth (1939), Basham (1959), Garrett (1963), Findlay (1966) and Hudson (1968). The colonization by fungi at 5 mm depth may be highly influenced by the competition and interaction between the fungi on the

surface layers of the post. The colonization at 25 mm depth may be influenced by the competition and interaction at 5 mm depth, but for the colonization at 45 mm depth the competition at 25 mm depth does not appear to be of any great significance. However, regarding the establishment of decay fungi their association with the sapstain fungi may be one of the most influential factors. This is discussed later in detail.

In interpreting the succession of fungi based on the results of periodic isolation two very debatable questions arise: (1) a few hyphae of a fungal species which may have penetrated the wood through ray cells are likely to be isolated repeatedly from below the surface layers and thus can assume the position of dominant colonizers although they in fact may not be so, (2) the physiologically inactive hyphae represented by resting bodies of an early colonizer are also likely to be isolated repeatedly and can be erroneously included in the results among the currently active and dominant fungi. The influence of these factors in interpreting the fungal succession based on the results of periodic isolations is very difficult to disregard completely. However, from the present results it appears that these factors may not be always very critical when the criterion for selecting dominant species is their ability to colonize at depth coupled with repeated isolation for a considerable length of time. The arguments with evidence, are given here to support this view. A very few fungi were in fact isolated repeatedly for a considerable length of time and still fewer fungi entered to 25 mm and 45 mm depth. Ceratocystis piceae and Phialophora sp. were the main fungi isolated from 25 mm and 45 mm depth along with a few species of Basidiomycetes. These microfungi have always preceded Basidiomycetes in the sequence of colonization, and were concurrently isolated with Basidiomycetes for several consecutive months. However, the microfungi were eliminated first from 45 mm depth and later in birch from 25 mm depth with the establishment of Basidiomycetes (white-rot fungi) and the advancement of the decayed area towards the surface. Shigo (1965) also reported that

column of decay caused by Hymenomycetes in living trees were always bordered by discoloured tissues that yielded bacteria, non-hymenomycetes or both. Both C. piceae and Phialophora sp. being essentially sapstain fungi might have colonized the rays along with other cells in the wood, as has been shown to happen in the test blocks in vitro. In the presence of white-rot fungi sapstain fungi might have autolysed or due to their parasitism by white-rot fungi have been eliminated. (Another sapstain fungus Botryodiplodia sp. showed similar probabilities which have been discussed in Section II,). It can therefore be assumed that C. piceae and Phialophora sp. were isolated from the posts as long as they were actively present. There were many other sapstain fungi which colonized the post on the surface and immediately below but did not penetrate further into the wood. After eighteen months of exposure when these posts were cut into several vertical sections no stain was noticed in the area colonized by white-rot fungi. Similar results were found in the test blocks with Botryodiplodia sp. and Polystictus versicolor

The above evidence supports the view that there is small chance of isolating any odd pieces of old fungal hyphae repeatedly. Further support to this view was given by the fact that after eighteen months, from the same posts, Butcher (1969) isolated a similar flora from below the surface layers by random sampling above and below the ground line (zone III), using a more sophisticated sampling technique for quantitative analysis.

It is difficult to assemble any evidence to show whether the presence of residual spores in the wood cells or live hyphae is the usual form of the fungus isolated. Certain interesting observations were made during the course of the laboratory tests on succession of fungi. Spores of Botryodiplodia sp. (the first fungus in the sequence) were normally present throughout the blocks of both Scots pine and birch. After colonisation by Polystictus versicolor for six weeks these spores were no longer observed. Griffith (1964) recorded similar observations, when he showed that the endoconidia of Ceratocystis spp. were highly susceptible to

attack by certain Basidiomycetes. However, immediately below the surface the intense fungal colonization may result in a very high population of spores of several fungal species. This can make a significant difference if the pattern of fungal succession is described from results of isolations made from various depths beneath the surface layers. Species very common in the superficial regions may be assigned greater importance than is warranted since these fungi are present in fact as dormant spores rather than actively growing mycelium.

It is therefore suggested that some of the factors influencing the isolation of fungi can be avoided by developing some suitable techniques. Butcher (1969) is presently engaged in an intensive study on fungal succession and his findings may help in understanding some of these problems.

The distribution and succession of fungi on debarked wood has been described by early workers on the results of periodic isolation of fungi from below the surface layers of the posts (Corbett and Levy 1963; Merril and French 1966; Greaves 1966; Kaarik 1967, 1968; Butcher 1968). Butcher (1968) in a more elaborate study on fungal succession isolated fungi from the sap wood stakes of Pinus radiata D. Don, after cleaning the surface with ethanol. This isolation from near the surface may include several surface colonizers but he did not make any distinction between superficial and internal series of isolations. In the present investigation fungal succession has been interpreted on the results of periodic isolations from the surface layers of the post and at three known depths (5 mm, 25 mm, 45 mm). The establishment of decay fungi below the surface layers has been assumed as the important stage in the succession of ~~wood-rotting~~ fungi. These fungi having been able to establish themselves first in the superficial layers have later been able to go further into the wood. Clearly this is not possible for the great majority of the surface flora isolated.

The variations in the succession of fungi is not only confined to different zones of a post but is also shown in the different depths in a zone. For example on the surface layers bacteria are the pioneer

colonizers and the sequence of colonization ends with the establishment of moulds and sapstain, but at 45 mm depth in the ground line zone the succession of fungi begins with sapstain stage and reaches to Basidiomycetes stage. The sequence of colonization in other intermediate depths also shows a little variation.The detailed information received on fungal colonization by using the technique to isolate fungi from different depths has given wider scope to interpret fungal succession from different points of view and to assess the phenomenon more accurately. Corbett and Levy (1963) earlier suggested the scope of studying the fungal distribution on fence posts with respect to depth from the surface and time of exposure. The 'two-chisel' technique has been used for obtaining isolates from a block of wood from immediately below the surface to an unknown depth by Greaves and Savory (1965), Butcher (1968). In this sampling procedure several fungi which are not capable of penetrating the wood beneath a few millimeters from the surface against the competition from other fungi or even certain other fungi with strong soft-rot ability which did not penetrate to any significant depth, receive almost similar attention in a grouped sample to a few dominant decay fungi and their associates which dominated near the centre of a post. It may be possible that a few moulds which penetrated to a depth very near the surface may get highly represented in the flora, if spores happen to be there. These fungi may be very different from those obtained from sampling at different depths. The results have shown that very few fungi actually become dominant colonizers and probably these fungi are more actively involved in the process of succession leading to the establishment of Basidiomycetes. The pattern that emerges is that of a large mass of fungi colonising the surface and superficial layers. After a time a smaller number of fungi are able to penetrate beyond 5 mm depth. Once these fungi have made the initial break-through, the Basidiomycetes appear and seem able to make a rapid development in the centre of the wood.

Surface layers

The succession of fungi on surface is discussed separately from the

succession below the surface. There is not much information available on the surface colonization on debarked wood. Some of the early work on logs and tree trunks related to the problem and the work done by Corbett and Levy (1963) on fungal colonization on fence posts nearest to the surface layers has been included in the discussion of the present results on surface colonization. The succession of organisms in the surface layers in all three zones is bacteria - mould - sapstain fungi. Several workers have suggested that bacteria are the primary colonizers followed by Fungi Imperfecti or non-Hymenomycetes (Etheridge 1961; Henningsson 1967; Maloy and Robinson 1968; Goods et al. 1968 and Shigo 1969). Corbett and Levy (1963) recorded the presence of bacterial slime along with moulds or soft-rot fungi in the initial infection on the fence posts. The fungi which dominated the flora were Penicillium spp., Trichoderma viride and Fusarium spp. Among the moulds, Cladosporium herbarum a sapstain fungus was present in all zones of the posts, but C. piceae and Phialophora sp., the other two dominant sapstain fungi, were present only at the ground line zone. These two fungi are essentially sapstain fungi but also produce soft-rot in hardwoods (Rösch and Liese 1968, Findlay and Levy 1969, Findlay 1969). In laboratory tests with these fungi, soft-rot was found very near the surface and mostly confined to the end surfaces of the blocks. These fungi have been included in the results as sapstain/soft-rot fungi to separate them from other typically sapstaining or soft-rot organisms, but in the following text they are included among the sapstain fungi to interpret fungal succession based on groups of microorganisms, since they only act in this way in softwoods.

On the surface layers fungi belonging to 26 genera colonized the posts during 10 months exposure but fungi from 20 genera persisted for less than 4 months which include 11 genera isolated for one or two months only. Several potential soft-rot fungi were amongst those isolated which did not persist for any appreciable length of time and could not be considered even as sub-dominant fungi. The failure of

several potential soft-rot fungi to make any appreciable progress even in the ground line zone is possibly because of lack of inoculum/and this potential leads to their replacement by 'sugar fungi'. The soft-rot that is seen in the surface layers under microscope may be due to the effect of several soft-rot fungi even with very low soft-rot ability which colonized the post in no definite sequence. The influence of soft-rot in the fungal succession is discussed later. Moulds remained as dominant fungi in above ground zones throughout the period of investigation but were replaced by sapstain fungi in the ground line zone probably due to the near optimum conditions which favoured the growth of some stronger competitors. Early colonization by moulds is likely to be due to the intensity of infection by spores and the rate of germination (Findlay 1940). Their prolonged dominance may depend on the following factors (1) their antagonistic action resulting in the inhibition of other fungi, (2) utilisation of the simple carbohydrates which may reduce the capacity of the surface layers to support the life of new colonizers (Garrett 1955), (3) they may initially utilize simple carbohydrates but later due to their cellulolytic activity may persist for a longer period (Hudson 1968) which is an advantage over many true"sugar fungi", (4) their possible reinfection from existing spores, (5) their tolerance to seasonal variations of temperatures and moisture content (Findlay 1966) and (6) probably due to their development in the superficial layers immediately below the surface. However, if the number of fungi which could fulfil most of these conditions are listed since Ward (1898) reported that Penicillium could bore through cellulose membranes and their spores were highly resistant to temperatures, the list would include most of the wood-inhabiting fungi, yet very few fungi are isolated in any one month. This suggests that available space, competition between the fungi and several other factors may also be involved in the prolonged dominance of certain fungi near the surface layers.

It may be of some interest to know whether the colonization of fungi on the surface layers is influenced by the micro-organisms already present there, by the presence or absence of surface nutrients, interactions

between fungi and host-fungus relation.

The first species of fungi to colonize the surface layers following bacteria can be influenced by the intensity of bacterial attack. In the zones above ground of both birch and Scots pine where bacterial attack was considerably less, the fungi which followed bacteria were Penicillium sp., Paecilomyces varioti and C. herbarum and later by Fusarium spp. At the ground line zone Fusarium spp. and Botrytis sp. were the first fungi to colonize surface layers, probably because their felty-woolly aerial mycelium enables them to grow over bacterial slime. Fusarium spp. later became established on the surface layers at the zones above the ground whilst other fungi except C. herbarum did not persist for any appreciable length of time, possibly due to the competition for space and nutrients. However in zone III where several fungi colonized through hyphal contact, these may have greater inoculum potential and are not influenced by the presence of Fusarium spp. Levy (1962) suggested that the moulds and other fungi growing on the surface are important precursors in the initiation of decay.

The influence of interaction between fungi on the fungal colonization is very difficult to assess especially on the surface layers where several fungi are involved in it. T. viride is known to inhibit the growth of several fungi including Basidiomycetes, but did not prevent the growth of certain fungi which later dominated the flora on the surface layers of Scots pine posts. However the influence of T. viride on growth of those fungi which were eliminated during its presence cannot be fully ascertained. For example Cytospora sp., C. globosum and a few other fungi which colonized Scots pine post immediately after T. viride, were eliminated within a short period. Whether this is entirely due to the antagonism of T. viride or not is difficult to assess. However if it is assumed that these fungi have been eliminated due to the interaction of T. viride, then the influence Cytospora and C. globosum could have exerted on the subsequent colonizers were made ineffective by their elimination. Shigo(1965)reported that Cytospora dicipiens restricted the growth of some non-Hymenomycetes and also some Hymenomycetes, and

C. globosum a soft-rot fungus can facilitate the entry of Basidiomycetes (Duncan 1960). This underlines the point that the influence of interaction between fungi needs more attention (Levy 1968).

The presence or absence of surface nutrients appears to be a critical factor which can considerably affect the colonization on the surface and eventually below the surface layers of the posts. A gradual increase in the number of fungal species on the surface layers was observed during the first few months of exposure which was followed by a fall in subsequent months. The fungal colonization represented by the number of species present reached a climax within 4 to 5 months of exposure, but afterwards the new species of fungi or even the old species eliminated earlier were very rarely isolated. Butcher (1968) made a very similar observation with the fungal isolates obtained from below the surface layers in pine stakes. In the present investigation a few new species of fungi were isolated at ground line zone towards the end of isolation series, e.g. Pestalotia Dactylosporium, Alternaria, Dendrophoma and Aposphaeria species from birch and Chaetophoma, Pestalotia, Trichoderma viride, Coniothyrium fuckellii from Scots pine posts. All these fungi were very rarely isolated from 'aerospora' and it has been assumed that most of them have colonized the posts through hyphal contact.

It, therefore, appears that fungal activity on the surface layers may reach a climax within a few months of exposure, and with depletion of nutrients not only the activity of existing fungi is greatly curtailed but also the surface layers are rendered uninhabitable for new fungi which generally colonize by deposition of spores. Garrett (1955) made a similar suggestion that "the succession of organisms does not improve rather depletes the capacity of the habitat to support further plant life, so that the end point of a succession of micro-organisms on a substrate is not a persisting climax association, but zero ". - It is therefore suggested that there are probably two phases in fungal colonization at ground line zone. In the first phase fungi developing both from air-borne spores and through hyphal contact from the soil are involved in the

colonization. During this phase the surface layer probably has the greatest fungal activity, heavy colonization, intense competition and may provide severe resistance to many other colonizers. The results of growth and competition between fungi may lead to the establishment of a few species and to the elimination of others and eventually to the depletion of nutrients from surface layers. Second phase of fungal colonization begins probably with depletion of surface nutrients and with the decline in fungal activity. In this phase fungi involved in the succession are mainly those which probably colonize the surface layers through hyphal contact. The success of these fungi may greatly depend on their inoculum potential. It is in the second phase that decay fungi enter into wood. It is thought that the depletion of nutrients on the surface layers may render greater difficulties for germination of spores of Basidiomycetes and the development of inoculum potential, and therefore there is the possibility that the infection of decay fungi could be through hyphal contact. However Findlay (1966) suggested that the greater number of infections in a post at ground line arise from spores. First isolation of decay fungi from birch after 4 months and from Scots pine after 6 months of exposure when the fungal activity on posts was very low, suggests that surface nutrients have very great influence on the fungal colonization and succession of fungi.

The colonization on the posts may also depend on the host-fungus relationship. Several fungi were only isolated from one of the two wood species, e.g. from birch · Coniothyrium fuckelii Stysanus sp. and Polystictus sp.; and from Scots pine Gliomastix, Chaetomium globosum, Botryodiplodia sp. and Peniophora sp. It cannot be said with any certainty that these fungi would always colonize the same wood species. Corbett and Levy (1963) also isolated Stysanus sp. only from birch and C. fuckelii from both birch and Scots pine. In the present work T. viride was among the dominant fungi in all the three zones of Scots pine but it was present for only a very short time in birch posts.

Corbett and Levy (1963) made a very similar observation, and Butcher (1968) using only pine stakes found T. viride as a dominant fungus. It is therefore suggested that if T. viride, an antagonist to several other fungi, and Stysanus sp., which could cause considerable weight loss due to soft-rot activity (Rösch and Liese, 1968), had any influence on the subsequent colonization of fungi on wood it may be very selective to one wood species only. Westerdijk (1949) suggested that substrates were selective and that this selectivity depended upon their chemical and physical properties.

Beneath the surface

The succession of fungi below the surface is different in the zones above ground and the ground line zone and also very different at 5 mm and 45 mm depths. The sequence of fungal colonization below the surface layers at the ground line and in zones above the ground line is discussed separately in the following pages.

Fungal colonization at depth occurred only near the top of the posts in zone I. In the middle of the post (zone II) fungi were not isolated from below the surface. Corbett and Levy (1963) also reported that zone II in contrast to the other two zones proved to be most unfavourable for fungal growth. The succession of fungi in zone I was from sapstain to moulds and very few fungi colonized below the surface layers. A similar pattern of succession in above ground zones was suggested by Corbett and Levy (1963) and Butcher (1968). A soft-rot fungus Cephalosporium sp. colonized the birch post for a short period. The presence of this fungus was also recorded by Corbett and Levy (1963) from birch fence posts and by Shigo (1962) from the cut ends of hardwood bolts. The soft-rot fungi were not isolated from Scots pine posts. Butcher (1968) made a similar observation on Pinus radiata. Although a few soft-rot fungi including Cephalosporium sp. colonized the surface of both species of post none of the soft-rot fungi was isolated from below the surface layers except Cephalosporium.

Corbett and Levy (1963) recorded very similar moisture contents below

the surface layers in both birch and Scots pine during the month of August. It was in the month of August that Cephalosporium sp. was isolated during the present series. The posts below the surface were too dry for the establishment of soft-rot fungi but for the penetration of some of these fungi into the wood, the structures of the timber may also be a liniting factor.

The succession of fungi at the ground line was more complete than it was in the above ground zones and it reached the Basidiomycete phase. With the present results the pattern of fungal succession can be suggested in two ways: a) based on the dominant fungi which were repeatedly isolated for a considerable length of time, b) based on both dominant and also sub-domiant groupsof fungi isolated for a short period. C. piceae and Phialophora sp. were the most dominant fungi on the post along with Basidiomycetes. Both these fungi not only dominated immediately below the surface but were the main fungi isolated along with decay fungi in the still deeper regions of the posts. These are mainly sapstain fungi but were also found to produce soft-rot cavities in birch blocks nearest to the surface layers. The soft-rot activity of these fungi have been reported earlier by Rösch and Liese (1968), Findlay and Levy (1969) and Findlay (1969). These fungi have been regarded in the text as sapstain fungi (c.f. page 87).

Taking into account only the dominant fungi, the succession of fungi below the surface in the ground line zone was sapstain to Basidiomycetes, but from the surface to the centre of the post the pattern of succession was from bacteria-mould-sapstain - Basidiomycetes. A similar pattern of fungal succession was suggested by Basham (1957, 58, 59 and 66), Shigo (1965, 1969), Good et al (1968), Maloy and Robinson (1968). The main sapstain fungi Ceratocystis piceae and Phialophora sp. preceded Basidiomycetes in both birch and Scots pine posts. A close association between these genera and Basidiomycetes in the fungal succession was also observed by several authors (Meredith 1959, 1960, Ueyama 1961, Shigo 1962, 1965). A succession pattern in which sapstain fungi preceded Basidiomycetes

has also been suggested by Findlay (1940, 1966). Mangenot (1952) in his studies on succession also placed the Phialophora fastigiata stage and the Melanomma stage which included Ceratocystis spp., before Leptoporus stage which included the Hymenomycetes. He also included Phialophora spp. among the fungi which often developed on the remains of other fungi. Phialophora sp. remained a dominant fungus on the surface layers in zone III throughout the period of investigation, which may also be due to its ability to develop on the remains of other fungi as suggested by Mangenot (1952).

Karkanis (1964) following up published work of Krapivina (1960) examined a number of staining fungi and found that C. piceae and Phialophora fastigiata were capable of causing stain in both softwoods and hardwoods, but could in addition produce soft-rot caveties in hardwoods. It is therefore likely that those fungi which can find a secondary source of nutrition such as Phialophora in birch can continue to colonise the wood after the nutrients are depleted and it can no longer act as a "sugar fungus". Fuller (1968) has isolated a species of Phialophora from soft-rotted hardwood stakes treated with a wood preservative (Chrome-Fluor-Arsenate) from graveyard tests in West Africa.

In taking into account several sub-dominant fungi the pattern of fungal succession below the surface in Scots pine was moulds ⟶ sapstain ⟶ soft-rot ⟶ Basidiomycetes. In birch the pattern of succession of fungi was very similar to Scots pine except no mould fungi were isolated before the sapstain fungi. In Scots pine the fungi included in the scheme are Botrytis sp., followed by C. piceae and Phialophora sp. among the sapstain fungi, Chaetomium globosum the soft-rot fungus and Peniophora sp. in Basidiomycete. In birch the sequence included the sap-stain fungi Aureobasidium pullulans, C. piceae and Phialophora sp. the soft-rot fungus Cephalosporium sp. and Basidiomycetes - Polystictus sp. and an unidentified species.

This succession pattern falls more in line with the pattern suggested by Corbett and Levy (1963), Merrill and French (1966) and Butcher (1968), except that they did not give any significance to staining fungi.

Corbett and Levy (1963) isolated several staining fungi from the posts but their order of appearance in relation to soft-rot fungi was a little different, and they concluded that the position of staining fungi was not clear. Butcher (1968) also isolated several sapstain fungi from the pine stakes but as their mean frequency values were very low he did not include the staining fungi in his suggested pattern of succession. Merril and French (1966) isolated Alternaria humicola the staining fungus among the early colonizers but did not isolate it in later isolation series.

In Scots pine certain moulds were also isolated along with the soft-rot fungus as secondary colonizers. Butcher (1968) and Corbett and Levy (1963) also made similar observations. Corbett and Levy (1963) proposed the following general pattern of colonization: Moniliales group I (Penicillium spp., Trichoderma viride, Botrytis sp.) ⟶ Sphaeropsidales (soft-rot fungi) ⟶ Moniliales group II (Gliocladiopsis sp., Cylindrocarpon sp., Memnoniella sp.) ⟶ Basidiomycetes (Coprinus sp. and unidentified species). In their proposed scheme of succession they also isolated certain moulds after the soft-rot stage but the species of moulds isolated during the present studies were very different. In birch moulds were not isolated after the colonization by soft-rot fungi. Corbett and Levy (1963) reported that Cephalosporium sp. and pycnidial fungi were isolated often on the same occasion. In the present study a similar association between Cephalosporium sp. and pycnidial fungi was observed. The fungal flora in both birch and Scots pine posts show some remarkable similarities with that described by Corbett and Levy (1963).

The suggested pattern of succession of fungi on living trees, trees killed by various agents, fallen branches, logs, pulpwood bolts and debarked wood, falls mainly into two school of thought, (a) the primary colonizers are Basidiomycetes and these are followed by non-Hymenomycetes and other Basidiomycetes (Findlay 1940, 1966; Chesters 1950, Mangenot 1952; Meredith 1959, 1960; Käärik and Rennerfelt 1957). (b) Non-Hymenomycetes especially Fungi Imperfecti are the pioneer colonizers probably associated with bacteria and Hymenomycetes follow these micro-

organisms (Etheridge 1961, Shigo 1962, 1965, Corbett and Levy 1963, Butcher 1968 and several others). The results of the present investigation support the latter suggestion on the pattern of succession of micro-organisms. In this also there are two schools of thought: several authors believe that soft-rot fungi are the precursors of Basidiomycetes in the fungal succession and others hold the view that the staining fungi precede Hymenomycetes in fungal colonization.

