A THESIS ENTITLED

Studies on Meria laricis. Vuill. - needle cast disease of larch.

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by

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MERIA LARICIS - Needle Cast of Larch

INTRODUCTION

(A) The fungus

Meria laricis Vuillemin - needle cast disease of larch is a serious disease in larch nurseries in Europe and North America. The fungus attacks the needles, causing browning and premature defoliation which can seriously weaken and even kill the seedling.

The disease was first described by Mer (1895) from material collected at a nursery near Nancy in 1890 and from subsequent examination of severe attacks occurring in nurseries around the Gerardmer forest during the summers of 1893 and 1894.

The pathogen showed a septate mycelium and produced 'bunches' of conidia projecting through the stomata near the mid-rib on the lower surface of the needle. Conidia were sometimes found in a similar position on the upper surface in severely attacked needles. No mycelium was found in the branches or even the short shoots, and it was suggested that infection in subsequent years resulted from conidia produced from mycelium over-wintering in the fallen

infected needles. This fact in turn suggested a control measure, namely the collecting and burning of all infected needles.

Mer considered that the pathogen attacked the weaker trees to a greater extent than the stronger, but this conclusion was not confirmed by Peace and Holmes (1933) who stated that disease damage did not seem to be dependent upon the health of the tree. Vuillemin (1896) described the fungus in detail from Mer's material and named it Meria laricis. The mycelium consisted of branched hyphae showing a definite sheath; these hyphae being inter-cellular with no haustoria. The unusual funnel-shaped lumena of the hyphal cells (used by a banking of protoplasmic contents on each side of the septa) were considered as a diagnostic character that could be used when fructifications (conidial clusters) were absent. Peace and Holmes (1933), however, found that this phenomenon was by no means constant, occurring only among the older hyphae.

Prior to spore formation, a thickened hyphal branch grew towards the sub-stomatal chamber and proceeded to swell into an aseptate mass. This mass underwent two transverse septations followed by several oblique

septations, eventually forming a two-layered tissue of cells with dense contents - an upper fertile layer, and a lower sterile layer which came to form a mucilaginous cup protecting the fertile cells. The fertile cells next elongated and the aseptate hyphae produced by each cell grew up through the stomatal ostiole. Each of these hyphae subsequently branched dichotomously in a more or less regular manner, each branch dividing into 1 - 3 cells. Each cell, just below the septum, produced a sterigma which cut off conidia consecutively, a new conidium being formed subsequent to the shedding of each mature conidium. Occasionally a second conidium was found to be forming before the first was shed.

The formation of conidia in succession from a single sterigma noted by Vuillemin was overlooked in appreciations and interpretations of his work by later workers (Peace and Holmes (1933) and Dreschler (1941)) Peace and Holmes showed that the phenomenon occurred in culture and while assuming that it also took place in nature made no observation of the fact. Assuming that they were the first to observe the phenomenon they used the diagnosis to throw doubt on the affinities between Meria and Ustilaginales suggested by Vuillemin.

To a certain extent this criticism of the

connections between Meria and the Ustilaginales is justified as this successive production of conidia from a phialide is a common feature of many of the genera of the Fungi Imperfecti.

Vuillemin further considered that the conidiophores of Meria were analogous with the promycelium of the Ustilaginales. The production of several basidiospores from each basidial cell in the Ustilaginales is very similar to spore production in Meria, but it must again be emphasised that spore production (from a phialide) in Fungi Imperfecti is very similar to Meria. This analogy could not therefore be disputed until nuclear behaviour at the time of spore delimitation was observed.

Several other resemblances between Meria and Ustilaginales also led Vuillemin to propound these affinities. Anastomosis between spores was not observed but anastomosis between fertile cells, a type of anastomosis occurring in Thecaphora in the Ustilaginaceae was common. Although the brand spores of Ustilaginales normally have thick walls and germinate after being released from the host, the genera Doassansia and Entyloma possess thin walled brand spores that germinate in situ. The elongated promycelium produced grows out

and ruptures the host epidermis and produces 'basidiospores' externally. This is very reminiscent of Meria with the production of hyphae by thin walled fertile cells and consequent formation of conidia outside the host. The fertile cells of Meria are considered as analagous with the brand spores of the Ustilaginaceae.

One of the difficulties encountered by Vuillemin in this consideration of affinities between Meria and the Ustilaginales was in connection with damage caused to the host by the fungus. Most Ustilaginales cause little if any noticeable damage to their host during the vegetative phase of their life history, the damage occurring usually during the reproductive stage, while the vegetative phase of Meria kills the host tissue. However, as the fructifications were formed such a short time after needle browning became apparent, Vuillemin considered that there was no differentiation in time or space between the vegetative and reproductive phases.

Seeking further affinities Vuillemin also suggested that the origin of spore bearing branches in Meria was identical with that of the origin of the ascus in some Ascomycetes. Asci were suppressed as distinct organs, however, as the special conditions of parasitism rendered

them superfluous, although this is equally true of many other Ascomycetes. In his view Meria indicated new affinities between the Ascomycetes and Ustilaginales and was one of the stages in a series of transformations which, by progressive adaptations to parasitism, led from the primitive Ascomycetes to the characteristic Ustilaginales. Meria was not, however, strictly attributable to either group. Consequently, Vuillemin created a new family, the Hypostomaceae, to contain Meria with Hypostomum, another parasite of conifers described in the same paper as the type genus. This family was considered to occupy a transitional position between the Ascomycetes and Ustilaginales as previously mentioned.

The most important diagnostic characteristic of this family is the formation of the nodule of fertile and sterile cells in the sub-stomatal chamber of the leaf prior to spore formation.

Another description of the disease was given by Hartig (1899), who, unaware of Vuillemin's work, named the causal fungus Allescheria laricis. However, since the name Allescheria had been used earlier by Saccordo and Sydow (Sylloge fungorum - v.14 p.464) for an

entirely different fungus. Allescheria Hartig was renamed Hartigiella by Sydow (ex Lindau), who made it the sole representative of a separate sub-family, the Hartigiellaceae which he distinguished by the production of a single conidium on each cell of the conidiophore - an example of careless observation. Lindau later withdrew this sub-family in subsuming H. laricis under the sub-division Botrydeae in the Mucidinaceae-Hyalosporae. He mentioned that the peculiar method of spore formation in the species had no counterpart amongst the Hyphomycetes. Vuillemin (1905) showed that Hartig's fungus was identical with the one he himself had described and that his name - Meria laricis had priority over the later Hartigiella. He also stressed once again the significance of the sub-stomatal mycelial nodule in the taxonomy of this fungus and the creation of a special family in which it could be placed.

The significance of this nodule, however, is not recognised by Hartig (1899), Saccordo (1899, 1902, 1913), Lindau (1910) and Fiori (1912), who all preferred to place Meria within the Fungi Imperfecti. In this they are followed by all the later workers. Further descriptions of the fungus by Hiley (1921), Peace and Holmes (1933) and Batko (1957) make little mention of its taxonomic position.

Peace and Holmes observed that the conidiophores that grew out in culture from stout hyphae were more or less identical with those formed in nature. Hence the hyphae from which the conidiophores originated were considered equivalent to the sub-stomatal masses formed in the needle - an equivalence tending to disagree with the morphological significance imputed to these masses by Vuillemin. Peace and Holmes suggest that the systematic position of the fungus is uncertain and that a decision concerning its true position could only be reached when the nuclear behaviour of the fungus had been correctly traced.

Wakefield and Bisby (1941) in a classification of recorded British Hyphomycetes into wet and dry spored types place Meria in the dry spored group - the first mention of this particular spore characteristic. Clements and Shear (1934) express the classification as

O. Moniliales F. Moniliaceae Macronemeae
Meria laricis

(B) The fungus in culture

A major contribution to the study of this fungus was the work of Peace and Holmes (1933), the first authors to study the fungus in culture as well as to investigate the disease in the field. In culture two distinct morphological

strains, named 'a' and 'b', of the fungus were evident.

In isolations made from nature the 'b' strain tended to occur more commonly than the 'a'. Various experiments to determine the effect of external conditions upon the growth of the fungus in culture were attempted, although the results obtained were rather irregular. Artificial infection experiments were also carried out with especial regard to the effects of humidity and sheltering of the seedlings during the early part of the year. As a result of these investigations and a series of field trials, a system of control using various sulphur sprays was recommended.

(C) Distribution

The disease was first reported from the Continent towards the end of the nineteenth century. Mer (1895) recorded occurrences at Nancy, Gerardmer and in the Vosges mountains, and Hartig (1895) stated that it was found throughout Germany. Baudisch (1903) reported it from Northern Austria, and Fiori (1912) from two localities in Italy. A single record from Norway was given by Jørstad (1925). All these records appear to refer to the disease on European larch (Larix decidua. Mill). The first records in this country were given by Hiley (1921 and 1925) when the disease was discovered on both European Larch and Western larch (Larix occidentalis Nutt.) in

Scotland. Peace and Holmes (1933) carried out a large number of inoculation experiments with different species of larch, but only obtained infection on European and Western larch, although one needle of Japanese larch (L. leptolepsis (Sieb. and Zucc.) Murr.) was infected. Neither the Korean larch (L. gmelini (Rupr.) Litvin) nor the Asiatic larch (L. sibirica Ledeb.) could be infected artificially.

The first American record appears to be that of Ehrlich (1942) who noted disease symptoms on L. occidentalis in Idaho and intimated that the disease was also well established in the Pacific Northwest.

Robak (1946) reporting from Norway noted symptoms on plantation trees, between 13 and 20 years old, and also the first occurrence of this disease on Japanese larch and Hybrid larch (L. eurolepis Henry) Langner (1951) in Germany noticed an unidentified fungus attacking certain Hybrid larches produced during breeding experiments. From his description of the disease symptoms the pathogen was probably Meria. In Czechoslovakia, Prichoda (1954) mentioned that the fungus was recorded on European larch in 1900, but not reported again until 1952.

Orlos (1951) has recorded the disease on Siberian and Asiatic larch.

It is apparent that until 1946 the disease was more or less confined to European larch and Western larch or was not sufficiently severe on the other larch species for the symptoms to be noticeable. However, in 1954, in Britain, outbreaks of the disease on Japanese and Hybrid larches were noticed (Batko 1955) in several Forestry Commission nurseries scattered over the country. As a result of these attacks the present investigation was undertaken.

THE FUNGUS IN CULTURE

(1) Materials and Methods

(a) Collection and storage of field material

Forestry Commission nurseries in various parts of the country sent material of European, Hybrid and Japanese larch considered to be attacked by *Meria*. This material was stored at 3°C over 60% Sulphuric Acid in a desiccator until isolations of the fungus could be attempted. Cultures of *Meria* isolated from diseased trees in 1954 by the Forestry Commission were also supplied by the Forest Pathologist.

(b) Isolation

Due to the comparatively slow growth of the fungus in culture, contamination of agar plates by other fungi during isolation of the pathogen was particularly evident. Consequently all attempted isolations were carried out in a special inoculation chamber which was thoroughly sprayed with 2% phenol thirty minutes prior to the isolation and again disinfected immediately afterwards. The following technique, a modification of that used by Plakidas (1948), gave the most consistently effective method of isolation.

From the diseased material needles showing about 50% browning were selected. These were sterilised in 0.1% mercuric chloride for five minutes, and then rinsed in

sterile water. Using a sterile scalpel the needles were cut into small segments and these were washed with two changes of sterile water. The segments were finally placed on the surface of 2% malt agar plates, about four segments to each plate and incubated at 25°C. After a week the plates were examined. If mycelium had grown out from the cut end of the needle segment it was examined under the microscope, and, if it showed typical Meria characteristics, transferred understerile conditions to a slope of 2% malt agar.

Later, as a result of experiments on the fungus in culture, the technique was slightly amended. The medium used was Dox-yeast, this giving a more rapid and even growth, and the plates were incubated at 20°C instead of 25°C. In spite of the precautions observed, much trouble was caused by contamination with other organisms. Normally six replicate plates from each batch of three needles were inoculated, firstly to overcome losses due to contamination, and secondly to obtain as many differing cultures of the fungus as possible.

Isolations from diseased material from the various nurseries were continued for a two year period. Attempts to store infected needles at 40% humidity in a desiccator over 50% sulphuric acid at 3°C, for any length of time, proved unsuccessful as isolations of Meria from this

material could not be made after fourteen days.

(c) Maintenance of stock cultures

A stock culture collection of Meria was obtained by the following single spore isolation technique, a modification of that used by La Rue (1920). Isolates from diseased needle segments were incubated on Dox-yeast agar slopes at 20°C for three weeks. Using a sterile paint brush spores from these cultures were transferred to a marked area on the surface of agar in a thin glass petri dish. With cultures showing a large spore concentration it was found advisable to dip the brush in sterile water before transferring the spores, in order to lessen their concentration in the agar plate. These plates were incubated for 48-60 hours at 20°C while the spores commenced germination and then examined under the microscope. Early attempts at this method using Difco plain agar gave slow and uncertain germination, but later Dox-yeast agar was substituted and the results were more successful.

Under the X10 eyepiece and X10 objective a field was found that contained only a single germinating spore. A brass dummy objective with a circular cutting edge corresponding in size to the ⅔" objective with a X10 eyepiece field, was sterilised in a flame and substituted for the normal ⅔" objective. This was slowly racked down

until the cutting edge was just below the surface of the agar and hence it removed a small disc of agar holding the germinating spore. This disc of agar was transferred, with a sterile needle, to a slope of Dox-yeast agar and incubated at 20°C. At least five germinating spores from each plate, i.e. from each single isolate were transferred by this method.

Each batch of five tubes was examined after three weeks' incubation and those showing differing characteristics, mainly of texture, colour and degree of discoloration of the medium, were kept for further examination. Sub-cultures were made from each selected tube, one maintained at 20°C and sub-cultured every two months, and the other kept in reserve at 0°-3°C and only sub-cultured every six or twelve months.

(2)

(a) Characterisation of strains

Peace and Holmes (1933) described two distinct strains of Meria in culture. One strain, faster growing and profusely sporing, was termed the 'a' strain; the other slower growing and sparsely sporing the 'b' strain.

These strains, however, were regarded as aggregates as within either there was a wide range of variation. Also saltant sectors tended to occur in the cultures unless these were of monosporous origin. Some of these saltants

were obviously intermediate between the two strains but a few could not definitely be classified as either 'a' or 'b'. One of the more outstanding was a fluffy white variant that produced few spores and did not discolour the medium. This variant was also isolated during the present investigation.

Before commencing work on the determination of strains of Meria a random sample of stock cultures was taken and this was used as inoculum in a preliminary experiment to determine the medium most suitable for growth. Each isolate was sub-cultured on the listed media and incubated for three weeks at 20°C. The incubated tubes were then examined and the colony size estimated on a purely arbitrary basis. (Table 1).

Table 1.

Medium: Agar slopes	Culture tubes				
	A	B	C	D	E
Dox + glucose	4	4	4	4	4
Dox yeast	1	1	1	1	1
Mannitol	5	5	5	5	5
Potato dextrose	2	2	2	2	2
Potato	3	3	3	3	3
2% Malt	3	3	3	3	3
5% Malt	1	1	1	1	1
2% Malt 40% Sucrose	4	4	4	4	4
Maize	3	3	3	3	3
Maize yeast	2	2	2	2	2
Oatmeal	5	5	5	5	5

Colony size: Arbitrary scale. Largest - 1
Smallest - 5

5% malt and Dox- yeast agar gave the largest colony size, and it was decided to use Dox-yeast for all culture work, as the initial establishment and growth of the fungus was more rapid on this medium than on 5% malt.

In order to investigate the presence of different strains of Meria, the stock culture collection was examined for differing morphological characteristics. All stock culture tubes were sub-cultured and incubated at 20°C for four weeks and the following morphological characteristics were then noted:

(a) Colour - the colour of the mycelium.

(b) Type of mycelium - (i) whether the mycelium was growing entirely on the surface of the agar or if the culture also showed submerged growth; (ii) general mycelial appearance whether mainly aerial-fluffy or mostly closely appressed to the agar surface - recumbent.

(c) Discoloration of the medium - a proportion of the stock culture tubes showed distinct discoloration of the agar. According to Peace and Holmes this discoloration was due partly to brown granular accretions produced by the Hyphal extremities and partly to the fact that the whole mycelium became green and that the green colouring matter diffused out into the agar. During the present investigation very little evidence of crystals was found. Extensive examination of plate cultures,

which showed the same discoloration phenomenon, revealed very few crystals which could not possibly account for the intensity of colour produced. The main part of the colour had therefore diffused out from the hyphae and consequently, preliminary attempts were made to determine the nature of colouring matter. Different solvents were added to a batch of tube cultures showing a particularly intense greenish black colour in an attempt to dissolve out the colouring agent. (Table 2). The tubes were shaken vigorously and allowed to stand for half an hour before examination. Very little colour was apparent in any of the solvents immediately after shaking with the exception of the tube containing water. After standing however, several of the tubes showed a diffusion of colours.

Table 2. The effect of different solvents on the substance causing discoloration of the medium in certain cultures of *Meria laricis*

Solvent	Degree of diffusion of colour
Water	Much
Absolute Alcohol	Little
Carbon tetrachloride	None
Petroleum ether	Very little
Ether	Very little

Since it proved impossible to extract any appreciable quantity of the pigment, further attempts to identify it were abandoned.

(d) Degree of deliquescence of culture surface - Peace and Holmes in their description of the two strains of Meria mentioned that the 'a' strain showed a deliquescent mycelial surface while the 'b' strain was characteristically dry. Wakefield and Bisby (1941) dividing the Hyphomycetes into dry and slime spored groups, placed Meria in the former, but obviously examined only a limited range of cultures. By examination of the stock culture tubes it was evident that the degree of deliquescence of the mycelial surface was dependent upon the number of spores formed. The spores are formed in definite clumps and isolates with a definitely wet surface have a large number of these clumps. (See Plate 5). In some cases these clumps coalesce so that the culture surface appears as one mass of spores. On the other hand the tubes with a dry mycelial surface have fewer clumps and, in one particular strain, a complete absence of spores. Plate cultures showing deliquescent areas within normally dry mycelium were examined microscopically and it was noticeable that the number of spore clumps in the wet areas was more than treble the number in the dry areas.

From the examination of a large number of different isolates it was possible to distinguish four distinct growth forms of the fungus. The description of these growth forms, from morphological appearance, is as follows:-

Strain a - a pink semi-aerial mycelium partially submerged. The mycelium is dry and there is no discoloration of the medium.

Strain b - A yellow-green recumbent mycelium entirely on the agar surface. Mycelium is dry and there is much discoloration of the medium; the colour passing through light to dark brownish green to almost black.

Strain c - A tan or tan pink recumbent mycelium growing on the surface of the agar only. The surface of the culture is deliquescent and there is no discoloration of the medium.

Strain d - A white fluffy mycelium which is half below and half above the agar surface. The mycelial surface is dry and there is no discoloration of the medium.

(See PLATE 1)

A more detailed morphological examination was carried out to determine further differences between these growth forms.

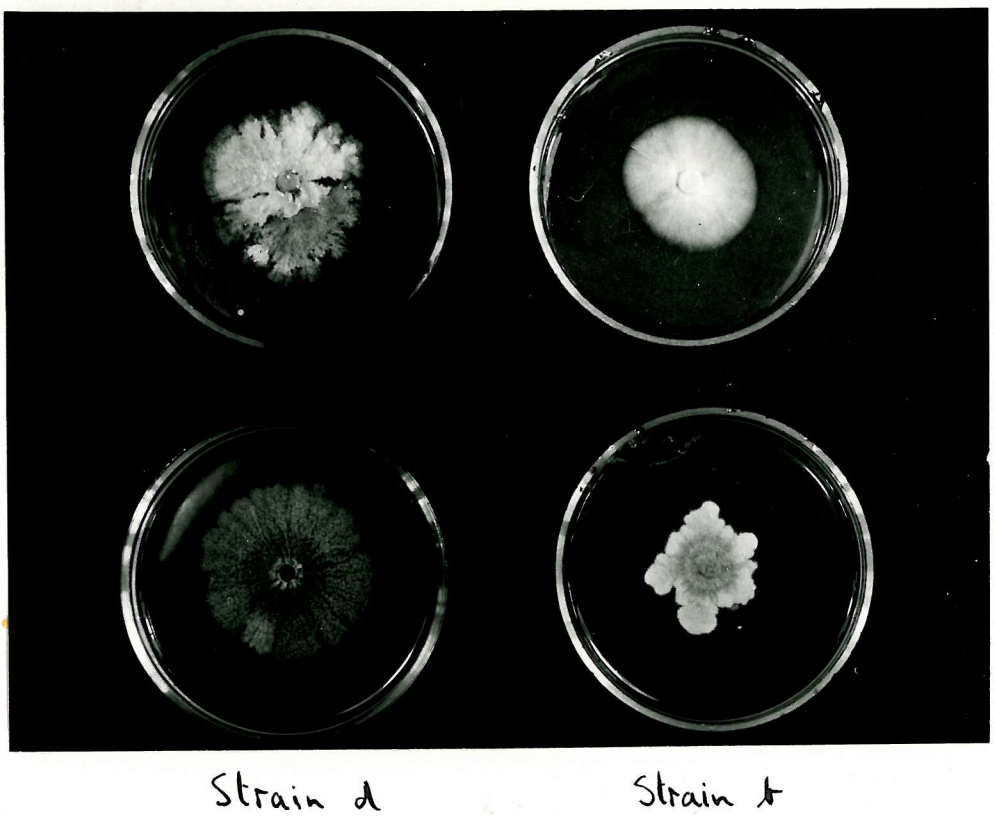


PLATE 1:

Appearance of the four cultural strains of Meria in plate culture.

Detailed morphological examination

(i) Spores

Peace and Holmes described two distinct spore types, macroconidia - 9μ long and constricted at the centre, and microconidia, formed on germination of the macroconidia 3.6μ long and not constricted at the centre.

During the current investigation both types of spore were found, together with many gradations between the extremes. The measurements here however, refer to the macroconidia only as these were the spores produced directly from the mycelium. The microconidia were formed only during germination of the macroconidia.

Care must be taken, however, in using spore number and size and diagnostic features for the separation of distinct strains. Dodsell and Christensen (1923), working on Helminthosporium and Johann (1923) with Fusarium noted that both the number and the size of spores is altered under differing cultural conditions. Peace and Holmes also noted that the spores of Meria show an increase in length in old cultures. Consequently the following observations were made on spores from recently isolated cultures grown under identical environmental conditions.

(a) Spore number

From microscopic examination of cultures obvious

differences in the number of spores formed by each growth form was evident. The deliquescent cultures of the c strain showed the greatest number of spores, while the dry cultures of the a and d strains showed the least.

A quantitative estimate of the number of spores produced by each strain was made as follows:

A single germinating spore isolate of each of the a, b and c strains was cultured on a slope of Dox-yeast agar. As no spores of the d strain were available, a very small hypha, equivalent in size to the germ tube of a spore, was used as inoculum. The tubes were incubated at 20°C for three weeks. A standard volume of water was added to each tube, which was then shaken to dislodge all the spores, and the number of spores present in 1 ml. of this suspension was counted under the low power of the microscope, using a haemocytometer. Five different drops were taken from each tube and a mean obtained. The results are given in Table 3.

Table 3: Mean spore number of strains of Meria laricis under standard growth conditions.

Strain	Mean number of spores
a	0
b	150
c	1280
d	0

Smaller volumes of water were added to replicate slopes of strains a and d but even then no spores were found. The microscopic examination of large numbers of plate cultures of both strains revealed very few spores in the a cultures and only a single spore in the d cultures, which was not viable.

(b) Spore size

Using the suspensions from the experiment above, at least fifty spores from each drop were measured and a mean length was obtained.

Table 4: Mean spore length of strains of Meria laricis under standard growth conditions.

Strain	Mean spore length μ
a	-
b	10
c	8
d	-

(ii) Mycelium:

All the strains had septate multinucleate hyphae. It was apparent from a cursory examination that the growth rates of the strains were different, and a preliminary experiment to measure this difference was set up.

Using a flamed cork-borer, of diameter 9 mm., discs of mycelium and agar were cut from well established plate cultures of each strain. These discs were transferred, under sterile conditions, to the centre of fresh plates and incubated at 20°C. Five replicates from the same culture of each strain were used and the diameter of these cultures was measured after twenty-four days.

Table 5: Mean diameter of colonies of Meria strains after twenty-four days.

Strain	Mean diameter in cms.
a	21.7
b	29.3
c	31.7
d	23.4

The colony diameter bears a relationship to the relative amounts of aerial and submerged mycelium. Strains b and c produce mainly surface growths but in strains a and d growth is mainly submerged.

(2)

(b) Morphology and Cytology of the fungus in culture

Few observations on morphology and cytology of the mycelium have been made by previous workers. Vuillemin (1896 A), describing the mycelium in the needle, mentioned that the hyphae were septate and

In a later paper (1896 B) Vuillemin described Meria in more detail. The hyphae were branched with some anastamoses, usually 2.5 - 4μ and occasionally up to 10μ in diameter. The hyphal walls were soon transformed into a gelatinous sheath and there was a narrow lumen. This lumen, however, dilated in contact with the septum due to an uneven thickening of the wall, and the protoplasmic contents of the cell were banked at each end. Vuillemin used this observation as a method of identifying the parasite if typical fructifications were absent. Peace and Holmes, however, found this character was shown only by some of the older hyphae, and fact confirmed by the present worker.