There is a good deal of controversy regarding Basidiomycetes as primary colonizers in logs and fallen branches. In a living tree parasitic Basidiomycetes can be the early colonizers. The subsequent sequence of colonization by non-hymenomycetes may only occur when the tree trunks are killed by these primary colonizers (Mangenot 1952). In fallen branches and logs Basidiomycetes can also be the primary colonizers (Findlay 1940, 1966; Chesters 1950 and others). In logs and fallen branches the hyphae or the spores of certain Basidiomycetes may reach the wood through cracks in bark (Findlay 1966) and grow under a protective shield where competition from non-hymenomycetes is of little significance. These Basidiomycetes might have been included among the primary colonizers when the bark falls off. It may also be possible that certain parasitic Basidiomycetes which were already present may survive as saprophytes or the primary colonizers on bark may continue as secondary colonists in the wood (Chesters 1950). In debarked wood the possibility of primary colonization by Basidiomycetes is very doubtful unless there is recolonization by the dormant parasitic Basidiomycetes which had been already present in the timber. Wälchli (1968) working on the distribution of fungi on avalanche fences in Switzerland reported that isolates of wood from various areas and heights up the mountains showed similar flora. Levy questioned that this may be due to the same fungi being present in the timber though in a dormant stage, the infection having been introduced with the timber. The isolation of fungi from Tywarnhale mine has also shown that fungal infection of timber is largely due to the

fungus being present when the wood is taken underground. The present work suggests [illegible] that non-Hymenomycetes are the early colonizers and these are followed by Hymenomycetes.

The dominance of C. piceae and Phialophora sp. from the surface into the wood in all the depths, their presence along with Basidiomycetes, their colonization immediately before the decay fungi, and finally the presence of certain soft-rot fungi for a very short period posed the questions whether soft-rot fungi have any great significance in the fungal succession or whether the present techniques are suitable for their isolation from the wood.

Duncan (1960) has suggested that the various fungi capable of producing soft-rot were the pioneer colonizers on preservative treated poles and piling. These fungi do not cause any loss in strength but are believed to facilitate the entry of decay fungi. Several other authors have noticed the presence of certain soft-rot organisms on untreated wood prior to the colonization by Basidiomycetes (Mangenot 1952, Shigo 1962, Corbett and Levy 1963, Butcher 1968, Merrill and French 1966,) and was also noticed here However, none of the authors have clearly mentioned the dominance of soft-rot organisms at any stage of fungal succession. Mangenot (1952) mentioned in his proposed sequence of colonization of fungi the presence of Chaetomium in Mortierella ramanniana stage which preceded Hymenomycetes, but in Mortierella stage the main fungi mentioned were Phycomycetes and moulds.

Merrill and French (1966) observed soft-rot under polarized light in pine stakes after 6 weeks of exposure but their isolates accounted for 92% mould fungi. Butcher (1968) isolated Streptomyces sp. the only soft-rot fungus among the dominant fungi isolated from ground line zone in pine stakes. In his 54 weeks record of periodic isolation this fungus was present for about 12 weeks, and its frequency of isolation was the lowest : compared to other fungi isolated during the same period. In the present investigation the proved soft-rot fungi which were isolated both from the surface and immediately below the surface, persisted for a

very short time. If it is assumed that the soft-rot fungi were rarely isolated not due to faults in technique but because they were not there, any great significance of soft-rot fungi in the fungal succession is not very convincing. There can be no doubt that soft-rot fungi occur on the surface of posts in ground contact, but their role in facilitating the entry of decay fungi is debatable. However, it has to be borne in mind that when fungi are not always isolated it is no proof that these organisms are not involved in the process of succession.

If the change in the substrate brought about by the presence of soft-rot fungi is of much significance in permitting the entry of decay fungi into the posts, the question then posed is what factors are involved in this process. In spite of this the position of soft-rot still presents a considerable problem. On the same site as the test posts there has been a long term trial of preservative treated fence posts since 1958, Levy (1965). Corbett (1963) observed soft-rot in the Scots pine posts up to 10 cells below the surface and in the birch posts up to 30 cells below the surface in untreated controls after 8 months exposure.

These posts failed after 3 - 5 years exposure and in each case the decay was presumably due to a Basidiomycete. In a series of posts treated with an experimental fluorchrome salt mixture the birch posts failed within three years by primarily soft-rot attack which had rotted the wood through to the centre. Similar examples of hardwoods treated with similar chemical mixtures have been shown to fail from through and through soft-rot attack in Germany (Becker 1968), West Africa (Fuller 1969 ). and New Zealand (Belford 1963).

This suggests that soft-rot fungi need a stimulus for growth which may be provided by the chemical salts. Alternatively they may normally be inhibited from further development by (1) interactions with other fungi or (2) the faster growing 'sugar fungi' which remove surplus nutrients, and the faster growing Basidiomycetes which utilize the cell wall materials. This is clearly an aspect of fungal succession that required further investigation and until this is done it is premature to

assign any great significance to a possible role they may play in fungal succession.

The results of this investigation have shown that several factors are involved in the colonization of Basidiomycetes in fence posts which may either act jointly or separately depending on the existing conditions. These factors are as follows:

1. The decay fungi may enter the posts when fungal activity on the surface of the post has declined with the depletion of nutrients in the surface layers. It has been mentioned earlier that after about 4 or 5 months of exposure the surface layers of the posts are seldom colonized by any fresh fungal spores probably due to the depletion of nutrients on the surface. Basidiomycetes were isolated from the post during this time. It appears therefore that during this period when the resistance from the surface colonizers is at its lowest level the decay fungi may get easy access to the wood below the surface layers. Butcher (1968) isolated Basidiomycetes from stakes after 4 to 6 months of exposure. He suggested that Basidiomycetes invaded the wood when soft-rot activity had declined but the activity of other fungi was at its greatest. The colonization by Basidiomycetes in Scots pine was observed in the present experiment after the posts had been in the ground for 6 months and during this time fungal activity was at its lowest.

2. The association between sapstain fungi and Basidiomycetes may be an important factor for the colonization by Basidiomycetes but it may not be possible to generalize this association due to lack of sufficient data. Findlay (1966) also mentioned that "the sapstaining fungi include such a range of species that generalisations about their ecology are difficult". However, Basidiomycetes were isolated immediately following the colonization of C. piceae and Phialophora sp. It has been mentioned before that several workers have isolated these staining fungi and other sapstain fungi either along with or before the colonization by decay fungi - (Findlay 1940; Meredith 1959, 1960; Ueyama 1961; Shigo 1962, 1965). However, Radvan (1951) mentioned that Pine sapwood with living Ceratocystis sp., was more resistant to decay than clean wood (quoted from Findlay 1959).

3. The colonization by C. piceae and Phialophora sp. followed by Basidiomycetes may lower the pH of the substrate and thus make it unsuitable for the growth of other fungi (Mangenot 1954), and may permit the colonization by Basidiomycetes. Several fungi (11 to 13 in number) colonized immediately below the surface layers of the posts but only a very few fungi (4) colonized at 25 mm and 45 mm depths, suggesting that probably the change in pH of the substrate has eliminated the competitors of Basidiomycetes by inhibiting their growth. Butcher (1968) has also shown that the mean valuesof pH decrease in untreated pine stakes from below ground zones during 12 months of exposure. This is clearly a matter requiring further investigation.

4. The presence of staining fungi may provide an extra source of nitrogen for the increased growth of Basidiomycetes. Aureobasidium pullulans ,a staining fungus has been shown to scavenge nitrogen from the substrate and also atmospheric nitrogen (Millbank 1969). Increased hyphal growth of Polystictus versicolor a white-rot fungus, has been shown in the presence of Botryodiplodia sp. a sapstain fungus (see p. 119 ).

The parasitism of staining fungi by Basidiomycetes may also be a source of certain nutrients for the rapid growth and colonization by Basidiomycetes. The presence of P. versicolor inside the cells of Botryodiplodia has been observed (Fig. 11 p. 133) which suggests possible parasitism or saprophytism by P. versicolor. Similar suggestionsof parasitism by wood-rotting Basidiomycetes of other wood inhabiting fungi came from Barnett (1964). Griffith (1964) also stated that endoconidia of Ceratocystis spp. were highly susceptible to attack by certain Basidiomycetes.

Basidiomycetes first colonized the centre of the post and then migrated back towards the surface. Corbett and Levy (1963) also made a similar observation. C. piceae and Phialophora sp. were isolated concurrently with Basidiomycetes for the same period but in the later series of isolations the staining fungi were not isolated from the centre of the post. Six months later the posts were cut vertically, and white-

rot was seen in the centre, and stain towards the surface. A similar effect was also seen in blocks of wood during the laboratory test of succession with Botryodiplodia sp. and P. versicolor. This also suggests a possible parasitism by Basidiomycetes of staining fungi

5. The development of 'inoculum potential' in the soil substrate near the post may also be an important factor responsible for the penetration of decay fungi into the wood. It has been discussed earlier that when the nutrients from the surface layers are depleted the fungal attack on the post is only through hyphal contact. A decay fungus growing near the post may have a better chance of colonizing the post first provided it has the 'inoculum potential'. Corbett and Levy (1963) also suggested that Basidiomycetes present in the soil at the base of a post at the time of its insertion in the ground were the early colonizers of ground level wood.

6. Interaction and antagonism between the fungi can also lead to penetration of wood by Basidiomycetes, or certain antagonistic fungi may affect their colonization. A sapstain Discula pinicola was isolated from the surface layers of birch posts at the same time that Polystictus sp. and an unidentified species of Basidiomycetes were isolated from below the surface. This fungus was found to be antagonistic to several other fungi but was overgrown by P. versicolor (see p.142 ). The fungi which are antagonistic to other fungi but not to Basidiomycetes, can facilitate the entry of Basidiomycetes by inhibiting the growth of several fungi which otherwise might have inhibited the growth of decay fungi.

It has been extensively reported in the literature that certain antagonistic fungi can inhibit the entry and growth of Basidiomycetes in the wood (Rishbeth 1952, 1959; Lindgren & Harvey 1952; Etheridge 1957; Bourchier 1961; Whittaker 1962; Shields & Atwell 1963; Basham 1966; Shields 1968; Scheffer 1969). In the laboratory test a similar effect was also noticed, in which Trichoderma viride inhibited the entry of P. versicolor in birch and Scots pine blocks. However, in the fence posts a similar action of T. viride was not confirmed. This may be because

interaction between a large number of fungi on the surface layers may neutralize the effect of certain inhibitors. The presence of antagonistic fungi may delay the entry of Basidiomycetes into the posts, but it has not been possible to confirm this effect under field conditions. Butcher (1968) has suggested that T. viride inhibits the growth of certain other fungi but he has also not mentioned its effect on the entry of Basidiomycetes into the wood. It has been observed that several fungi are eliminated from the flora when certain other fungi begin to dominate the tissues of the wood. The effect of interactions and competition between fungi on the succession is however not clearly understood and may be difficult to assess but its significance in the colonization of subsequent fungi cannot be denied and it requires further investigation.

7. Certain Basidiomycetes able to suppress the growth of other fungi both on the surface and immediately below the surface can possibly enter the wood without much difficulty. Polystictus sp. isolated from birch posts suppressed several moulds and Discola pinicola an antagonist to certain moulds, sapstain and soft-rot fungi (c.f. Section III). This may, in part, explain its ability to colonize the post earlier than other decay fungi. For entry into the wood Basidiomycetes capable of suppressing the growth of other fungi may not depend very much on the level of fungal activity on the surface layer of the post. Butcher (1968) also isolated an unidentified Basidiomycete which was antagonistic to other fungi. He suggested that this fungus was able to compete favourably with other organisms due to its antagonistic action. His earlier suggestion that Basidiomycetes invade the wood when the fungal activity is at its greatest, was probably based on the isolation of this antagonistic fungus at the time of greatest fungal activity. It appears therefore that the entry of decay fungi into the wood and their colonization may also significantly depend on their physiology.

The isolation of Basidiomycetes is a difficult problem in this work. The medium suggested by Russell (1956) was found very satisfactory for this isolation. The identification of these fungi poses much greater

problems. Two species were identified from their fruit bodies formed on the posts after about eighteen months. Peniophora sp. was isolated from Scots pine and Polystictus sp. and an unidentified Basidiomycetes Culture No. IC 101) from birch posts. All the decay fungi isolated from the posts produced white-rot. Kaarik (1967) isolated 26 species of Basidiomycetes from spruce and pine posts and most of these were white-rot fungi. Butcher (1968) isolated five species of Basidiomycetes of which four were white-rot fungi. Corbett and Levy (1963) isolated two species of Basidiomycetes from a birch post situated in the same experimental plot as the present work. Brown-rot fungi were not isolated from the posts. Butcher (1968) also made a similar observation that white-rot fungi were the early colonizers amongst Basidiomycetes and suggested that white-rot fungi generally precede brown-rot fungi.

The isolation of a very large number of species of Basidiomycetes by Kaarik (1967) from spruce and pine posts situated in three different localities shows that probably the sequence of colonization by Basidiomycetes is a complicated process. However, the present investigation and the information available from the earlier works (Findlay 1940, Mangenot 1952, Corbett and Levy 1963, Shigo 1965, Butcher 1968 and others) reveals that very few species of Basidiomycetes are generally involved in succession of fungi in any one locality. Findlay (1966) has suggested that several species of Basidiomycetes may grow in the same log but rarely more than one species of Basidiomycetes is isolated from the same area in a piece of wood.

The general conclusions drawn on the basis of results and discussion are given later.

## SECTION II

## The effect of the interaction between Trichoderma viride and Botryodiplodia sp. on the decay of Scots pine and birch wood by Polystictus versicolor

### Introduction:

In the study of the distribution and succession of fungi on Scots pine and birch fence posts, interactions between certain micro-fungi and Basidiomycetes were occasionally observed in agar plate culture. The effect of these interactions either leads to the inhibition or establishment of the Basidiomycetes. T. viride and Botryodiplodia sp. were frequently isolated together from different regions of the posts. Their presence was confirmed from examination of sections cut from wood below the surface of Scots pine posts. The association between T. viride and Botryodiplodia sp. may affect the establishment of Basidiomycetes in the post and the present experiment was designed to determine whether this was the case.

Several authors have reported that the growth of Basidiomycetes is inhabited by T. viride. In the present ecological studies it was observed that the succession of fungi in Scots pine usually followed the course-T. viride to a sapstain fungus to Basidiomycetes sp. To study the effect of this prior infection on the establishment of Basidiomycetes a test model was studied in the laboratory using isolates of T. viride, Botryodiplodia sp. and P. versicolor (a white rot fungus); all of which had been found on the posts.

There are many limitations in such laboratory tests. The conditions under which fungi are exposed with test blocks can never directly simulate field conditions. In the laboratory the moisture content and temperature of the wood can be kept constant, fungi are grown on artificial media, and most important the influence of other organisms is absent under these conditions. The lack of knowledge of the organisms and their effects render it impossible to be sure of achieving the natural balance with mixed

cultures.

The following experiments may help us to gain an understanding of how interactions between certain fungi may pave the way for the establishment of Basidiomycetes since, without these interactions Basidiomycetes may not invade the wood.

Literature:

Interactions and associations between fungi have received considerable attention in the last few decades. D'Aeth (1939) surveyed the previous literature and classified the principal types of interactions as occurring, (a) in the soil, (b) on or in a host plant, and (c) on artificial media. The soil interaction is more complex where numerous micro-organisms may interact in various ways and the interaction may have a depressing effect on certain fungi through the secretion of specific inhibitory or toxic substances by other fungi. On the other hand, interaction may be beneficial, through the interlocking of metabolic requirements or the secretion of stimulatory substances. In the case of a host plant attack by one pathogen may allow the entrance of more virulent parasite, which could not by itself have initiated the attack.

Findlay (1939) found that the rate of decay caused by Basidiomycetes in stained pine wood was slightly greater than that of unstained wood, but the difference was not of any significance. Barlund (1950) studied the interaction of decay fungi on wood blocks in the laboratory and pointed out that when certain wood-inhabiting organisms invade the wood first they may prevent others from invading by removing from the wood substances necessary for growth. Basham (1959) suggested that more than one fungus could be involved in the course of decay of balsam fir trees killed by spruce budworm disease, and pointed out that the interaction may be the rule rather than the exception in such diseases.

Several authors have reported interactions between Basidiomycetes and other fungi. Whittaker (1962) recorded significantly lowered weight losses due to three Basidiomycetes in the presence of Coryne sarcoides; Stillwell (1966) had shown that the growth of 31 species of Basidiomycetes were inhibited in the presence of Cryptosporiopsis sp. A strong antagonism of Scytalidium sp. to Poria carbonica on malt extract agar was shown by Ricard & Walter (1968) and they attributed this partly to the production of an antibiotic substance by Scytalidium sp. Bourchier (1961) had shown that the presence of Retinocyclus abietis in the wood block increased the rate of decay by Stereum pini but when Tympanis hypopodia and Coryne sarcoides were initially inoculated to the blocks the growth of S. pini was inhibited.

The interaction between Trichoderma sp. and certain pathogenic fungi and wood rotting Basidiomycetes has received considerable attention. Weindling (1934) and Weindling and Emerson (1936) isolated a substance gliotoxin from T. viride which was found to be highly toxic to the other fungi, later another fungistatic substance viridin was isolated from T. viride by Brian et al.(1946). These toxic substances could be inactivated by other substances of metabolic importance (Brian et al. 1946, Cavallito and Bailey, 1944). The metabolic detoxification of Dinitrophenol intreated fence posts by Fusarium oxysporum which thereby permitted the growth of Coprinus sp. was reported by Madhosingh (1961). Duncan and Deverall (1964) listed several non-Basidiomycetes capable of decomposing wood preservatives. Biological control of diseases in plants and protection of timbers in storage has been suggested by several authors. Baines (1937) (recorded by Wood & Tveit, 1955) has shown that the antagonistic effect of Trichoderma reduces potato scab caused by Actinomyces scabies if added as a suspension in developing tubers. Rishbeth (1952) has shown that the development of Fomes annosus may be checked or entirely prevented if the living tissues of the cut stump are first invaded by Trichoderma or Peniophora gigantea. Rishbeth (1959) has also shown that the treatment of

stump surfaces with ammonium sulphamate favours the growth of T. viride and Penicillium spp., which tend to suppress colonization by Fomes annosus. He pointed out that in formalin-treated stumps "blue stain" fungi were dominant, often in close association with T. viride. Shields and Atwell (1963) found that when T. viride was allowed to become established in sodium-fluoride treated birch blocks, no decay was caused by Polyporus adustus, P. hirsutus, P. versicolor (syn. Polystictus versicolor) or Stereum purpureum. Shields (1968) and Scheffer (1969) have found that by inducing Trichoderma growth on freshly cut rough bolts (by spraying four per cent water solution of sodium fluoride or five per cent ammonium bifluoride) decay is often reduced in bolts during storage.

The inhibitory effect of T. viride on several other fungi, including a few sapstain fungi (Ceratocystis, Aureobasidium pullulans and Discula pinicola) was recently reported by Vasilev (1969).

The studies mentioned above were mostly concerned with testing interactions between an antagonist and individuals of a range of fungi (plant pathogens, wood destroying fungi, sapstain fungi). On an exposed fence post several fungi may interact (or influence) each other. The antibiotics or toxins from one fungus may be inactivated or detoxified by the secretion from another, thereby opening the way for a third fungus.

The detoxification of toxic substances from one fungus by the action of another fungus which succeeds the first has received very little attention. Glasert et al (1961) experimented with Discula brunneo-tingens, Stereum Sanguinolentum and Trichoderma lignorum (syn. T. viride). The wood samples infected with each species were subjected to the action of the other two and weight losses occurring in the samples were recorded. Results showed that (a) the sterilization of wood samples infected with any one species had no inhibiting effect on the subsequent activity of the other species, (b) the presence of live T. lignorum did not prevent slight discolouration by D. brunneo-tingens but did prevent decay by S. sanguinolentum and (c) the wood infected with live D. brunneo-tingens mycelium did not prevent the activity of other two species.

In the present experiment an attempt has been made to study the process of succession of fungi leading to the establishment of Basidiomycetes, in the laboratory with special attention given to the effect of interaction between the test fungi. The results do not entirely agree with Glasert et al. (1961)

Materials and Methods

One centimetre orientated cubes (Greaves & Levy 1965) of birch and Scots pine were exposed in succession to monocultures of T. viride, Botryodiplodia sp. and P. versicolor, in 2.5 % malt agar plates. The cubes were placed with transverse face in contact with the medium. T. viride and Botryodiplodia sp. were isolated from Scots pine fence post and P. versicolor obtained from Forest Product Research Laboratory (culture No. FPRL - 28A). A species of Polystictus had been isolated from the posts and this particular culture appeared to be similar to the culture obtained from F.P.R.L. Sterilization of the blocks was carried out with propylene oxide using the technique described by Greaves (1966).

The experiment consisted of two parts. In part A birch and Scots pine blocks were used, which had no previous infection. In part B, the blocks used were cut from a Scots pine post which had already been infected naturally by T. viride and Botryodiplodia sp. in the field. In both parts of the experiment two replicate sets of 5 blocks each were used for determining the average rate of decay in each exposure.sequence. Hereafter a set of 5 blocks will be mentioned as "set" or set of blocks in the text. The rate of decay was measured by a loss in dry weight of each set. This was determined by keeping the set for 24 hrs., at 107°C. before weighing. The five blocks in each set were weighed together because in a preliminary control where blocks were weighed individually and also together no significant difference in the average weight losses was found. However it is more accurate to weigh individual blocks.

In part A of the experiment the oven dry weight of each set was first measured before exposing to the fungi. But in part B, the blocks

with naturally infected fungi were placed on the medium as inocula, therefore oven dry weight of these test blocks could not be determined prior to the inoculation. In order to calculate the actual weight loss in each set due to the fungal decay, the mean initial moisture content was determined with a matched replicate set of blocks.

Part A:

The sets of birch and Scots pine blocks were exposed to the successive fungi in two series: in Series I: T. viride ⟶ Botryodiplodia sp. ⟶ P. versicolor; and in Series II: Botryodiplodia sp. ⟶ T. viride ⟶ P. versicolor. For each wood species 14 sets were used in each series of exposure.

Series I: 14 sets of sterilized blocks of each wood species were exposed to T. viride for 3 weeks. After 3 weeks, 2 sets were used to measure the rate of decay by T. viride, 6 sets were sterilized and exposed to Botryodiplodia sp. for 3 weeks and the other 6 sets were exposed to Botryodiplodia sp. for 3 weeks without being sterilized. After 3 weeks exposure to Botryodiplodia sp. 2 sets from each subseries (sterilized and unsterilized) were removed to measure the rate of decay, 2 sets from each subseries were sterilized and exposed to P. versicolor for 6 weeks and the other two sets from each of the subseries were exposed to P. versicolor for the same period without being sterilized. After 6 weeks exposure to P. versicolor the rate of decay was measured for each set and the average decay rate was calculated from two replicates in each exposure sequence. (see Table 14 ).

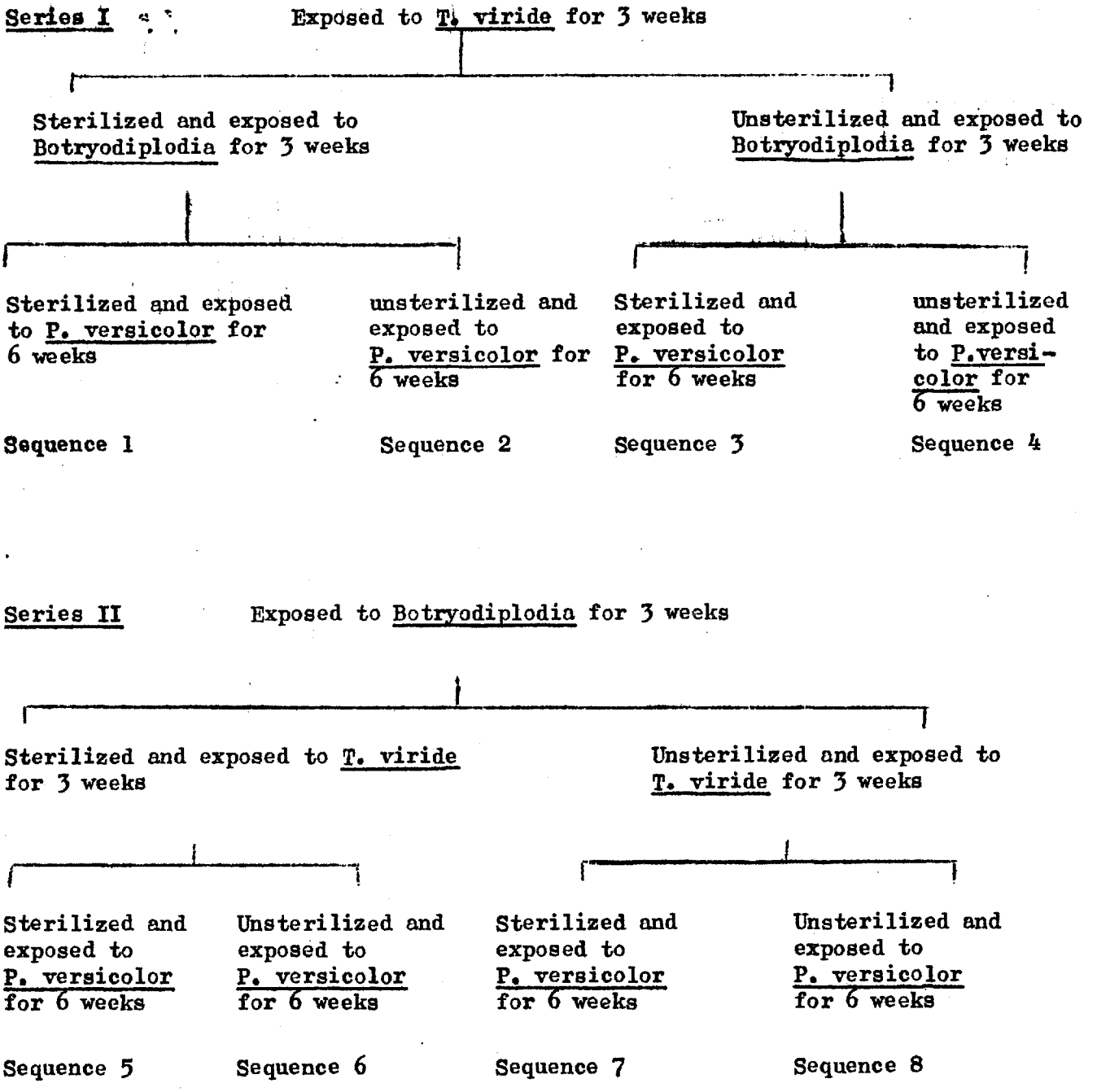
Series II: 14 sets of sterilized blocks from each species of wood were exposed to successive fungi in a similar manner to those in series I but they were exposed in the sequence Botryodiplodia ⟶ T. viride ⟶ P. versicolor.

Control The sterilized sets of blocks from each wood species were placed on the medium without any fungal inoculum. Three replicate sets

Table 14

Exposure sequence of the blocks of birch and Scots pine -

- on T. viride, Botryodiplodia sp. and P. versicolor



were removed at weekly intervals for 8 weeks and the difference in the oven dry weight was measured.

The various sequences of fungus exposure to which the sets of blocks of both timbers were subjected are summarized in Table 14.

The numbers 1 to 8 represent the end of each exposure sequence after the sets have been finally exposed to P. versicolor for 6 weeks. (The sequence numbers are referred to in the results).

Part B:

It has been mentioned earlier that in this part of the experiment the blocks used were cut from a natural infected Scots pine post obtained from the experimental plot. The sets of blocks with live T. viride and Botryodiplodia sp. were exposed to P. versicolor in the following series and the results are based on two replicate sets.

Series 1: 6 sets of blocks were placed on malt agar for 6 weeks. After six weeks 2 sets removed to determine the weight loss, two sets were sterilized and exposed to P. versicolor for 6 weeks and two sets were exposed to P. versicolor for 6 weeks without sterilization and the weight losses were determined.

Series 2: Three sets of blocks were sterilized and placed directly over P. versicolor for 6 weeks and another three sets were exposed to the fungus for the same period without prior sterilization.

Series 3: Mean percentage of moisture content for a set was determined by weighing individually 6 sets of replicates and re-weighing after keeping in the oven for 24 hrs, at 107°C. The mean percentage of moisture content per set was subtracted from the results of weight losses in series 1 and 2 to get the actual approximate rate of decay.

Series 4: In the course of the investigation the blocks in certain sequences showed increase in dry weight due to the salt uptake. This

led to studies to determine the mean increase in dry weight of the sets of blocks to incorporate the necessary correction in the weight loss values, and to find out how far the presence of T. viride and the sapstain fungus could alter this increase in weight.

The naturally infected unsterilized and sterilized blocks of Scots pine, and the blocks of Scots pine and birch without any previous infection were used to determine the increase in weight. The blocks were placed on malt agar and the increase in dry weight was calculated each week from three replicate sets of blocks for about 8 weeks.

Preparation of slides for microscopic examination:

Sections were cut from some blocks of all sequences in Parts A and B (described previously) using a sledge microtome. Sections were examined to study the distribution of fungi in the blocks and degradation of walls.

Transverse and longitudinal (RLS & TLS) sections about 10 micron thick were stained in safranin and picro-aniline blue (Cartwright 1929) and mounted in Euparal or Canada balsam. The staining procedure was very slightly modified from the method recommended by Cartwright. The sections were stained in 0.5 % aqueous safranin for about 5 mins. and in picro-aniline blue the sections were heated gently to the point of simmering for about 3 mins., and left to cool down for some time before washing in water. The sections were then washed quickly in 30, 50, 70 and 90 % ethanol, and dehydrated in absolute alcohol.

Photomicrographs were taken to show details of cell wall degradation and distribution of fungi in order that a comparative study could be made of blocks from all exposure sequences. Microscopic examination of sections was carried out using bright field illuminations, polarized light and phase contrast.

## RESULTS

### Part A

Series I: The mean losses of weight in birch blocks after incubation with the fungi in the various exposure sequences are shown below :-

| | | | | | |
|---|---|---|---|---|---|
| Sequence 1: | *T. viride* | $\xrightarrow{+S}$ | *Botryodiplodia* sp. | $\xrightarrow{+S}$ *P. versicolor* | 21.85 % |
| Sequence 2: | *T. viride* | $\xrightarrow{+S}$ | *Botryodiplodia* sp. | $\xrightarrow{-S}$ *P. versicolor* | 21.50 % |
| Sequence 3: | *T. viride* | $\xrightarrow{-S}$ | *Botryodiplodia* sp. | $\xrightarrow{+S}$ *P. versicolor* | 9.25 % |
| Sequence 4: | *T. viride* | $\xrightarrow{-S}$ | *Botryodiplodia* sp. | $\xrightarrow{-S}$ *P. versicolor* | Nil |
| Sequence 5: | *Botryodiplodia* sp. | $\xrightarrow{+S}$ | *T. viride* | $\xrightarrow{+S}$ *P. versicolor* | 8.95 % |
| Sequence 6: | *Botryodiplodia* sp. | $\xrightarrow{+S}$ | *T. viride* | $\xrightarrow{-S}$ *P. versicolor* | Nil |
| Sequence 7: | *Botryodiplodia* sp. | $\xrightarrow{-S}$ | *T. viride* | $\xrightarrow{+S}$ *P. versicolor* | 11.0 % |
| Sequence 8: | *Botryodiplodia* sp. | $\xrightarrow{-S}$ | *T. viride* | $\xrightarrow{-S}$ *P. versicolor* | 0.90 % |
| Control: | Sterilized Sets of blocks | $\longrightarrow$ | | *P. versicolor* | 35.0 % |

Summary of the results:

The results show that in control blocks when *P. versicolor* was the only fungus present, the rate of decay had been 35 % which was higher than in any other sequence. In the sequences where *T. viride* was sterilized and followed by *Botryodiplodia* sp. $\xrightarrow{\pm S}$ *P. versicolor* the rate of decay was about 22 %, which was about 13 % less than the control, or 63 % of the normal rate of decay when the rate of decay in control blocks was taken as standard. In the sequences where *T. viride* was sterilized immediately before the exposure to *P. versicolor* the rate of decay was 10 % which was 25 % less than the control, or 29 % of normal rate of decay. In the sequences where *P. versicolor* followed *T. viride* without any sterilization between the exposure there was no decay. This indicates that the introduction of *Botryodiplodia* after sterilization of *T. viride* doubled the rate of decay by *P. versicolor* over that when it is not introduced.

Series II: The mean losses in weight in Scots pine blocks after incubation with the fungi in the various sequences are shown below :-

Sequence 1: *T. viride* $\xrightarrow{+S}$ *Botryodiplodia* sp. $\xrightarrow{+S}$ *P. versicolor*: 7.58 %

Sequence 2: *T. viride* $\xrightarrow{+S}$ *Botryodiplodia* sp. $\xrightarrow{-S}$ *P. versicolor*: 7.13 %

Sequence 3: *T. viride* $\xrightarrow{-S}$ *Botryodiplodia* sp. $\xrightarrow{+S}$ *P. versicolor*: 3.9 %

Sequence 4: *T. viride* $\xrightarrow{-S}$ *Botryodiplodia* sp. $\xrightarrow{-S}$ *P. versicolor*: Nil

Sequence 5: *Botryodiplodia* sp. $\xrightarrow{+S}$ *T. viride* $\xrightarrow{+S}$ *P. versicolor*: 3.5 %

Sequence 6: *Botryodiplodia* sp. $\xrightarrow{+S}$ *T. viride* $\xrightarrow{-S}$ *P. versicolor*: Nil

Sequence 7: *Botryodiplodia* sp. $\xrightarrow{-S}$ *T. viride* $\xrightarrow{+S}$ *P. versicolor*: 4.05 %

Sequence 8: *Botryodiplodia* sp. $\xrightarrow{-S}$ *T. viride* $\xrightarrow{-S}$ *P. versicolor*: Nil

Control: Sterilized sets of blocks $\longrightarrow$ *P. versicolor*: 13.25 %

Summary of the results:

For Scots pine blocks the trend of the results was same as in birch but the weight loss was much reduced.

Note: (a) The sign $\xrightarrow{+S}$ indicates that sets were sterilized prior to exposure to successive fungi, and the sign $\xrightarrow{-S}$ stands for the sets not sterilized between the exposure.

(b) In both series neither *T. viride* nor *Botryodiplodia* sp. caused any loss of weight in test blocks regardless of their exposure sequence. Losses of weight were recorded only after exposure of blocks with *P. versicolor*.

(c) The loss of weight in each sequence after 3 weeks exposure to *P. versicolor* was also determined, but as the trend of weight losses were the same as after 6 weeks exposure (except that they were not as great) the results have not been included in the text.

Part B

The blocks from old Scots pine fence post with live *T. viride* and *Botryodiplodia* sp. were very dry. The mean moisture content of the blocks at the time of starting the experiment was 10 %. When the blocks were placed on moist cotton wool, they first turned bluish in colour and later

became very dark. A few clusters of T. viride spores appeared after some time. It appears that the fungi were in a dormant state and their activity was revived with the increase in moisture content. However, when several blocks (15 to 20 in number and not sterilized) were placed on malt agar Botryodiplodia sp. was more often found to grow first and partially suppress T. viride, but in several other plates T. viride was found to suppress Botryodiplodia sp. It is therefore suggested that after the dormant period when the resting stage of both these fungi regain their activity due to the changedconditions Botryodiplodia sp. can grow earlier than T. viride and may partially suppress the growth of T. viride, (Fig.4

The mean losses in weight in Scots pine blocks (naturally infected with T. viride and Botryodiplodia sp) after 6 weeks exposure to P. versicolor in different sequences are shown below :

Series 1

a) Set of blocks placed on the medium for 6 weeks $\xrightarrow{+S}$ P. versicolor: 13.2 %

b) Sets of blocks placed on the medium for 6 weeks $\xrightarrow{-S}$ P. versicolor: 0.98%

Series 2

a) Sets of sterilized blocks placed directly $\longrightarrow$ P. versicolor: 7.3 %

b) Sets of unsterilized blocks placed directly $\longrightarrow$ P. versicolor: 12.64%

No significant weight loss was found when the unsterilized blocks were placed on the medium as inocula for 6 weeks. The loss in weight was only due to P. versicolor.

The results show that when the blocks (naturally infected) were sterilized and exposed to P. versicolor the loss of weight was 7 % which was about 50 % lower than the normal rate of decay (13.25 %, mentioned before) in Scots pine blocks which had no previous infection before exposure to P. versicolor. In the sequences where unsterilized blocks were placed on P. versicolor directly (Series 2b) or after being first on the medium for 6 weeks then sterilized and placed on P. versicolor, (Series 1a the losses of weight were near normal. The weight loss was very greatly reduced in the sequence where the blocks were first placed on the medium and then

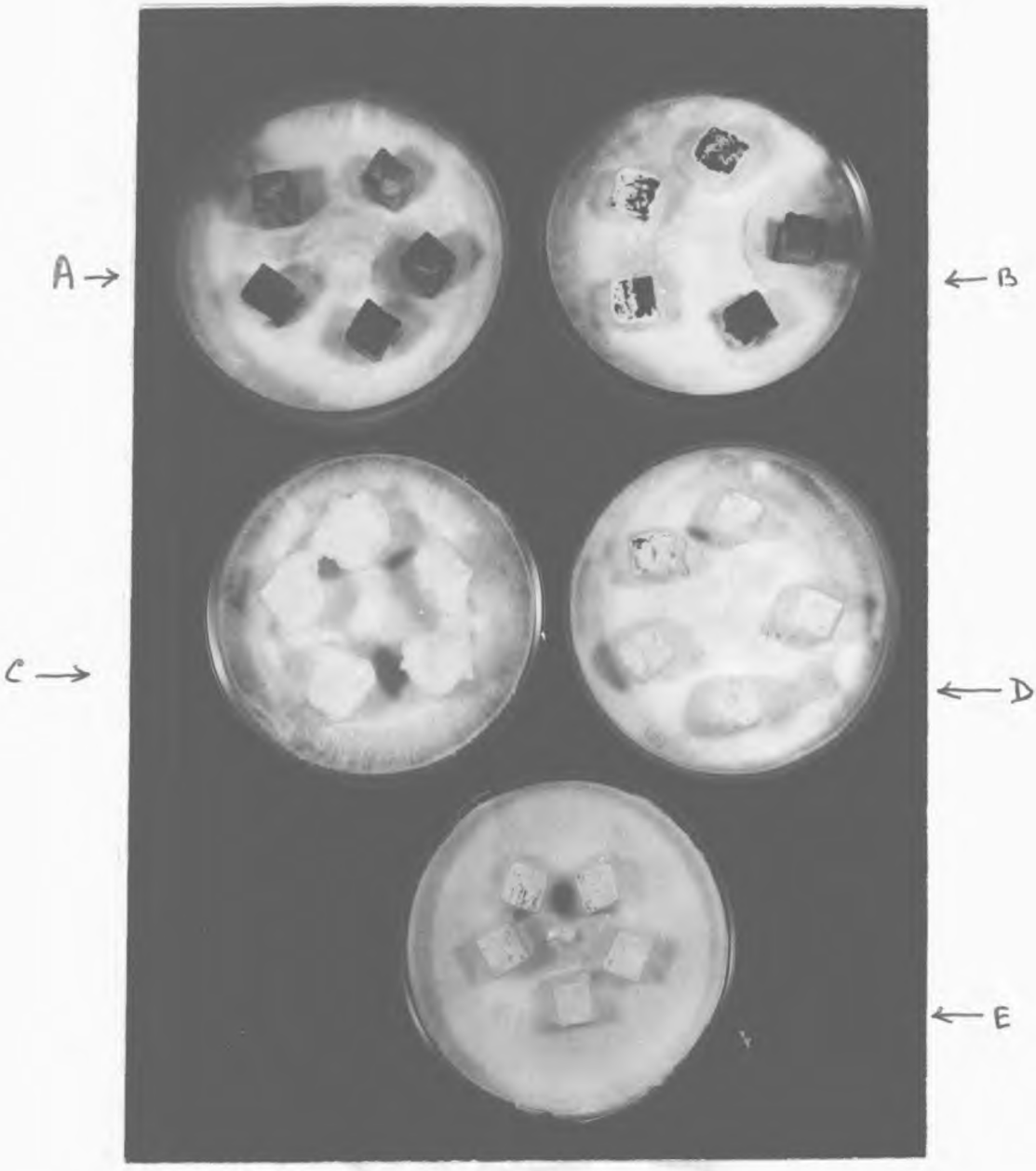



Fig. 4 - A, B & D. Blocks of Scots pine naturally infected by Botryodiplodia sp. and T. viride, kept on malt agar for 3 weeks and then exposed to P. versicolor. The figure shows that after 8 days the blocks where T. viride developed first P. versicolor was suppressed but in the blocks where Botryodiplodia sp. has grown first these blocks were attacked by P. versicolor.

C - Naturally infected blocks exposed directly to P. P. versicolor after 8 days the blocks are fully attacked by P. versicolor.

E - Naturally infected blocks were sterilized and exposed to P. versicolor, after 4 days the blocks were attacked by P. versicolor

exposed to P. versicolor without being sterilized between the exposures.

It appears that when T. viride and Botryodiplodia sp. regain their activity, the interaction between them increased the rate of decay by P. versicolor in certain conditions and decreased in others.

A similar trend in the resultswith reduced value was obtained in the exposure sequences of 3 weeks duration.

Series 3

The mean moisture content of the test blocks was 10.1 % by weight.

Series 4

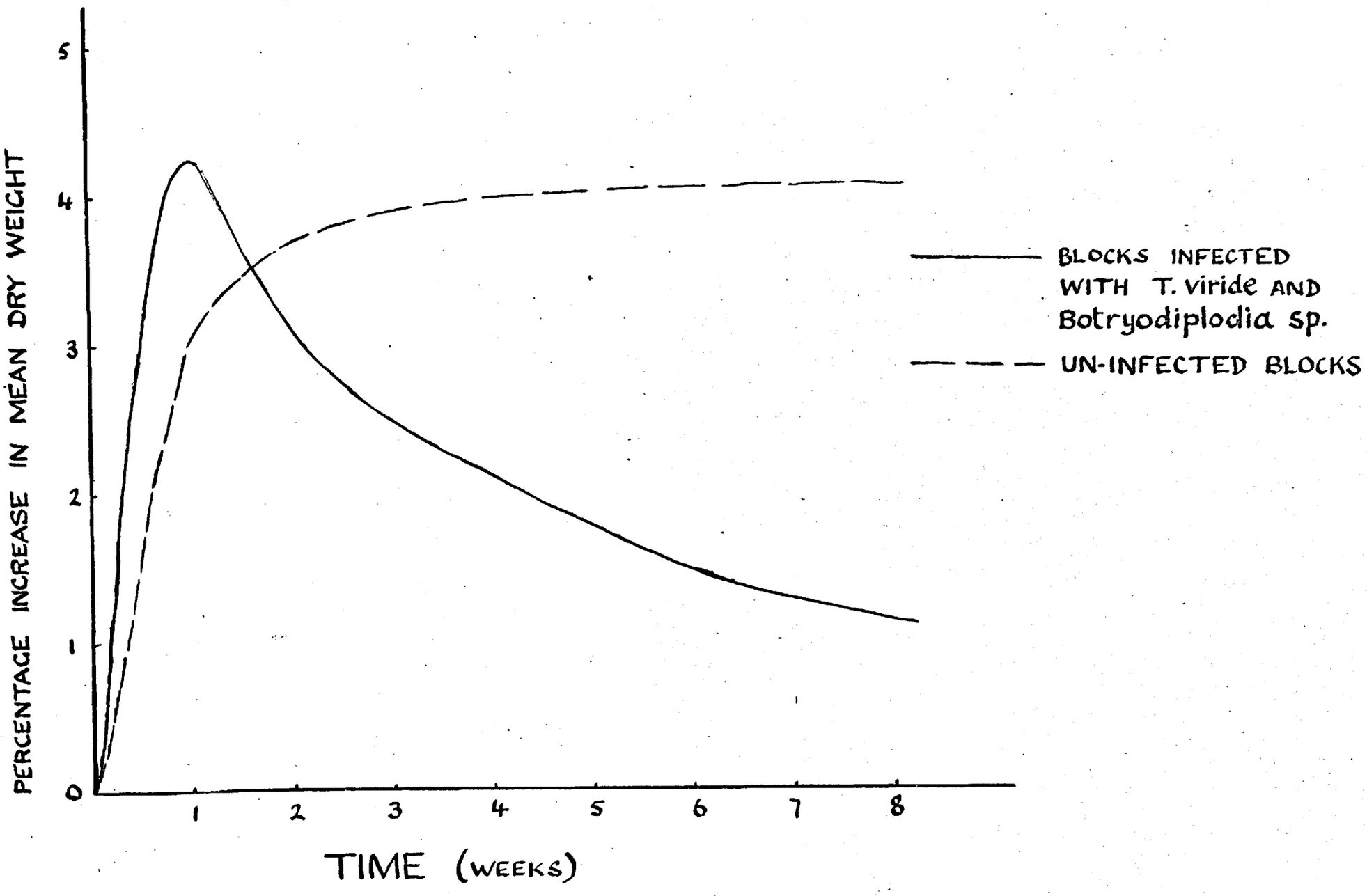
The increase in dry weight due to salt uptake in unsterilized Scots pine blocks (naturally infected) was 4.25 % after one week and it gradually decreased to 1.14 % at the end of 8 weeks (Fig. 5 ). In the sterilized blocks both naturally infected and without previous infection the increase in weight ranged between 3.0 to 4.5 % and remained fairly constant for 8 weeks. In birch blocks there was no increase in weight throughout the same period.