A. Methods

Detailed microscopic examination of plate cultures of Meria was not successful owing to the density of the Dox-yeast medium. As colony growth was slow and irregular on water agar, the following technique, a modification of that of Rees and Jinks (1952) was devised using a thin smear of Dox-yeast agar.

Cleaned microscope slides were sterilised in a large covered crystallising dish in an 80°C oven for at least one week. Petri dishes, each containing a damp filter paper and the two halves of a microscope slide, were autoclaved at 15 lbs./sq.in. for ten minutes. In the

sterile inoculation chamber the centre of each sterile slide was painted with a thin film of Dox-yeast agar using a sterile paint brush. The slides were then placed face upwards on the half slides in the petri dish and inoculum, either spores or a small piece of mycelium, was placed at one end of the agar smear. These dishes were incubated at 20°C for varying lengths of time, two weeks giving the best results. The resulting slide smears were then fixed and stained as follows:

(i) Fix in acetic-alcohol (with a few drops of aqueus ferric chloride to act as mordant) - 24 hours.

(ii) Stain in a drop of weak aceto-carmine in ferric acetate.

Attempts were made to obtain permanent mounts but these were unsuccessful.

B. Results

The mycelium consists of septate hyphae (PLATE 2) that branch in an irregular manner, although the overall effect is a regularly advancing hyphal edge. This is especially true of the b and c strains, where the hyphae tend to branch in one plane only. The a and d strains do not show such a distinct edge as the hyphae immediately begin to branch in several planes - those pointing downwards giving the submerged mycelium and the upwardly directed ones giving the colonies their typical fluffy

appearance. Anastamoses between branches are very common and all colonies quickly attain the tangled mycelial state. (PLATE 3).

The cells vary in length from 8μ - 18μ and are between 2μ and 4μ in diameter. The cell wall is fairly thin (see PLATE 2) in contrast to the thick walls described by Vuillemin and Peace and Holmes for the mycelium in the needle. The banking of the protoplasmic contents of the cell at the septum was only observed once in a c strain agar smear culture after thirteen days' incubation.

Normally the protoplasm is granular and evenly distributed. The cells are multinucleate; the nuclei being minute (PLATE 4). They are either evenly distributed amongst the cytoplasm or tend to be aggregated in the centre of the cell. Owing to their small size and the fact that they are buried in the granular material of the cytoplasm, it is difficult to get an accurate count of the number in each cell. The numbers appear to vary between three and nine or ten. Occasionally apparently uninucleate cells are found. The cells in the hyphal coils where abstriction of conidia is occurring show a reduction in nuclear number compared with those of the rest of the mycelium. This possibly indicates that nuclear fusion occurs prior to spore formation (see later section).

The only differences between the different strains of Meria were in their methods of branching, to give recumbent colonies in b and c strains and aerial and submerged colonies in a and d strains.

In general the observations of Vuillemin and Peace and Holmes have been confirmed but it must be remembered that their observations were confined to the fungus within the larch needle.

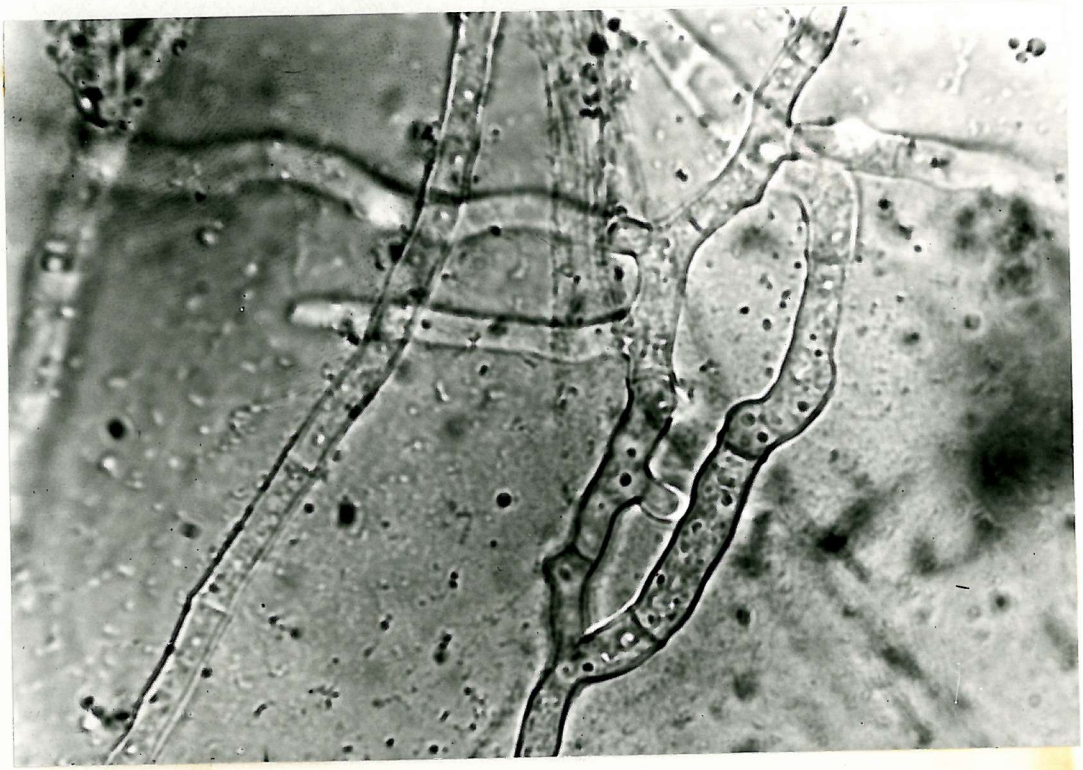


PLATE 2: Strain a showing septate hyphae.

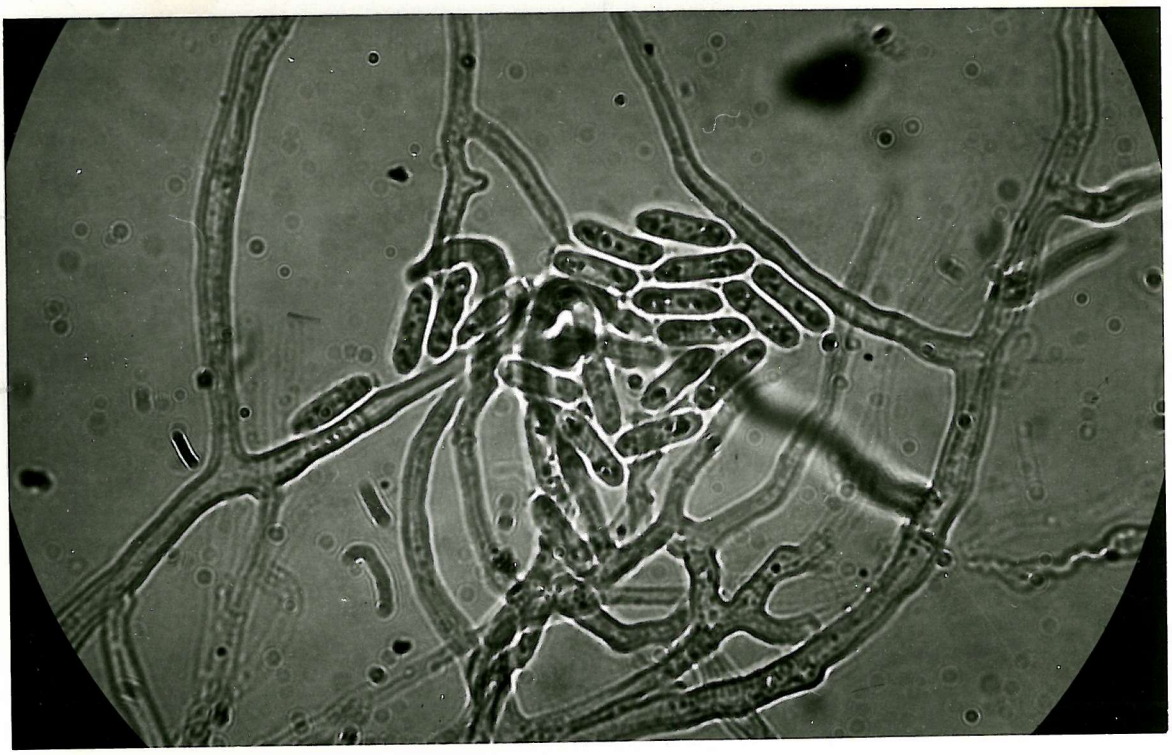


PLATE 3: Strain c hyphal anastomoses.

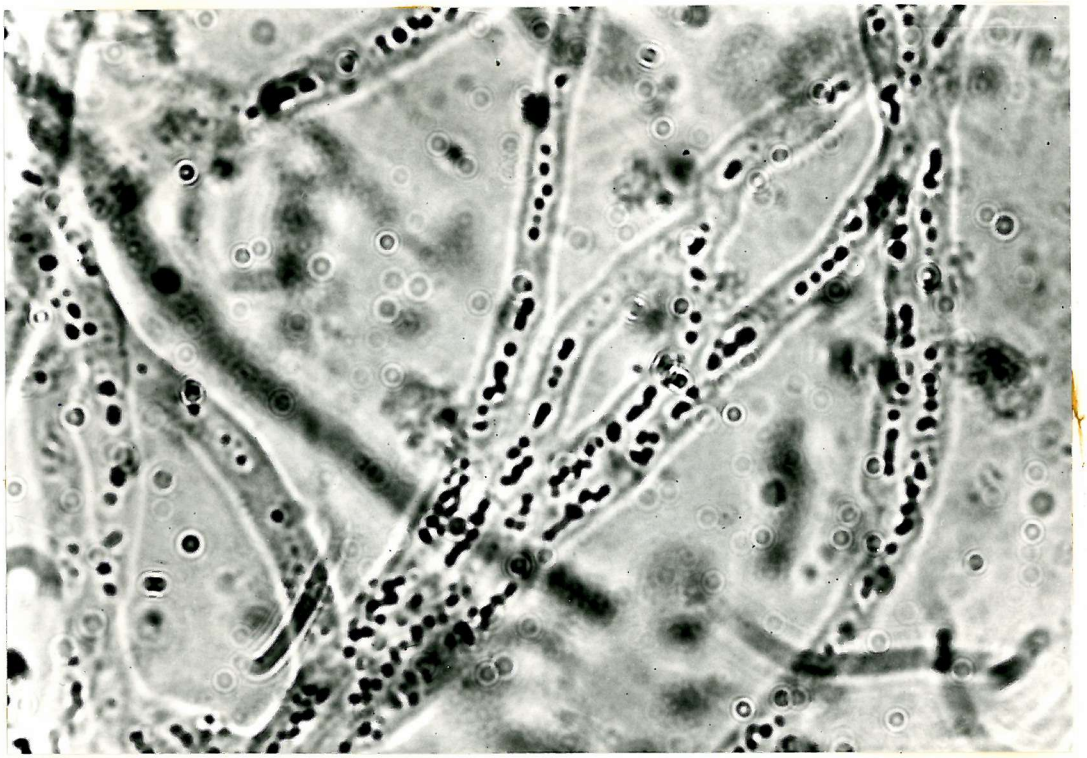


PLATE 4: Strain a multinucleate cells near colony edge.

GROWTH STUDIES ON THE FUNGUS IN CULTURE

Peace and Holmes are the only previous workers to study the growth of the fungus in culture. They used various techniques and found that optimum temperature for growth was between 15° and 20°C, although some growth was evident at 5°C.

To provide information for the investigation of the disease in situ and to help with artificial inoculation studies, the growth of fungus in culture was studied.

Materials and methods

Stock plate cultures of each strain, derived from single spore isolations, were used as inoculum in these experiments. Agar plugs were used as inoculum as a large number of these plugs could be cut rapidly from the agar of a plate culture using a sterile cork borer of diameter 9 mm. Each plug was then transferred with a sterile pointed scalpel to the centre of a test plate and incubated.

In the plate experiments the diameter of the colony was measured daily using a circular scale which consisted of a series of concentric circles, differing in diameter by 1 cm., drawn on graph paper to prevent inaccuracies arising by measuring two diameters. For some treatments tube cultures were also inoculated so that the morphology of the culture could be studied.

(1) Effect of composition of the medium on colony size.

From a preliminary tube experiment it was found that 2% Malt, Potato dextrose and Dox-yeast agars gave the best growth. A plate experiment was set up to record this growth difference. Five replicate plates of each strain on each medium were inoculated as outlined above and incubated for twenty-four days at 20°C. The colony diameter was measured and the mean of the replicates was used to construct the histogram. (PLATE 5)

Table 6: Mean colony diameter after 24 days of strains grown on different media.

Medium	Mean diameter of colony in mm.			
	a	b	c	d
2% Malt	19.4	21.8	18.5	18.9
Potato dextrose	17.4	22.0	25.5	17.9
Dox-yeast	21.0	34.4	33.0	21.3

Overall growth was best on Dox-yeast agar and the colonies had a more robust appearance than on the other media. The b and c strains showed the largest colony size on all media with the exception of the malt agar where all colonies were of a similar size.

A duplicate experiment using agar slopes was set up at the same time and the tubes were examined to

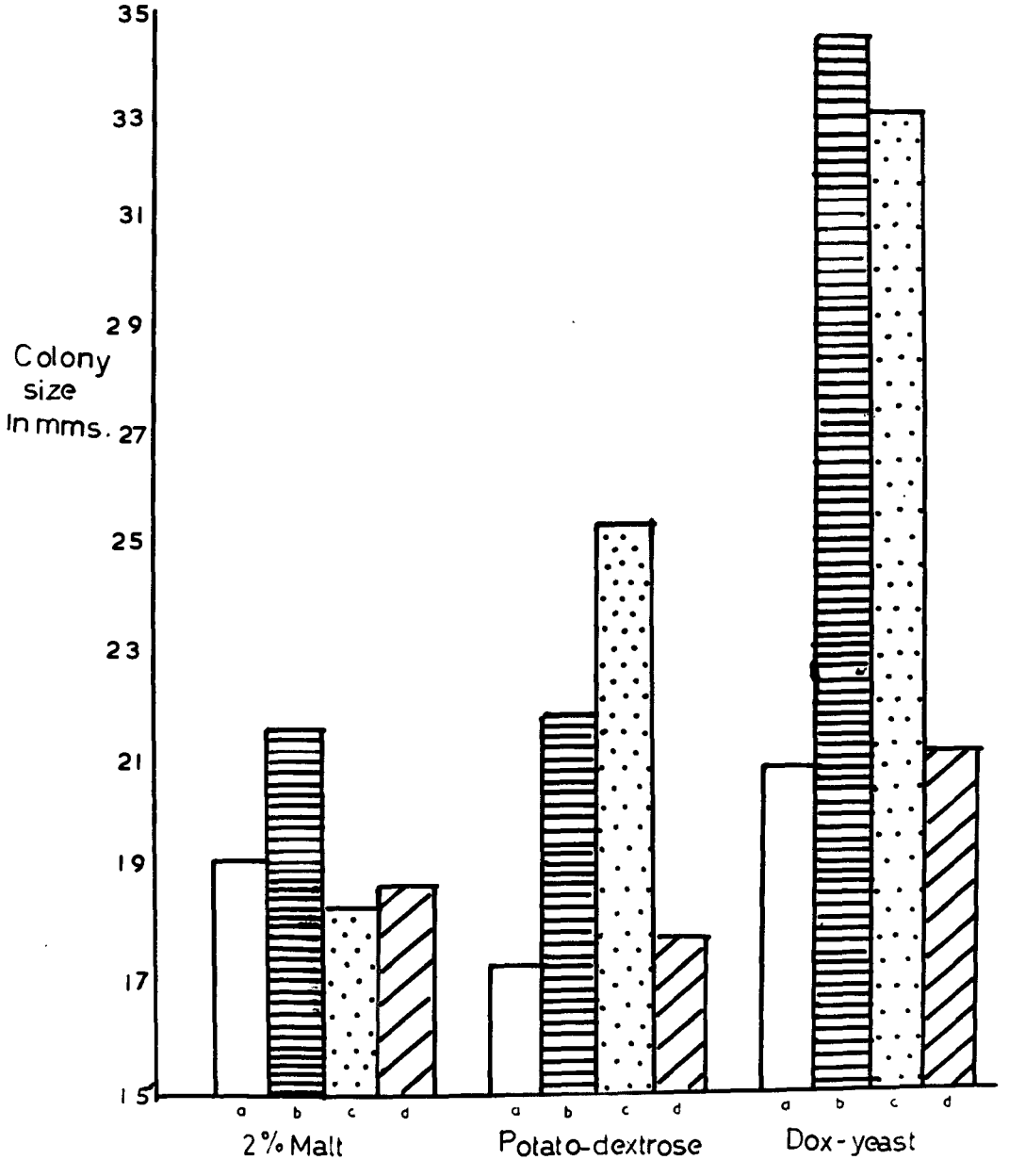


PLATE 5: The effect of different media on colony size after 24 days.

discover any morphological differences. Apart from the smaller colony size shown above, all treatments produced colonies typical of the strain concerned, although the d strain on Potato dextrose agar had less aerial mycelium than the other two media.

(2) Shake and plate culture

Brown (1923), in his studies on fungal growth, mentioned that the diameter of a fungal colony does not necessarily bear any relation to the amount of mycelium present. This was certainly obvious by examination of Meria colonies. The b and c strains showed the largest colony diameter due to the fact that the a and d strains had much submerged and aerial mycelium. Peace and Holmes (1933) measured the weight of mycelium by dissolving the agar with hot water, but it was found to be more convenient to grow the fungus in a liquid medium in flasks.

50 ml. of Dox-yeast medium, without agar, was added to 350 ml. conical flasks which were plugged into cotton wool and autoclaved as usual. The initial inoculum was obtained by homogenizing three slope cultures of each strain in a Waring blendor using 250 ml. of sterile water. 0.5 ml. of the resulting suspension was added to each flask with a sterile pipette. Three replicates of each strain were inoculated and then incubated for twenty-four

days at 20°C. Every other day the flasks were shaken to mix up the contents and to allow more air to dissolve in the medium.

Using a Buchner funnel and filter pump the contents of the three flasks were filtered through a filter paper. Because of inaccuracies this method of filtration was altered. The contents of the flasks were poured through weighed porcelain crucibles containing a wad of glass wool to retain the mycelium. These were dried overnight in the oven at 80°C, cooled and reweighed.

Table 7: Mycelial weight of strains grown in liquid culture.

Strain	Total mycelial weight in grammes
a	0.0792
b	0.0596
c	0.0526
d	0.0932

The colonies with the largest diameter, those of b and c strains, actually have the smallest mycelial weight, while the colonies with the smallest diameter a and d strains, have the greatest mycelial weight. The mycelium formed by the d strain is approximately 50% heavier than that of both band c strains and 20% heavier

than the a strain. The growth in all flasks was fairly diffuse although there was a tendency for the formation of mycelial mats. The medium in the b flasks was slightly discoloured.

The most serious disadvantage of this method of studying growth rates is the lack of oxygen available to the mycelium. This can be partially overcome by shaking the flasks every day - an inconvenient practice in long term experiments. As two workers in the University department were both interested in fungal growth, a shaker was designed (Ballance 1959) that would shake continuously three hundred 350 ml. flasks. It consisted of four flat trays connected above each other and arranged about a pivot in pairs. The bottom pair were connected to a geared down electric motor via an eccentrically arranged rod. This gave a reciprocating shaking movement of about 40 cycles per minute and was run continuously during an experiment.

An identical experiment to the one described above was set up, using the shaker, and run for a similar length of time. The mycelium was filtered and weighed as previously but, because growth was much greater, the mycelium from individual flasks was filtered and a mean mycelial weight for each strain obtained.

Table 8: Mean mycelial weight of strains grown in shake culture.

Strain	Mean mycelial weight in grammes
a	.1223
b	.1072
c	.1054
d	.1602

The ratio of the weights of different strains was as before but the morphology of the mycelium was entirely different. In all strains the mycelium occurred as pellets which were either completely smooth (d strain), or showed small fluffy projections, (a, b and c strains). The pellets of the a and d strains were large (up to 2 cm. in diameter) while those of the b and c strains were small (less than 1 cm. in diameter). Similar results for other fungi have been recorded by Burkholder and Sinott (1945). The mycelial colour of all strains was identical with the previous experiment.

The medium from all strains was examined for spores, a few were found in the b strain and many in the c strain flasks, but none were found in the a nor d cultures.

This was obviously a more accurate and satisfactory method of determining the effect of different factors on growth, than that using solid media but only if sporulation

was not being considered. Also various other physical factors such as temperature could not be investigated using this method. One advantage of the method was the production of fairly large amounts of mycelium in a short time. A disadvantage, however, was the difficulty of preventing contamination, mainly by bacteria and yeasts, if large numbers of flasks were inoculated from one batch of mycelial suspension. Owing to the small size of the sterile inoculation chamber only twenty flasks could be inoculated without opening the door. Re-sterilization after every batch was impracticable, but otherwise strictly sterile conditions were observed in order to prevent this casual infection.

PLATE 6 shows a comparison of the growth of *Meria* in solid and liquid culture.

(3) Effect of temperature on growth and morphology

(a) Growth Peace and Holmes discovered that the optimum temperature for growth of *Meria* was between 15°-20°C and that slow but continuous growth would occur at 5°C. Since it was not possible to operate the shaker at a number of temperatures the plate culture technique (page 32) was employed to compare the effect of temperature on the growth rates of the different strains.

Plates of each strain were placed in incubators at

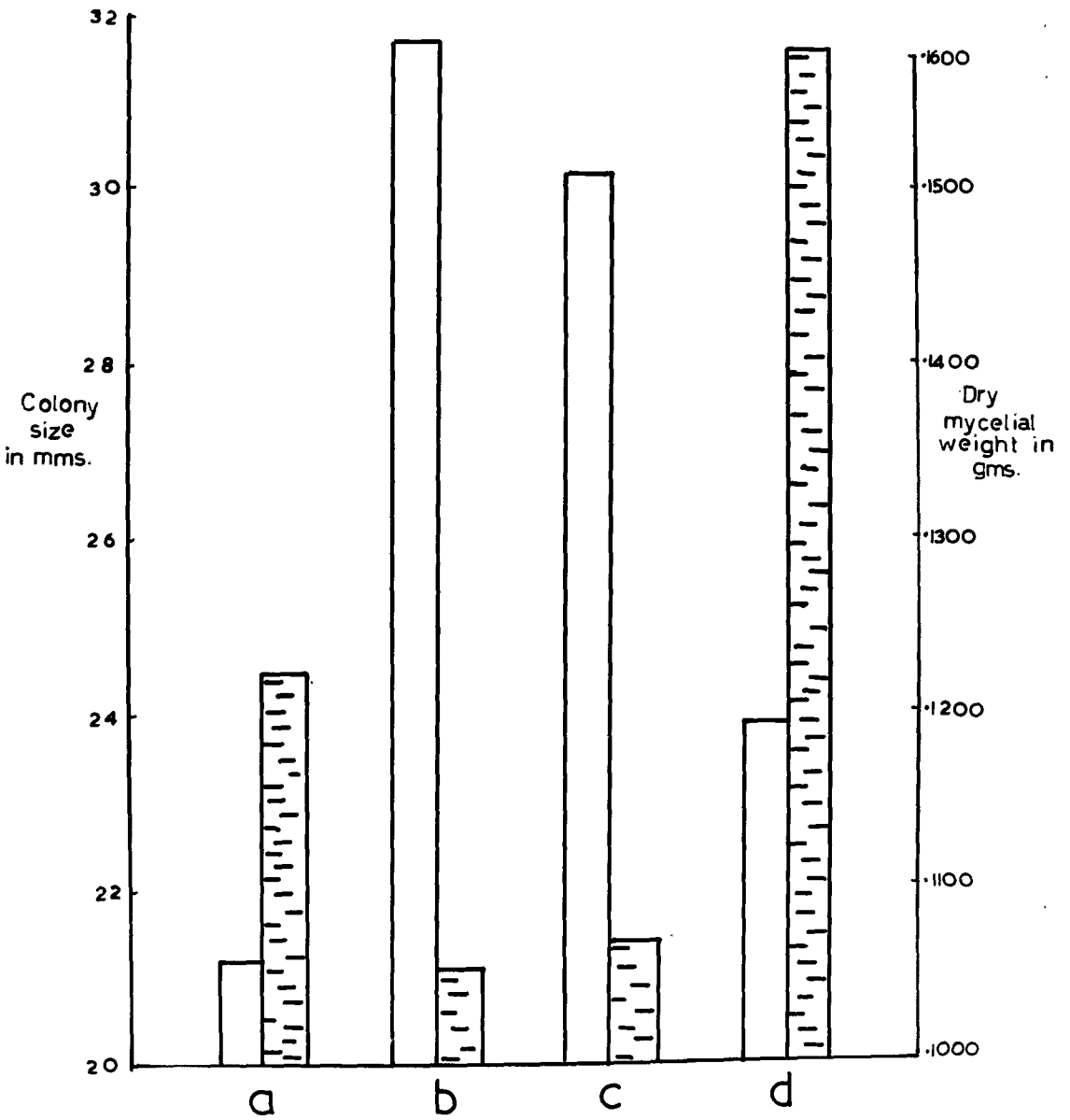


PLATE 6: Comparison of growth of Meria in solid and liquid shake culture.

25°C, 20°C, 15°C and 10°C and in a refrigerator at 0-3°C, three replicate plates at each temperature. The colony diameter was measured after twenty four days (TABLE 9).

Table 9: Effect of temperature on growth of different strains after 24 days.

Strain	Mean Colony diameter in mm. after 24 days				
	Temperature °C				
	0-3°	10°	15°	20°	25°
a	10	15.5	19.7	21.6	16.1
b	10	19.8	23.2	26.3	17.3
c	9.2	17.0	23.7	31.7	23.2
d	9.2	14.0	17.1	23.7	11.0

Inoculum diameter = 9 mm.

(See PLATE 7)

All strains show a fairly regular increase in growth as the temperature rises to 20°C, the optimum. There is a fairly rapid falling in rate between 20° and 25° and several slope cultures incubated at 30°C showed no growth whatsoever. All strains also showed slow but continuous growth between 0° and 3°C. Consequently it was practicable to maintain duplicates of the stock culture collection on agar slopes in the refrigerator - these only needing sub-culturing once every six or twelve months.

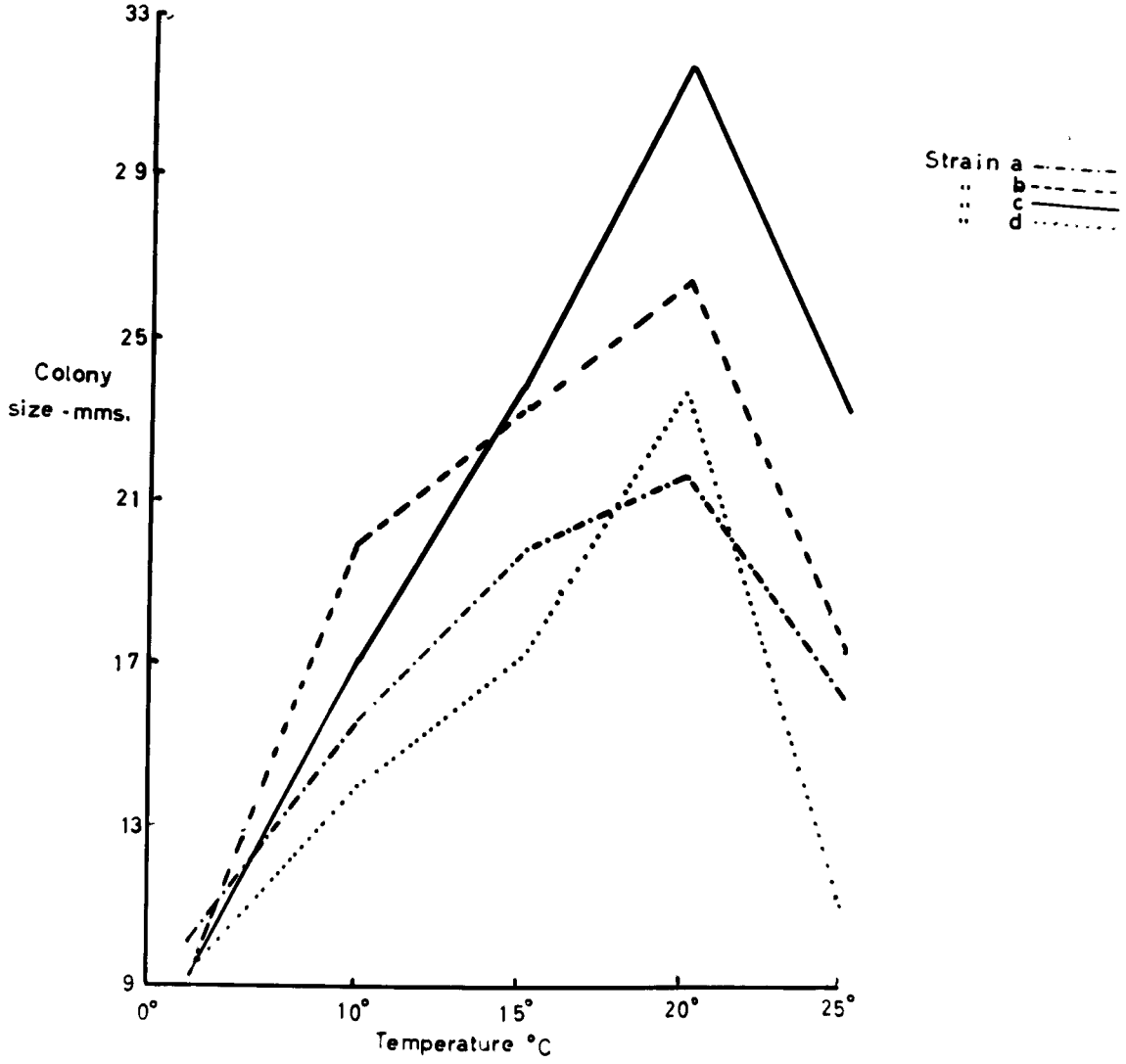


PLATE 7: The effect of temperature on growth. Colony size after 24 days. - Inoculum diameter 9 mm.

(b) Morphology A comparable experiment to study the effect of temperature on the morphology of the strains, in tube culture, was set up. All the strains, with the exception of the b strain, showed their typical characteristics at all temperatures.

At 0-3°C and 10°C the mycelium of the b strain was yellow in colour: after two months the medium had turned an intense greeny-black and there were deliquescent spots on the mycelium which, when examined, were found not to be spore clumps however.

Since prolonged incubation at low temperatures affected the morphology of one strain it was decided to investigate the effects of such incubation more fully. Several slope cultures of each of the different strains were left in the 10°C incubator for eight months. Owing to its construction the humidity inside the incubator remained fairly high and the agar did not dry up. These tubes were examined morphologically and if they showed abnormal characteristics there spores were also examined. The results were as follows:

a strain Both slopes showed large pink wet colonies although there were no spores present.

b strain One slope showed a lime yellow mycelium with many black deliquescent dots. These were found not to be spore clumps but there were a large number of

spores in this culture possibly due to the long incubation period.

One slope showed a brown mycelium and no discoloration of the medium. The number and size of spores present were similar to those of the above slope.

c strain In one slope there was a tan discoloration of the medium and a completely dry non-sporing mycelial surface.

d strain All the d strain cultures remained true to type.

All strains abnormal at 10°C reverted to the normal type when sub-cultured and grown at 20°C. This effect of prolonged growth at low temperatures on the morphology of the mycelium is interesting and its significance will be discussed later.

(4) The effect of pH on growth

Most fungi are tolerant of a fairly wide pH range, and a preliminary experiment was set up to discover if this were true of Meria and also to see if the different strains were separable by different pH optima.

Dox-yeast agar was made in the normal way, and after steaming, was poured into batches of test tubes. The pH of these was adjusted, using $\frac{N}{100}$ Hydrochloric acid and 20% KOH, to give a range from 1 to 10: the liquid agar being tested with pH paper. The tubes were then

autoclaved and before the agar had solidified the tubes were again tested to find any alteration in pH. All of the tubes showed a difference in pH but a suitable range from pH3 to pH10 was available, the tested tubes being discarded. The slopes were inoculated, incubated at 20°C for twenty-four days, and examined.

(See PLATES 8 and 9)

Some mycelial growth was apparent in all tubes. The more acid media pH3.1 and 4, were liquid jelly and the colonies were small and submerged, while the extreme alkaline media, pH9.2 and 10 were very solid and the colonies were compact and on the surface. From a superficial examination it appeared that there was a difference in optimum pH between the strains. Strains a and c showed the most robust growth and largest colony at pH4, while strains b and d had optimum colony size about pH6.

As mentioned in the previous section on the effect of temperature on growth, some of the cultures of a given strain tended to show characteristics of a different strain under certain conditions (10°C). This was also evident here in colonies grown under highly alkaline conditions - pH9.2. Two especially were very noticeable on a cursory examination.

1. Strain c at pH9.2 - most of the colony showed pink

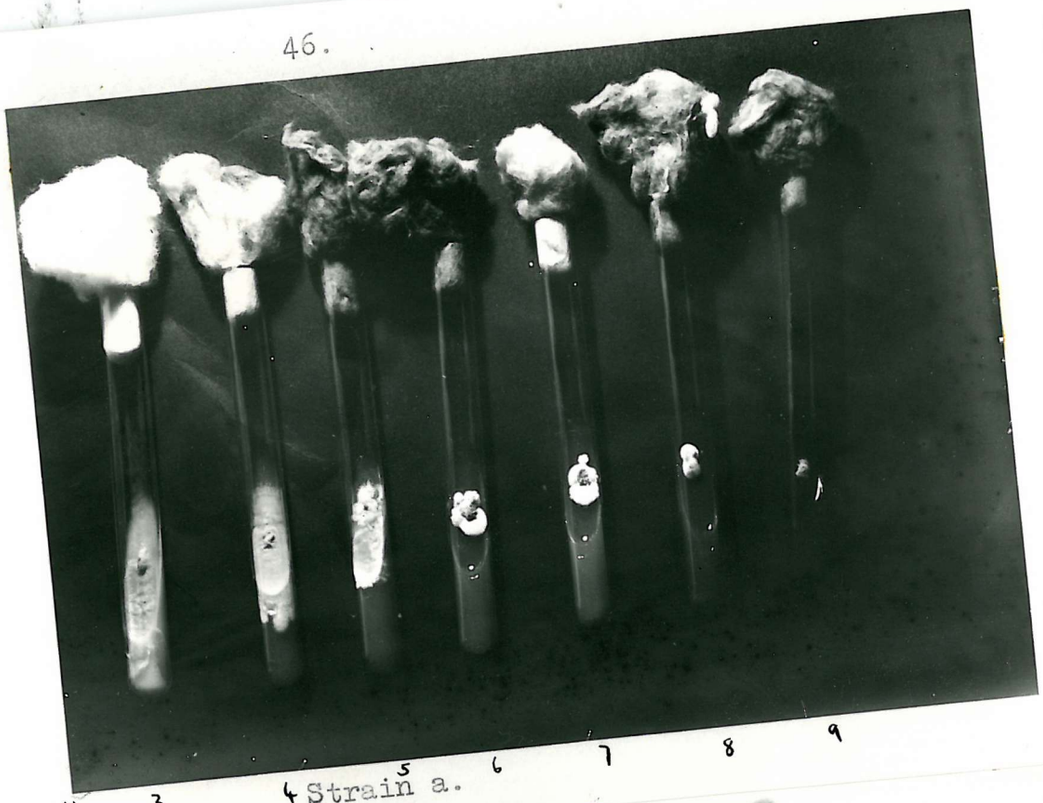
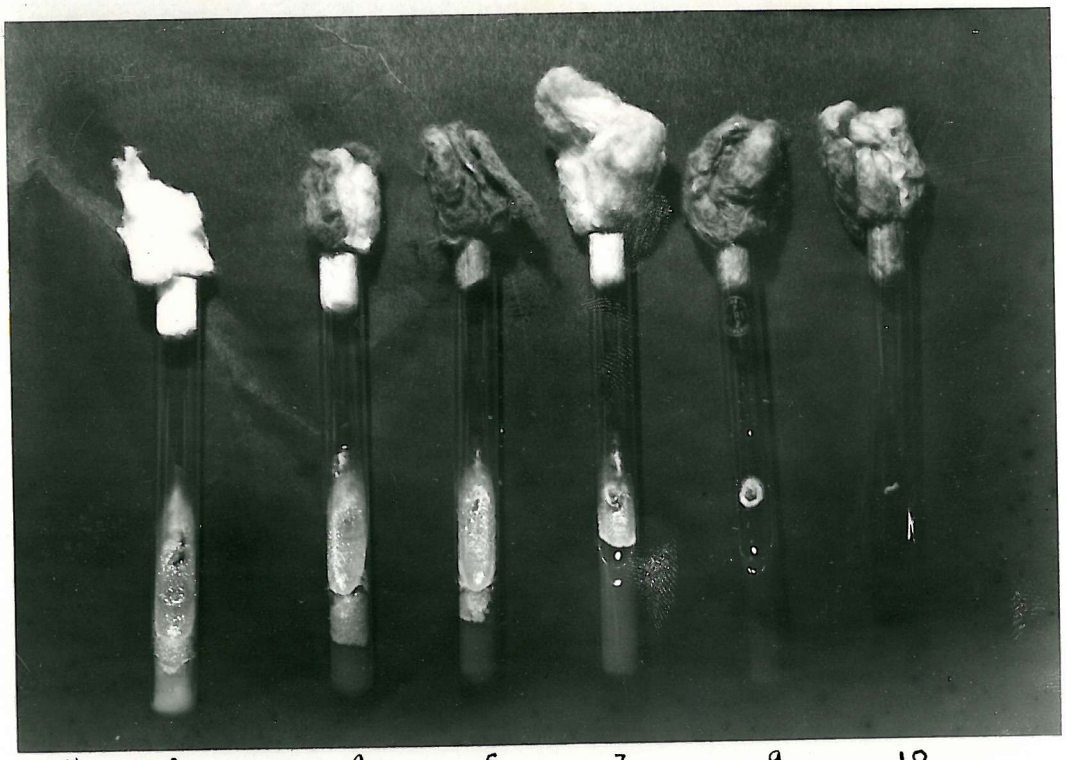
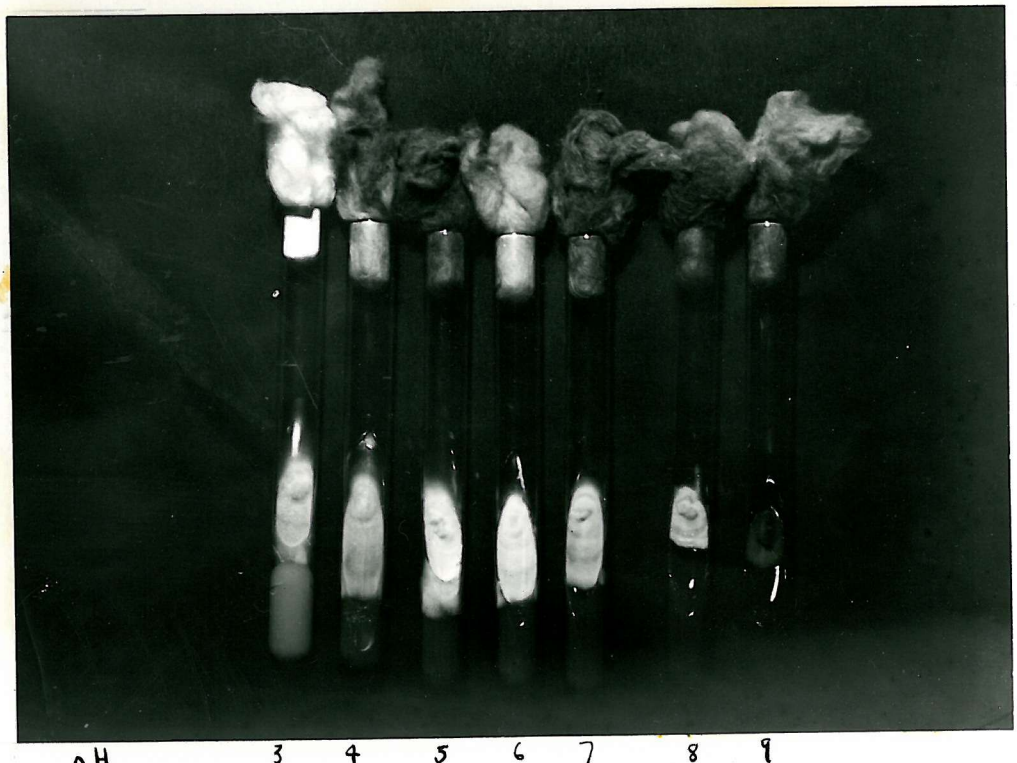


PLATE 8: The effect of pH on growth in slope culture.



Strain c.



Strain d.

PLATE 9: The effect of pH on growth in slope culture.

aerial mycelium typical of the a strain, and there was only a very small deliquescent area near the top of the slope. When recultured under normal conditions the whole colony grew as a pink aerial mycelium - a typical a strain. A definite change of strain had occurred, not a temporary and reversible change such as that found in the low temperature experiments.

2. Strain d pH9.2 - a large area of the mycelium was greeny brown in colour and there was a distinct discoloration of the medium beneath the colony. A small section near the base of the slope showed typical b strain characteristics. Several recultures from all parts of the slope, under normal conditions, showed distinct b strain characteristics, although some slopes had a distinctly brownish mycelium. Here again it appeared that an irreversible strain change had occurred.

This is the only recorded instance of any abnormal appearance of the d strain. It remained remarkably stable in culture throughout the whole period of experimentation.

A quantitative experiment to find the optimum pH for growth was set up using the flask shake culture technique. Replicate flasks of pH1 to pH10 were autoclaved. As previously, the pH of the medium was tested after autoclaving and a set of flasks showing a suitable range from pH2 to pH8 was inoculated with mycelial suspension and shaken for fourteen days The mycelium

from each flask was filtered, dried and weighed and a mean dry weight obtained for each treatment.

Table 10: Effect of pH on growth - Mean mycelial dry weight after fourteen days.

pH after auto-claving	Mean dry Mycelium weight in gms.			
	Strains			
	a	b	c	d
2.1	.0148	.0371	.0217	.0170
3.1	.1030	.0578	.0636	.1262
4.3	.1206	.0794	.0665	.2932
5.4	.2769	.0706	.0510	.2711
6.1	.1537	.0327	.0210	.2751
7.0	.0172	.0187	.0060	.0500
8.2	.0105	.0106	.0113	.0053

(See PLATE 10)

The previous observation, that Meria shows a wide range of tolerance to pH, was confirmed -all strains showing growth from pH2.1 to pH8.2. This was the only range tested as it proved difficult to adjust the pHof the flasks before autoclaving, to obtain a larger range after autoclaving.

All strains show a preference for acid conditions, especially strains b and c whose optimum is pH4. These two strains are also the most tolerant as the range of mean

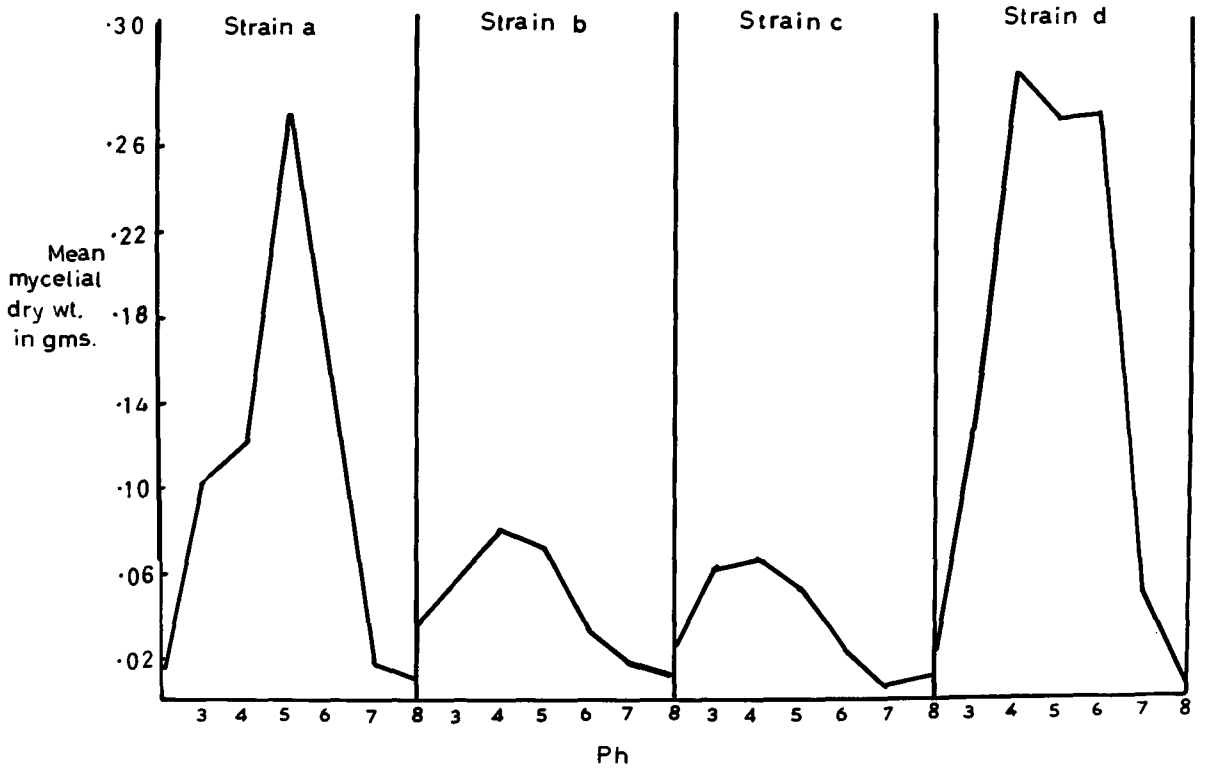


PLATE 10:

The effect of pH on growth in shake liquid culture after 14 days.

mycelial weight over the whole pH scale is fairly low. Strain a shows a very definite optimum at pH5 with a fairly rapid fall in growth rate under more alkaline conditions Strain d has little growth at the extremes, pH2 and pH7 and 8, but over the central range, pH 4 - 6 is relativdy insensitive to pH changes.

It is obviously not possible to separate the strains by pH optimum as this is very similar in all. However, it can be said that strain d shows remarkably similar growth at pH4, pH5 and pH6, which drops rapidly outside these limits.

(5) <u>The effect of different sugars and different concentrations of sucrose on growth</u>.

Cochrane (1958) reports that most cultivable fungi can utilise glucose and many can grow on fructose, mannose or galactose as a carbon source. Sucrose is another good carbon source but is not so universally available as maltose which is formed in nature during starch hydrolysis.

In Dox-yeast agar sucrose at a concentration of 1.5% is used as the carbohydrate source. A shake culture experiment was set up to test firstly the preference of the different strains for a particular sugar, and secondly the concentration of sucrose giving optimum growth.

Dox-yeast, minus sucrose, was used as a basal

medium and the sugars to be tested were added. For the first part of the experiment 2% glucose, 2% fructose and 2% sucrose were used and three replicate flasks for each treatment were inoculated. For the second part the concentrations of sucrose were 5%, 10% and 20%, again three replicates being used. As a control one set of replicates contained the basal medium only without sugar. The inoculum used was a mycelial suspension obtained as follows: Mycelium of each strain grown in Dox-yeast liquid medium was washed several times under sterile conditions with sterile water to remove all residual traces of sugar. This mycelium was then homogenized as previously described, in a Waring blendor, and 0.5 ml. of the suspension added to each flask. The flasks were shaken for fourteen days, the mycelium from each flask was filtered and a dry weight obtained.

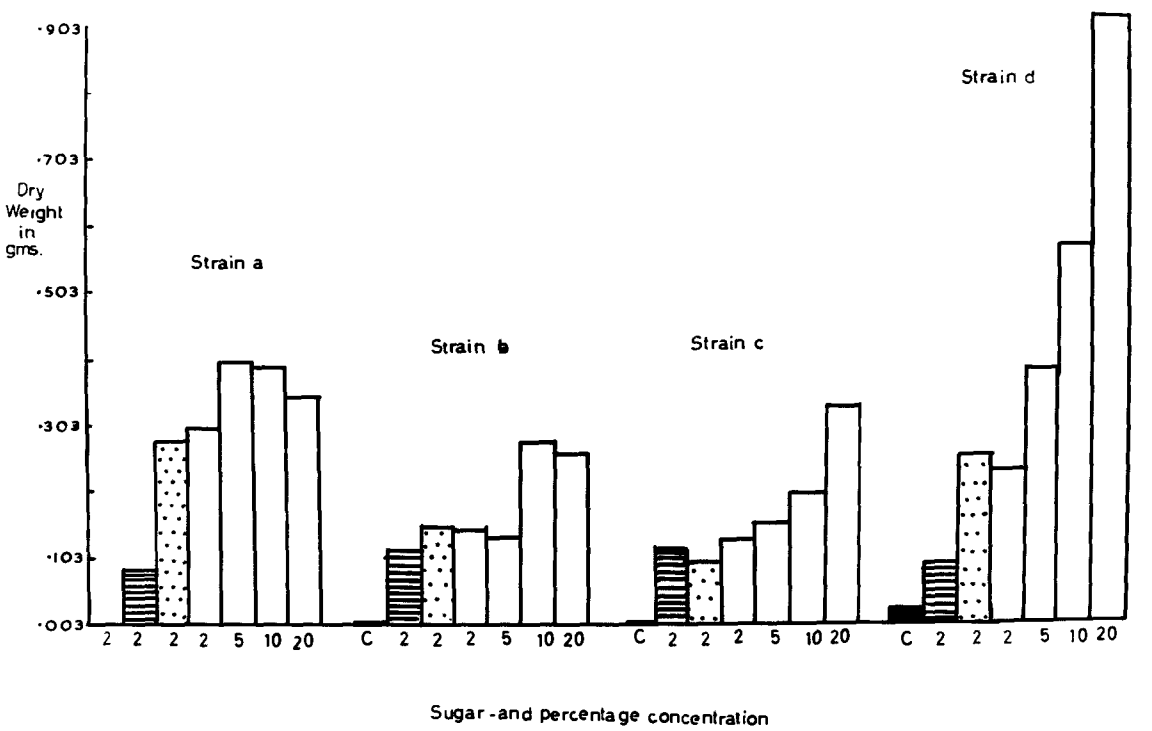
(See Table 11 and PLATE 11)

Strains a and d show an undoubted preference for fructose as opposed to glucose while strains b and c can utilise sugar equally well. All strains, however, grow as well on their best monosaccharide as they do on 2% sucrose.