It was earlier mentioned that in birch blocks there was no change in dry weight in presence of T. viride and Botryodiplodia sp. and there was no loss in weight in Scots pine blocks in presence of these fungi. The increase in weight in Scots pine block was possibly due to the salt uptake by capillary action. The gradual decrease from the gained weight in the unsterilized Scots pine was probably due to the utilization of these salts by the fungi. This is worthy of further investigation.

Microscopic study of the interaction of Botryodiplodia sp., T. viride and P. versicolor on birch and Scots pine

The sections cut from the blocks of each sequence, the preparation of which has already been described (see page 112 ) were examined under the light microscope using bright field, phase contrast and polarized light illumination.

FIG 5. COMPARISON OF THE MEAN DRY WEIGHTS OF INFECTED AND UN-INFECTED SCOTS PINE BLOCKS ON 2·5 % MALT AGAR WITH TIME.



Colonization of fungi

In sequence 4, 6 and 8 in which P. versicolor followed live T. viride there was no attack by the decay fungus.

In sequence 1 and 2 where blocks were first exposed to T. viride followed by Botryodiplodia after sterilization the weight loss by P. versicolor was similar. In sequence 2 where P. versicolor followed live Botryodiplodia without being sterilized between the inoculation there were more hyphae present in the sections and these were much more evenly distributed than in sequence 1, where there was a patchy distribution of the hyphae present, in birch and fewer hyphae in Scots pine. In both cases P. versicolor hyphae were present throughout the blocks. The colonization was mainly in fibre cells and ray-parenchyma in birch. In Scots pine the distribution of hyphae on the longitudinal surface was restricted mainly to ray-parenchyma and springwood tracheids but in the transverse surface of the blocks from sequence 2, the summerwood tracheids were also colonized.

In sequences 3 and 7 where T. viride and Botryodiplodia were not sterilized between the inoculation, but sterilized only before exposure to P. versicolor the weight loss was similar in both cases, but much lower than in sequences 1 and 2. The colonization in the surface layers was very dense (even more than in sequences 1 and 2, and in the control blocks) and was not only confined to fibres and ray-parenchyma but in birch several vessels were also heavily colonized. The fungus did not penetrate to any depth. The colonization only took place near the surfaces and radial and tangential sections taken from near the centre of the blocks showed little penetration of hyphae, even though the transverse face was presented to the fungus.

In sequence 5 where the blocks were sterilized between each inoculation the colonization by P. versicolor was in patches and very much restricted to ray-parenchyma in the tangential sections. In transverse sections both fibres and rays were colonized in birch, and parenchyma and springwood tracheids in Scots pine.

Decay of blocks by P. versicolor.

T. viride and Botryodiplodia colonized the blocks mainly near the surfaces but did not cause any significant decay. Decay was only due to P. versicolor. There was a difference in the degree of decay in each sequence but the decay pattern was very similar in all and could be compared favourably with the decay in control blocks which were exposed directly to P. versicolor.

In sequences 1 and 2 the birch blocks showed a very great disintegration of ray-parenchyma, vessels and $S_1$ and $S_2$ layers of fibre cell walls within about 50 $\mu$ from the surface. Decay was much less in the centre of the blocks. The blocks from sequence 2 were very severely decayed especially at the longitudinal edges where the primary wall and even middle lamella were also disintegrated. In control blocks the disintegration of primary wall and middle lamella was found widespread in very large number of cells. Decay was spread more uniformly in sequence 2 than in sequence 1 where there was severe decay in patches. In Scots pine decay of cell wall was less in both the sequences and was restricted to ray-parenchyma and springwood tracheids. In the transverse sections of the block from sequence 2 the colonization was found in summerwood tracheids also.

In sequences 3 and 7 the decay was only found on the surface layers. Severe disintegration of cells was found along the edges in birch blocks but elsewhere only the $S_2$ layers of the fibres were slightly affected. In sequence 5 the cells were disintegrated only at the edges of the blocks but in the rest of the blocks a very little decay was noticed.

In Scots pine the decay was not of any significance in sequences 3, 5 and 7. A very few ray-parenchyma cells on the surface layers were slightly decayed and rest of the cells were sound. A large number of bore holes were noticed in the radial and tangential sections.

The bore holes formed by P. versicolor in both birch and Scots pine blocks were larger in diameter in sequence 2, than in the blocks from other sequences or even in the control blocks. Several of these bore holes

in thick walled tracheids of Scots pine crossed the entire tracheid wall in radial section, passing through the $S_2$ layer. (Fig. 6 ). The bore holes are 2 to 3 times larger in diameter than the width of the penetrating hyphae. Several bordered pits in the summerwood tracheids of Scots pine were completely decayed in the blocks from sequence 2. Similar features were not noticed in the blocks from other sequences. The pit membranes in the thin walled tracheids were often found to decay after six weeks exposure to P. versicolor but rarely in the case of the pits from thick walled tracheids (Fig. 7 )

The effect of P. versicolor on the distribution of Botryodiplodia in the blocks.

The hyphae of Botryodiplodia was widely distributed in the cells of the blocks as separate hyphae when it was the only fungus growing in the blocks or as densely packed mycelia. There was no significant change in the distribution of this fungus in the blocks from sequences 1 to 8 except in the sequence 2. The blocks from this sequence (T. viride $\xrightarrow{+S}$ Botryodiplodia $\xrightarrow{-S}$ P. versicolor) were destained throughout except the face which was in contact with the medium. The hyphae of Botryodiplodia were absent from the radial and tangential sections except near the face which was in contact with the medium. In presence of P. versicolor these hyphae were either destained, or disintegrated or were found to anastomose to form a pseudo-parenchyma. These pseudo-parenchyma were more prominent near the rays (Fig. 8 ) It is difficult to give a precise reason for the formation of pseudo-parenchyma other than the presence of P. versicolor. It might be due to the presence of enzymes from P. versicolor or it could be a physical effect due to the overcrowding of hyphae in the cells.

The disappearance of most of the pigmented hyphae from the section might be due to autolysis of the fungus together with the "bleaching" in presence of P. versicolor.

The cells which were colonized by Botryodiplodia were not attacked by P. versicolor but the neighbouring cells were severely decayed (Fig.9 ). In presence of live Botryodiplodia sp. a very unusual type of attack by P. versicolor was noticed in the radial sections of birch blocks. A row of very closely formed bore holes were found on either side of the hyphae of Botryodiplodia, almost throughout the hyphal length of this fungus. In the adjacent fibre cells of the wood where this fungus was absent bore holes were scattered (Fig. 10 ). The other interesting feature noticed was the presence of P. versicolor hyphae inside the hyphae of Botryodiplodia (Fig. 11 ) In several cases the hyphae of P. versicolor passed through six or seven cells of the host hyphae. This may be due to the development of a parasitic or saprophytic habit of P. versicolor in the blocks. Most probably the hyphae of P. versicolor migrated through the hyphae of Botryodiplodia when the latter was encountered by chance. However parasitism by P. versicolor can also be possible (Griffith 1964).

## Discussion

Botryodiplodia sp. and T. viride were together isolated from the fence posts. Microscopic examination of the sections prepared from blocks of old Scots pine post also revealed an association between these fungi. A similar association between T. viride and a sapstain fungus has been reported by Rishbeth(1959). In the laboratory tests it was shown that the presence of actively growing T. viride inhibited P. versicolor whether it preceded or was succeeded by Botryodiplodia. A similar observation was made by Glasert et al (1959) using different fungi in the sequence. That actively growing T. viride inhibits the growth of Basidiomycetes has been also reported by Rishbeth(1952, 1959), Shield and Atwell (1963), Shield (1968) and Scheffer (1969). From the present results it appears that the antibiotics of T. viride left in the wood can also greatly reduce the decay by Basidiomycetes even if T. viride has been killed, provided the antibiotics have not been inactivated by another fungus before the attack by Basidiomycetes. The results of the present work have been discussed here. The figures for

rate of decay have been quoted from the results obtained with birch blocks. In Scots pine the trend in rate of decay was the same with reduced values.

In sequences 3, 5 and 7 when T. viride was grown in the blocks in association with or after sterilization of Botryodiplodia and was subsequently sterilized before exposure with P. versicolor, the rate of decay was about 9 % when the standard decay rate was 35 %. Microscopic examination of the sections from these blocks had also shown that the decay was confined to the surface of the blocks. From this it could be inferred that the antibiotics produced by T. viride in wood remained effective even after the fungus was dead, and were able to reduce the activity of P. versicolor and in consequence the decay. Other possible reasons for the slow rate of decay such as depletion of certain nutrients from the substrate (Barlund 1950) or a partial mechanical barrier by dead mycelium of the earlier colonist or the change in pH of the substrate, did not appear to be of any significance on the light of subsequent results.

In sequences 1 and 2 (the blocks infected with T. viride were sterilized and then exposed to Botryodiplodia followed by P. versicolor) - the rate of decay in birch was about 21 %. When the results of sequences 1 and 2 are compared with the results of sequences 3, 5 and 7, it appears that in sequences 1 and 2, the introduction of Botryodiplodia in the succession after T. viride was sterilized, had increased the rate of decay by over 125 % as compared to the rate of decay value obtained in sequences 3, 5 and 7, in which Botryodiplodia was not introduced in the succession after T. viride was sterilized. The microscopic examination of the sections from the blocks of these sequences also reveals the fact that in sequences 1 and 2 decay of cell wall is much greater than sequences 3, 5 and 7. Glaser et al (1959) using different species of fungi and wood - suggested that the sterilization of wood samples infected with one species had no inhibiting effect on the subsequent activity of the other species.

This suggests that certain substances secreted by Botryodiplodia sp. have detoxified the antibiotics of T. viride which were left by this fungus before it was killed. This detoxification may have paved the way for the growth of P. versicolor and thus resulted in the increase in rate of decay. This increase in rate of decay would not have been possible otherwise. The inactivation of toxic substances from T. viride was also suggested by Cavallito and Bailey (1944) and Brian (1946). The detoxification of several wood preservatives by non-Basidiomycetes has also been suggested by Madhosingh (1961) and Duncan and Deverall (1964). However, very little information is available on the detoxification of toxic substances from one fungus by another fungus, which might be involved in several interactions between fungi resulting in the establishment of more virulent pathogens or decay fungi (D'Aeth, 1939, Basham, 1959).

It may be also possible that Botryodiplodia had stimulated the development of P. versicolor either by producing certain stimulatory substances or by removing certain substances from the wood, as Bourchier (1961) had shown an increased rate of decay by Stereum pini in presence of Retinocyclus abietis. However it has been mentioned earlier that the presence of Botryodiplodia in association with live T. viride does not have any considerable effect on the rate of decay by P. versicolor. Findlay (1939) reported that the presence of sapstain fungus does not significantly increase the rate of decay in pine by Basidiomycetes. Radvan (1951) found that Pine sapwood with living Ceratocystis sp. was more resistant and with dead sap-stain fungus less resistant to decay than clean wood.

Botryodiplodia may provide some extra nitrogen to P. versicolor when both the fungi are growing together. This may increase the mycelial growth of P. versicolor and eventually the rate of decay. This would be similar to the reported action of Aureobasidium pullulans (Millbank 1969) as a scavenger of nitrogen and possibly even a fixer of atmospheric nitrogen. The presence of P. versicolor hyphae inside the cells of Botryodiplodia, suggests that P. versicolor may utilize the hyphal content of Botryodiplodia. In sequence 2 where P. versicolor followed live Botryodiplodia the mycelial growth was more than in the blocks from sequence 1, probably because of the autolysis of Botryodiplodia in the sequence 2. Although mycelial growth

in sequence 2 was more than in sequence 1, the rate of decay was similar in both the sequences. This suggests that the mycelial growth may not be directly proportional to the enzyme production in P. versicolor. There is certain biochemical evidence to suggest that it is not (King 1969).

The interaction between the fungi could play an important role in fungal succession. T. viride and Botryodiplodia sp. colonize the Scots pine posts much earlier than Basidiomycetes. One can postulate that T. viride may prevent the entry of Basidiomycetes as long as it is active in production of antibiotics. However if the growth of T. viride slows down or stops due to the depletion of nutrients on the surface of the fence posts, an increased growth of Botryodiplodia sp. or some other sapstain fungus may make the entry of Basidiomycetes possible. These suggestions are based on the results of Part B of the experiment with natural infected blocks. In these blocks T. viride and Botryodiplodia sp. were probably in an inactive stage due to the low moisture content. When the activity was induced by supplying water and nutrients, there seemed to be an almost equal chance for one fungus to regain activity earlier than the other and partially suppress it.

The rate of decay (in Series 2a of Part B, see p. 115 ) where the natural infected blocks were sterilized and exposed to P. versicolor for 6 weeks, had been taken as standard. A very low rate of decay in these blocks might be due to the presence of the antibiotics which had been secreted earlier by T. viride (although the fungus might have been inactive for a long time). In the Series (b), when the blocks were first kept for 6 weeks on the medium as inocula, then sterilized and transferred to P. versicolor for the same length of time, the rate of decay was increased. This increase may be due to the detoxification of the antibiotics by Botryodiplodia which has probably grown more vigorously than T. viride. A similar increase in the rate of decay was found when the blocks were exposed to P. versicolor without prior sterilization (Series 2b).

It has been suggested by several authors that Trichoderma and a few other fungi can be used as biological control (Daines 1937, Rishbeth 1952,

1959, Shield 1968, Scheffer 1969). Several other species of fungi were also found to inhibit the growth of Basidiomycetes (Whittaker 1962, Stillwell 1966, Ricard and Walter 1968). It may be interesting to know more on the effect of certain sapstain fungi on those antagonistic fungi which inhibit the growth of Basidiomycetes. However, more thorough investigation on the interaction of fungi is necessary to understand various problems related to the ecology of fungi on wood.

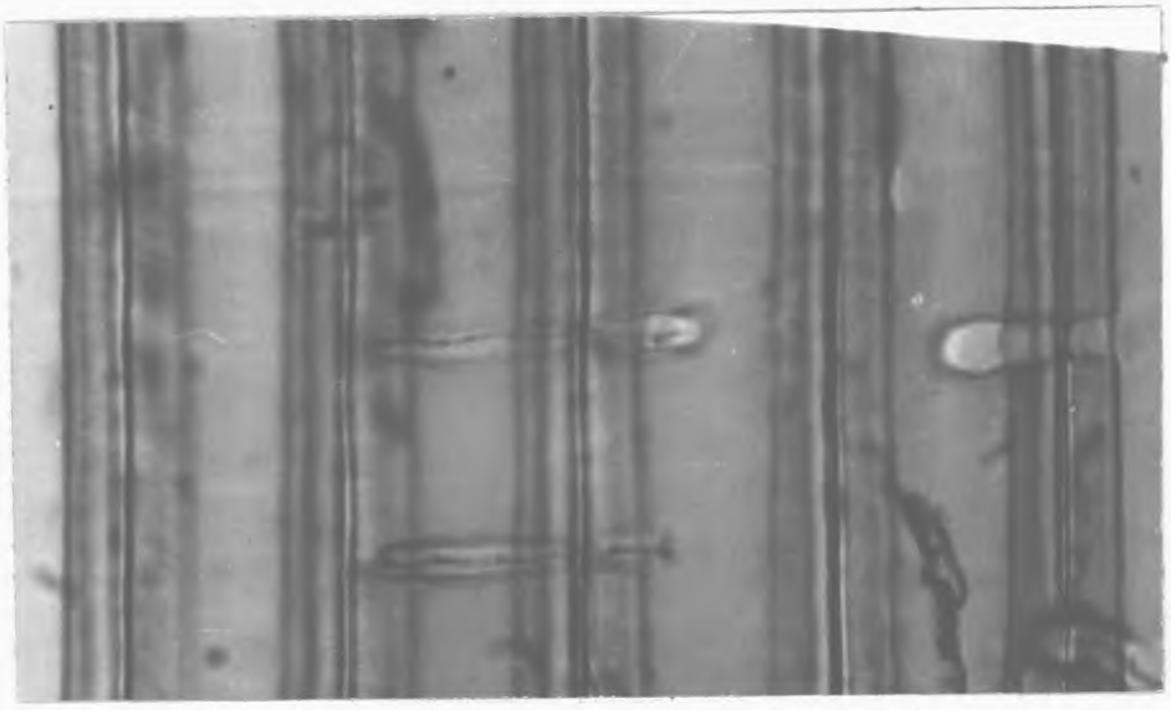


Fig. 6A - RLS Scots pine showing P. versicolor hypha crossing the entire width of the two adjacent tracheid wall - Sequence 2 - 500 X

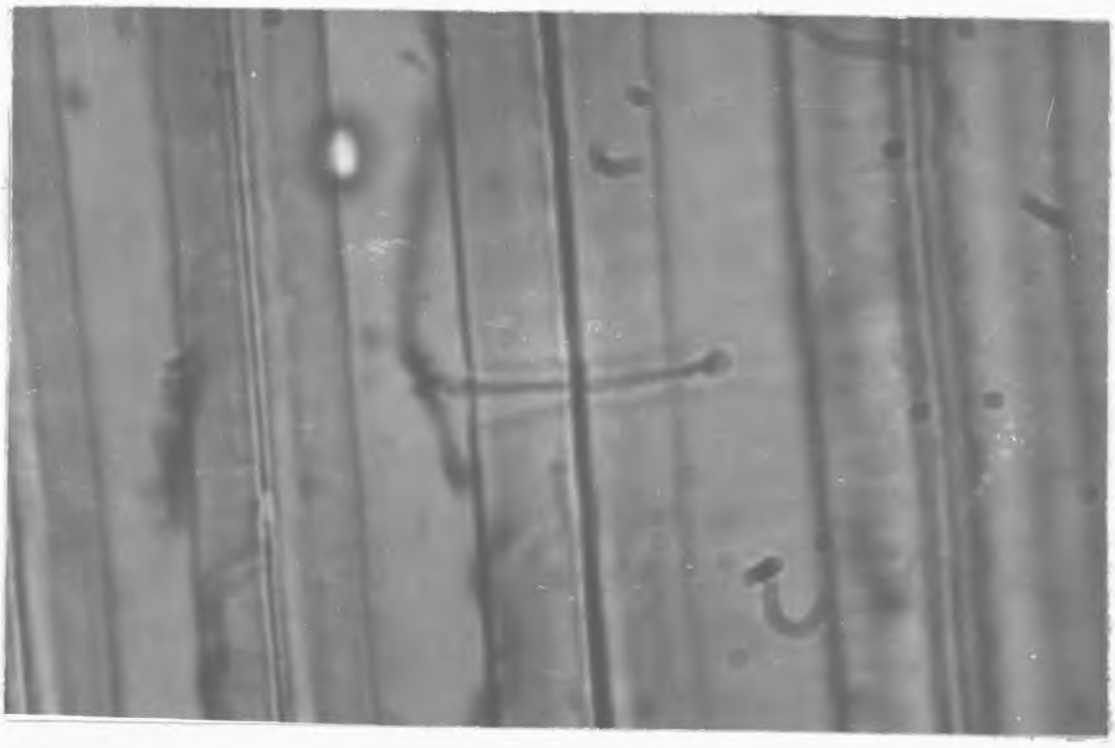


Fig. 6B - RLS Scots pine showing P. versicolor migrating through the tracheid wall - Sequence 2 - 500 X

Fig. 6C - RIS Scots pine showing bore hole formation by a hypha of P. versicolor - Sequence 2 - 500 X

Fig. 7A - RIS Scots pine showing large bore holes formed from bordered pit by P. versicolor in thick walled tracheids - Sequence 2 - 320 X

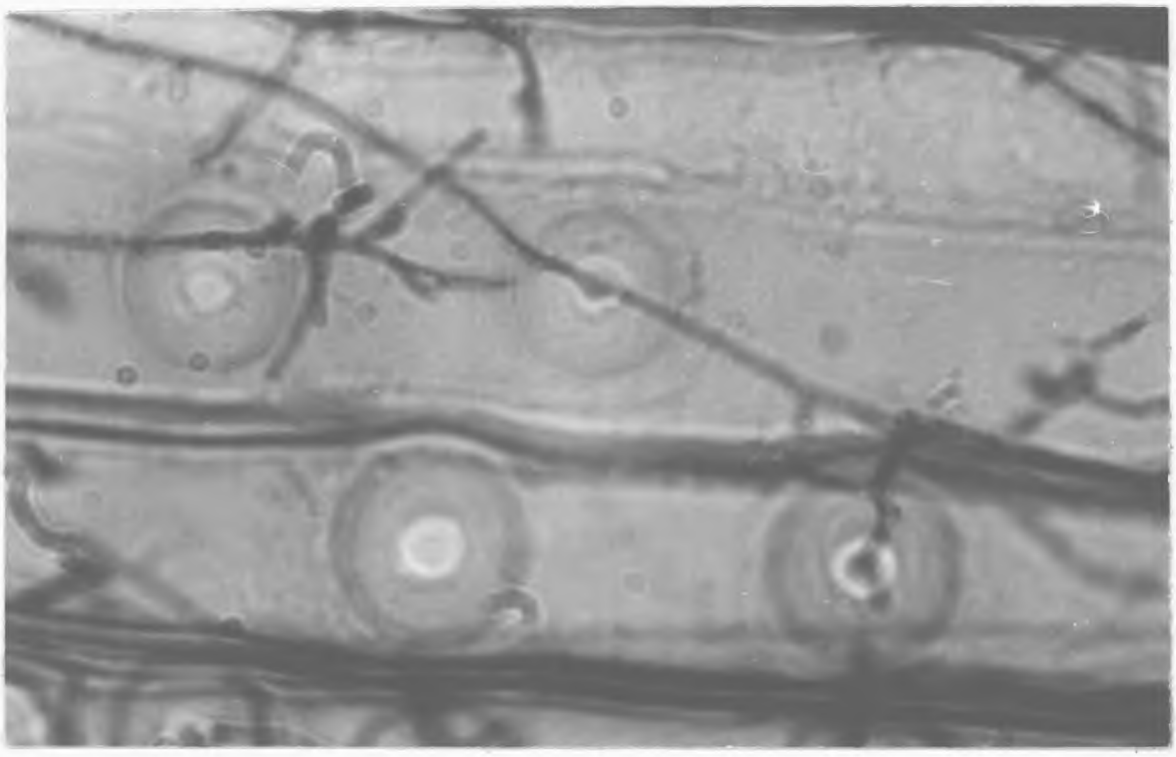


Fig. 7B - RIS Scots pine showing normal bordered pits penetrated by P. versicolor hyphae - Sequence 1 - 787 X