Table 11: The effect of different sugars and different concentrations of sucrose on growth after fourteen days

Treatment	Mean dry mycelium weight in gms.			
	Strains			
	a	b	c	d
Control no sugar	0	.0033	0	.0230
2% Glucose	.0838	.1138	.1107	.0920
2% Fructose	.2740	.1467	.0845	.3000
2% Sucrose	.2965	.1453	.1273	.2831
5% Sucrose	.3951	.1322	.1516	.3804
10% Sucrose	.3879	.2730	.2457	.5688
20% Sucrose	.3435	.2566	.3260	1.0998

Growth on the higher concentrations of sucrose is variable. Strain a shows a slight though probably not significant depression of growth at 20% sucrose, otherwise growth under all treatments is similar. Strain b shows definitely increased growth at 10% and 20% sucrose, while the other treatments give a practically similar mycelial weight. Strain c increases its



Treatment for each strain is:

Control: 2% Glucose; 2% Fructose; 2 5, 10, 20% Sucrose

PLATE 11: The effect of different sugars and different concentrations of sucrose on growth after 14 days.

growth by about one third from 10% to 20% sucrose. The most interesting results were shown by strain d where the growth rate increases enormously with every higher concentration of sucrose.

In only two strains, b and d, were any recognisable amounts of mycelium formed in the control flasks. The fact that a certain amount of sugar is necessary for growth and that strains a and d prefer fructose to glucose may have a bearing on the infective ability of particular strains - a point which will be discussed later.

It is obviously not practicable to separate the different strains on their ability to utilise different monosaccharides and different sucrose concentrations. However the method could be used to distinguish between strains c and d, both of which show proportionally increased growth at 10% and 20% sucrose and strains a and b which do not. This also suggests that the osmotic pressure of the cell contents of the mycelium of these two strains is higher than that of strains a and b.

(6) The effect of different vitamins on growth

Most fungi able to grow in artificial culture are given a vitamin source or simple substances from which vitamins can be synthesised. There are five vitamins quoted (Fries 1948) as being necessary for fungal

growth and many others which may affect growth, depending on the synthetic ability of the fungus.

Thiamin, biotin, riboflavin, pyridoxine and inositol are the most important vitamins concerned in fungal growth and a preliminary tube culture experiment was set up to determine their effect on Meria.

The medium used was ordinary dox. Seven separate batches of agar were made. One contained the correct quantities of all the vitamins tested, and one consisted of the basal medium without any vitamins. The remaining five contained all the vitamins except the particular one under test. Agar plugs, cut from plain agar and sugar cultures, were used as inoculum, and the tubes were incubated at 20°C for twenty-four days.

Examination showed distinct differences in colony size within the four strains under different treatments and also between the strains, and a more detailed experiment using a larger vitamin range and flask shake culture, was therefore set up. All glass apparatus was cleaned with chromic acid and analar chemicals were used wherever possible. The medium was dox with the correct quantities of vitamins added (Table 12) before autoclaving. This was possible as ascorbic and folic acids, which are destroyed by heat, were not used.

Three replicate flasks of each treatment were inoculated, the treatments including a batch with all the vitamins added, one with the basal medium only and one of the full Dox-yeast medium for comparative purposes. The inoculum for each flask was 0.5 ml. of homogenised mycelial suspension, obtained from plain agar and sugar cultures. The flasks were shaken for one month to ensure a substantial mycelial growth in all treatments, filtered and the mycelium dried and weighed.

Table 12: Concentrations of vitamins used in gms/litre.

Vitamin	Concentration gms/litre
Nicotinic acid	200
Pantothenic acid	400
Pyridoxine	100
Inositol	5,000
Thiamin	100
Riboflavin	250
Biotin	5
Choline Chloride	400

(See Table 13 and PLATE 12)

Table 13: Mean dry mycelial weight of strains of Meria grown under differing vitamin conditions.

Treatment	Mean dry mycelial weight gms.			
	Strains of Meria			
	a	b	c	d
Dox-yeast	.2286	.1738	.1333	.3439
NO vitamins	.0106	.0102	.0096	.0098
Minus nicotinic acid	.0171	.0207	.0455	.0388
Minus Pantothenic acid	.0303	.0145	.0535	.0632
Minus Inositol	.0463	.0124	.0337	.1529
Minus Pyridoxine	.0304	.0147	.0834	.1295
Minus Thiamine	.0218	.0074	.0239	.0740
Minus Riboflavin	.0204	.0208	.0220	.0227
Minus Biotin	.0201	.0256	.0275	.0512
Minus Choline Chloride	.0189	.0218	.0254	.0482
All Vitamins	.0213	.0255	.0267	.0621

The results of this experiment must be interpreted with caution as only the b strain shows the replicates containing all vitamins with a larger dry weight than the other treatments. This is due partially to the disagreement between the replicates but mainly to

The treatment for each strain is:

C_1 - No Vitamins; 1 - Minus nicotinic acid; 2 - Minus Pantothenic acid; 3 - Minus Inositol; 4 - Minus Pyridoxine; 5 - Minus Thiamine; 6 - Minus Riboflavin; 7 - Minus Biotin; 8 - Minus Choline Chloride; C_2 - All vitamins

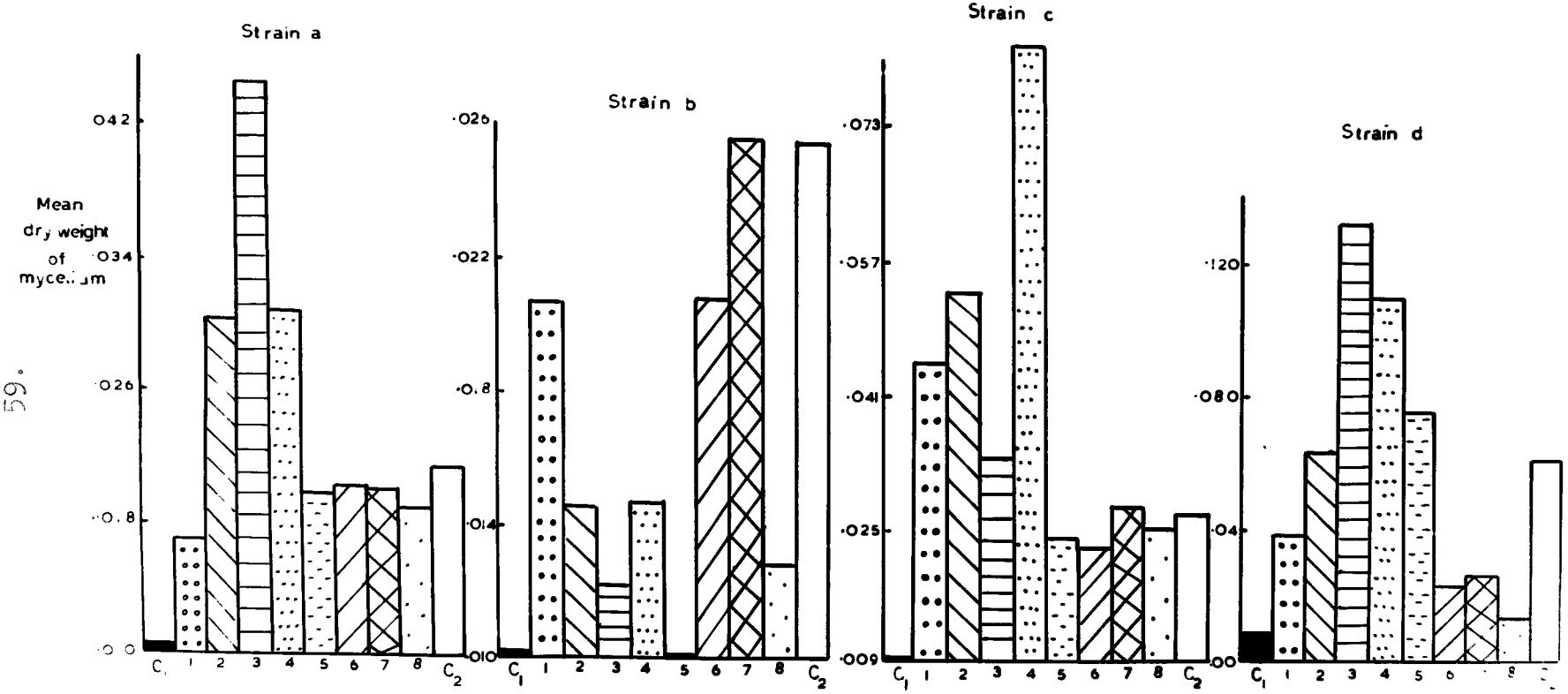


PLATE 12: The effect of vitamins on growth in shake culture.

the type of inoculum used in some of the flasks. Insufficient inoculum from plain dox cultures was available and a small quantity of mycelium from Dox-yeast shake culture was used after many washings with sterile water. Obviously sufficient vitamins were carried over into the test media to give a greater growth than would have been expected. However, the following trends occur. Choline chloride is apparently not necessary for adequate growth of a and c strains, but is necessary for b and d. Strain b is the only strain that requires thiamin for adequate growth. All strains show some growth, though a very limited amount, without vitamins.

The most interesting of these trends is the partial requirement for choline chloride of strains b and d and the fact that it is not necessary for the growth of a and c strains. This indicates that strains b and d and strains a and c have some physiological connection and supplies additional proof for a tendency that has already shown itself during other growth studies.

SUMMARY

Study of Meria laricis in culture has revealed that the species is divisible into four distinct cultural strains. These strains can be identified by mycelial colour and type, the number of spores they produce and

their length and their differing growth rates.

In all strains the mycelium is a tangled mass of branched and anastamosed hyphae. The hyphae are septate and multinucleate with dense cytoplasm. More definitive growth studies were made in an attempt to distinguish between the strains and experiments concerning the effects of the composition of the medium, the temperature, the pH, different sugars and different concentrations of sucrose, and different vitamins on growth in culture were carried out.

DISCUSSION

Four distinct cultural strains of Meria have been recognised. These have been designated a, b, c and d, and can be identified by various cultural characteristics. Peace and Holmes described two strains a and b which appear to correspond with the present a and b strains, but also mentioned the occurrence of a further sparsely sporing variant with a white fluffy mycelium which corresponds to the current strain d. The recognition of these strains will be important when considering the pathogenic aspects of this disease and may in fact account for the sudden appearance of Meria on previously resistant tree species.

All strains remain stable during sub-culturing under normal cultural conditions but growth for long periods

at 10°C produced reversible strain changes while growth under differing pH conditions produced permanent strain changes. At 10°C there was a tendency for certain a strain cultures to take on c strain characteristics (a deliquescent surface mycelium) and some c strain cultures showed b strain characteristics (discoloration of the medium and dry mycelial surface). All these reverted to normal type when sub-cultured and grown at 20°C. This indicates that the cultural strains are not definite genotypes but aggregates containing nuclei of some or all of the different strains. The dominance of particular strain nuclei gives the observed strain characteristics but this dominance can be upset under certain cultural conditions.

During the pH experiment two permanent strain changes occurred, both from cultures grown at pH9.2. A strain d culture changed to strain b and a strain c culture changed to strain a. Strain d remained remarkably stable through frequent sub-culturing and this was the only change of strain recorded for this strain. The rapid fall in growth, measured by dry weight of mycelium, shown by strain d shake culture between pH6 and 8 could account for this strain change. The d strain nuclei were adversely affected under these alkaline conditions, shown by the rapid fall in growth, while those of the b strain, being slightly more tolerant, were

able to exert their effect. This effect however was permanent unlike the temperature effect. If the strains are interchangeable under differing cultural conditions, it is probable that they will also interchange during passage through the needle.

The significance of these strain interchanges and of the strains in relation to the pathogenic aspects of Meria will be discussed later.

SPORE FORMATION AND GERMINATION

Vuillemin (1896) describes in detail the formation of spores of Meria when pathogenic in the larch needle. A thickened hyphal branch grows into the sub-stomatal chamber, swells into an aseptate mass and then undergoes various septations to form a two layered tissue. The upper, fertile layer is protected by the cup-shaped mucilaginous sterile layer. Cells from the fertile layer elongate, undergo septation and protrude from the stomatal pore, subsequently branching in a regularly dichotomous manner. Each branch divides into 1-3 cells and each cell forms a sterigma just below the septum. Spores are cut off consecutively from this sterigma, a new one beginning to form after the first is abstricted. The spore is unicellular with a constriction in the centre.

Peace and Holmes confirmed these observations and described spore formation in culture. Conidiophores, similar to those found in nature, are produced from specialised hyphae. These hyphae are thicker and contain more cytoplasm than the normal vegetative hyphae and are probably equivalent to the sub-stomatal masses found in nature. The number of cells in each conidiophore is variable and each sterigma produces an indefinite number of spores successively. The spores are identical to

those produced in nature and prior to germination a septum forms across the constriction - an observation not recorded by Vuillemin, Saccardo or Lindau.

Dreschler (1941), describing an unknown disease of nematodes, called the parasite Meria coniospora using as one of the major reasons the similarity of spore production to that of Meria laricis. Conidiophores are pushed out through the integument of the nematode and become divided by septa into cells of a uniform length. Each cell forms a short sterigma, at the distal end, which abstricts small conidia consecutively.

The present investigation has confirmed earlier observations and more detail of spore formation of the fungus in culture is available.

Spore formation in culture

Method - The slide smear cultures used for the cytological examination of Meria were kept under the same conditions, 20°C and a high humidity, for periods from ten to twenty days. It was found that during the interval spore formation had commenced in various parts of the culture, and using the aceto-carmine staining technique previously described (p.27) details of this process could be observed.

1. Observations on spore formation: Strains b and c

Spores are produced from hyphae exactly similar in

appearance to vegetative hyphae apart from a decrease in nuclear number. This disagrees with Peace and Holmes' observations where larger and more granular hyphae acted as conidiophores. These were observed under adverse conditions in starved and staled cultures, but were by no means predominant and were never present in normal cultures. The conidiophores were formed in two different ways.

(a) A hyphal branch would become septate and each cell would form a sterigma at the distal end and cut off spores consecutively, usually one every twenty-four hours. (PLATES 13 and 14). The conidiophore cells were multinucleate, although they contained fewer nuclei than the normal vegetative cells. The conidia were also multinucleate (see PLATE 18) - an important fact to be considered when discussing the taxonomy of the strains.

(b) A hyphal branch would coil into a circle or partial circle, septation would occur and spores would be cut off from sterigmata as previously into the inside of the coil. (PLATE 15). Usually, however, neighbouring hyphal branches would anastamose with the original circle and these would also begin to cut off spores (PLATE 16). This method of spore production accounts for the spore clumps, especially obvious in the c strain at low magnifications (PLATE 17).

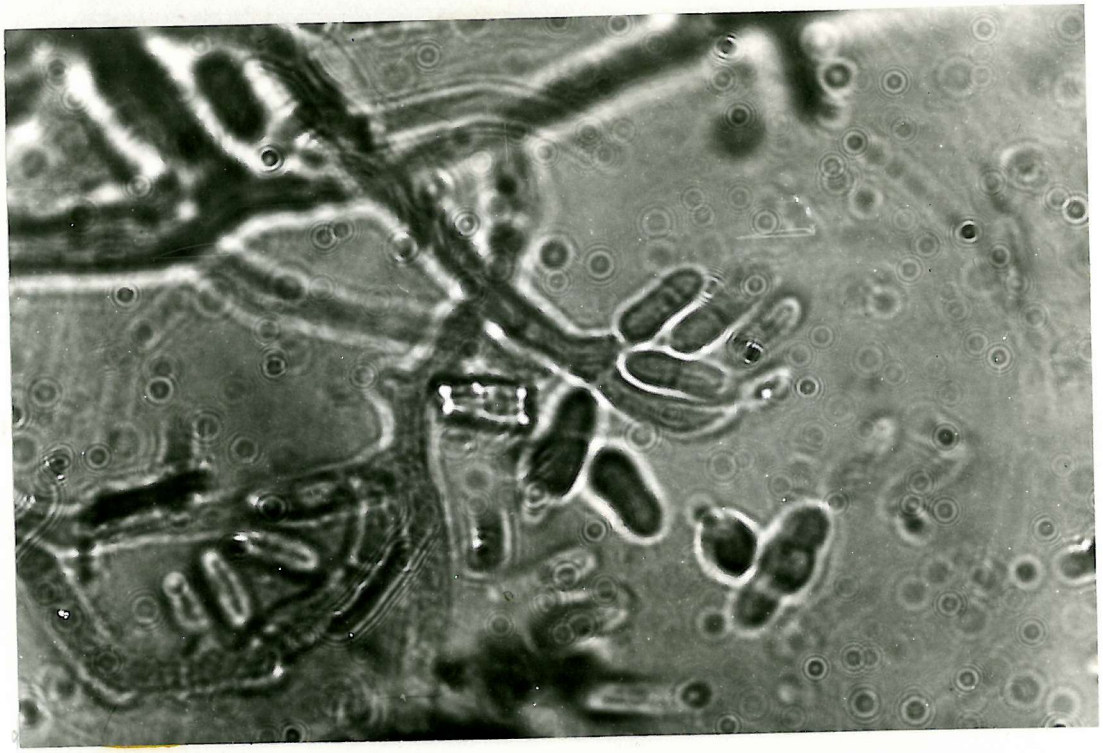


PLATE 13: Strain b after 10 days: spore formation showing septation.

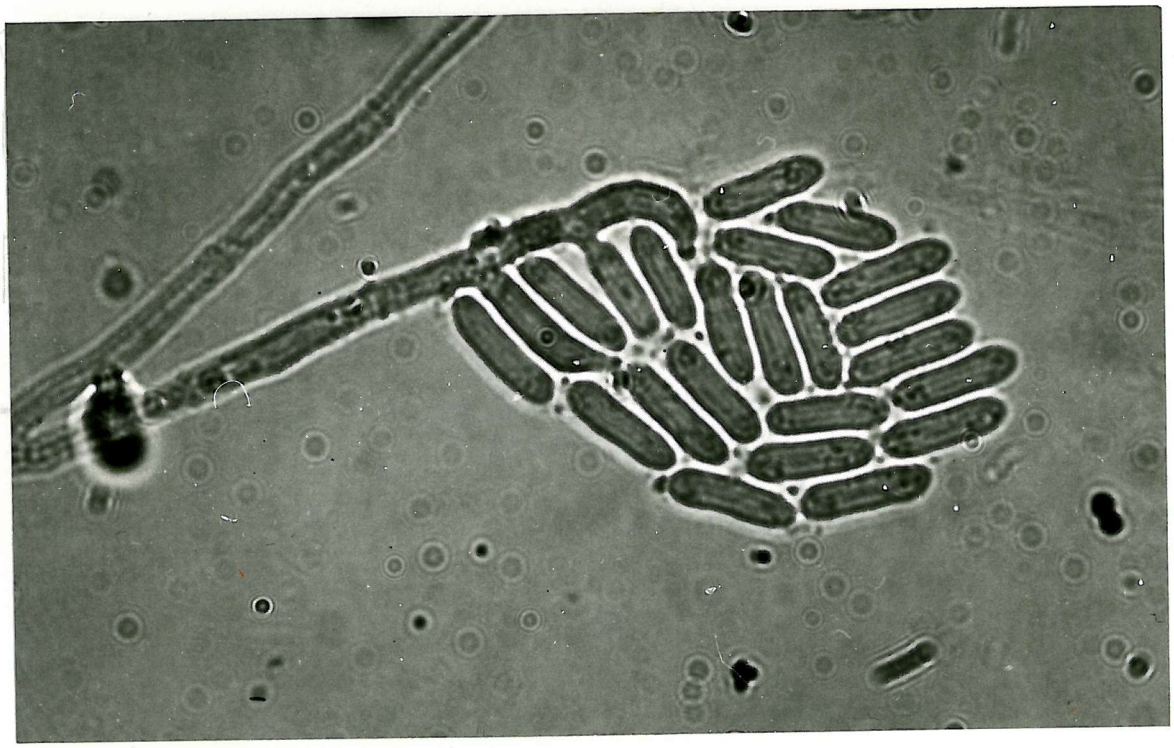


PLATE 14: Strain b after 16 days: three initials abstricting many spores.

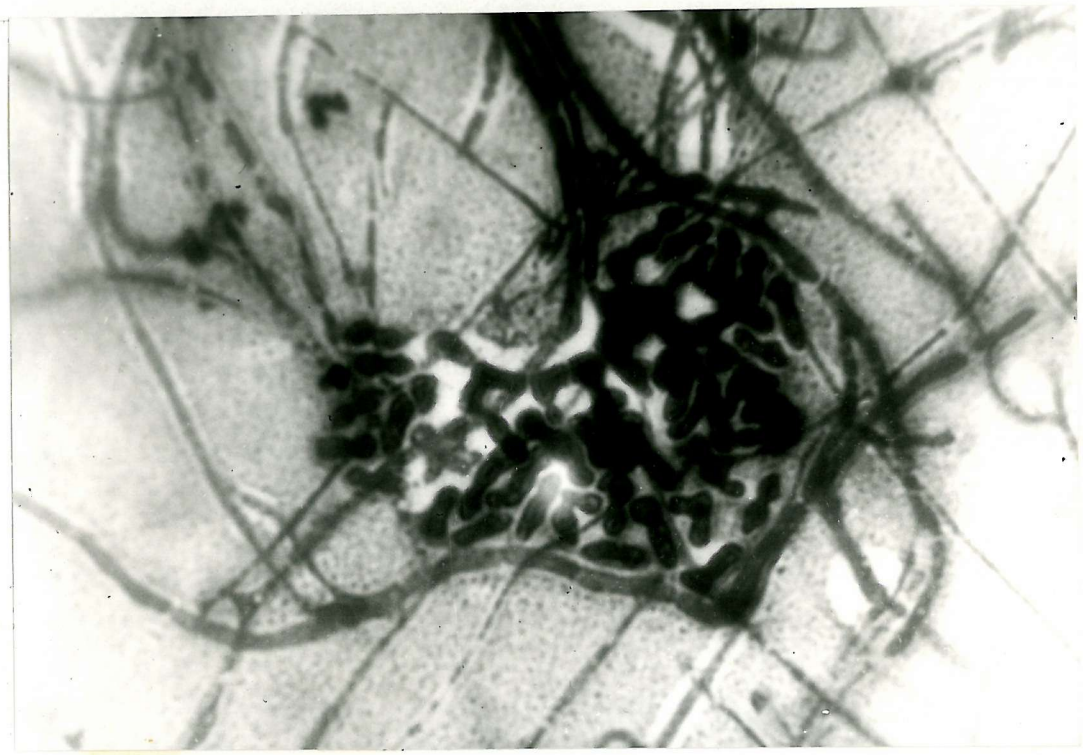


PLATE 15: Strain c - early stage of spore formation within coiled hyphae.

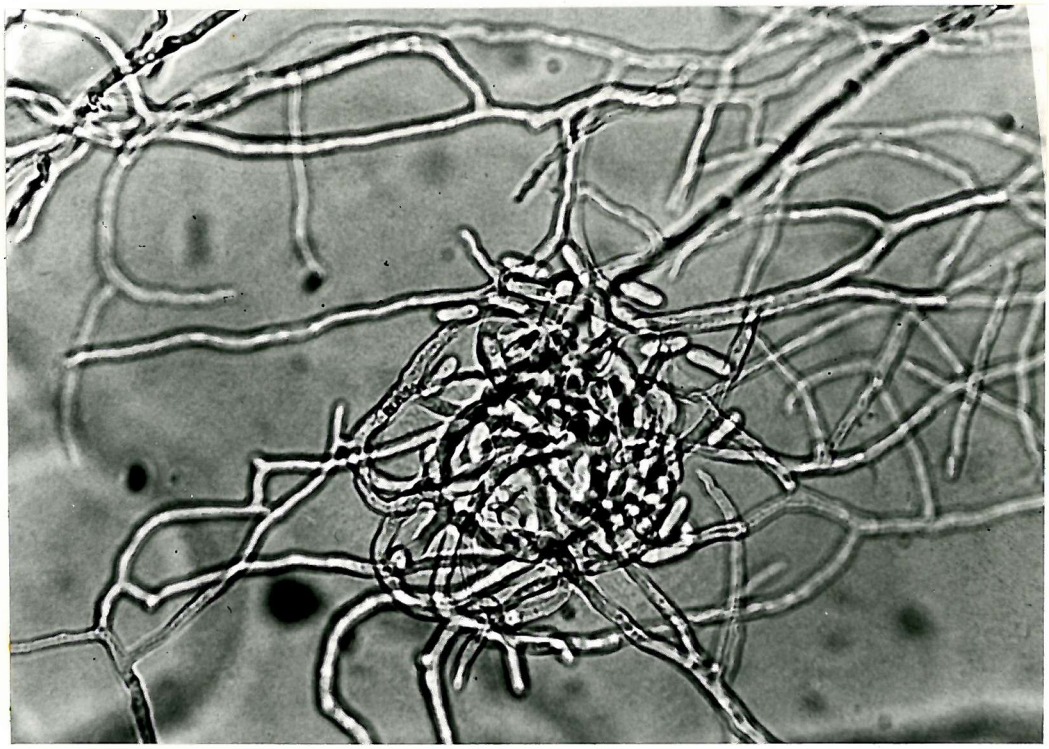


TABLE 16: Strain b after 11 days - a more advanced stage of hyphal coiling and spore formation.

PLATE 17: Strain c after 13 days showing spore clumps.

There appears to be no definite reason why one or other of these conidiophore formations should arise. Method (b) occurs rather more frequently than method (a) especially in c strain cultures. However, neither method is confined to any particular part of the culture, although there is a tendency for method (a) to appear more obviously at the colony edge. Method (a) is very similar to that observed in the needles (Vuillemin) and by Peace and Holmes and Dreschler in culture. Method (b) is possibly a modification occurring under favourable conditions in culture with the production of large numbers of spores. Isolated instances of spore formation at the extreme colony edge have been observed (PLATE 19) but this is unusual.

On one occasion one b strain culture on plain agar that had been incubated for twenty-one days developed aerial spore clumps, resembling the conidiophores of Aspergillus. Normally all conidiophores are produced in the same plane as the mycelium or agar, these however were borne aloft on short aerial hyphae (PLATE 20).

Peace and Holmes record that the conidia become septate prior to germination. The time of septation is however variable. In the majority of instances septation does occur prior to germination, especially

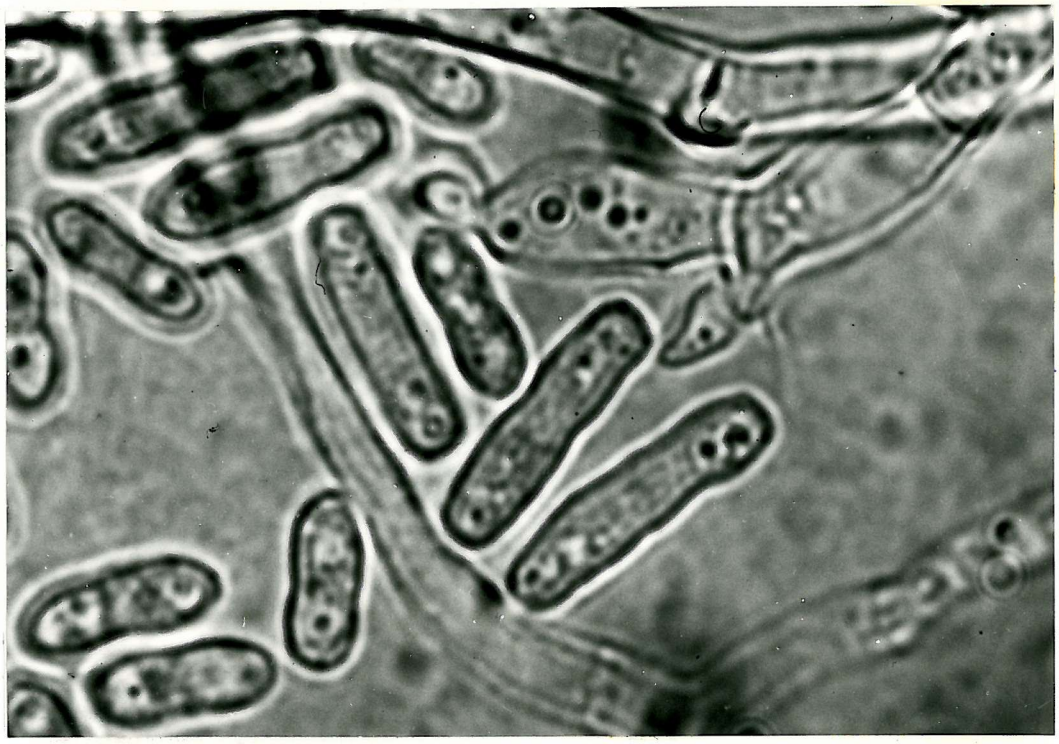


PLATE 18: Strain c after 12 days showing nuclei within the spores and the spore forming initial.

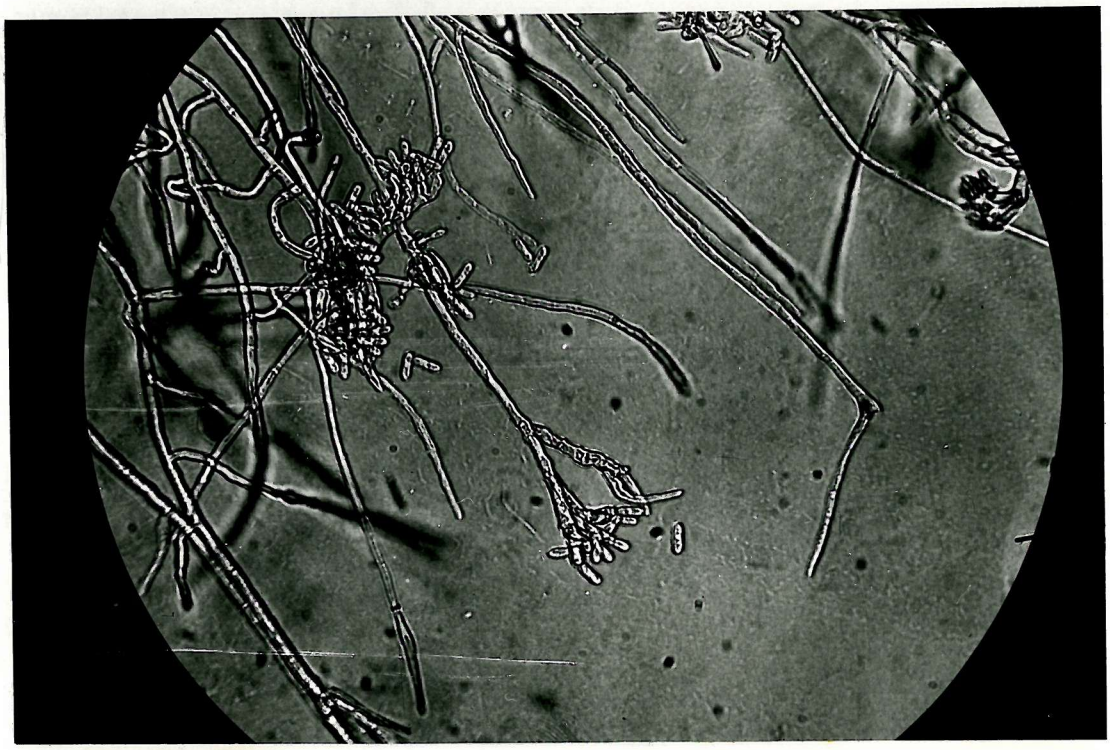


PLATE 19: Strain b after 14 days - spore formation at the colony edge.

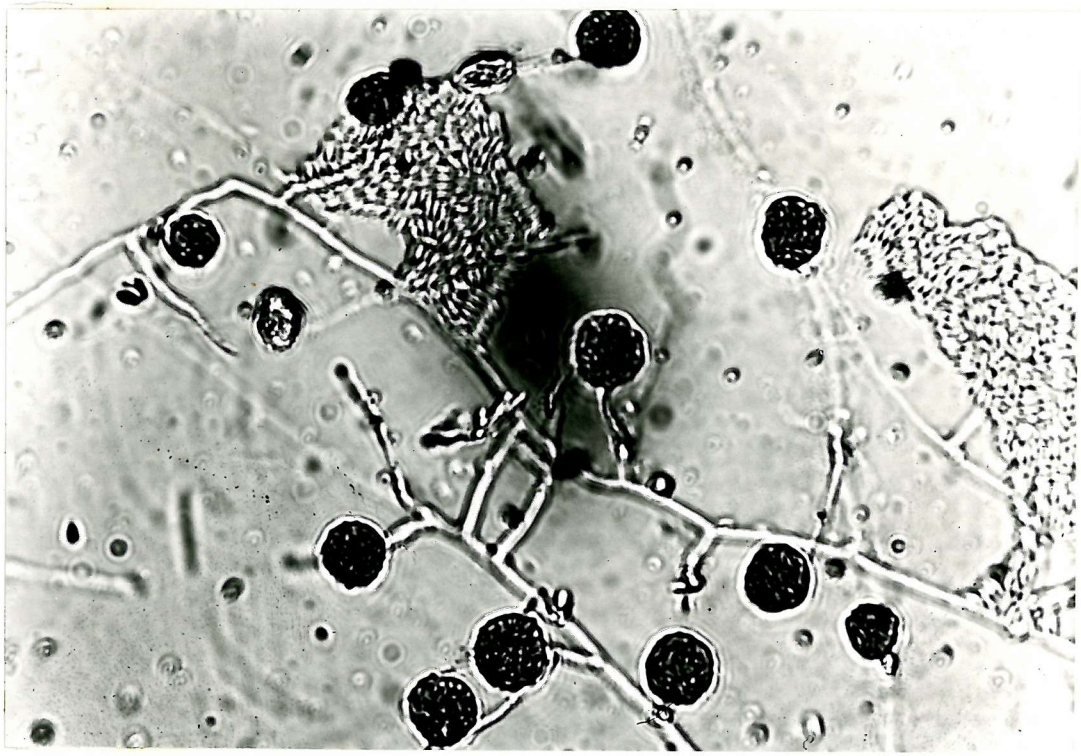


PLATE 20: Strain b after 21 days showing aerial spore clumps.

in the c strain. Conidia of the b strain, however, often form a septum immediately after abstriction or even before abstriction (See PLATE 13).

Vuillemin describes the spores as being granular throughout. Saccardo (1902) and Lindau (1910) mention that the ends of the spore each contain a large oil globule. Peace and Holmes state that although oil drops do occur the ends of the spores are usually granular. Recent observations partially confirm both views. Circular semi-transparent areas around the edges of which the nuclei are found, occur at the ends of the spores. These are not oil globules but may be vacuoles as they appear to contain no granular contents. The remainder of the spore is filled with granular cytoplasm, the nuclei often being obscured by aggregations of the granules. (PLATE 21)

Owing to the small size of the spores it was difficult to observe accurately the number of nuclei in each spore. Generally the numbers varied between two and ten, and these were usually aggregated at the ends, although spores were occasionally found where all the nuclei were aggregated in the centre. The number of nuclei in each spore was reduced prior to spore germination. In a c strain germination experiment 43% of the spores showed a single nucleus, 49% two

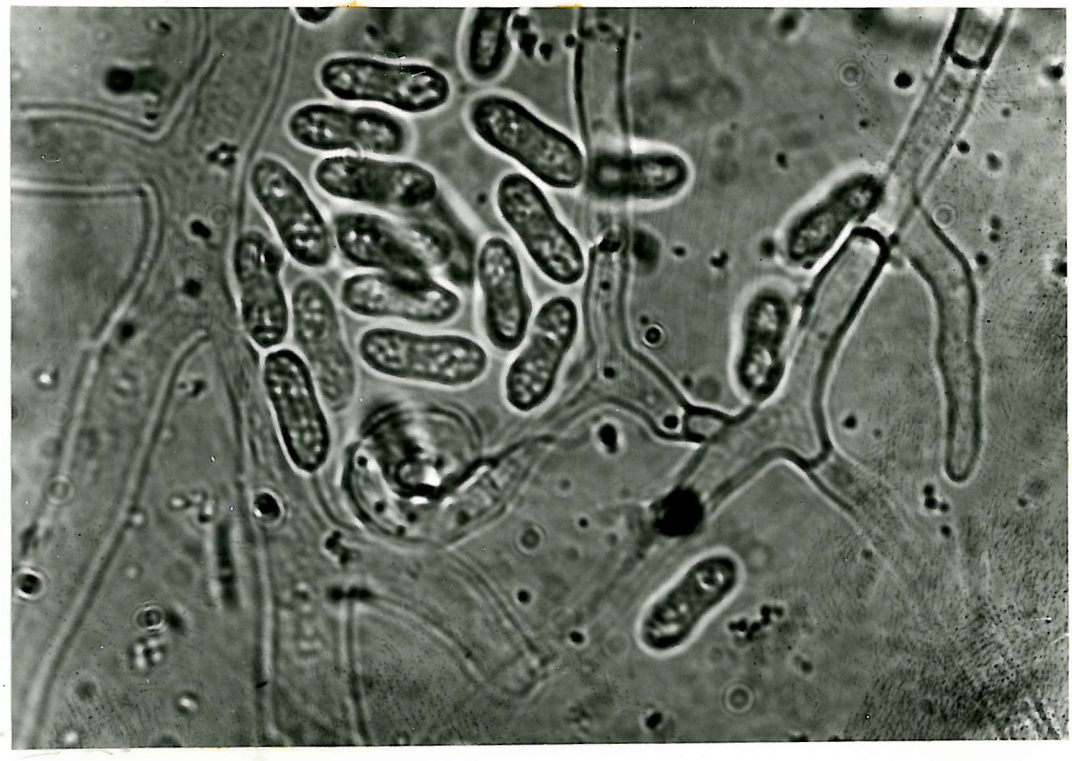


PLATE 21: Strain b after 16 days showing apparent spore vacuoles and granular cytoplasm.

nuclei and only 8% showed three or more nuclei, whereas it was very unusual to find a uninucleate spore in a spore clump. It was not possible to observe if the reduced number of nuclei in the germinating spores, when compared with the newly formed spores was a result of nuclear fusion or degeneration of some of the nuclei.

2. Spore formation: Strains a and d

Both strains a and d were cultured by the slide smear technique for observations on spore formation but no instances were found in either. In one d culture twenty-one days old there were branches that appeared to be forming conidia at their tips although no spores were found in their vicinity, but this was the only observation made of this type.

Strain a when originally isolated, showed a small amount of sporulation and some of the earlier experiments on spore germination (see next section) used spores of this strain. However, the ability to sporulate was very rapidly lost in culture, usually after one sub-culture, a phenomenon not uncommon in pathogenic fungi grown artificially. This created difficulties both with spore germination tests and later with tree inoculation, as only a few isolates of the a strain were made during the later stages of the work and consequently few a strain spore suspensions were available for tree inoculation.

A. Factors affecting spore formation

It was decided to investigate factors affecting spore formation in culture as these would have a bearing on spore formation in nature. The factors affecting spore formation in the fungi include the physical factors of temperature humidity and sometimes light; however carbohydrate and nitrogen availability also affect sporulation which occurs at low concentrations, although the nitrogen source may also have an effect. To a pathogenic fungus such as Meria, the physical factors will be more important than the chemical, as the chemical composition of the host remains fairly uniform.

(1) The effect of temperature on spore formation

The slope cultures used in the experiment for the effect of temperature on growth (p. 39) were investigated for spore number and size. A standard volume of water was added to each tube which was vigorously shaken to dislodge all the spores. A drop of this suspension was examined under the high power of a microscope using a haemocytometer. The number and size of the spores was recorded. Neither strain a nor strain d produced conidia.

Table 14: Effect of temperature on spore number in 1 ml. of spore suspension.

Temperature °C	No.of spores in 1 ml. of suspension	
	Strain	
	b	c
0	0	0
10	0	320
15	52	1580
20	150	4240
25	1	2020

20°C is the optimum temperature for the formation of conidia in both strains b and c. This is the same temperature as the growth optimum. The c strain, being more vigorous from the sporing point of view, produces spores at both 10° and 25°C where growth is slow, but the b strain produces spores over a limited range (15° - 20°) only.

In general the spores of both strains appear to be longer at higher temperatures. The b strain at 15°C shows 48% spores 6μ in length, the longest (4%) being 8μ. However at 20°C the smallest spores are 8μ (24%) and 48% are 10μ in length. The c strain spores are similar but the difference in length is

not so marked and probably not significant. Spores at 10°, 15° and 20° are 8μ long while 48% of the spores formed at 25° are 10μ in length. This result emphasises the fact that spore measurement studies must be made under standard environmental conditions. The range of temperatures over which sporulation occurs is important as the information can be used firstly during artificial inoculation experiments and secondly while attempting to devise a forecasting system for Meria attack.

(2) The effect of humidity on spore formation

During experiments on the effects of different factors on spore germination the humidity effect was investigated. Due to the technique described below it was possible to incubate the cultures for as much as one month. These were then examined microscopically and an arbitrary assessment of mycelium growth and sporulation was made.

The technique is a modification of that used by Snow (1949). Small square 'staining pots' with flat glass lids were stored in crystallising dishes and sterilised at 80°C for several days. The lids were cooled and painted, under sterile conditions, with a smear of Dox-yeast agar in their centre and then replaced at 80°C for several days. The pots were removed from the oven and a few drops of a solution

giving a known humidity were placed in the well. The lid, with its edges smeared with silicone grease and with the dried agar face downwards was placed over the well and the pot left to equilibrate at 20°C for twelve hours. A spore smear was made on the agar of each lid and the pot was incubated at 20°C. Due to the thin glass lid, germination and subsequently mycelial growth and sporulation, could easily be observed under the low power of the microscope without disturbing the contents of the pot.

Table 15: Arbitrary assessment of mycelial growth and spore formation of Meria under differing humidity conditions. Scale: 1 - 3.

Humidity %	Mycelial growth and sporulation: Scale 1 - 3					
	Strains					
	a		b		c	
	M	C	M	C	M	C
100	1	-	1	2	1	-
99	3	-	3	-	2	1
98	2	-	2	3	1	3
90	2	-	-	-	3	-

Lower humidities were not used as it was found, from preliminary experiments, that no germination occurred below 90% relative humidity.

100% relative humidity was unsatisfactory for growth and only the b strain sporulated. 99% R.H. gave the best mycelial growth in all strains but only the c strain showed a little sporulation. 98% R.H. gave maximum sporulation in both b and c strains and while at 90% R.H. growth was good, in a and c strains no spores were found by any culture. Sporulation therefore occurs over a very limited range of relative humidities - 98 and 99%, although some sporulation will occur in the b strain at 100%. It is doubtful whether this slight difference between the b and c strains will have any effect upon inoculum available for infection in nature. The humidity will fluctuate rapidly over these small limits and b and c conidia will be formed and probably released at the same time. This means that the relative abundance of b and c inoculum in nature will depend entirely on relative numbers of trees or needles infected with strains b and c in any particular area.

(3) The effect of light on spore formation

It has long been recognised that fungi can be divided into different groups depending upon their reaction to light (Hawker 1957).

Slope cultures of the four strains were given the following light treatments for one month at 20°C,

by means of a 60 watt pearl tungsten filament lamp installed close to the glass insulating door of the incubator. Spore size and number were counted and the morphology of the colony noted.

Light conditions:

(i) Continuous light

(ii) Continuous dark - tubes were placed in a light tight but not air tight tin

(iii) Normal day - this was in fact exposure to sixteen hours light in every twenty-four.

Table 16: Effect of light on spore numbers in 1 ml. of spore suspension.

Treatment	Numbers of spores in 1 ml. of spore suspension			
	Strain			
	a	b	c	d
Continuous light	10	270	1080	No spores formed
16 hour light	8	10	1350	do.
Continuous dark	12	50	3340	do.

All cultures were normal morphologically, with the exception of strains b and d grown in continuous darkness. There was less pigmentation of the b mycelium than usual, and the d strain was less fluffy and showed a slight browning of the mycelium. The

former effect was probably due to the darkness but both may be caused by the continuous high humidity in the enclosed tin.

This is one of the few experiments where the a strain has sporulated. However, the number of spores produced is negligible and there is no difference between the treatments. The b strain shows greater sporulation in continuous light than in the other treatments, but again the difference is probably not significant. The c strain shows the greatest sporulation in continuous darkness with a diminution of numbers of spores in sixteen hours light and a further reduction in continuous day. This large number, in continuous darkness, may again be due to the humidity conditions in the enclosed tin but, as there is a definite decrease in numbers formed as the total exposure to light increases, it is more likely to be a definite darkness effect.

The effects of light on conidial formation are of little consequence in nature where the host is subject to normal day and night and conidial formation will depend more upon temperature and humidity conditions. The different light treatments had no effect on the size of the spores of either strain b or strain c. The measurements showed their normal

spread with optimum 10 µ for b strain and 8 µ for c strain. As a result of these responses, Meria will actually be placed between different light response groups. The fungus will sporulate during light and even in continuous light, but forms a larger number of spores in complete darkness.

(4) The effect of different sugars and different concentrations on sucrose in spore formation

Most fungi will give maximum sporulation at fairly low sugar concentrations. The tube cultures inoculated to observe the effect of sugars and sucrose concentrations on growth (p.51) were examined to assess the numbers and size of conidia present. Unfortunately the b strain slopes became infected with Penicillium sp. and these were discarded. There was no conidial formation in strains a and d. The results were therefore confined to strain c.

Table 17: The effect of different sugars and different concentrations of sucrose on numbers of spores in a 1 ml. spore suspension - strain c.

Treatment	Numbers of spores in 1 ml. suspension
	Strain c
2% Fructose	1560
2% Glucose	480
2% Sucrose	1280
5% Sucrose	1580
10% Sucrose	740
20% Sucrose	6

(See PLATE 22)

STRAIN C

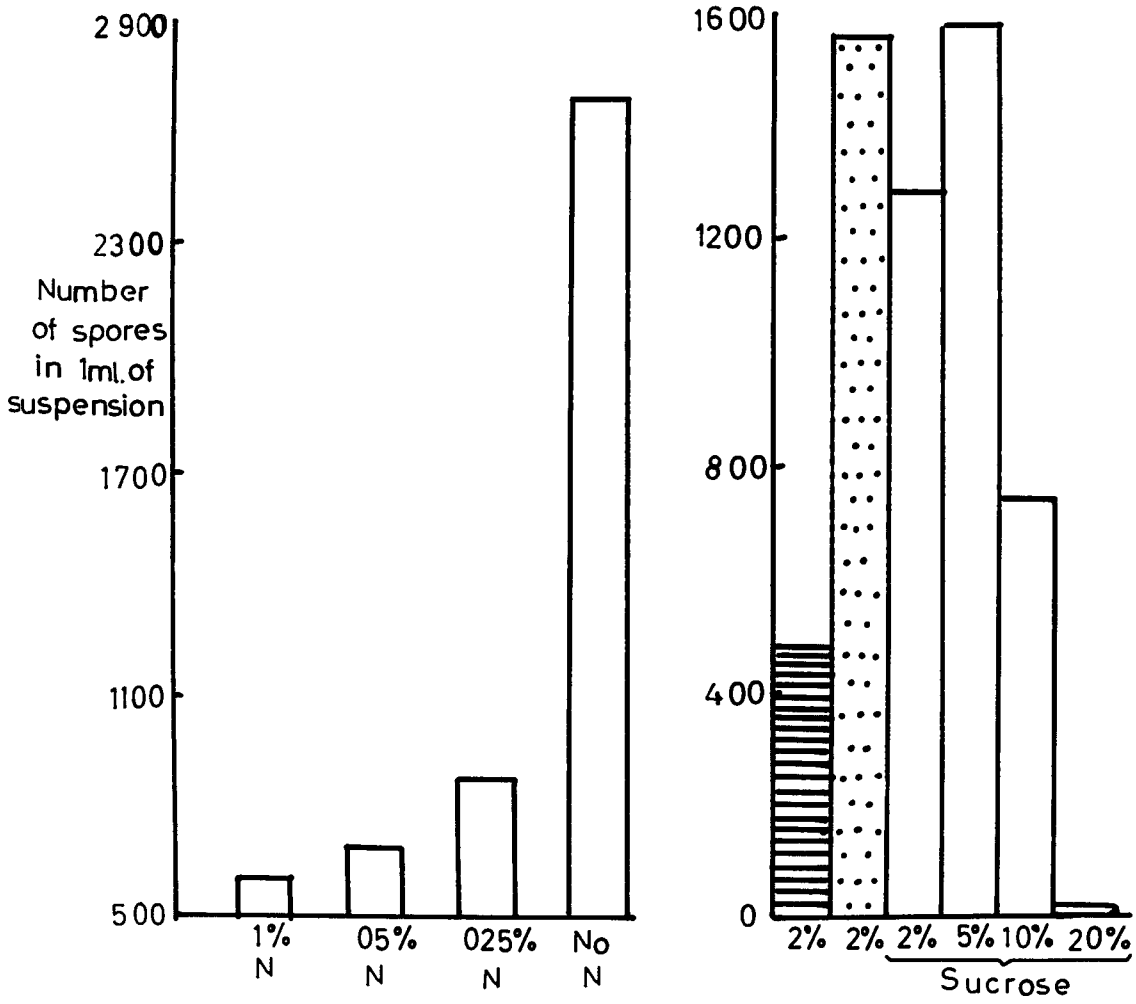


PLATE 23:

The effect of concentrations of sodium nitrate on sporulation.

PLATE 22:

The effect of different sugars and different concentrations of sucrose on sporulation.