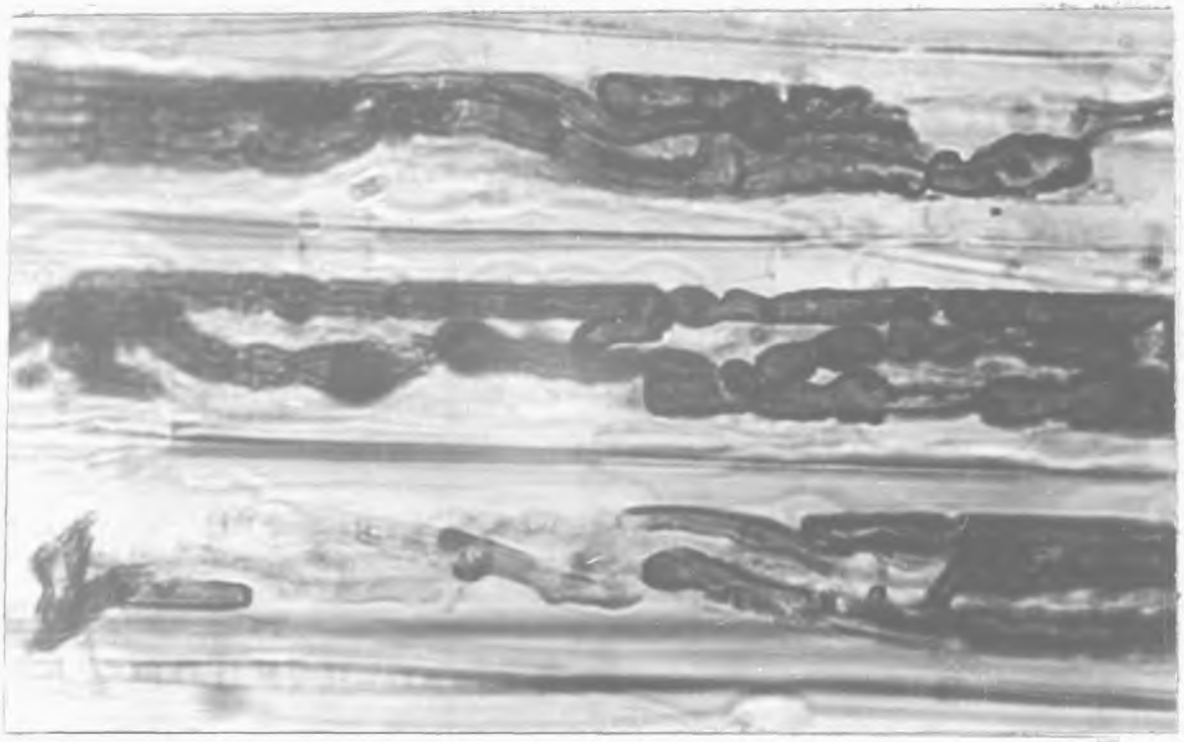


Fig. 8A (above)
8B (below)

RLS Birch showing disintegration of hyphae of Botryodiplodia sp. which leads to the formation of pseudoparenchyma. Sequence 2 - 500 X

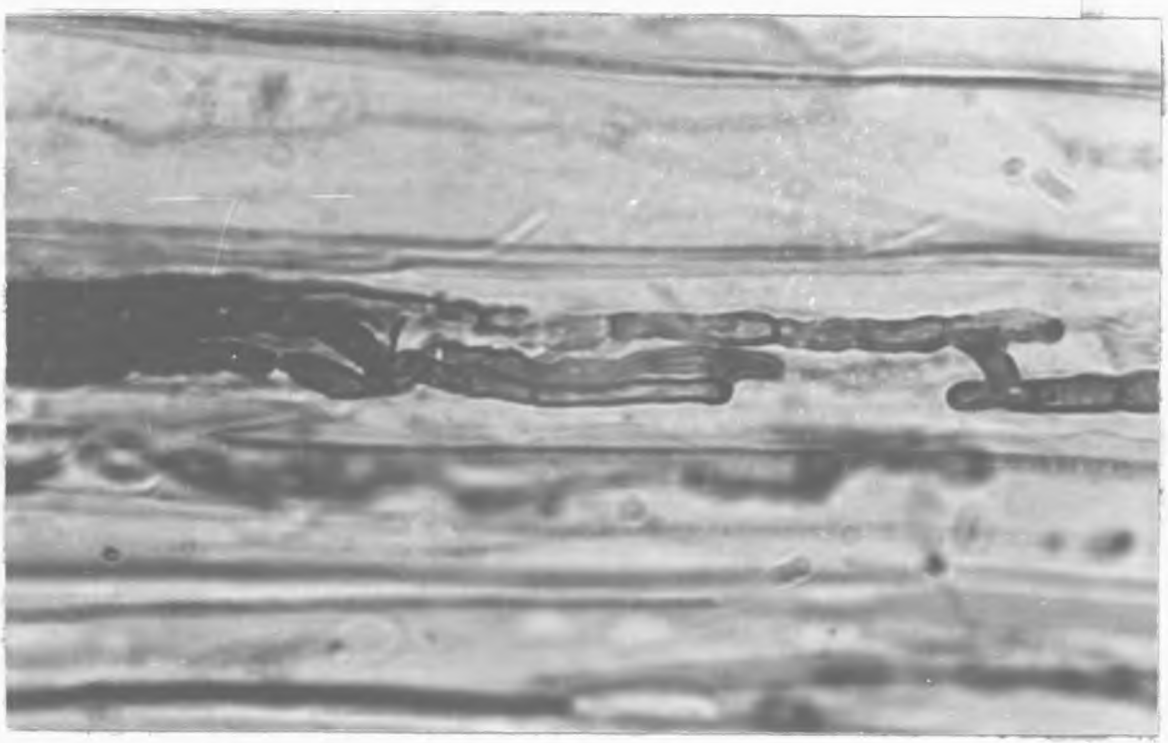


Fig. 8C - RIS Birch showing pseudoparenchyma like structure.
Sequence 2 - 500 X

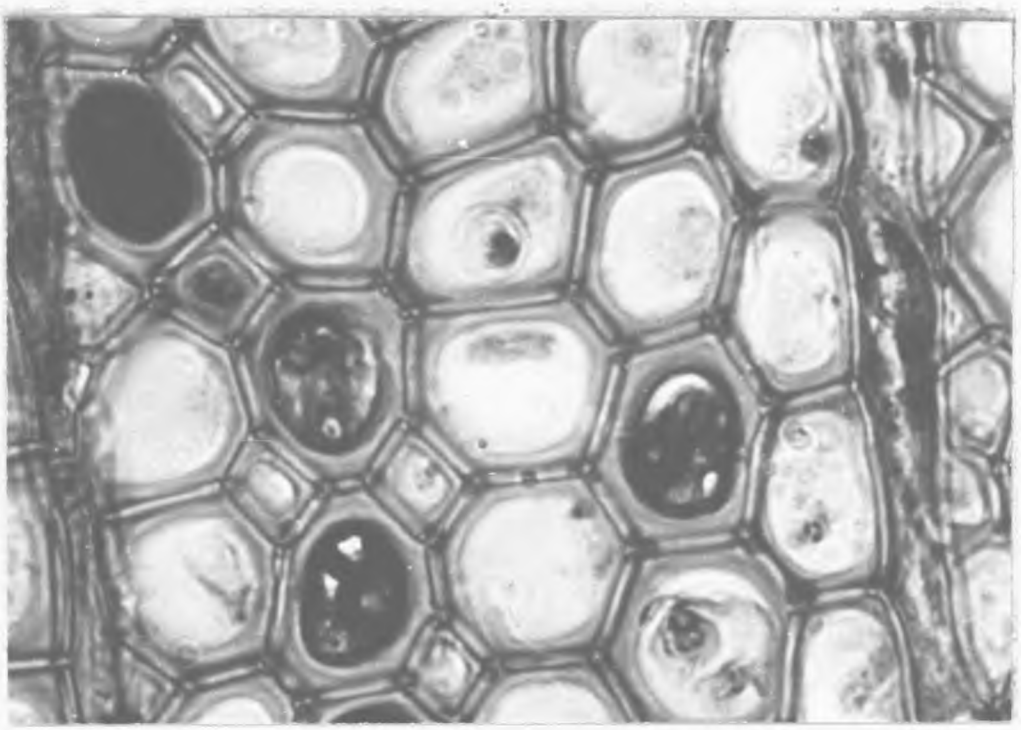


Fig. 9 - TS Birch showing cells colonized by (sterilized) hyphae of Botryodiplodia sp. are not attacked by P. versicolor. Note the degradation of wall in other cells. Sequence 1 - 500 X

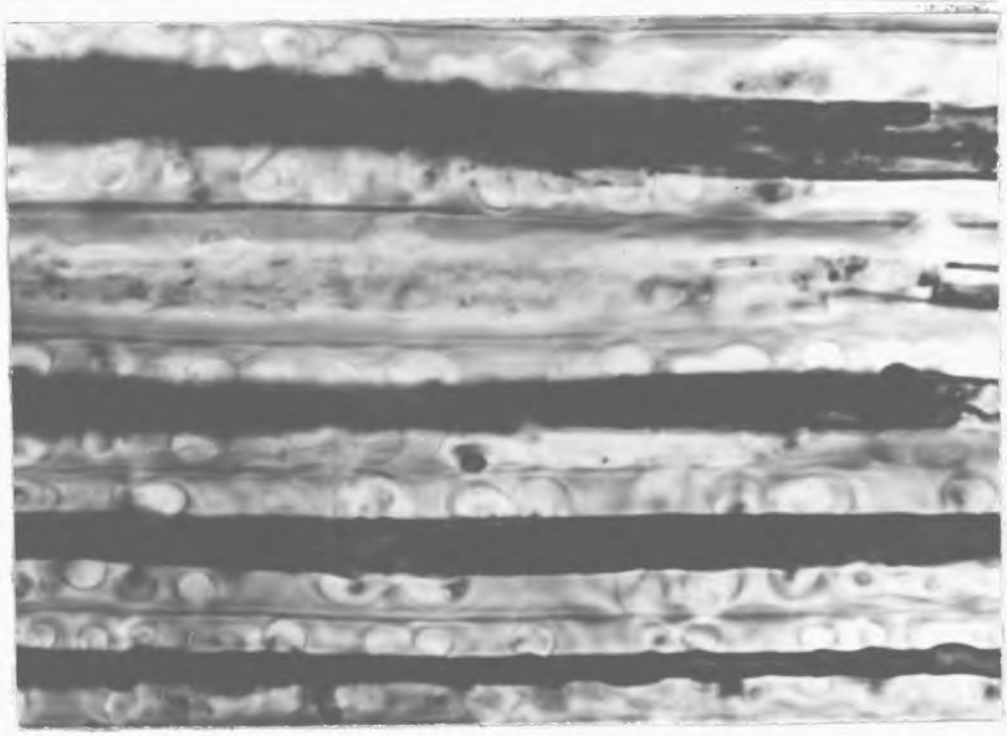


Fig. 10 - RLS Birch showing bore holes in rows formed by P. versicolor on both the sides of the lumen with live Botryodiplodia sp. Sequence 2 - 500 X

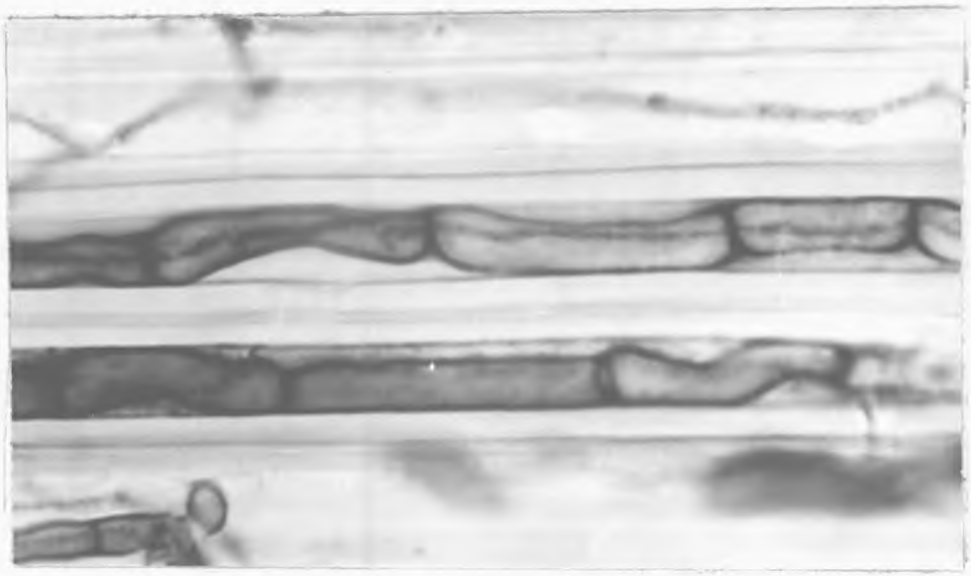


Fig. 11A - RIS Birch showing P. versicolor hypha inside the hypha of Botryodiplodia sp. Sequence 2 - 720 X

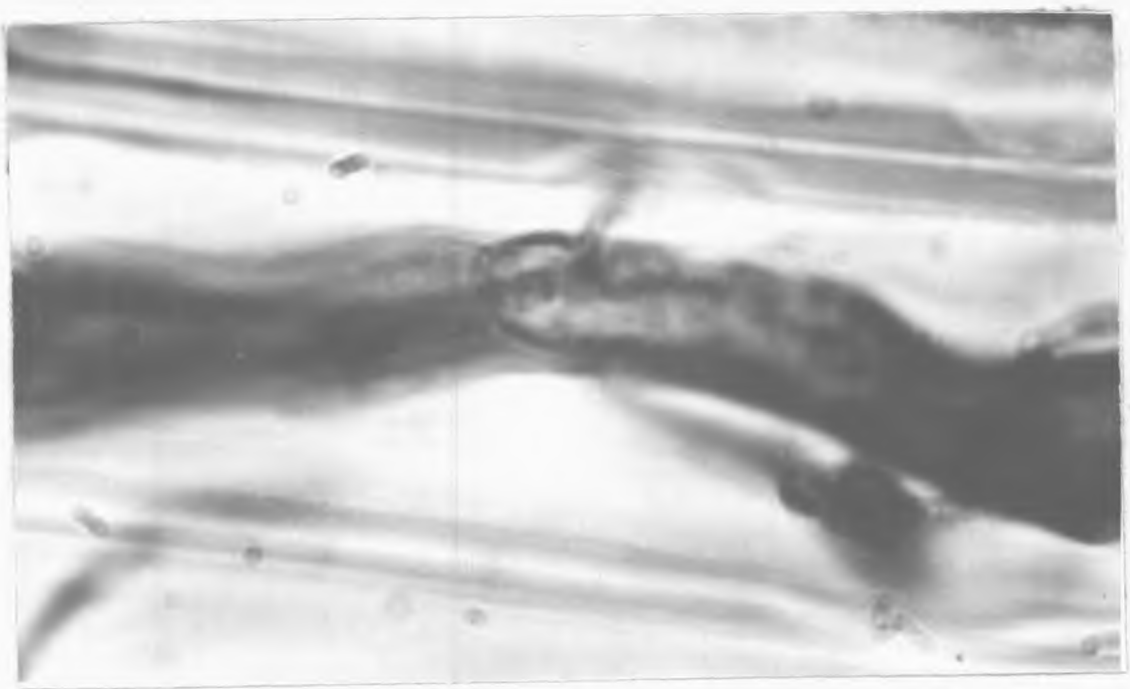


Fig. 11B - RIS Birch showing the penetration of P. versicolor hypha into Botryodiplodia. Sequence 2 - 1250 X

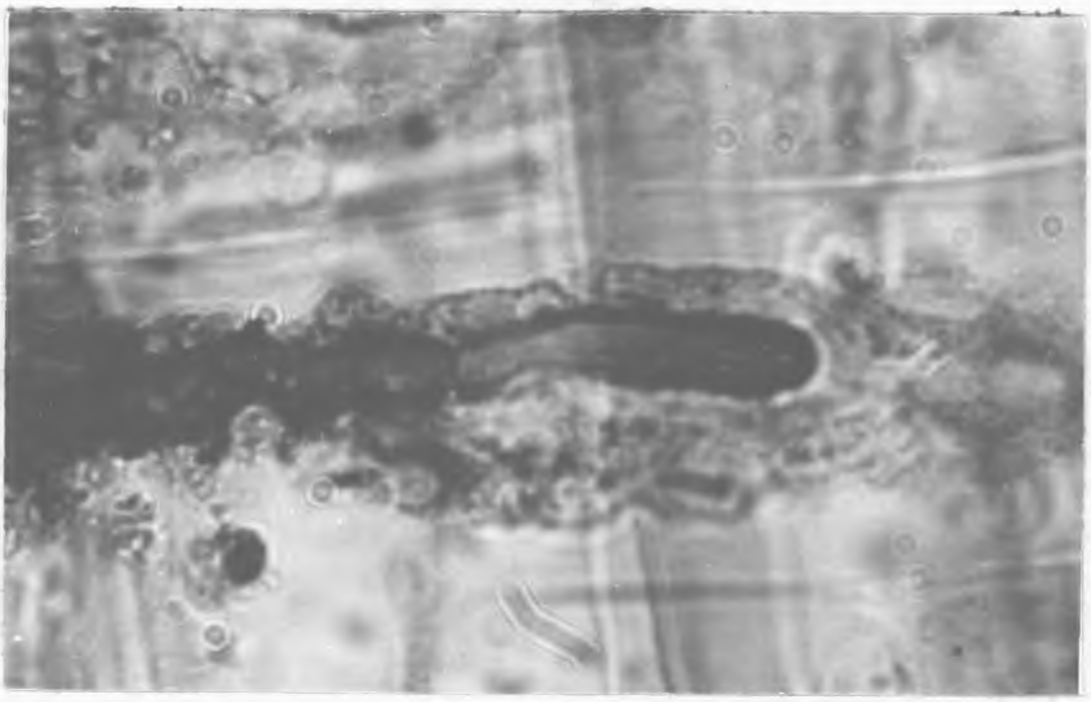


Fig. 11C - RLS Birch showing Botryodiplodia hypha surrounded by the hyphae of P. versicolor. Sequence 2 - 787 X


Fig. 11D - P. versicolor hyphae growing over the colony of Botryodiplodia sp. 3 weeks old culture

## SECTION III

## Interaction and antagonism between some fungi isolated from birch and Scots pine fence posts.

### Introduction

The phenomenon of antagonism and interaction between fungi has received much attention during the last three decades. D'Aeth (1939) stressed the need for a uniform terminology and suggested that the word "interaction" should include all the possible effects peculiar to a mixture of micro-organisms. "Antagonism" could be reserved for the type of interaction in which one fungus in the mixture would exert an adverse influence on the other. The term "association" should be applied when fungi existed together without exerting any harmful mutual influence. He further pointed out that as the succession continued repeated associations and interactions of organisms occur. The antagonistic effect of micro-organisms was earlier reviewed by Waksman (1937 and 1941).

Wood and Tveit (1955) reviewed the work done on antagonism between antagonists and pathogens and the use of antagonistic fungi in the control of diseases. They stated that it was unlikely that these methods of cohtrolling diseases by direct use of an antagonist would compete with ordinary fungicidal treatments except in isolated cases under special conditions. Several workers have demonstrated the possibility of biological control of wood inhabiting fungi. Garrett(1950) mentioned that "the saprophytic survival of a root inhabiting fungus in dead infected host tissue is curtailed by the competition and antagonism of associated saprophytes". The replacement of Fomes annosus from the root and stump of pine by Trichoderma viride has been reported by Rishbeth(1950). The possibility of protecting freshly cut stumps and logs against attack from certain wood-rotting Basidiomycetes, by allowing the prior growth of Trichoderma on the wood has been reported by Rishbeth(1952, 1959), Lindgren and Harvey (1952), Shields and Atwell (1963), Shields (1968) and Scheffer (1969). The Forestry Commission in the U.K. has developed Rishbeth's

work and made further investigations on Peniophora sp. on pine stumps as a fungus likely to compete with and suppress Fomes annosus. A technique has been evolved to infect pine stumps with Peniophora sp. and an extensive trial is being made of its effectiveness as a practical method of controlling Fomes annosus in the forest.

Several other fungi which inhibit the growth of wood-rotting Basidiomycetes have been extensively studied by several workers. Etheridge (1957), Bourchier (1961), Whittaker (1962) and Basham (1966) have reported that Coryne sarcoides, which produces an antibiotic, can inhibit the development of several species of wood-rotting fungi. Certain other members of Fungi Imperfecti, such as Cryptosporiopsis sp. and Scytalidium spp., have been mentioned by Stillwell (1966), Klingström and Beyer (1965), and Ricard and Walters (1968) as inhibiting the growth of several Basidiomycetes. Henningsson (1967a) has pointed out that temperature had a decisive effect on the development of interaction phenomena between decay fungi.

These studies have shown that certain members of lower fungi can inhibit the growth of several wood-rotting Basidiomycetes. In earlier work the antagonistic reaction of the lower fungi was tested mostly against Basidiomycetes. However, not much attention has been paid to determining the effect of antagonists on a range of wood inhabiting fungi (from moulds to Basidiomycetes), with a view to correlating their role in the succession of fungi.

During isolation of fungi from birch and Scots pine fence posts different types of interaction were often observed between the numerous isolates occurring in the original isolation plates from the many particles of wood present. These interactions ranged from suppression of one or several fungi by another fungus to antagonism in which inhibition zones formed around the antagonist. The interactions were either of short duration where eventually one fungus was found to overgrow the others or the interactions were found to persist throughout the entire life of the interacting fungi, keeping a permanent demarcation zone between them.