2% Fructose gives better sporulation than 2% Sucrose, but the difference is probably not significant. Glucose gives poor sporulation. Spore formation is at its maximum at 5% sucrose and there is a steep decline in numbers formed at 10% and 20% sucrose. The cultures have in fact become more mycelial as growth is actually better in 10% and 20% sucrose than in 5% sucrose. Spore size is not affected by any of these treatments.

Meria shows the normal response to sugar concentrations producing most spores at fairly low concentrations of sucrose, while maximum growth occurs at higher concentrations of sucrose.

(5) The effect of nitrogen concentrations on spore formation

Many fungi absorb nitrogen in the form of ammonium salts but most of the mould fungi can also utilise nitrates. While some facultative parasites (Ophiobolus graminis) require organic nitrogen for growth, others will grow well on a nitrate source. Sporulation in culture tends to be at its maximum at low concentrations of nitrogen, although the actual source of nitrogen may also exert an effect. The nitrogen in Dox- yeast medium is in the form of sodium nitrate, and as growth on this medium is satisfactory the effect of different

concentrations of sodium nitrate on sporulation was investigated.

Slope cultures with differing concentrations of sodium nitrate were inoculated and incubated at 20°C for one month. The numbers and length of spores present were measured.

In Dox-yeast agar the concentration of sodium nitrate is about 0.2% and consequently lower concentrations were used for the experiment. The results are confined to strain c as neither strain a nor strain d showed any sporulation and sporulation was sparse in strain b with practically no difference between treatments.

Table 18: Effect of concentrations of sodium nitrate on spore number in 1 ml. of suspension.

Concentration of sodium nitrate	No. of spores in 1 ml. of suspension
	Strain c
0.1%	600
0.05%	660
0.025%	860
No $NaNO_3$	2660

(See PLATE 23)

Sporulation, in culture, tends to be at its maxiumum at low concentrations of nitrogen, although the actual source of nitrogen may also exert an effect.

There is a small but definite increase in spore numbers with a decrease in the concentration of sodium nitrate in the medium. Maximum sporulation occurs in the absence of sodium nitrate. As the chemicals used in the preparation of the medium were not pure it is probable that there was a certain concentration of nitrogenous impurities, in the form of nitrates, present. This very low concentration would account for the large spore production.

The conidia were of normal length in all treatments. It is difficult to equate the effect of nitrogen concentration on sporulation in nature. It must again be emphasised that the most important factors affecting the sporulation of Meria in nature will be the purely physical ones of temperature and humidity.

(6) The effect of vitamins on sporulation

During experiments concerning the effect of vitamins on growth in shake culture slope cultures were prepared by the addition of 3% agar agar to the solutions and these were inoculated with the different strains of Meria. After twenty-eight days at 20°C spore number and size were noted.

Table 18: The effect of vitamins on conidial number in 1 ml. of suspension.

Vitamin missing	Number of spores in 1 ml. of suspension			
	a	b	c	d
Inositol	0	0	330	0
Nicotinic acid	0	34	210	0
Pantothenic acid	0	37	450	0
Pyridoxine	0	44	200	0

Spores were not formed in a or d cultures and the number of spores formed under different vitamin treatments by strains b and c did not significantly differ. Spore size in both strains was similar to the normal - b strain mostly between 10 and 14 μ and c strain between 6 and 8 μ. Unfortunately no results are available for the other vitamins used in the growth experiment owing to destruction of slope cultures before examination.

B. Factors affecting spore germination

An investigation of factors affecting germination seemed essential as a prerequisite for the study of the epidemiology of Meria.

There are many difficulties inherent in the study of spore germination concerned with methods of sampling (McCallan and Wilcoxon 1939) and methods of recording results (Wellman and McCallan 1942). Tomkins (1932) suggested that three criteria should be employed in germination studies: (i) latent period of germination - i.e. average time for germ tubes to appear, (ii) percentage of spores germinating, (iii) rate of elongation of germ tubes, and that (i) and (ii) would give the most useful information regarding the effect of environment on germination. McCallan and Wilcoxon (1932) after commenting on means of assessing variation in germination experiments suggest that the chi-squared test should be applied to the results to discover if in fact the deviations in results are not simply due to sampling errors.

In the present investigation the following methods were employed in order to eliminate as many errors as possible.

(a) Sampling Spores were obtained from culture tubes incubated for twenty-eight days at 20°C by means

of a sterile camelhair brush and transferred rapidly to a marked area of the experimental vessel.

(b) Counting The counting of spores was carried out as far as possible without disturbing or opening the vessel in which they were germinating to avoid contamination during long term experiments. As Neria germinates slowly six or twelve hour observational periods were found to be most suitable. The method consisted of microscopic examination of many low power fields of the inoculated area and the counting of percentage number of spores showing germination. At least 100 spores were counted.

(c) Germination Germination was regarded as having occurred if (i) a germ tube was visible, (ii) a spore forming initial was present, as many conidia on germination immediately produced further smaller or microconidia from a spore forming initial (see PLATE 34). As the spores were small these criteria presented observational difficulties but the formation of a septum across the middle of the spore was also an indication that germination had commenced or was about to start. This criterion could not be used alone, however, as spores were often abstricted with septation completed.

A preliminary experiment to investigate the

effect of age of the isolate on both the formation and germination of spores was carried out. Three tube cultures of strain c, the isolates differing in age by one year, were made and incubated at 20°C for twenty-eight days. Using the technique mentioned above a smear of spores from each tube was transferred to the surface of a marked area of Dox-yeast agar in a thin glass petri dish. As the same time a count was taken of a number of spores present in a 1 ml. spores suspension made by pouring 10 ml. of water into each culture tube. (See PLATE 24). The thin glass petri dishes were examined while still closed, every twenty-four hours and the percentage spores germinated was counted. (See PLATE 25).

The age of the isolate has a decided effect on both the number of spores formed and the percentage germination. The 1955 isolates produced a small number of spores with a low percentage germination while the 1957 isolates produced a large number of spores with a 52% germination. In consequence in all further experiments on spore germination, spores of the most recent isolate of each strain available, were used.

Gottlieb (1950) suggested that the following factors were important in germination of fungal spores - water, nutrient availability (especially in pathogenic

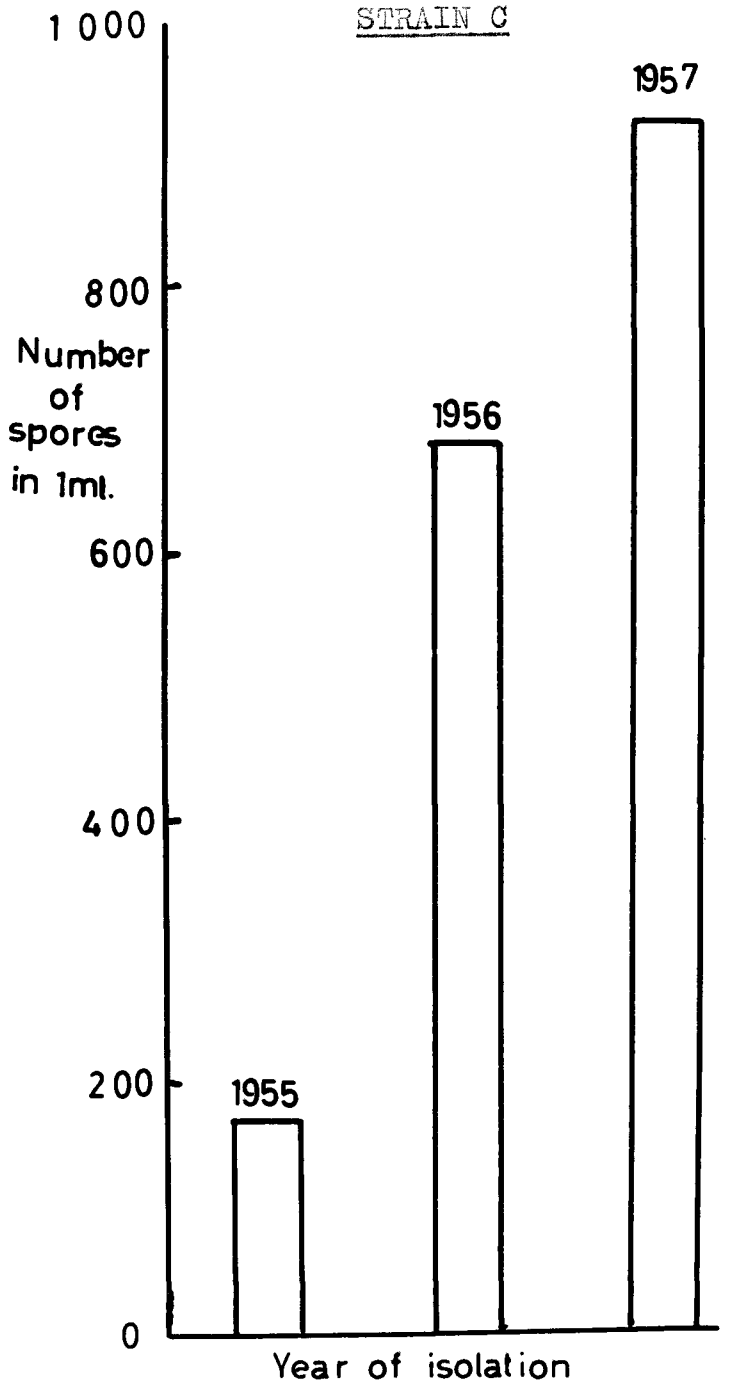


PLATE 24: The effect of the age of the isolate on spore formation.

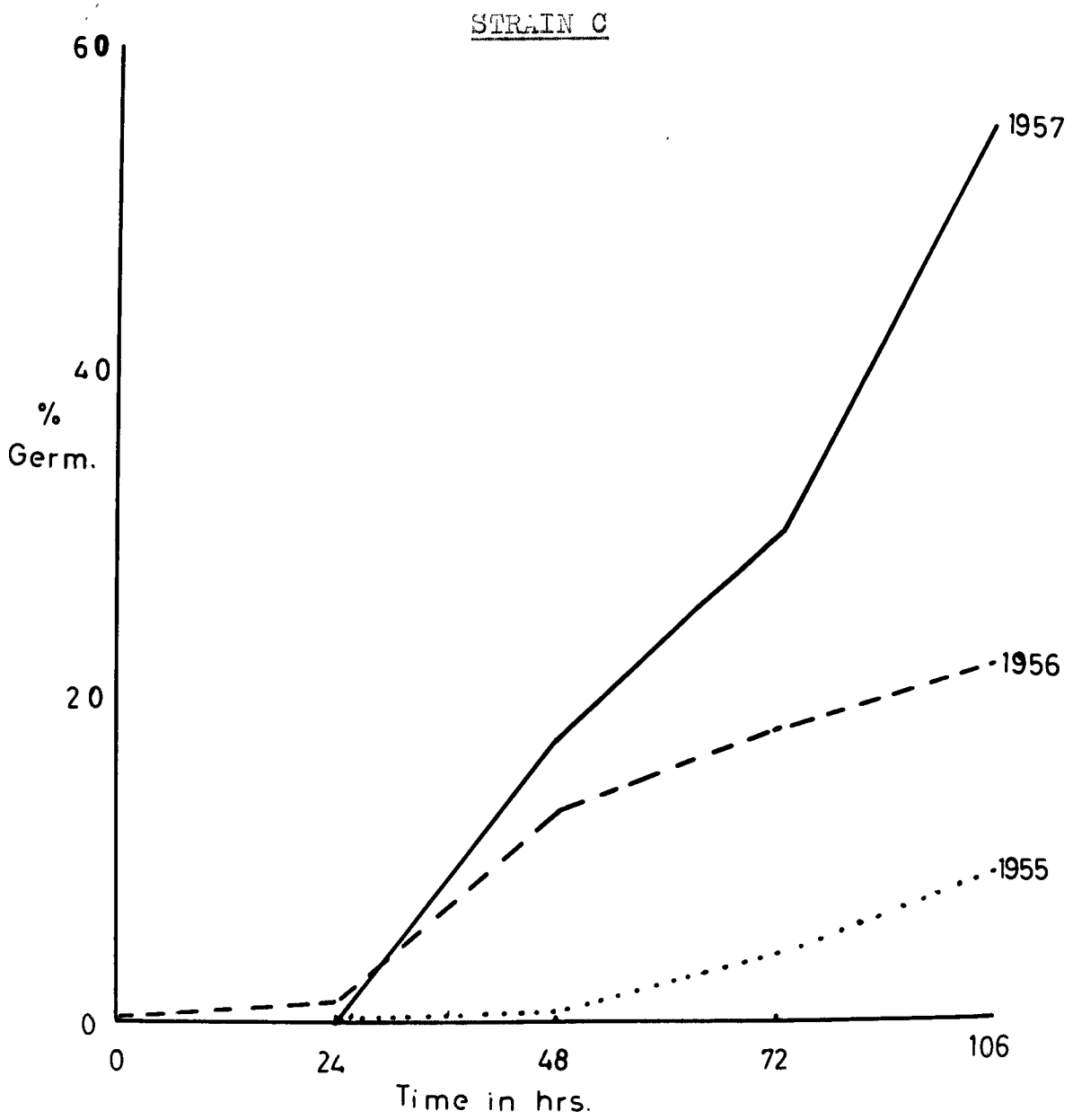


PLATE 25: The effect of the age of the isolate on spore germination.

fungi) oxygen and carbon dioxide, temperature, pH, dormancy, radiation and senescence - the relative importance of each of these factors depending on the fungus studied.

Peace and Holmes were the only previous workers to attempt germination experiments with Meria. One experiment to test the effect of temperature on germination was unsuccessful but another, to test the viability of spores under wet and dry conditions, indicated that spores did not germinate freely after drying with calcium chloride for one hour and were killed after drying for two days. This result helped to explain the check in Meria epidemics at the onset of dry weather.

The factors that exerted the major effect on germination of Meria, especially from apathogenic viewpoint, were considered to be nutrient, including larch needle extract, temperature and humidity.

(1) The effect of nutrient on germination

Gottlieb (1950) suggested that some form of carbohydrate, especially glucose or sucrose, frequently stimulated germination in many fungi, although sufficient nutrient to do this is removed from the culture medium when taking the spore sample.

Preliminary experiments using water agar gave poor germination results possibly as Meria has very small spores and therefore insufficient nutrient for good germination. The effect of various nutrient media on germination was consequently investigated.

Thin walled petri dishes containing water agar, water agar plus 2% sucrose and Dox-yeast agar were inoculated with c strain spores and incubated at 20°C for eighty-four hours, observations on germination being made every six hours.

Table 20: Effect of different culture media on germination of spores using strain c

Time hrs.	Percentage Germination		
	Water agar	Water agar + 2% sucrose	Dox-yeast
12	0	0	0
18	0	0	12
24	2	14	17
30	3	15	18
36	3	16	24
42	3	18	25
48	3	19	27
54	3	21	29
60	3	24	30
66	3	31	35
84	5	31	37

(PLATE 26)

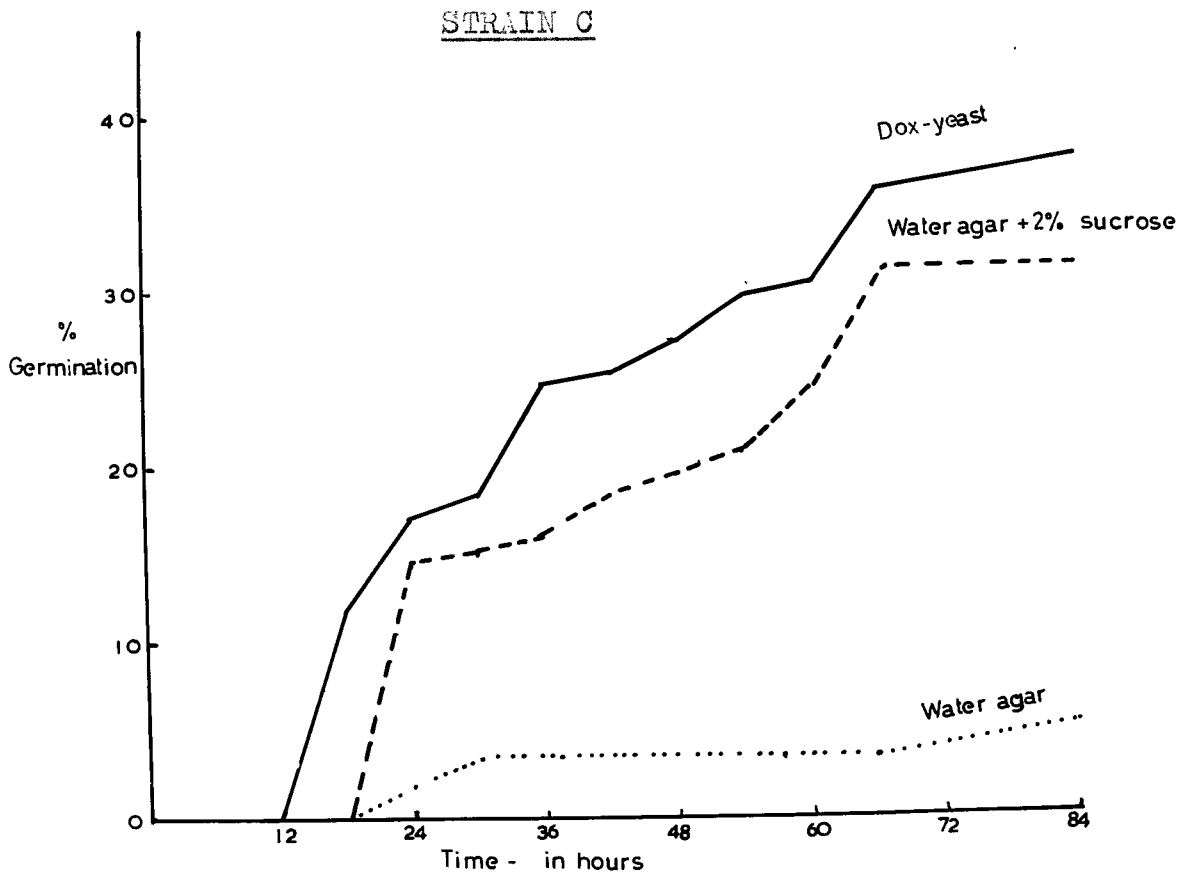


PLATE 26: The effect of different culture media on spore germination.

Best germination is given by Dox-yeast agar but the addition of sucrose to water agar improved germination considerably, probably as the small spores (9μ x 3.6μ) contain insufficient stored nutrient for good germination on water agar. Two other points are evident (a) nutrient decreases the time lag before the commencement of germination, (b) the final germination percentage figure is not very high.

It is interesting to note that germination is complete only after eighty-four hours whereas most fungi have completed germination in twelve hours (Pinkerton 1936).

As a result of this experiment all other germination experiments were carried out using Dox-yeast agar, and observed periodically for at least eighty-four hours.

(2) <u>The effect of temperature on germination</u>

Using the technique described above thin walled petri dishes containing Dox-yeast agar were inoculated with spores from a, b and c strains. Two replicate plates of each strain were then incubated at each of the temperatures 0^{o}, 10^{o}, 15^{o}, 20^{o} and $25^{o}C$ and examined at six hourly intervals for a total of seventy-two hours. The percentage germination of each replicate was recorded and the mean result tabulated (Table 20).

Table 21: Effect of temperature on germination of strains a, b and c.

Time	Temp	a	b	c	Time	Temp	a	b	c	Time	Temp	a	b	c
	0	0	0	0		0	0	0	0		0	0	0	0
	10	0	0	0		10	4	0	0		10	4	0	0
12 hrs	15	6	0	0	18 hrs	15	6	0	0	24 hrs	15	10	3	4
	20	6	0	0		20	13	0	0		20	15	1	6
	25	0	0	1		25	6	0	1		25	9	0	1
	0	0	0	0		0	0	0	0		0	0	0	0
	10	4	0	0		10	5	0	0		10	6	8	0
30 hrs	15	12	3	8	36 hrs	15	13	8	11	42 hrs	15	15	10	11
	20	24	1	8		20	27	6	24		20	29	10	25
	25	18	0	3		25	20	0	6		25	31	0	8
	0	0	0	0		0	3	0	0		0	5	0	0
	10	13	12	3		10	14	20	3		10	14	21	9
48 hrs	15	16	22	11	60 hrs	15	17	22	29	72 hrs	15	18	24	37
	20	33	10	40		20	36	12	44		20	55	25	45
	25	33	4	18		25	34	5	22		25	72	9	34

(PLATE 27)

All strains, with the exception of a, which shows a little germination at 0°C, commence germination at 10°C but this may be delayed for up to forty-eight hours. Strains b and c show optima at 20°C but strain a shows an optimum at 25°C. This experiment was one of the few in which spores of strain a were available for experimentation.

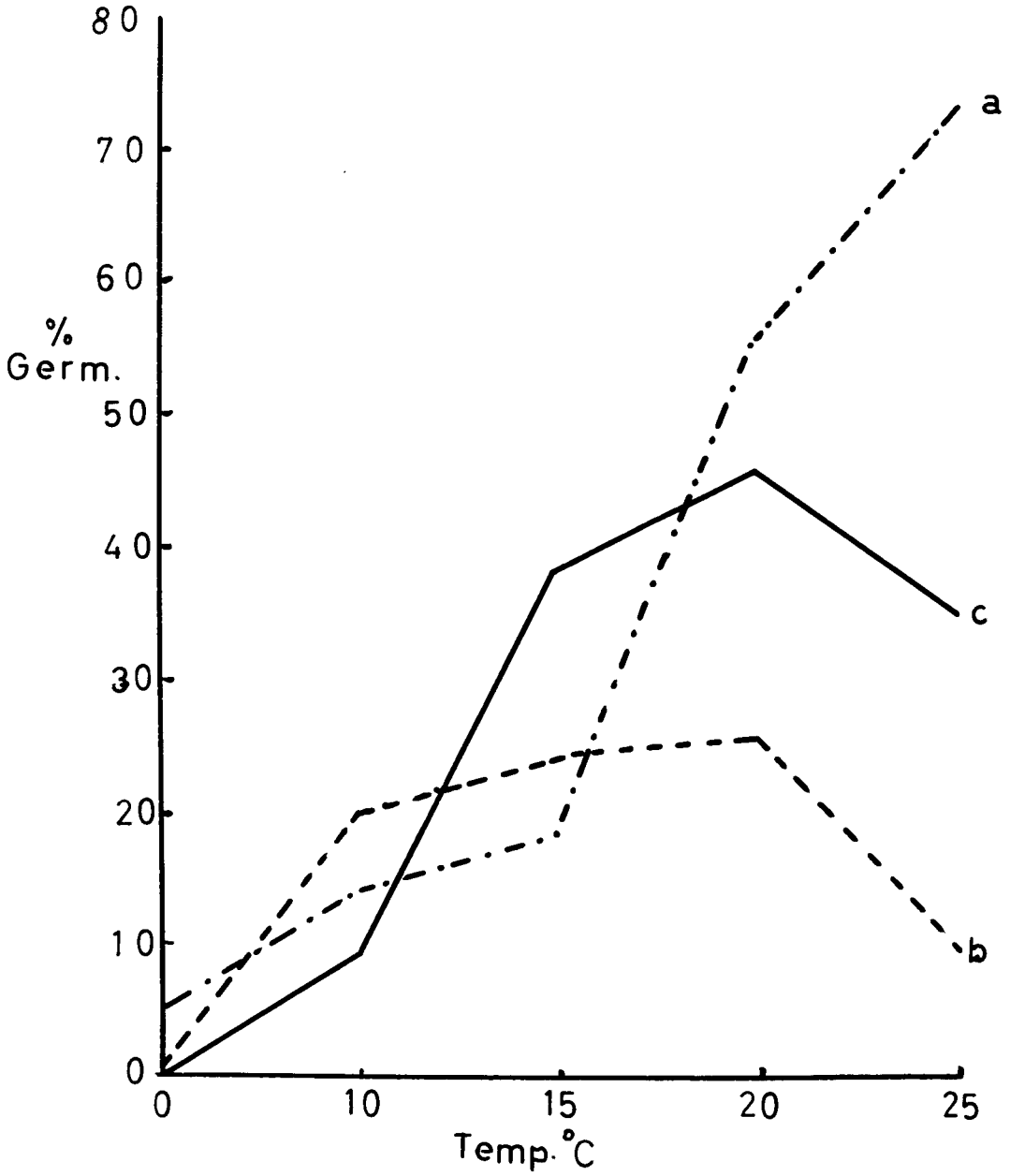


PLATE 27: The effects of temperature on germination after 72 hours.

It is also evident that strain a shows the better final percentage germination than either strain b or c.

(3) Effect of relative humidity on germination. It is important in epidemiological studies of pathogenic fungi that the exact humidity requirements for germination should be determined as these are useful, together with temperature data, for devising a forecasting system or a scheme of preventative measures.

Gottlieb (1950) suggested that water was the primary factor in spore germination and many spores require only water to germinate. Certain fungi require liquid water (Puccinia coronata) while others need a very high relative humidity (100% Perenospora nicotianae). The powdery mildews on the other hand can germinate with relative humidity of only 75%.