Antagonism between bacteria and fungi was very often observed but no detailed study of such antagonism was undertaken. Discula pinicola (Naoum) Petr., and some other fungi which showed strong antagonism to various members of the wood flora, were tested against the common representatives of mould, staining fungi, soft-rot and white-rot fungi in pure culture, with a view to studying the effect of this phenomenon in the succession. The test fungi were all originally isolated from the posts.

Method:

Antagonism of Discula pinicola was tested against pure cultures of Trichoderma viride, Paecilomyces varioti, Fusarium sp., Ceratocystis piceae, Chaetomium globosum, Botryodiplodia sp. and Polystictus sp. These fungi were isolated either from Scots pine or birch posts, and were grown on 2.5 % malt agar and incubated at 25°C.

In the first series of tests a petri dish was inoculated in the centre with D. pinicola and the test fungus at four points to the side. In the second series the antagonist and the test fungus were inoculated opposite to each other. The cultures were incubated at 25°C and observed regularly for two weeks and then occasionally for about 6 weeks. The tests were repeated. Temperature and pH of the medium were kept constant. The experiment was also attempted on Abrams medium with 10 gm of cellulose powder per litre, but was not successful because D. pinicola did not grow appreciably in this medium.

The antagonist, being a very slow growing fungus, was grown for five days before the inoculation of test fungi. When inocula of both antagonist and test fungi were plated simultaneously, the test fungi were often found to over-grow the antagonist. Perhaps the antagonistic reaction of D. pinicola is shown only after the build up of sufficient inoculum potential.

Result

The cultural characteristics of D. pinicola compared favourably with the description given by Lagerberg et al (1927) for the same species. They reported that this fungus caused blue stain in pine and spruce. However, the staining reaction of this strain was tested on orientated blocks of birch and Scots pine and found to cause very little stain in 6 weeks.

D. pinicola was found to be antagonistic to all the test fungi except Polystictus sp. which on the other hand grew over the antagonist within two weeks (Figs. 12 ). The antagonistic reaction between D. pinicola and other fungi persisted throughout the life of the fungi leaving a permanent demarcation zone between the antagonist and test fungi, where the hyphae of test fungi did not penetrate. In the test the demarcation zone varied between 0.4 to 1.2 cm in different species except for Fusarium sp. where it was even less than 0.4 cm. The zone separating the two fungi was established within about two weeks from the time of inoculation and no significant change was observed later. (Fig. 16).

A mutual antagonism was observed between D. pinicola and C. piceae. The margin of both the approaching colonies became flat along the demarcation zone. (Fig. 17 ). It appeared that growth of these fungi retarded on reaching the area where the secretion from both might have mixed in the medium. A more pronounced mutual antagonism between D. pinicola and an unidentified very slow growing fungus (culture No. IC102 ) was also observed. In this case it appeared that probably the antagonistic action of the unidentified fungus was more than that of D. pinicola. However, it was difficult to comment definitely on which fungus was more antagonistic (Fig. 18 ).

The interaction between D. pinicola and T. viride was different from the other interactions. No fungal hyphae or germinating spores were observed in the zone separating the two fungi, but Trichoderma spores were found to be produced all over the colony of the antagonist after about two or three weeks (Fig. 16 ). The possible explanation for this

phenomenon could be that T. viride spores deposited over the colony of D. pinicola germinated and later suppressed the growth of D. pinicola. Germination of T. viride spores on the colony of D. pinicola might be due to the lack of any antagonistic substance in the medium under the D. pinicola colony (secretion of antagonistic substances might be limited to young hyphae from where they diffuse into the medium around the colony) or to T. viride being parasitic on D. pinicola. Parasitic behaviour of T. viride has been earlier reported by Garrett (1958). However D. pinicola was antagonistic to the original colony of T. viride but new colonies of Trichoderma developing from spores on the mycelium of D. pinicola could possibly inhibit the growth of the latter. The inhibitory effect of T. viride on the growth of D. pinicola was also reported by Vasilév (1969).

Detailed work on the antagonism between bacteria and fungi was not attempted, but certain observations made on such antagonism are recorded here. In the plates inoculated with the wood samples from the field, colonies of bacteria and several different fungi generally develop in the same plate. In the plates with heavy bacterial infection very few fungal colonies were formed, but in the plates with few bacterial colonies antagonism between bacteria and certain fungi were often observed. Two interesting antagonisms were between bacteria and Cytospora sp., and bacteria and Alternaria sp. However, the demarcation zone between these fungi and bacteria was very narrow and was never crossed by these fungi. Bacteria were often overgrown by Fusarium spp., Botrytis sp. and several other moulds. The fruit bodies of Cytospora sp. were formed more abundantly and earlier along the demarcation zone between the fungus and the bacteria than in the rest of the fungal colony. (Fig. 19 ). There was profuse mycelial growth of Alternaria sp. along the demarcation zone but as the hyphae could not cross the zone, the mycelial mat became dense and high. (Fig. 20).

Discussion:

The antagonistic action of several fungi inhibiting the growth of pathogens and wood-rotting fungi has been extensively discussed by Wood and Tveit (1955), Etheridge (1957), Bourchier (1961), Henningsson(1967) and several others. In most of these cited instances the inhibitory fungi were antagonistic to Basidiomycetes. D. pinicola on the contrary was not antagonistic to Basidiomycetes but showed antagonism towards several other fungi, many of which could have inhibited the growth of Basidiomycetes. The presence of such types of antagonistic fungi in the wood flora could possibly facilitate the entry of Basidiomycetes by eliminating some of their competitors, where otherwise Basidiomycetes had less chances of colonization. D'Aeth (1939) cited similar instances where one parasite by its prior attack on a plant allowed the entrance of a more virulent parasite, which could not by itself have initiated the attack. The fungi producing antibiotics and toxic substances which could inhibit the growth of Basidiomycetes, when eliminated from the wood flora by the antagonistic fungi, might pave the way for the entry of Basidiomycetes in the wood in a manner similar to that of Fusarium and other non-Basidiomycetes in reducing the toxic component of wood preservatives in a substrate, thus enabling the Basidiomycetes to cause substantial decay (Madhosingh1961, Duncan and Deverall 1964, Shields 1967).

However, it is suggested that the fungi which could inhibit the growth of organisms antagonistic to Basidiomycetes and which have no influence on Basidiomycetes themselves, could possibly affect the pattern of succession of fungi in exposed timber. The role of antagonism between the fungi in the succession has been pointed out by Macauley and Thrower (1966). They suggested that the replacement of Mucorales and Penicillium spp. on leaf litter might not be due to the nutritional requirements but due to antagonism. Hudson (1968) has discussed the association of several fungi in the process of fungal succession in the substrate other than wood. The association of Fungi Imperfecti with the colonization by Hymenomycetes on wood and the fungi associated with the detoxification of preservatives

have received some attention in recent years. To understand many complex phenomena involved in the succession of fungi adequate knowledge of antagonism and interactions between wood-inhabiting fungi is necessary. The effect of antagonism and interaction on fungal succession may be difficult to assess but must be considered in the further development of the ecology of fungi on wood in soil contact.

In many instances nutrient availability and space to grow appear to be the most important factors in succession, but clearly the disappearance of certain species and replacement by others must have been brought about by other means. It is in this field of investigation that further knowledge of interaction and antagonisms is necessary to explain the observed changes in the flora.

Fig. 12A - Polystictus sp. growing over the colony of D. pinicola which is in the centre of the plate - 2 weeks old culture

Fig. 12B - Polystictus sp. growing over the colonies of Penicillium spp., P. varioti and C. herbarum in malt agar medium - 3 weeks old culture. (Dark spots and very light coloured area the colonies of non-Basidiomycetes.

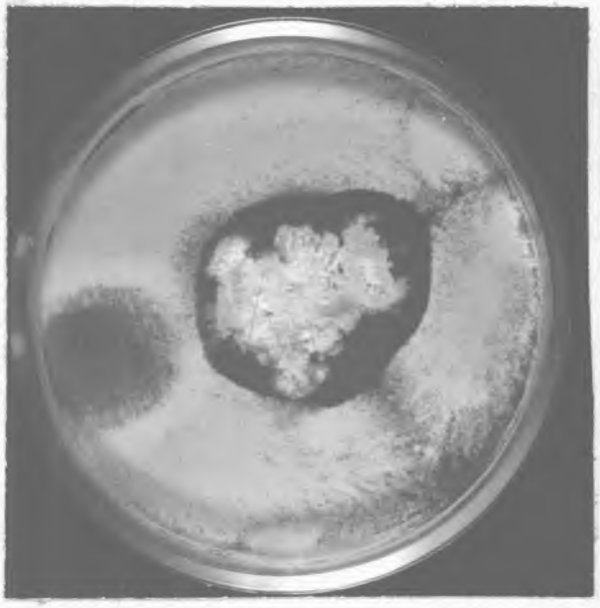


Fig. 13 - Antagonism between D. pinicola (in the centre) and P. varioti - 4 weeks old culture

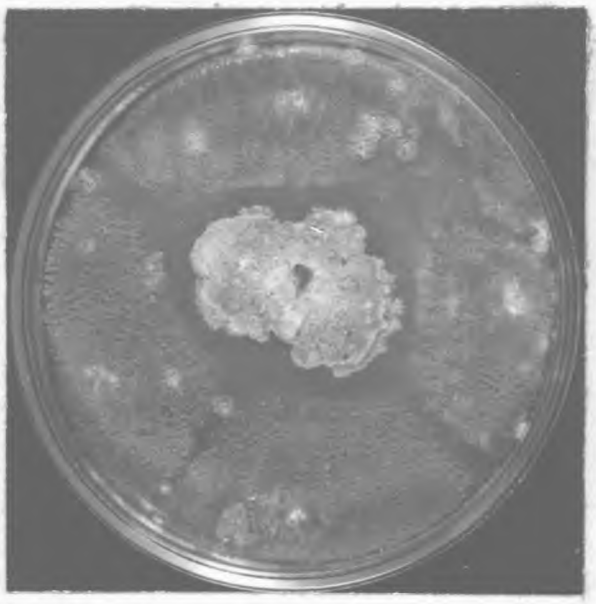


Fig. 14 - Antagonism between D. pinicola (in the centre) and C. globosum - 4 weeks old culture

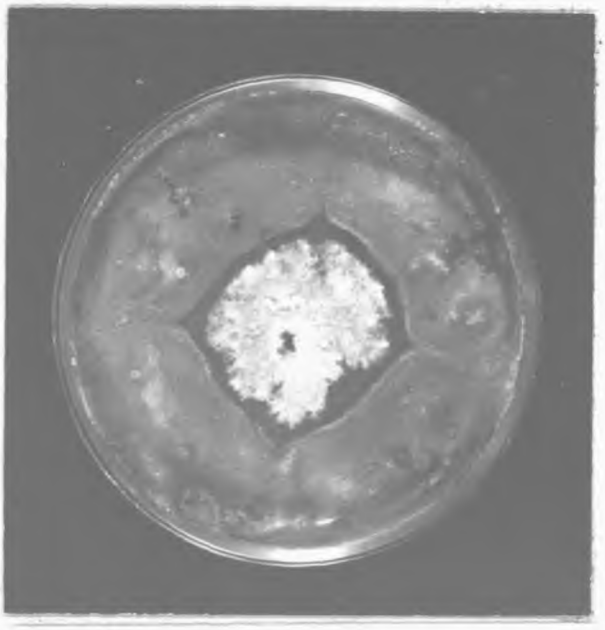


Fig. 15 - Antagonisms between *D. pinicola* and *Botryodiplodia* sp. - 4 weeks old culture

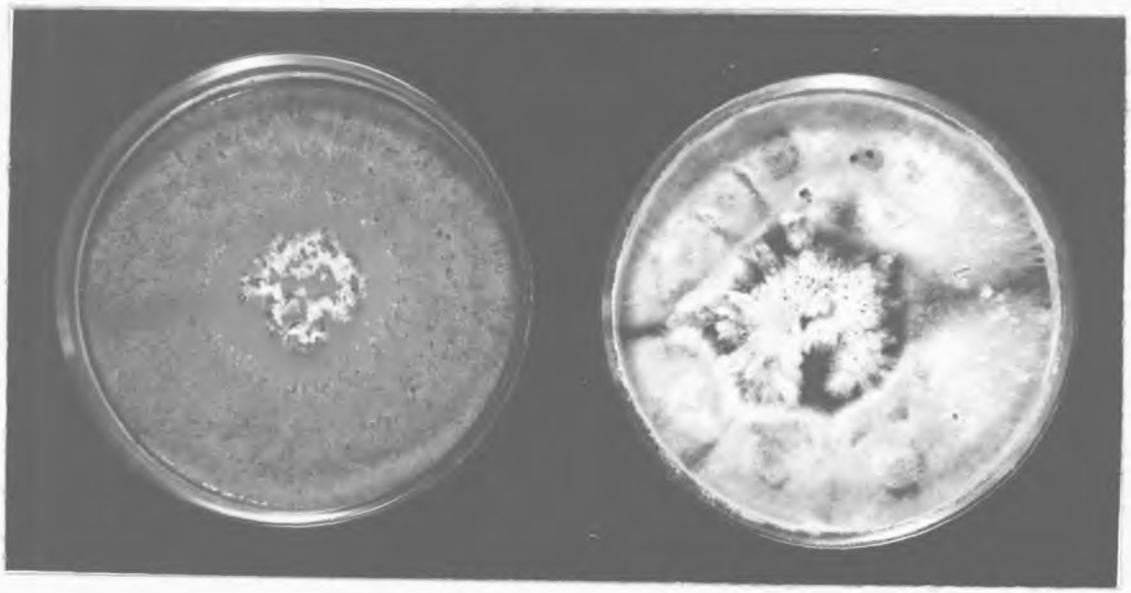


Fig. 16. Left - Antagonism between *D. pinicola* and *T. viride*. Right - Antagonism between *Fusarium* sp *D. pinicola*. 4 weeks old culture

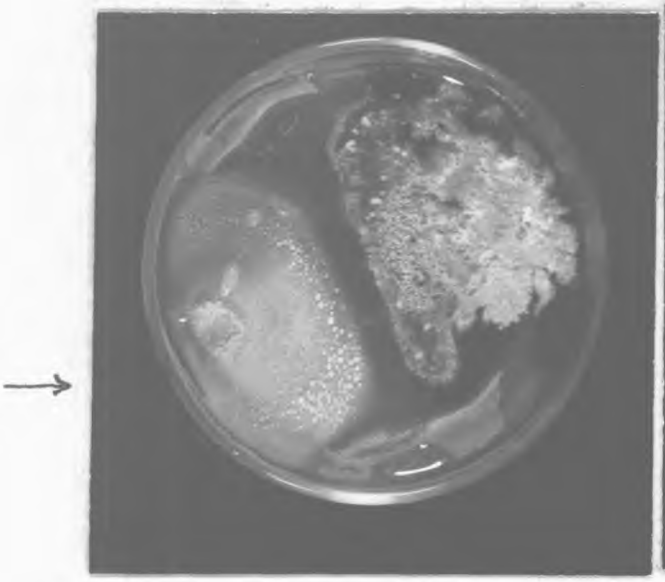


Fig. 17 - Showing mutual antagonism between D. pinicola and C. piceae. (The colony of C. piceae is pointed with arrow).

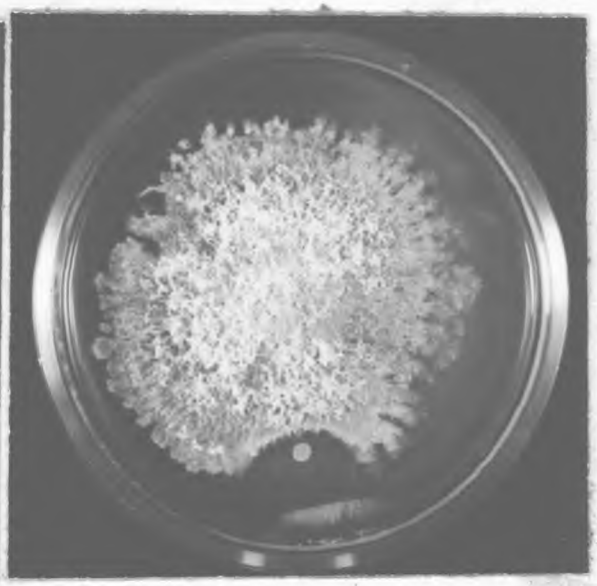


Fig. 18 - Antagonism of an unidentified fungus (small colony) and D. pinicola (large colony)

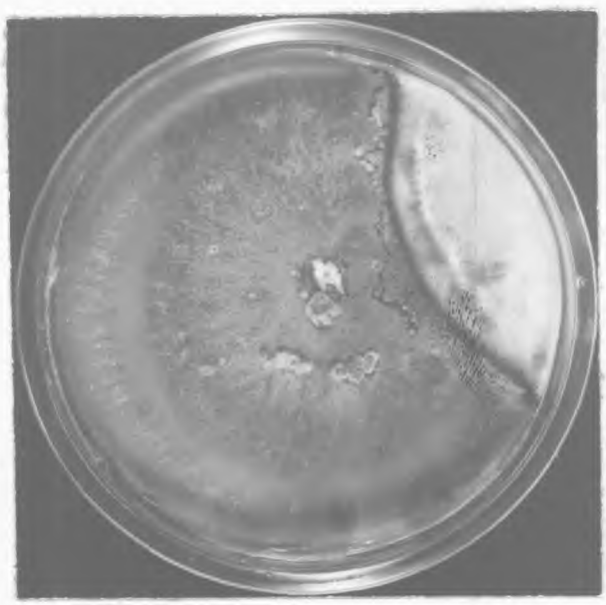


Fig. 19 - Antagonism between bacteria and Cytospora sp. (darker colony). More fruit bodies are formed along the zone near the bacterial colony.

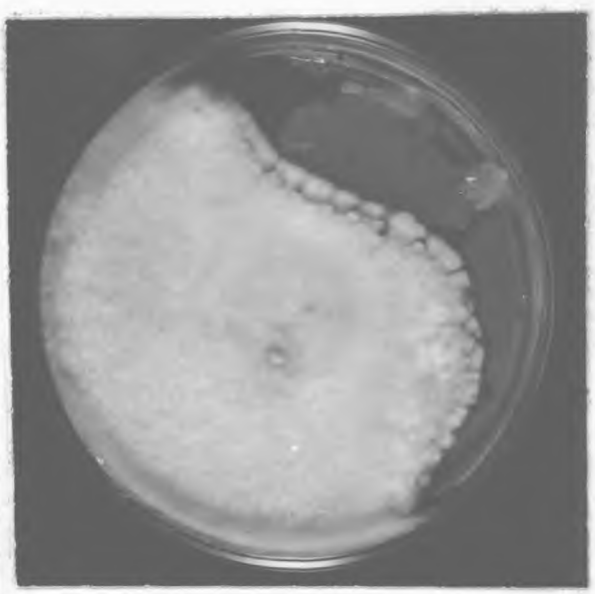


Fig. 20 - Antagonism between the bacteria growing below the medium and Alternaria sp. growing over the medium.

## Section IV

## Decay capability tests of some fungal species

### Introduction

This section deals with the tests done in the laboratory to examine the cellulolytic and soft-rot ability of some of the fungal isolates obtained during the ecological study on the fence posts. A comparative decay of the sapwood of Scots pine (Pinus sylvestris) and birch (Betula sp.) by the fungi causing white-rot (Polystictus versicolor), brown-rot (Coniophora cerebella) and soft-rot fungi (Chaetomium globosum) was also studied. The results obtained in the study of comparative decay, being very similar to those described earlier by several authors, have not been included in the text. The soft-rot fungus C. globosum showed some interesting patternsof attack, and is therefore briefly discussed in this section. Wood decay by soft-rot, white-rot and brown-rot fungi has been extensively discussed in the literature by Bailey & Vestal 1937, Savory 1954, Cartwright & Findlay, 1958; Corbett and Levy, 1963, Levy 1965, Greaves 1966, Wilcox 1968 and several others. Findlay (1956) reviewed the work on the problems of timber decay since 1942.

### Material and Method

The cellulolytic activity as assessed by the formation of a clearing zone in the medium, was tested for several fungi isolated from the fence posts during the ecological studies. The fungi were inoculated in petri-dishes containing Abrams medium to which 10 gm. of Whatman cellulose powder per litre of solution were added, and observations were recorded after one week of inoculation. Soft-rot ability of these fungi was tested by exposing for 15 weeks, the orientated centimetre cube blocks of birch and Scots pine to test fungi grown in the medium described above. The transverse faces of the blocks were in contact with the medium. Radial longitudinal sections nearest to the surface layer of these blocks were prepared, and examined under polarized light. In the investigation on comparative decay the birch and Scots pine blocks were exposed to

P. versicolor (F.P.R.L. No. 28A), C. cerebella (F.P.R.L. 11E) and C. globosum (I.C. 81), grown on 2.5% malt agar medium for 1, 3, 6 and 12 weeks. Preparations were made of stained (staining procedure - Cartwright 1929) and unstained, transverse, radial and tangential longitudinal sections, and were examined under microscope. C. globosum did not produce any decay in the blocks in malt agar medium. C. globosum was later subcultured on Abrams medium with added cellulose powder for 4 consecutive times at weekly intervals before the blocks were exposed to the fungus. Fuller (1969) suggested that this fungus would probably produce more cellulase enzyme and thus greater decay if the soft-rot ability of this fungus was tested from the culture previously grown on a cellulose rich medium. This led to the examination of the behaviour of this fungus in wood decay by subculturing it several times in cellulose rich medium before the test for its soft-rot ability.

Results

The results of cellulolytic and soft-rot ability tests and sap staining ability of the fungi grown on Abrams medium with cellulose powder are summarized in the following table. The table also includes the staining reaction of the fungi in 2.5% malt agar medium.

| | Cellulolytic activity | Soft-rot cavity | | Staining effect on (AC) | | Staining effect on malt | |
|---|---|---|---|---|---|---|---|
| | | Birch | S.pine | Birch | S.pine | Birch | S.pine |
| Aposphaeria sp. | - | - | - | + | - | + | + |
| Alternaria (tenuis) | + | - | - | - | - | + | ? |
| Cephalosporium sp. | + | - | - | - | - | - | - |
| Ceratocystis piceae | + | + | - | + | - | + | + |
| Chaetomium dolichotrichum | + | + | - | - | - | - | - |
| Chaetomium globosum | + | + | + | - | - | - | - |
| Coniothyrium fuckellii | + | + | - | - | - | - | - |
| Dactylosporium sp. | + | - | - | - | + | + | + |
| Gliomastix sp. | + | - | - | + | + | + | + |
| Pestalotia (funeria) | + | + | - | - | - | - | - |
| Phialophora (fastigiata) | + | + | - | + | ? | + | + |
| Phialophora sp. | + | + | - | + | - | + | + |

Note 1. AC = Abrams medium with added cellulose; (+) = present; (-)= absent; (?) = negligible

The mean weight losses in birch and Scots pine blocks in six weeks exposure to Chaetomium globosum in 2.5% malt agar medium and Abrams medium with 10 gm. Whatmans cellulose powder, are given below. The weight losses for similar exposure to Polystictus versicolor in malt agar medium are also given as a comparison. The loss in dry weight was determined with five replicate sets and in each set five blocks were weighed together. The results are as follows:

| Fungus | Medium used | Weight loss in Scots pine blocks | Weight loss in birch blocks |
|---|---|---|---|
| C. globosum | 2.5% malt | Nil | 0.7% |
| | Abrams + cellulose | 2.6% | 27.2% |
| P. versicolor | 2.5% Malt | 13.3% | 35% |

The morphological structures of C. globosum did not show any change after being subcultured four times in Abrams with cellulose medium.