This experiment presented difficulties as most of the conventional methods rely on a short total germination time and tend to use non-sterile apparatus. Meria has a long total germination time, allowing easy contamination, and also requires a nutrient source for best germination. A modification of the method devised by Clayton (1942) was used but proved unsuccessful as examination of the spore smears by

removal from the apparatus every six hours caused contamination. Another unsuccessful method was to float sterile strips of cellophane, impregnated with Dox-yeast agar and dried, with a spore suspension on their surface, on solutions giving known humidities contained in sterile petri dishes. Finally a modification of a method devised by Snow (1949), was used, which has already been described (p.78). The pots were examined after sixty hours.

Table 22: Effect of humidity on germination of strain b and c spores after sixty hours.

Solution	Humidity at 20°C	b % germ.	c % germ.
Water	100	24	19
.2M Sucrose	99.62	30	23
.7M Sucrose	98.65	10	7
Sat. $ZnSO_4$	90	0	0
Sat. NH_4Cl. + KNO_3	72.6	0	0
Sat. $Na_2 Cr_2 O_7$	52.0	0	0
Sat. Ca Cl_2	32.3	0	0

Germination would only occur at humidities above 90% and germination was better at 99% relative humidity than 100%.

As a result of this experiment a fuller investigation of germination in the upper humidity range was carried out.

Table 23: Solutions giving known relative humidities above 90%.

Solution	Humidity
Water	100
.2M Sucrose	99.62
.7M Sucrose	98.65
1M Sucrose	98.03
Saturated Oxalic acid	96.
Saturated Na_2SO_3	95
Saturated K_2HPO_4	92.
Saturated $ZnSO_4$	90.

As the results of the experiment gave a lower total percentage germination than obtained previously, the time for the second experiment was extended to 108 hours and observations were taken every twenty-four hours, but otherwise the procedure was identical.

See Table 24 (over) and PLATE 28

Table 24: Effect of humidity on germination of spores of strain b and c.

Time in hours	Humidity	b	c
12	100	7	0
	99.6	4	0
	98.6	0	0
	98	0	0
	96	0	0
36	100	26	15
	99.6	31	17
	98.6	15	7
	98	11	0
	96	9	0
60	100	58	25
	99.6	71	36
	98.6	27	10
	98	26	0
	96	20	0
84	100	67	27
	99.6	84	41
	98.6	49	12
	98	34	0
	96	26	0
108	100	73	41
	99.6	93	57
	98.6	67	15
	98	39	0
	96	30	3

No germination was recorded at 95% humidity or lower and these are not included in the table.

Germination only occurs at relative humidities above 95%. The maximum germination is at 99.6% R.H., 100% R.H. giving the next best germination percentage. Strain b gave better germination than strain c, the

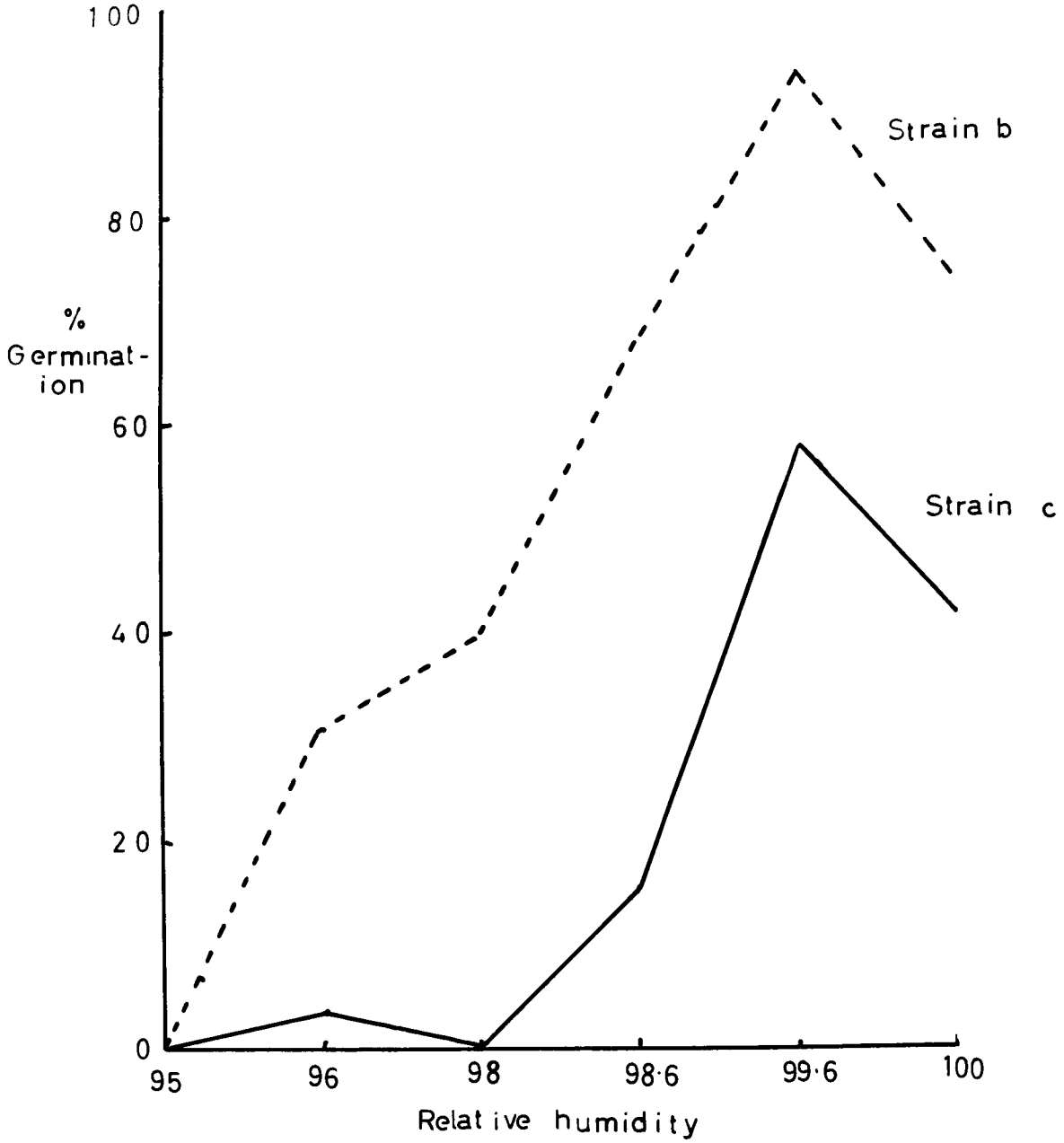


PLATE 28: The effect of humidity on germination after 108 hours.

opposite result to the effect of temperature experiment (p.97), and the maximum germination percentage of strain b was the highest recorded in any germinatinn experiments.

(4) Effect of larch needle extract on germination

Gottlieb (1950) suggested that pathogenic fungi germinate better on exudates of leaves or even bruised leaves of their host plants, than on artificial media. Turner (1953) using oats, has described a method of extraction which was modified for use with larch. This method and the results of the effect of larch extract on germination and growth of Meria will be described in detail in a later section on pathogenicity.

(5) Observations of the effect of exposure of the spores to $0^{\circ}C$ before germination at $20^{\circ}C$

During the experiment to test the effect of nutrient on germination (p.94) plates of Dox-yeast agar were kept at $0^{\circ}C$ for twenty-four and forty-eight hours before incubation at $20^{\circ}C$ to test the effect of exposure to cold on spore germination. The results are shown in Table 25.

(See PLATE 29)

Table 25: Effect of exposure of spores of c strain to 0°C before germination at 20°C

Time in hours	Percentage germination		
	Spores not exposed to 0°C	Spores exposed to 0°C for 24 hours	Spores exposed to 0°C for 48 hours
12	0	0	9
18	12	20	13
24	17	23	23
30	18	27	31
36	24	26	34
42	25	32	35
48	27	34	41
54	29	35	42
60	35	37	44
66	35	37	45
84	37	41	64

Exposure to 0°C prior to germination increases the final percentage germination of strain c spores. Exposure to 0°C for forty-eight hours practically doubles the final percentage germination when compared with spores germinated immediately at 20°C. It appears, from the graph, that exposure to 0°C also decreases the time for the commencement of germination as after forty-eight hours at 0°C, 9% of the spores have germinated after twelve hours at 20°C whereas no germination is found in either of the other two treatments.

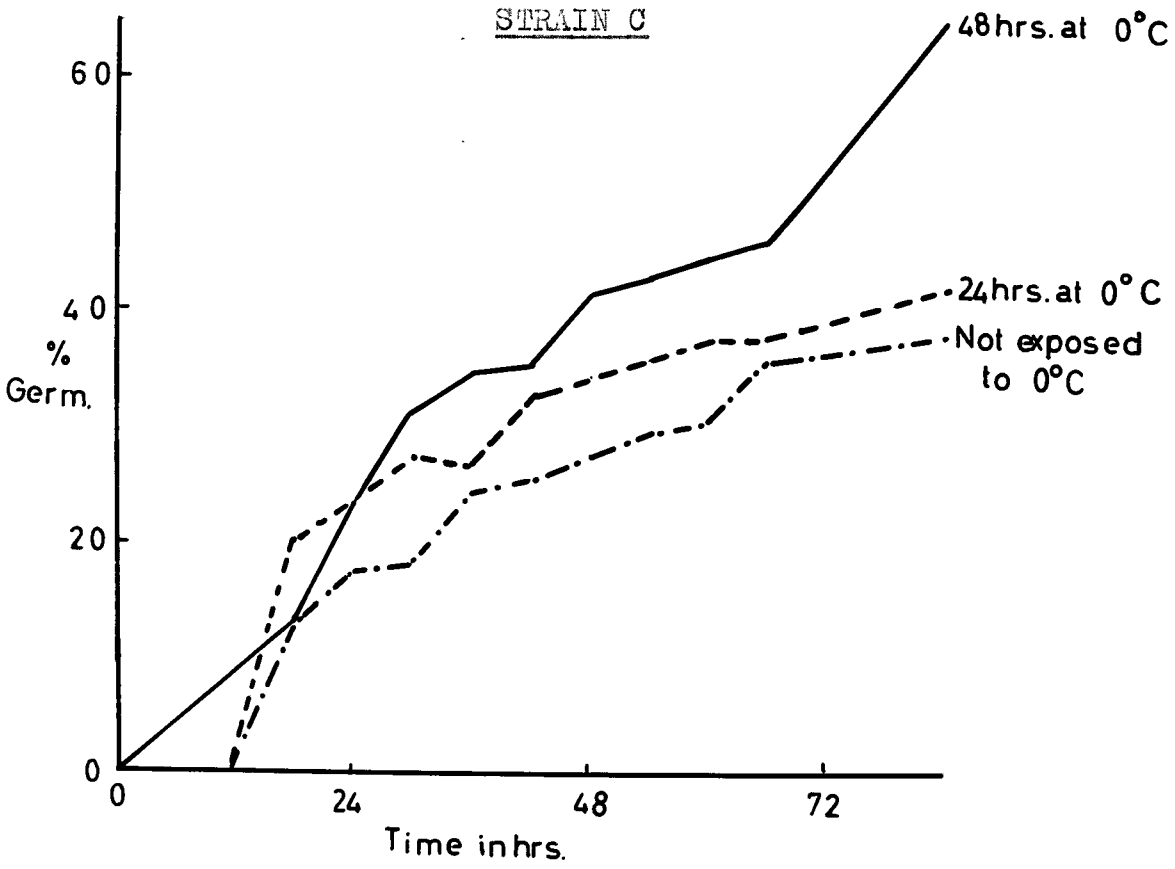


PLATE 29: The effect of exposure to 0°C before germination at 20°C.

It is doubtful whether this phenomenon will have any marked effect on the infective ability of Meria in nature.

The plates exposed to 0°C were then incubated for two weeks at 20°C until sufficient mycelium appeared to enable distinct morphological cultural differences to be detected. This was to discover if any strain changes had occurred similar to those already observed in the effect of long exposures of the mycelium to 10°C.

The plate exposed to 0°C for forty-eight hours showed distinct areas of fluffy pink mycelium (a strain characteristics) within the c strain areas. (PLATE 30). On sub-culturing and typing, these mycelia proved to be a strain colonies. Here an irreversible strain change had occurred unlike the reversible changes of the previous experiment (p.44).

Morphology of germination

Peace and Holmes are the only previous workers to have observed germination of the spores. They found that during germination the spores, placed in water, swelled at one end and a septum formed at the constriction. The larger cell darkened and formed a germ tube which either produced mycelium directly or a sterigma producing further large spores or masses of small spores. The large spores were termed

TABLE 30: Strain c after 48 hours at 0°C prior to spore germination and incubated at 20°C for 12 days.

Islands of strain a mycelium amongst strain c mycelium.

macroconidia and small spores microconidia, which tended to be produced under poor nutrient conditions. Occasionally both cells of the original spore produced germ tubes.

During the current investigation these observations were confirmed and further points noted. Information for the morphology of germination was obtained by microscopic examination of all experimental material used in spore germination experiments, and records were made either photographically or using the camera lucida.

Results (a) the septum sometimes formed before detachment of the spore from the sterigma. (See PLATE 13)

(b) the majority of spores, especially those of strain b, when germinated on Dox-yeast agar produced germ tubes or spore forming initials from both cells, although if one cell was cutting off spores, the other normally produced a germ tube only. PLATE 31

(c) Certain spores, when germinated on Dox-yeast agar, persisted in a state of partial germination and cut off consecutively many macroconidia from a single sterigma. PLATES 32 and 34

(d) Microconidia and many gradations between

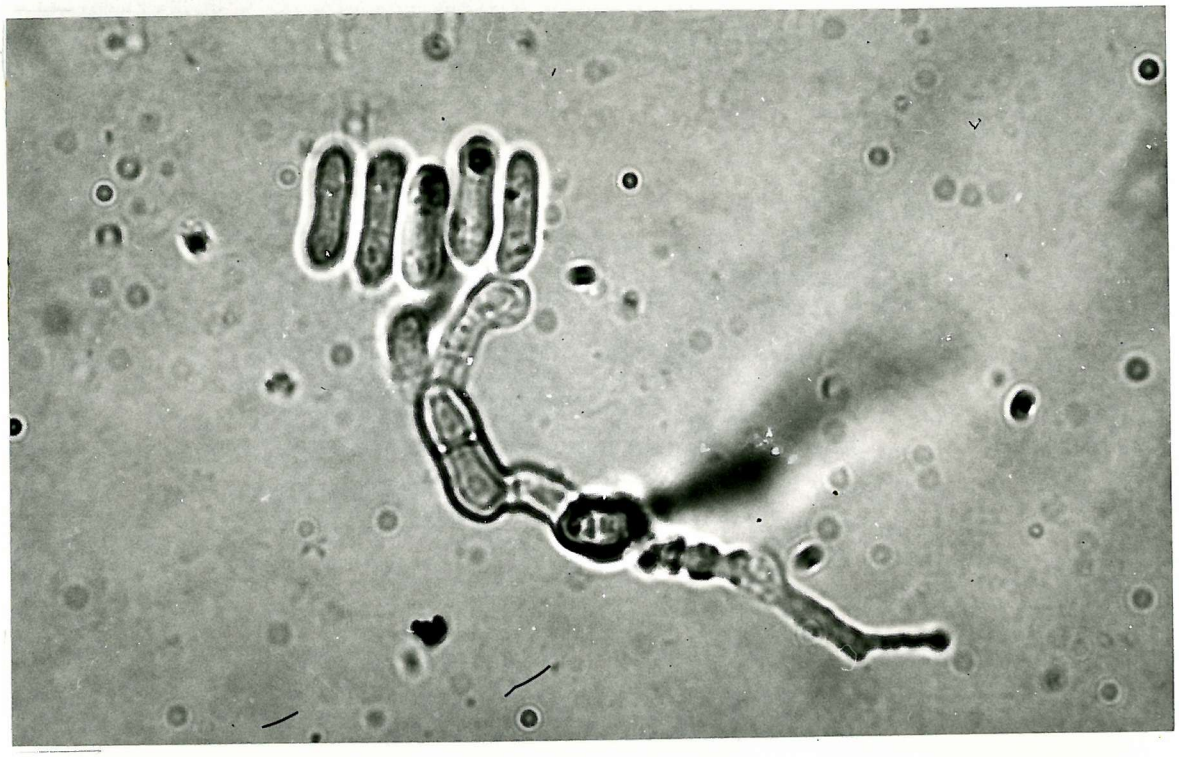


PLATE 31: Strain b after 15 days. Spore germination with one cell forming a germ tube while the other cuts off further conidia.

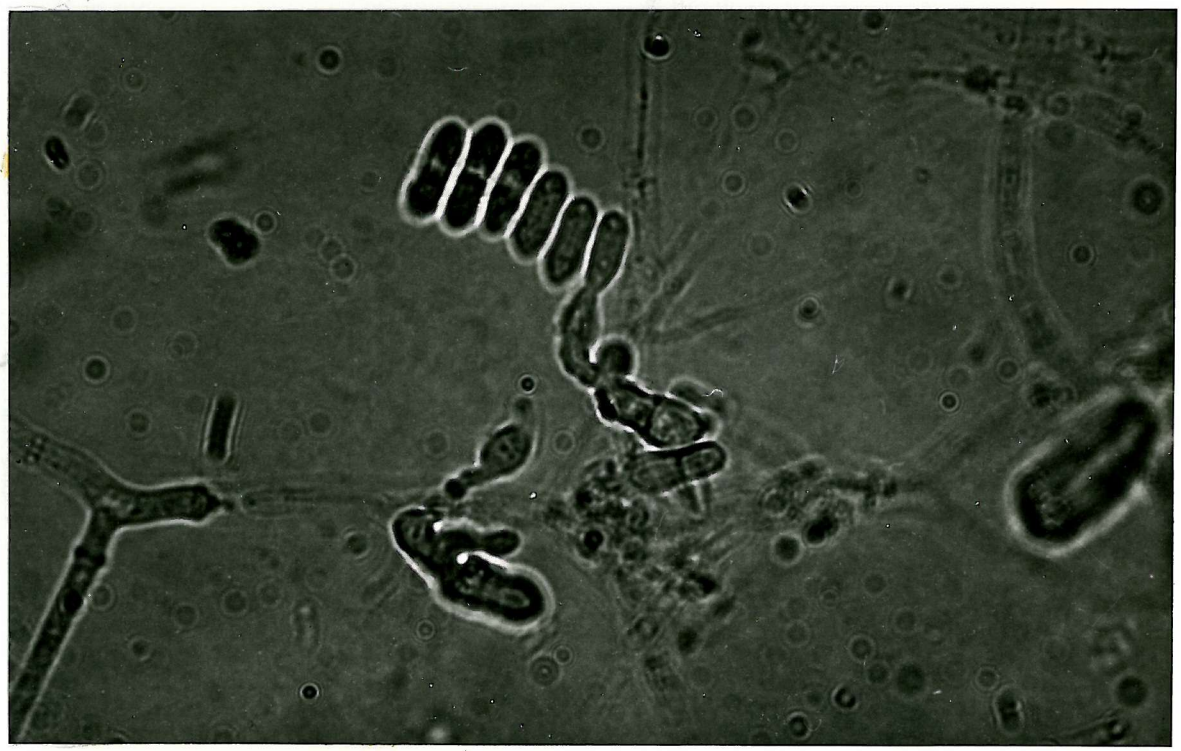


PLATE 32: Strain b after 15 days. Spore germination showing continuous production of macroconidia from a single sterigma.

these and macroconidia were also observed in many cultures but were confined to cultures with low nutrient concentration. Germination of the microconidia was never observed and it was difficult to decide if the macroconidia, produced from the original macroconidia, did in fact germinate, as no consecutive observations were made on a single germinating spore.

(e) Few spores showed the darkening of the germinating cell described by Peace and Holmes, both cells being equally granular. PLATES 33 and 34.

Under any one treatment most of the situations described above can usually be discovered. The formation of microconidia, which are sometimes round but often the same shape as the macroconidia, under conditions of low nutrient, appears to be the only stable phenomenon but it is possible that these are just macroconidia improperly formed owing to lack of nutrient.

One interesting observation is the long periods of time that elapse, in some cases ten, twelve or fourteen days before the formation of a distinct mycelial mass visible to the naked eye. There appears to be a retardation of growth occurring after initial germination as microscopic examination of the cultures

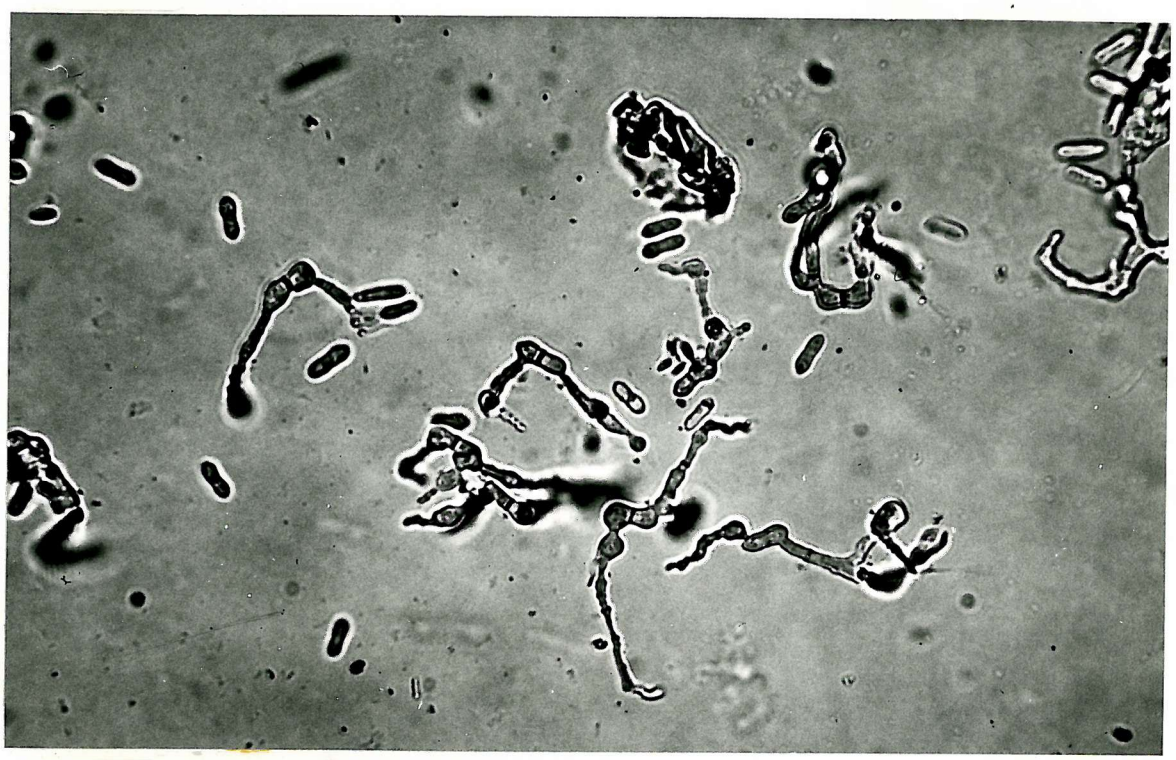
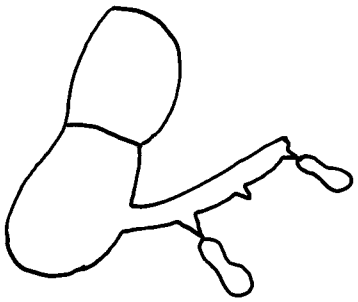
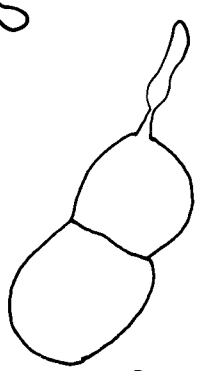


PLATE 33: Strain b after four days showing spore germination.

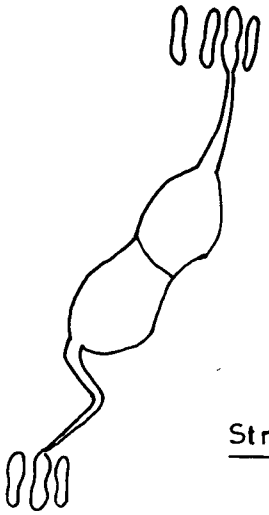
Germination to form further spores



Strain a 72hrs.



Strain b 84hrs.



Strain b 6days

PLATE 34:

reveals many isolated spores with short non-astamosed germ tubes or sterigmata still cutting off further spores. The reason for this retardation of growth is not known and was not investigated.

The morphology of germination of Meria on larch needles will be dealt with in a later section.

ARTIFICIAL INOCULATION

Peace and Holmes were the only previous workers to attempt artificial inoculation experiments. Their experiments were of three types, firstly an attempt to determine the host range of Meria among the genus Larix, secondly the effect of temperature and humidity on infection of single specimens of susceptible species and thirdly larger experiments on a field scale connected with sheltering, transplantation and Meria attack.

At the time (1933) Meria had only been recorded in nature infecting Larix decidua, Larix occidentalis and on a single leaf of a decidua x Kaemferi hybrid and the authors therefore attempted to inoculate many other different species and races of larch. Inoculation was carried out by spraying or brushing the needles with a spore suspension prepared by macerating the diseased needles in water. A spore suspension prepared from cultured material, by adding water and brushing the surface, did not give such certain infection. These methods gave 80-100% success with European larch and Larix occidentalis, but only a single needle of Japanese larch was infected and the Siberian larch (L. sibirica Ledebour) and Korean larch (L. Gmelini Pilger var.) could not be infected.