Results of microscopic examination of comparative decay:

C. globosum in malt agar medium did not degrade the cell wall of birch and Scots pine, but in Abrams cellulose medium, the $S_1$ and $S_2$ layers of fibre wall in birch were completely decomposed (Fig. 21 ). In Scots pine the $S_2$ layer of a few tracheids was attacked.

P. versicolor heavily degraded the cell wall of fibres in birch. Near the surface layers and especially towards the edges of the blocks some of the fibre cells lost the primary wall and middle lamella. In Scots pine some of the spring wood tracheids were heavily decomposed but in the summer wood tracheids uniform thinning of cell walls occurred near the surface layers.

C. cerebella caused very little decay in Scots pine. In several springwood tracheids and also in some summerwood tracheids the lamination effect (as shown by Greaves 1966 in Scots pine due to C. globosum) was observed, but in the present work it was not known whether this effect was due to a fault in sectioning. The wall degradation in birch was also

very little. The main reason for little decay in the blocks could be due to waterlogging as the blocks were in contact with the medium. However, the colonization in the blocks was very heavy.

The cell wall degradation in birch by C. globosum was almost of similar intensity to that of P. versicolor except that the primary wall and middle lamella were not attacked by C. globosum.

Microscopic examination of decay due to C. globosum:

These are the results of 3 & 6 weeks exposure of birch and Scots pine blocks on C. globosum after the fungus was subcultured four times in Abrams medium with cellulose powder.

In birch, in 3 weeks the $S_1$ and $S_2$ layers of fibre cells were completely decomposed very near the surface layers and in 6 weeks the decay proceeded towards the centre of the block (Corbett 1963 - type I decay) (Fig. 21 ). In addition the erosion of cell wall proceeded from the lumen towards the primary wall (Corbett 1963 - type II decay) (Fig. 22 ). This fungus also formed a very different type of cavity in the present test. Some of the cavities formed are diamond shaped (Fig. 23 ), a similar type of cavity was noticed in Phialophora sp. (probably P. fastigiata) isolated from the fence post which is similar to observation made earlier by Findlay and Levy (1969). The fungus also produced elongated cavities (Fig. 24 ) the shape of which were comparable to the cavities noticed in Phialophora sp. isolated from the fence posts.

In Scots pine cell wall degradation by C. globosum was very little. The cavities were elongated and the younger cavities were formed along with the migration of the hypha (Fig. 25 ). Another type of structure which was comparable to the cavity was often noticed (Fig. 26). These structures could also be erosion of $S_2$ layer in the area where the fungus was lodged. C. globosum formed 'T' branches (Corbett and Levy 1963, Levy 1965) in the $S_2$ layer of the tracheid wall. The fungal hypha from near the $S_3$ layer crossed the lumen of the cell and formed the 'T' branch in $S_2$ layer (Fig. 27). In this test an interesting feature was noticed that C. blobosum hyphae often migrate through the primary wall and middle lamella

region (Fig. 28 ). The fungal hyphae then enter the $S_2$ layers of the wall adjacent tracheid and form a pair of cavities opposite each other (Fig. 29). It also appeared that the fungal hypha migrating through the $S_2$ layer of the cell wall formed a branch (like 'T' branch) which crossed through the middle lamella, primary wall and $S_1$ layer of the adjacent cell and formed a cavity like structure in the $S_2$ layer nearer the $S_3$ layer of the cell wall (Fig. 30 ). Another type of degradation of cell wall was noticed where the fungus was found to attack the $S_2$ layer of the cell near the $S_3$ layer but without affecting the $S_3$ layer in the beginning (Fig. 31 ).

Discussion:

The results on the pattern of degradation of cell wall by Chaetomium globosum when the fungus was subcultured several times in a cellulose rich medium shows that although the general morphology of the fungus does not change in the treatment this treatment can bring some change in the physiology which may result in abnormal behaviour in the cell wall. It is too premature to draw any conclusion from the results except to say that this fungus can produce different types of cell wall degradation with this treatment. It would be interesting to investigate the effect of preconditioning the fungi to different types of culture medium (with a stimulation of different enzyme systems), on the degradation of cell wall by some soft-rot organisms. The soft-rot cavities formed by the test fungi are under investigation.

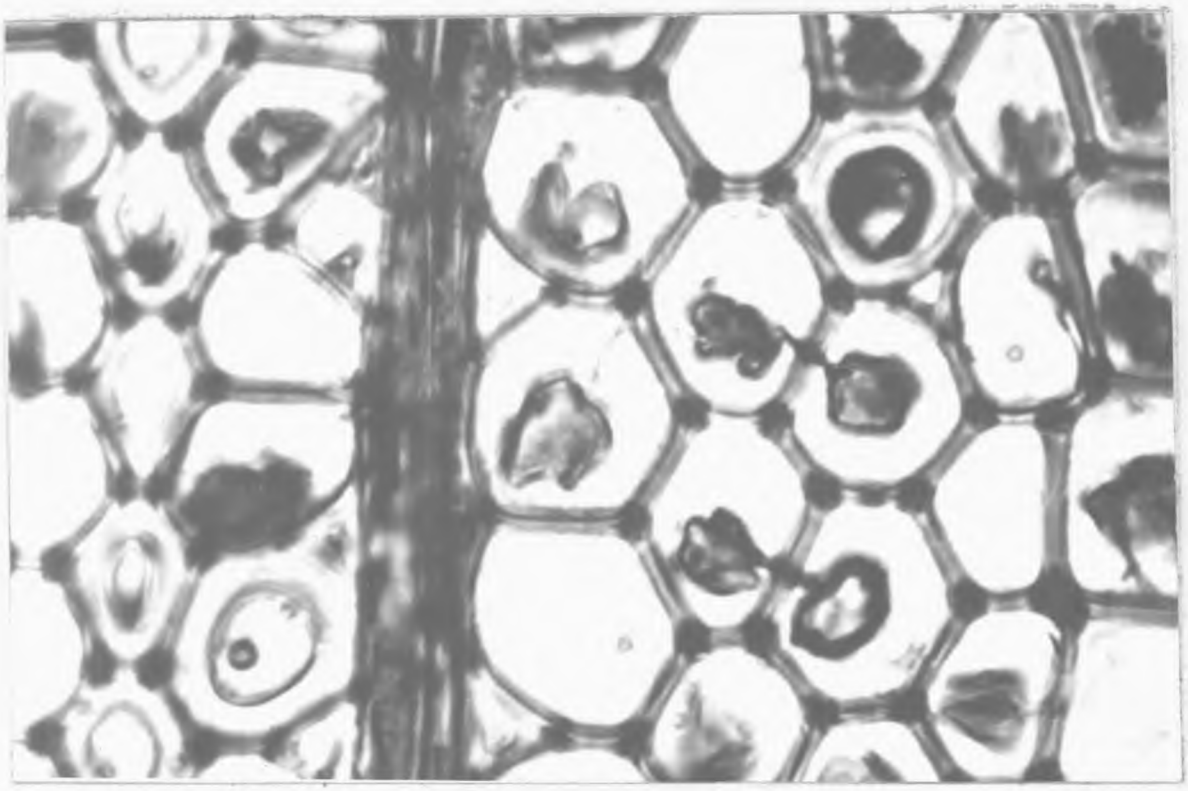


Fig. 21 - T.S. Birch showing heavily degraded secondary wall layers by C. globosum in 3 weeks - 500 X

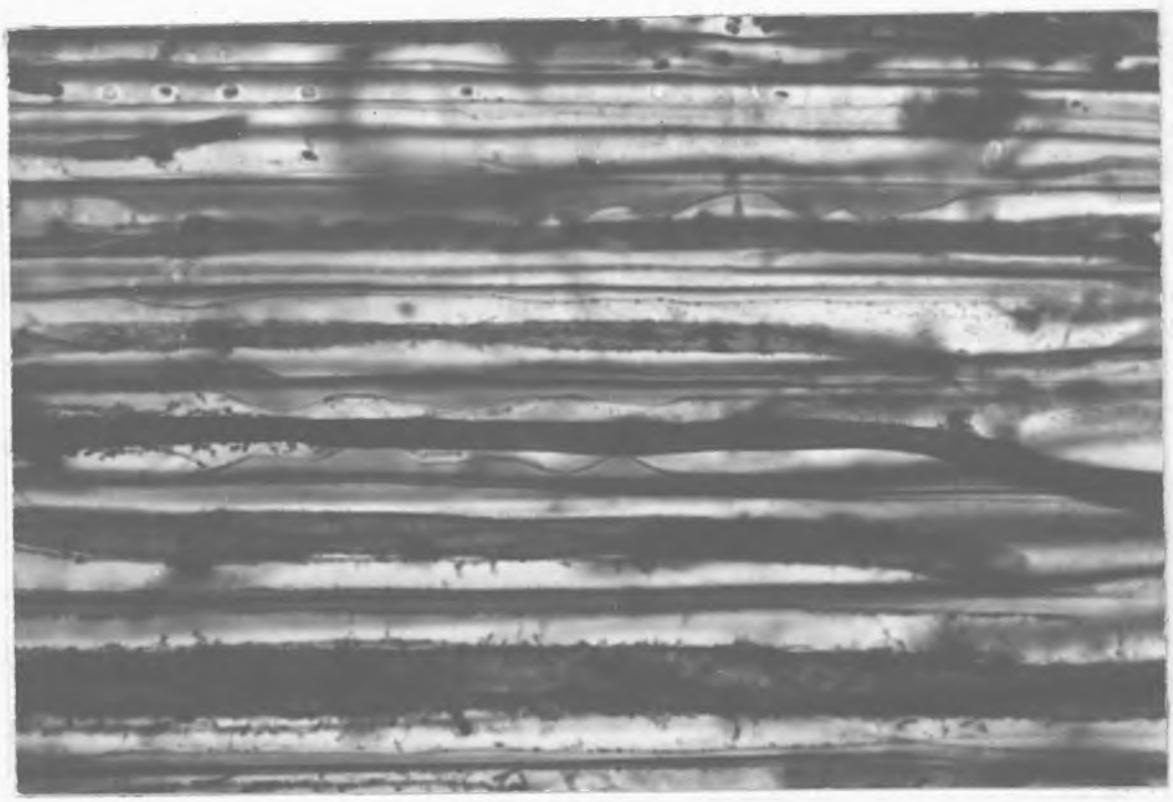


Fig. 22 - RLS Birch showing lumen erosion by C. globosum in 3 weeks - 500 X

Fig. 23 - RIS Birch showing diamond shaped cavities formed by C. globosum in 3 weeks - 787 X (Polarized light)

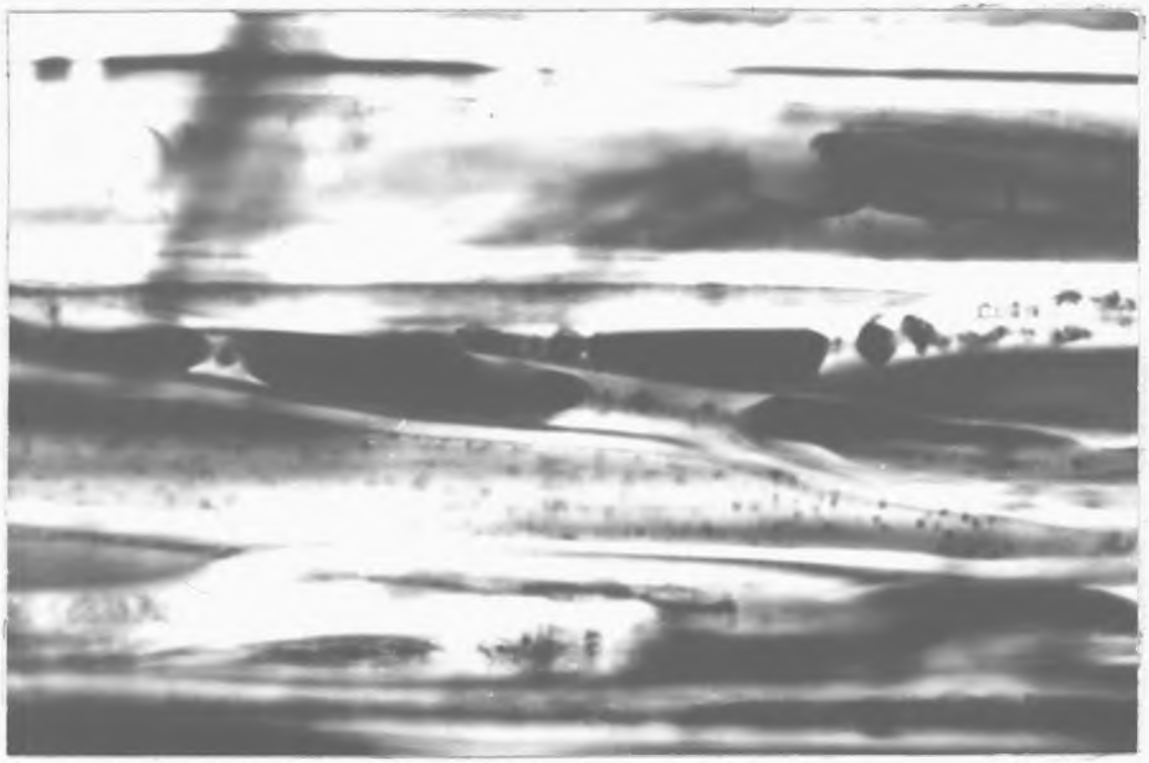


Fig. 24A.- RIS Birch showing chain of elongated and small cavities formed by C. globosum in 3 weeks - 500 X (Polarized light)

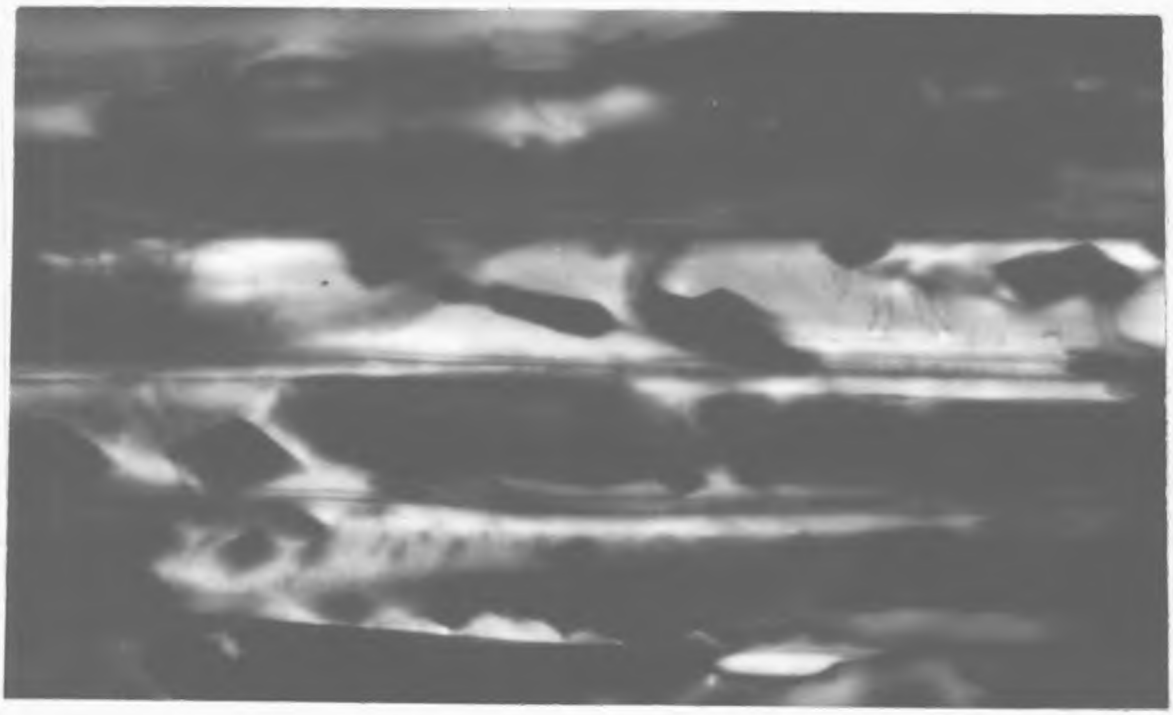


Fig. 24B - RIS Birch showing cavities of different shape formed by C. globosum in 3 weeks - 500 X (Polarized light)

Fig. 25A - RIS Scots pine showing developing cavities formed by C. globosum in 6 weeks - 1250 X

Fig. 25B - RIS Scots pine showing developing cavities formed by C. globosum in 6 weeks - 1250 X (Polarized light)

Fig. 26 - RIS Scots pine showing cavities formed by C. globosum in 6 weeks - 500 X

Fig. 27 - RIS Scots pine showing 'T-branch' formed by C. globosum in 6 weeks - 787 X

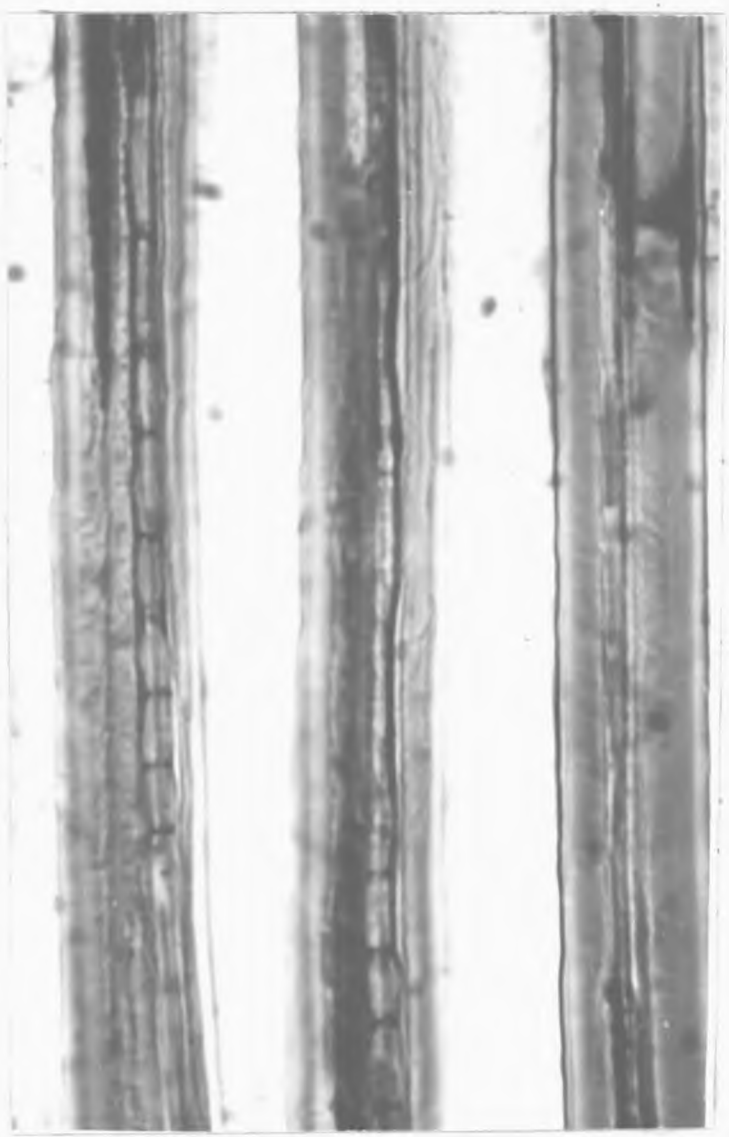


Fig. 28 - RIS Scots pine showing hyphae of C. globosum in the middle lamella and primary wall region - 6 weeks - 500 X

Fig. 29A - TS Scots pine showing colonization by C. globosum in the middle lamella and primary wall region, and forming cavities opposite to each other in the adjacent tracheid walls - 6 weeks - 320 X

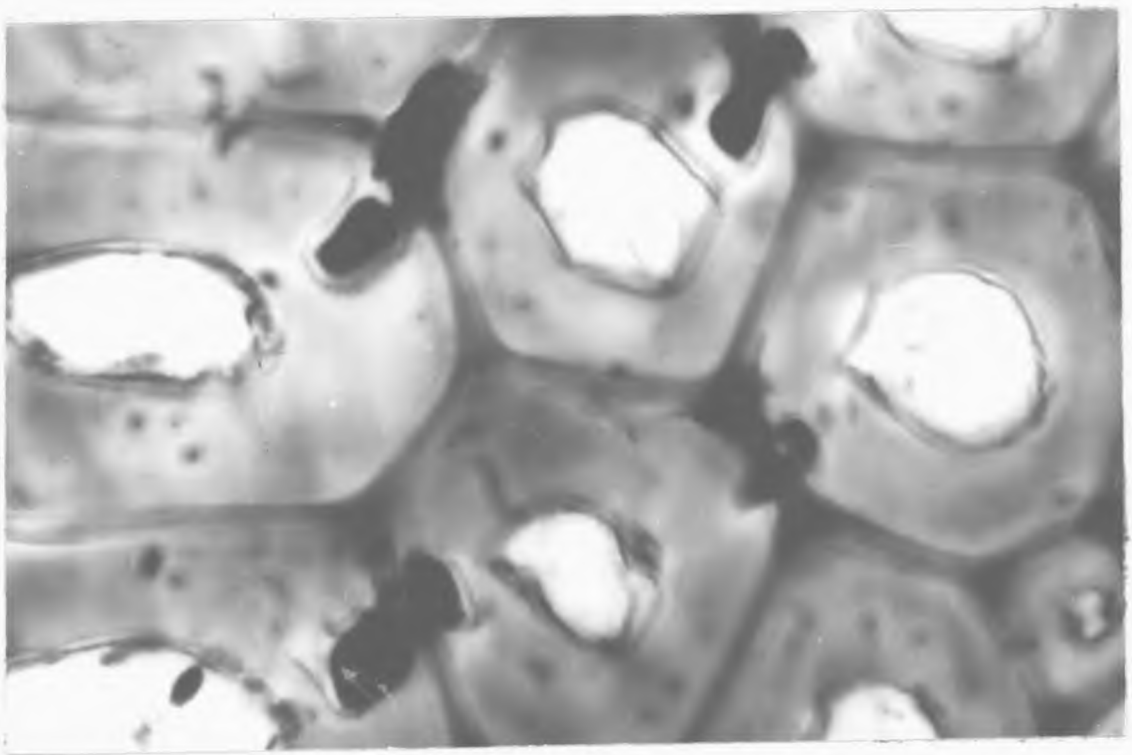


Fig. 29B - TS Scots pine showing cavities opposite to each other in the adjacent tracheid walls - 6 weeks - 787 X

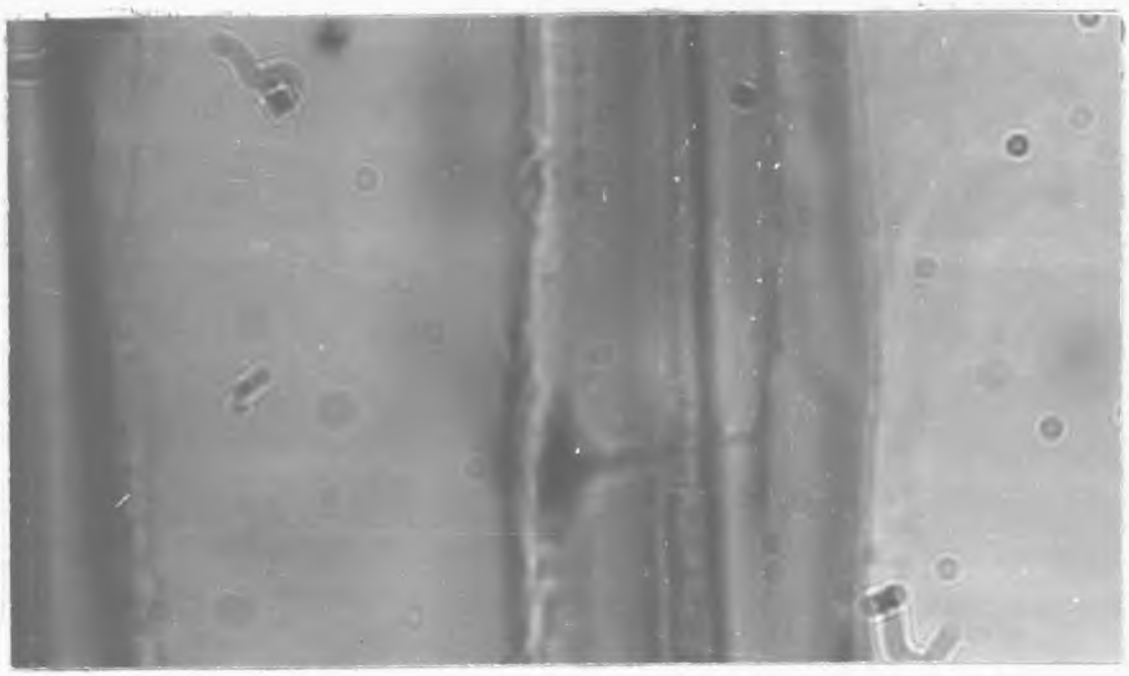


Fig. 30 - RIS Scots pine showing migration of hypha of C. globosum through $S_2$ layer, and the branch given off from this hypha forming cavity like structure - 6 weeks - 1250 X

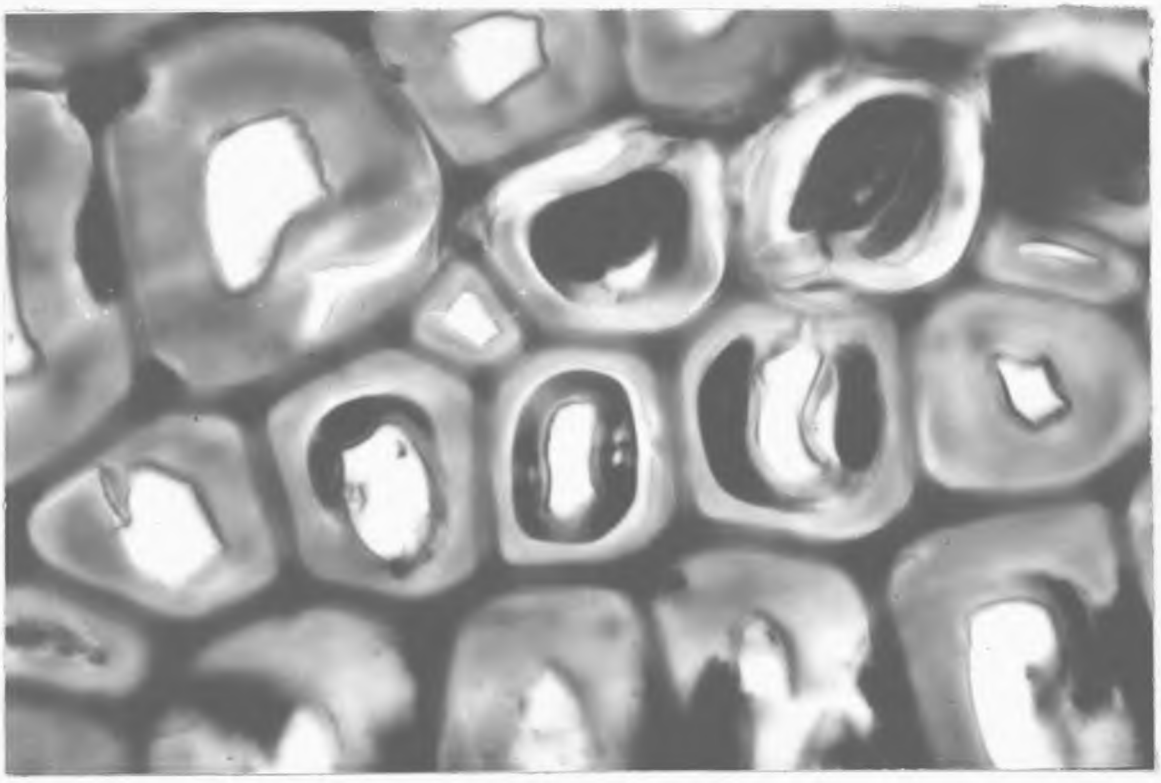


Fig. 31 - TS Scots pine showing degradation of cell wall by C. globosum in 6 weeks - 500 X

## General discussion and conclusions

The work was undertaken at the end of 1967. Until then very little information was available on the seasonal distribution and succession of fungi on debarked wood. Corbett and Levy (1963) gave some general information on the distribution of fungi in fence posts situated in three different environments and suggested a pattern of succession of fungi. Further information was added after Butcher published his papers in 1968. The present project was planned to study several aspects of the successional colonization of fence posts by fungi. The results have been discussed earlier in four different sections: I - seasonal distribution of fungi and fungal succession on birch and Scots pine fence posts, II - laboratory tests of fungal succession, III - interactions between the fungi which could affect the fungal colonization, and IV - wood destroying ability of certain fungi associated with the fungal succession. From the current investigation the following general conclusions can be drawn :

1. A technique has been established that is suitable for isolating fungi in depth from a post in the field.
2. The isolation can be carried out in the open air with very little evidence of chance contamination from air-borne spores.
3. Most of the fungi colonizing the superficial layers of the post do not penetrate below the surface. The general pattern that emerges is that a large number of fungi colonize the surface and superficial layers, and after a time a smaller number of fungi are able to penetrate beyond 5 mm. depth. Once these fungi have made the initial break-through, the Basidiomycetes appear and seem capable of developing rapidly in the centre of the post, where competition is low. Later the Basidiomycetes migrate towards the surface layers.
4. The establishment of fungi on the surface and immediately beneath the surface layers is highly influenced by the competition and interaction between the fungi, but at 25 mm and 45 mm depths

association between the fungi is of greater significance and competition is largely absent.

5. The intensity of bacterial ~~attack~~ infection on the surface layers of the posts has some apparent effect on the subsequent colonization by certain species of fungi.

6. The succession of fungi does not reach beyond the mould and sapstain stage in above ground zones, but it reaches the Basidiomycete stage in the ground line zone. The pattern of fungal succession at the ground line zone from the surface to the centre of the post is bacteria - mould - sapstain - soft-rot - Basidiomycetes, although the position of the soft-rot fungi in this sequence is of some doubt.

7. The fungal activity is very high on the surface layer for the first 4 or 5 months of exposure, when the number of species reaches a climax. After 5 months the number of species declines and colonization by fresh fungal species including those which had been already eliminated from the post is very rare. It is therefore suggested that the succession of fungi occurs in two phases. In the first phase both air-borne spores and fungal hyphae from the soil are involved in succession. The second phase begins with the depletion of surface nutrients and the fungal colonization may be mainly through hyphal contact. The surface layers in this phase are probably not very suitable for the growth of fungi developing from spores.

8. Basidiomycetes usually enter the wood in the second phase of succession when the number of other fungi on the surface layers is greatly reduced. The entry by Basidiomycetes into the wood during the first phase of succession when there is intense activity on the surface, may depend on the ability of the decay fungus to suppress other fungi by its antagonism or by the interaction between the non-Hymenomycetes.

9. The colonization and development of Basidiomycetes is associated with the presence of the sapstaining fungi Ceratocystis piceae and

Phialophora sp. Parasitism and saprophytism of these organisms by Basidiomycetes appears to be involved in their development. With the establishment of decay fungi most of the non-Hymenomycetes are eliminated from the zone.

10. Certain sapstaining fungi which are also able to decompose cellulose have a greater chance of persisting for longer periods than many other non-Hymenomycetes and these staining fungi can have some significant role in the fungal succession.

11. There is some evidence that interaction and association influences the fungal succession but it is difficult to assess this clearly. For example, in a test Discula pinicola was found to be antagonistic to several fungi but not to P. versicolor. Interaction of D. pinicola with other fungi may facilitate the entry of P. versicolor by suppressing the growth of those fungi which could have inhibited the colonization by P. versicolor.

12. Tests of fungal interaction have shown that T. viride when growing actively inhibits the growth of P. versicolor but not Botryodiplodia and when T. viride was killed the antibiotics left in the blocks retarded the growth of P. versicolor to a very great extent. However, when Botryodiplodia was introduced after T. viride was killed, the growth and rate of decay by P. versicolor was much higher than in the sequence where the sapstain fungus was not introduced. This shows that Botryodiplodia by its metabolic activity can possibly inactivate the antibiotics of T. viride when the latter fungus is either killed or inactive. The presence of sapstaining fungi may be one of the factors influencing the entry of Basidiomycetes in a post colonized by T. viride.

13. The colonization by fungi depends on (1) the intensity of spores present and their rate of germination and (2) the mycelium contact. Their establishment as dominant fungi would depend on several factors including their interactions and associations with these fungi.

14. Chaetomium globosum produced much greater weight loss when it was grown on a cellulose medium and was subcultured successively for 4 to 6 times at weekly intervals in the cellulose rich medium, before finally testing its soft-rot ability. The pattern of attack, the cavities formed and the decay in the cell wall show some considerable difference when the fungus was tested with and without being subjected to subculture. The general morphology of the fungus does not change in the treatment. Hyphae of the fungus which has been subcultured several times often migrate through the middle lamella and primary wall region and later attack the $S_1$ and $S_2$ layers of fibres and tracheids. The fungus forms characteristic 'T' branching in Scots pine. In birch the cell wall decomposition was very heavy and the loss in weight due to decay was very high.

## SUMMARY

The investigation of fungal ecology on birch (Betula sp.) and Scots pine (Pinus sylvestris) fence posts revealed that the fungal colonization followed a definite sequence. The establishment of fungi in the posts was influenced by several factors. To study this aspect of fungal ecology techniques were developed to obtain fungal isolates in the field from the surface layers and known depths of the posts. The isolation of small particles of decayed wood and their inoculation in the petri dishes containing different media was carried out in the field. The periodic isolations of fungi from the surface and at 5, 25 and 45 mm depth have shown that a very large number of fungi colonized the surface layers of the post and at 5 mm depth but very few fungi penetrated beyond 5 mm. The competition and interactions between the fungi were near the surface layers and beyond that the association between fungi was of greater significance in the establishment of Basidiomycetes. The pattern of fungal succession was postulated for three zones, (a) at ground line, (b) 18 inches above ground line, and (c) six inches below the top of the post. A punch card for the description of cultures of wood inhabiting Fungi Imperfecti was evolved.

Interactions between certain fungi isolated from the posts during this study were tested. In one of the tests where the blocks of wood were exposed to Botryodiplodia sp., Trichoderma viride and Polystictus vercicolor in succession in different sequences showed that Botryodiplodia sp. was able to overcome the inhibitory effect of T. viride in the establishment of P. versicolor. In another test on interactions a sapstain fungus Discula pinicola was found antagonistic to some moulds, sapstain and soft-rot fungi but was overgrown by Polystictus sp. The influence of interactions between fungi in the fungal succession is discussed in the text.

In the soft-rot ability test of some of the fungi isolated from the posts it was found that several test fungi produced soft-rot cavities in hardwood but the same fungi did not form any soft-rot cavities in soft-wood. The soft-rot ability of Chaetomium globosum when tested after the fungus had been subcultured several times in Abrams medium with 10 gm. of Whatman cellulose added was found to produce different types of cavities and degradation of cell wall in birch and Scots pine.

## APPENDIX I

An attempt was made to develop a punch card system for generic identification of the common wood inhabiting fungi, based on some of the gross culture characters, and the physiological and microscopic characters. The characters used in describing the cultures of Fungi Imperfecti have been mentioned earlier (c.f. p. 24 ). A description of the culture characters of some fungi isolated during the ecological studies is given in the following pages. It can be seen from the descriptions that certain fungi showed very significant variations in the gross culture characters e.g. Fusarium spp. and Cephalosporium sp., but many other fungi showed greater consistency within the genus. It appears from the results that it is possible to develop the punch card for description of cultures of wood inhabiting Fungi Imperfecti on a similar pattern to that described for the cultures of wood-rotting Basidiomycetes by Cartwright and Findlay (1958). However, a further investigation is needed in this work before a card for the description of wood inhabiting Fungi Imperfecti can be fully devised.

## APPENDIX I

## DESCRIPTION OF CULTURE CHARACTERS OF SOME OF THE MEMBERS OF FUNGI IMPERFECTI

| | COLOUR | CHANGE IN COLOUR | UNIFORM OR VARIED | OUTLINE | ELEVATION | TEXTURE | MARGIN | GROWTH RATE | COLOUR OF MEDIUM | CONIDIA | PYCNIDIUM | HYPHAE | SPORES | NO. OF CELLS IN A SPORE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FUSARIUM sp. | 4 | 21 | 23 | 24 | 28 | 30 | 34 | 38 | 44 | 47 | - | 56 | 59 | 63 |
| | 7 | 14 | 23 | 24 | 28 | 30 | 34 | 36 | 44 | 47 | - | 56 | 59 | 63 |
| | 3 | 19 | 23 | 24 | 28 | 30 | 35 | 37 | 44 | 47 | - | 56 | 59 | 63 |
| FUSARIUM sp. | 3 | 19 | 23 | 24 | 28 | 30 | 35 | 37 | 44 | 47 | - | 56 | 59 | 63 |
| | 4 | 21 | 23 | 24 | 28 | 30 | 34 | 35 | 44 | 47 | - | 56 | 59 | 63 |
| | 3 | 19 | 23 | 24 | 28 | 32 | 33 | 37 | 44 | 47 | - | 56 | 59 | 63 |
| FUSARIUM sp. | 3 | 19 | 23 | 24 | 28 | 30 | 35 | 37 | 44 | 47 | - | 56 | 59 | 63 |
| PAECILOMYCES VARIOTI | 4 | 21 | 22 | 24 | 27 | 29 | 33 | 37 | 45 | 47 | - | 56 | 59 | 61 |
| | 4 | 21 | 22 | 24 | 27 | 29 | 34 | 37 | 45 | 47 | - | 56 | 59 | 61 |
| PENICILLIUM sp. | 7 | 21 | 22 | 24 | 27 | 29 | 33 | 37 | 45 | 47 | - | 56 | 59 | 61 |
| | 7 | 21 | 22 | 24 | 27 | 29 | 33 | 37 | 45 | 47 | - | 56 | 59 | 61 |
| | 7 | 21 | 22 | 24 | 27 | 29 | 33 | 37 | 45 | 47 | - | 56 | 59 | 61 |
| PENICILLIUM sp. | 7 | 21 | 22 | 24 | 27 | 29 | 33 | 37 | 45 | 47 | - | 56 | 59 | 61 |
| CLADOSPORIUM HERBARUM | 7 | 21 | 22 | 24 | 28 | 31 | 35 | 36 | 43 | 47 | - | 56 | 60 | 63 |
| | 7 | 21 | 22 | 24 | 28 | 31 | 35 | 36 | 43 | 47 | - | 56 | 60 | 63 |
| | 7 | 21 | 22 | 24 | 28 | 31 | 33 | 36 | 43 | 47 | - | 57 | 60 | 63 |
| | 7 | 21 | 22 | 25 | 28 | 31 | 35 | 36 | 43 | 47 | - | 57 | 60 | 63 |
| | 7 | 21 | 22 | 24 | 28 | 31 | 33 | 37 | 43 | 47 | - | 57 | 60 | 63 |
| | 7 | 21 | 22 | 25 | 28 | 31 | 33 | 37 | 43 | 47 | - | 57 | 60 | 63 |
| ALTERNARIA sp. | 3 | 16 | 22 | 24 | 28 | 30 | 34 | 35 | 44 | 47 | - | 57 | 60 | 63 |
| AUREOBASIDIUM PULLULANS | 7 | 16 | 22 | 24 | 26 | 32 | 33 | 36 | 43 | 47 | - | 57 | 60 | 61 |
| | 7 | 16 | 22 | 24 | 26 | 32 | 34 | 35 | 43 | 47 | | 57 | 60 | 61 |
| | 7 | 16 | 22 | 24 | 26 | 30 | 34 | 36 | 43 | 47 | | 57 | 60 | 61 |
| BISPORA sp. | 3 | 16 | 23 | 24 | 28 | 30 | 34 | 34 | 41 | 47 | | 57 | 60 | 62 |
| PHIALOPHORA Sp. | 10 | 21 | 22 | 24 | 28 | 30 | 33 | 39 | 43 | 47 | | 57 | 60 | 61 |
| | 10 | 21 | 22 | 24 | 27 | 31 | 33 | 38 | 43 | 47 | | 57 | 60 | 61 |
| | 10 | 21 | 22 | 24 | 28 | 30 | 33 | 39 | 43 | 47 | | 57 | 60 | 61 |
| | 10 | 21 | 22 | 24 | 28 | 31 | 34 | 40 | 43 | 47 | | 57 | 60 | 61 |

# APPENDIX I (CONTINUED)

| | COLOUR | CHANGE IN COLOUR | UNIFORM OR VARIED | OUTLINE | ELEVATION | TEXTURE | MARGIN | GROWTH RATE | COLOUR OF MEDIUM | CONIDIA | PYCNIDIUM | HYPHAE | SPORES | NO. OF CELLS IN A SPORE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CEPHALOSPORIUM sp. | 3 | 21 | 22 | 24 | 26 | 32 | 33 | 36 | 41 | 47 | - | 56 | 59 | 61 |
| | 3 | 19 | 22 | 25 | 26 | 32 | 33 | 37 | 41 | 47 | - | 56 | 59 | 61 |
| | 9 | 21 | 22 | 25 | 26 | 30 | 35 | 37 | 41 | 47 | - | 56 | 59 | 61 |
| | 3 | 21 | 22 | 24 | 28 | 30 | 36 | 36 | 41 | 47 | - | 56 | 59 | 61 |
| | 9 | 21 | 22 | 24 | 26 | 32 | 33 | 37 | 41 | 47 | | 56 | 59 | 61 |
| | 3 | 19 | 22 | 24 | 26 | 32 | 33 | 36 | 41 | 47 | | 56 | 59 | 61 |
| CEPHALOSPORIUM sp. | 9 | 21 | 22 | 24 | 26 | 32 | 36 | 35 | 41 | 47 | | 56 | 59 | 61 |
| BOTRYTIS sp. | 10 | 21 | 23 | 24 | 28 | 30 | 34 | 37 | 41 | 47 | | 56-57 | 59 | 61 |
| | 10 | 21 | 23 | 24 | 28 | 30 | 34 | 37 | 41 | 47 | | 56 | 59 | 61 |
| TRICHODERMA VIRIDE | 3 | 17 | 22 | 24 | 28 | 30 | 34 | 37 | 41 | 47 | - | 56 | 59 | 61 |
| | 3 | 17 | 22 | 24 | 28 | 30 | 34 | 37 | 41 | 47 | - | 56 | 59 | 61 |
| CATENULARIA sp. | 7 | 21 | 22 | 24 | 27 | 31 | 33 | 38 | 43 | 47 | - | 57 | 59 | 61 |
| | 7 | 21 | 22 | 24 | 27 | 31 | 33 | 38 | 43 | 47 | - | 57 | 59 | 61 |
| GEOTRICHUM sp. | 2 | 21 | 22 | 24 | 27 | 29 | 34 | 38 | 41 | 47 | | 56 | 59 | 61 |
| VERTICILLIUM sp. | 3 | 10 | 22 | 25 | 28 | 30 | 34 | 39 | 41 | 47 | - | 56 | 59 | 61 |
| TUBERCULARIA sp. | 2 | 19 | 22 | 24 | 28 | 30 | 33 | 40 | 41 | 47 | | 56 | 59 | 61 |
| ASCOCYTA sp. | 10 | 16 | 23 | 25 | 28 | 32 | 35 | 40 | 41 | 48 | 50 52 55 | ? | 59 | 62 |
| DENDROPHOMA sp. | 10 | 21 | 22 | 25 | 27 | 30 | 34 | 39 | 43 | 48 | 50 52 55 | 57 | 59 | 61 |
| APOSPHAERIA sp. | 10 | 21 | 22 | 24 | 28 | 30 | 33 | 40 | 43 | 48 | 50 53 55 | 57 | 59 | 61 |
| | 10 | 21 | 23 | 25 | 28 | 30 | 34 | 40 | 43 | 48 | 50 53 55 | 57 | 59 | 61 |
| EPHELIS (MEXICANA) | 3 | 21 | 22 | 24 | 28 | 30 | 34 | 37 | 45 | 49 | | 56 | 59 | 61 |
| PEYRONELLAEA sp. | 7 | 21 | 23 | 24 | 28 | 30 | 34 | 38 | 43 | 48 | 50 52 55 | 57 | 60 | 60-63 |

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