Attempts were then made to discover if resistance of these larches to Meria was physiological but the results proved inconclusive.

From general observations of spread of disease in larch nurseries Peace and Holmes considered that the weather is the most important factor influencing severity of an attack, and that moisture was the most important climatic component. Investigations on these lines were attempted and with trees inoculated and left in the open infection only occurred on those inoculated during showery weather. Individual trees were then inoculated and placed under bell jars of varying arrangements, to give varying evaporation rates, and again infection only occurred where the evaporation rate was low. Peace and Holmes concluded that the evaporation rate was more important than humidity which accounted for non-infection in nature during hot weather when evaporation rate was high.

From a temperature experiment infection was found to occur between 10 and 25°C but not at or above 30°C.

Sheltering the seedlings grown in nursery beds by means of lath screens to prevent infection, gave anomalous results, while the manurial treatment of the seedlings and their geographical source has no

bearing on Meria attack. Artificial inoculation experiments are obviously important in the study of Meria for several reasons:

(a) The conditions under which Meria attack will occur can be accurately determined and a possible forecasting system devised.

(b) The appearance of disease symptoms on the three different larch species inoculated simultaneously could give an indication of the susceptibility of the particular species and also the virulence of particular Meria strains.

(c) It may be possible to distinguish between the different strains by their disease symptoms on particular species of larch or their severity of attack.

In consequence the experiments were divided into three distinct series.

(1) Greenhouse experiments.

Material and methods

Specimens of European, Hybrid and Japanese larches one year old were supplied by the Forestry Commission and were planted in six inch pots and stored in a cold greenhouse. In order to supply a constant source of experimental material, about 20 trees of each species, just after needle fall, were placed in

a temperate greenhouse and exposed to overhead illumination by fluorescent tubes. Dormancy was broken after about two weeks and needles were produced which reached full size in four to five weeks but remained light green and immature. It was also possible to break dormancy by exposing defoliated trees to illumination in a cold greenhouse during winter months but the needles formed remained small and soon withered. As far as possible the small scale experiments were carried out with the trees left in the cold unlighted greenhouse that developed needles under natural seasonal conditions.

Inoculation technique

Various techniques were used, the most successful being of modification of that of Peace and Holmes using artificial cultures. Slope cultures of the required strains were incubated for 28 days at 20°C and, using sterile water, spore suspensions were made by agitating the submerged surface of the culture using a rubber policeman. The suspensions from ten tubes were transferred to an atomiser and used as inoculum. Before spraying with inoculum the trees were first sprayed with water with a Flit gun, and after inoculation were transferred into inoculation bins (with glass lids) placed over a wet sand tray to maintain a high humidity. (See PLATE 35)

PLATE 35: Showing inoculation bins on a sand tray, containing inoculated trees.

To ensure that infection could occur the bins were placed in a shaded area and regularly sprayed with water to maintain the humidity. Forty-eight hours was the minimum incubation period underneath the bin and sixty hours was preferable. However, under summer conditions this encouraged the growth of various other fungi, especially Botrytis, and the incubation time was varied according to prevailing conditions.

Infection was more certain if a 0.1% solution of water agar was substituted for the sterile water used to make the suspension and this was subsequently used.

After the appropriate time the trees were removed from the bins and placed under individual cellophane covers to prevent cross-infection on the crowded greenhouse benches. This again led to contamination under high humidity conditions and so larger wooden frames covered in cellophane and polythene sheets that could take four or six similarly treated pots, were substituted.

The major difficulty with the polythene frames was the rapid rise in temperature to above 30°C causing death of spores (see later section on field experiments), which was partially overcome by placing

the pots under the greenhouse staging or in a shaded area outside the greenhouse. Under summer conditions most trees could be left uncovered in the open after their initial incubation period, provided that the overnight temperature did not drop below 10°C and retard infection, and humidity remained high.

To test the infectivity of different Meria strains on European, Hybrid and Japanese larch

Isolates of strains a, b and c from each tree species were grown in slope culture and spore suspensions were made as explained above. Each of these suspensions was sprayed on three similar trees of each larch (European, Hybrid and Japanese). The inoculated trees were incubated for forty-eight hours and then placed in the open under individual cellophane covers. As insufficient inoculation bins were available for eighty-one trees the experiment was performed in two halves, firstly using a and b fungal strains and secondly strain c.

The trees were watered regularly and examined twenty-seven days later for disease symptoms. Disease symptoms, described by most authors, consist of a yellowing and eventual browning (Mer 1895) of the infected needles. This browning starts from the tip and may eventually cover the whole needle, but the needle is usually shed before this occurs (Vuillemin 1896).

Table 26: Nurseries giving inoculum source for artificial inoculation experiments (single spore isolates)

Nursery	Tree Species	Fungal Strain
Fleet	EL	a
Fleet	HL	
Fleet	JL	
Coed-y-Brenin	EL	b
Benmore	HL	
Glenfinart	JL	
Glenfinart	EL	c
Fleet	HL	
Glenfinart	JL	

When the needles are brown fructifications can be found protruding through the stomata on the lower needle surface (Mer 1895) but these must not be confused with stomatal wax (Peace and Holmes) or grains of sand or resin (Prichoda 1954) and microscopic examination, after staining with cotton blue, is essential. These fructifications occur alongside the midrib on the lower needle surface but in severe infections can also be found in a similar position on the upper surface (Mer 1895). Mer did not mention if fructifications were found on both green and brown parts of the needle but Vuillemin (1896) suggested that the fructifications and the mycelium

were confined to the green parts. He also noted later (1905) that if the needles were kept under damp conditions fructifications would occur from both surfaces. Hiley (1921) recorded that the fructifications were confined to the browned parts of the needle and suggested that Meria attack could be distinguished from frost damage by the following criteria:

(a) youngest needles are not affected but only those a few inches from the shoot apex

(b) the needle does not die over its whole length at the same time

(c) fructifications are present.

He noted that infection occurred on both the leaders and short shoots of the nursery trees but tended to be confined to the leaders in six to seven year old trees.

In the present investigation browning of the needle for at least one third of its length, from the tip downwards, and confirmed by microscopic identification of the fructification, was used as an indication of infection. Occasionally, under autumn conditions in a non-heated greenhouse, the browning was confined to small isolated spots, also observed by Batko (1955) and infection was confirmed by microscopic examination. To confirm infection and

to investigate any change of strain that may have occurred in passage through the host, the infected needles were used in attempts to re-isolate the fungus (see method on p.12)

From this first experiment some infection was evident, especially on European larch, but insufficient to give any analysable results and consequently this experiment was repeated several times as trees and inocula were available. The experimental method and as many environmental conditions as possible were kept identical throughout the whole experimental series.

Although Peace and Holmes obtained a very high percentage (80 - 100%) success in their inoculation experiments they were using spore suspensions derived from infected needles, a method not possible for the current investigation. Success in all artificial inoculation experiments was very limited and no experiments using spore suspension obtained from needles were attempted. The overall results of this series of infection experiments run over a period of two and a half years are given in the following table.

Table 27: Summary of results of infectivity experiments.

Fungus		Tree species infected		
Strain	Isolate from	EL	HL	JL
a	EL	✓	x	x
	HL	✓	x	x
	JL	✓	x	✓
b	EL	✓	✓	✓
	HL	✓	✓	x
	JL	x	x	✓
c	EL	x	x	x
	HL	✓	✓	x
	JL	x	x	x

✓ - infection x - no infection

The following trends can be seen:

(i) European larch is more susceptible to *Meria* attack than either Hybrid or Japanese larch

(ii) It is possible that strain b is the most virulent as that isolated from a European larch infected both Hybrid and Japanese larches. These results will be considered more fully in the section dealing with nursery attacks.

Table 28: Detailed results of the infectivity experiment.

Tree species	Fungus type	No. of replicates infected	No. and position of infected areas	No. of infected needles	Remarks	Fructifications present	Re-isolation of Meria - & strain
EL	ELa	2	4 - shoots scattered about seedling bottom and middle	10	Poor infection. Small brown areas only. Others caused by damage to needle	✓	-
EL	HLa	1	3 - shoots at top usually leaders	8	Large brown areas present	✓	/ b
EL	JLa	1	3 - shoots at top and middle of tree	10	Typical infection mostly on leaders	✓	/ b
JL	JLa	2	5 - mostly top leaders	24	Yellow infection areas near base of top leader, white areas also present	✓	-
EL	ELb	2	6 - mostly top shoots and leader	35	Typical symptoms - much browning	✓	b c

(Continued overleaf)

Table 28 (contd.)

Tree Species	Fungus type	No.of replicates infected	No. and position of infected areas	No. of infected areas	Remarks	Fructifications present	Re-isolation of Meria & strain
EL	HLb	1	2 - top leader and few needles of top shoots	6	Typical symptoms although few needles infected	✓	b
JL	ELb	2	5 - top or middle shoots - top leader on 1 tree	15	Typical symptoms	✓	c
HL	ELb	2	4 - top leader and top shoot leaders	11	Typical symptoms	✓	c
HL	HLb	1	3 - top leader and next shoot leaders	8	Poor infection - isolated brown spots	✓	-
JL	JLb	2	12 - leaders of side shoots.Most of tree especially bottom and middle	32	Typical symptoms - also scattered white spots possibly due to scorching	✓	-
EL	HLc	1	2 - top leader and both trees	6	Typical symptoms but poor infection	✓	c
HL	HLc	1	3 - top leaders and one side leader near top	7	Little infection - isolated brown spots	✓	c

The following tendencies are noticeable:

(i) Infection in most cases was poor, only one or two of the three replicates showing disease symptoms. This is possibly caused by the loss of virulence of Meria by continual sub-culturing. These inoculation experiments were carried out for two and a half years still using the original strain isolates, to give a standard technique, and from results already recorded regarding production and germination of spores (p.91) from ageing cultures it is probable that all strains were less virulent and less capable of causing infection.

(ii) Infection tended to be confined to the upper parts of the tree, especially the top leader or leader of one of the topmost side shoots. This could have been due to the spraying technique although, as far as possible, the whole seedling was given an even spray of spore suspension.

(iii) Few re-isolations could be made although frucitifications were present. This was unusual as a fairly high proportion of success was obtained in isolations from diseased material received from the nurseries. Of the nine successful re-isolations four were strain b and five were strain c. These strains were identified by direct slope culture of the isolated fungus and not by taking single spore cultures as

previously mentioned (p.14) In five out of nine re-isolations a different strain of Meria was identified from the strain used as inoculum. The significance of this will be discussed later.

As a result of the poor percentage infection slight modifications were made to the method of obtaining spore suspensions for inoculum. Firstly a sterile 1% O.Y. agar solution was used in place of the water agar and after the suspension was made it was uncubated for 20°C for twenty-four hours before inoculation. Secondly using a modificatinn of a method discussed by Andrus (1941) the mycelium, together with a certain quantity of D.Y agar was removed from the culture tubes and homogenised, in 0.1% O.Y. agar solution, in a Waring Blendor. Neither of these methods gave an increased percentage infection and were later discarded.

The effect of time on the development of disease symptoms on different species of larch

From data sent from Forestry Commission nurseries it was noted that disease symptoms appeared at different times on different tree species and, using the information from the previous experiment this sequence was investigated. As the strain of Meria isolated from European larch appeared to be the most

virulent a large quantity of this inoculum was prepared. This was sprayed onto three replicates of European, Hybrid and Japanese larch which were then treated as before.

The first attempt was a failure as the temperature in the incubation bins rose over 32°C and a further batch of trees were lost due to Botrytis infection. However, using trees that had broken dormancy in a light chamber and leaving these in a temperate greenhouse the following results were obtained:

Table 29: Effect of time on appearance of disease symptoms

Infected tree	Meria strain	Date of inoculation	Date of appearance of disease symptoms	Re-isolated strain
EL	b	4.2.57	21.2.57	b
HL	b	4.2.57	26.2.57	c
JL	b	4.2.57	4.3.57	c

Disease symptoms appeared earlier on European larch than on either Hybrid or Japanese larches when inoculated at the same time. This possibly indicates that some substance present in European larch needles stimulates a rapid growth and reproduction of Meria or that initial infection of European larch occurs more rapidly than infection of Hybrid or Japanese due to anatomical or physiological reasons.

This confirms reported observations from the nurseries and indicates that nursery attacks may start from a single infection time, although probably from many sources, as Meria is capable of overwintering in the fallen needles and producing fresh conidia in the spring (Mer 1895). It was not possible to distinguish between the different strains of Meria by a cursory examination of infected needles as all strains gave similar disease symptoms.

The very low percentage infection in the whole series of these inoculation experiments means that many of the experiments have not given repeatable results, although conditions have been kept as stable as possible. The results so far listed are composite results collected from all the experiments run over a two and a half year period.

2. Field experiments

Field experiments have previously been performed by Peace and Holmes together with observations of the spread of Meria within normal larch nursery plots. In beds where diseased transplants were intermingled with un-infected trees infection occurred rapidly in the early summer. Similarly un-infected beds a few yards from a group of infected trees became heavily infected, although no infection was observed on a bed 20 yards

from the infection source. On the other hand in a different area slight infection occurred on beds at least 50 yards from an infection source. Peace and Holmes concluded that for rapid infection to occur the disease source must be abundant and near to the un-infected plants and then infection would take place in short steps from the origin. They observed that four seed beds within 20 feet of the infection source became infected at the same time and that even a four feet high hessian screen did not delay attack of a similar bed. Spacing of trees and seed beds was not suggested as a prophylactic measure by Peace and Holmes, who devised spraying techniques, but Prichoda (1954) suggested either a very wide spacing of trees and beds or removal of two year old infected trees to woods, during the growing season where infected needles were lost between grass and weeds and further infection was prevented. Both workers noted that spread of disease was stopped by the onset of dry weather.

In order to investigate the spread of Meria under natural conditions a series of field experiments were set up in three groups:

(a) To investigate distance of spread from inoculum source in discontinuous plots of trees,

(b) To investigate rate of spread of different

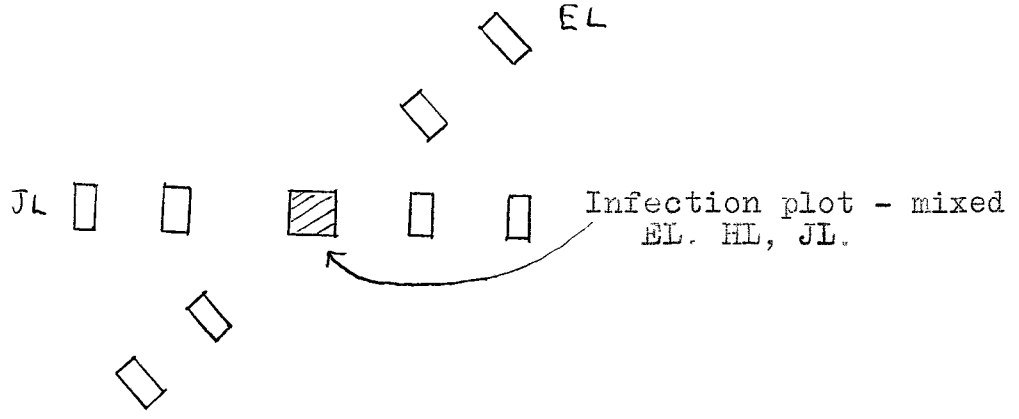
Meria strains in continuous plots of trees

(c) To investigate rate of spread from one tree species to another in continuous plots of trees. As the a and b strains of Meria appeared to be the most virulent from greenhouse experiments these strains were to be used as inocula in these field experiments.

Several thousand European and Japanese larch were sent by the Forestry Commission, but unfortunately only sufficient Hybrid larch were available to replenish potted stock of this species, and the experimental layout was modified so that this species could be planted when available.

The trees were planted during December and January 1956-57 in a shallow sheltered south facing valley of the Botany garden and were arranged in plots as indicated.

(a) To investigate spread of inoculum in discontinuous plots. Two areas of plots as shown -



(PLATE 36)

Strain a experimental plots.

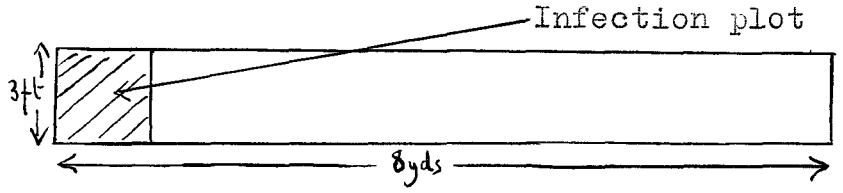
Strain b experimental plots .

PLATE 36: Series (a) experiments with incubation covers over infection plots.

To give dense cover the trees were planted four inches apart. The central plot was three feet square, and the smaller plots were 3' x 1'6" and the plots were three yards apart. Space was left in the second diagonal for the planting of Hybrid larch when these became available.

Two areas of these plots were planted side by side but ten yards apart in identical soil, to test spread of strain a and strain b.

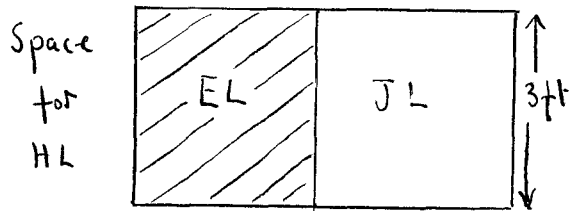
(b) To investigate the spread of different Meria strains in continuous plots -



(PLATE 37)

Four plots, two of European and two of Japanese larch were planted as shown. The trees in the infection plot were 4" apart but the remainder were 6" apart.

(c) To investigate rate of spread from European to Japanese larch in a continuous plot -



JL plots with incubation covers over the infection plot.

EL and JL plots showing anemometer.

PLATE 37: Series (b) experimental plots.

Two plots, planted as shown, were placed three yards from the far end of the plots in (b) above. To record the weather conditions throughout the experimental period meteorological equipment was borrowed from the Meteorological Office. A cup counter anemometer Mk.II was erected at tree height by series (a) and (b) plots - see PLATES 36 and 37, and a direct reading thermograph was used to record continuously wet and dry bulb temperatures in a Stevenson screen placed at the side of the infection plot in series (a) experiment (see PLATE 36). By means of a simple plastic grid designed by Hirst and Steadman (1956) rapid readings of relative humidities above 90%, between 75% and 90% and below 75% could be read directly from the recording paper in the thermograph.

Experimental details

In the early summer of 1957 (20th May) large quantities of inoculum of a and b strains were prepared using the standard method (p.118) and the infection plots of all series of experiments, after being sprayed with water, were sprayed with inoculum. Care was taken that the neighboroughing trees were not infected by chance, by covering these with sheets of polythene.

After inoculation the infection plots were covered by wooden frames covered in polythene sheeting

in order to maintain a high humidity. The actual inoculation period was a fairly warm humid day that had followed two days intermittent rain. The covers were sprayed with water and left. On checking the temperature inside the covers twenty-four hours later, using a maximum and minimum thermometer kept inside, the maximum temperature had reached 118°F, enough to kill the inoculum.

Attempts were made to spray the polythene covers continuously with water but these proved impractical and on a moderately sunny day the temperature inside the cover was at least 10°F above that of the surroundings.

Eventually hessian covers were substituted for polythene and these proved more effective, although frequent watering was necessary as drying out occurred very rapidly. Using hessian covers a second inoculation was made on 21st June. Rain occurred in the night and during much of the first day keeping the covers wet. The infection plots were covered for four days and watered twice daily. The thermograph indicated that in the screen the humidity did not fall below 75% for the whole of the incubation period and that the temperature fluctuated between 72° and 52°F.

Several examinations of trees in the infection

plots revealed no disease symptoms.

A third inoculation was attempted on 23rd August. The covers were left in placefor a week, being watered twice daily, and humidity was over 70% for six days. However the temperature in the screen rose to 83°F during the early incubation period. Again no signs of infection were found on periodic examination of the plots for the three weeks after removal of the covers.

A final attempt at infection was made on 31st September under cooler conditions but the overnight temperatures were down below 40°F and again no infection was evident.

The experiments were then discontinued as the trees began to shed their needles.

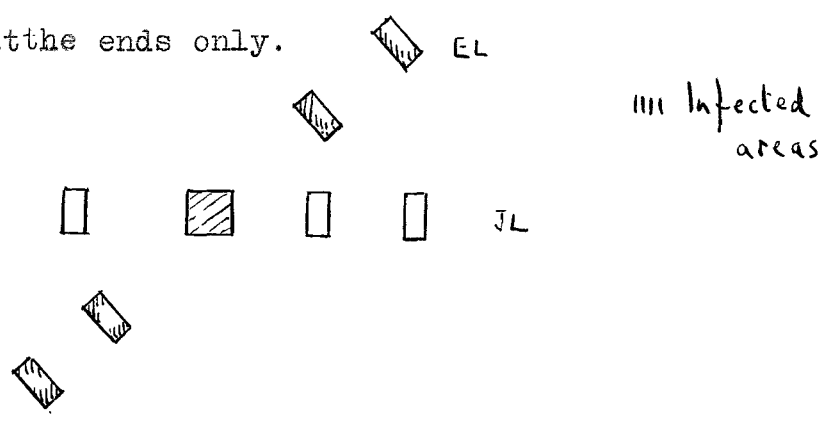
A reason for the failure of these experiments was probably the abnormal weather conditions of summer 1957 giving very high temperatures which killed the inoculum before penetration of the needles occurred. The temperatures in the sheltered valley were probably higher than those of exposed ground in the same area. In 1958 the experiments were restarted. The first inoculation took place on 19th May and the temperature under the covers remained within suitable limits (48°F - 71°F). The covers were kept in place for six days and watered twice daily. After removal the

humidity remained very close to 90% for the next seven days. Examination of infection plots showed some infection on both plots in series (a) experiments and the European larch plot infected with b strain of Meria in series (b) experiment.

Further attempts were made, at monthly intervals, to infect the non-infected plots but without success.

Series (a) experiments At the end of the summer all European larch plots in a and b strain experiments showed some infection This tended to be severe - i.e. many trees attacked and many needles on each tree infected, on the plots nearest the infection plot while only moderate or light on plots furthest from the infection plot.

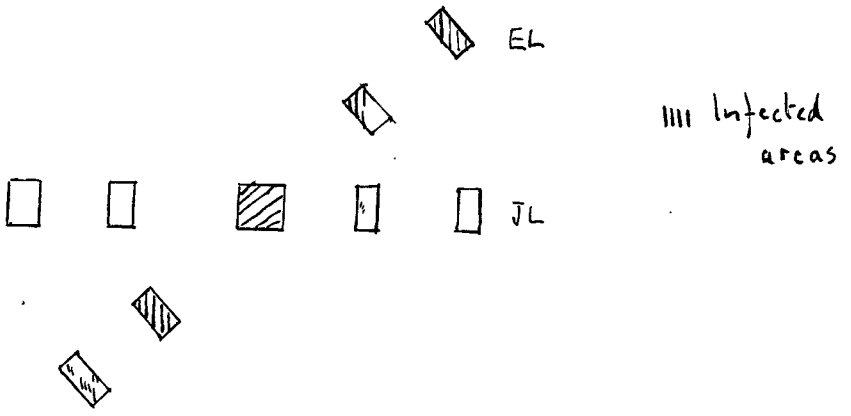
b strain experiment - European larch plots nearest infection plot showed severe infection at front and sides of plot especially on top leader shoots. One of the further plots showed moderate infection at the front of the plot while the other showed severe infection at the ends only.



Although some Japanese larch showed disease symptoms in the infection plot no Japanese larch in the other plots were infected.

a strain experiments - European larch plots nearest infection plot showed slight infection, one at the side, on the top leading shoots and the other scattered throughout the plot on top leaders and one side leader of a tree in the front. Both the farther plots showed very slight infection, only a few needles scattered throughout the top leaders of a few scattered trees.

a strain



Again, Japanese larch were diseased in the infection plot, but no disease symptoms were discovered on any of the other plots apart from a top leading shoot with three infected needles on a tree in the front of one of the nearest Japanese larch plots.

Unfortunately no records were made of the dates of appearance of disease symptoms on plots other than the infection plot - all recordings being made at the

end of August 1958. No isolations were made for the diseased material and so the strains of Meria have not been determined.

Series (b) experiments

The infection plot of one plot of European larch inoculated with b strain of Meria showed moderate infection, a scattering of trees, mostly with infected top leaders, in early June. At the end of August slight infection was noticeable on trees up to three yards from the infection source. On plants scattered throughout this three yards the top leaders showed several infected needles (usually three or four). There was a slight tendency for the number of infected needles per tree to be greater nearer the infection source but, at a cursory examination, infection could still only be called slight. No records of the rate of spread of infection in this plot are available and no re-isolations of the pathogen were made.

Series (c) experiments

After repeated monthly inoculations no infection was obtained on the European larch areas with either a or b strains of Meria.

Discussion of results to field experiments

The results of these experiments are poor and mostly inconclusive although certain trends can be

recognised.

(i) The result of greenhouse experiments suggesting that b strain is more virulent is confirmed in the series (a) experiments, where infection by this strain is much more severe than that of the a strain. This difference could have been due to local environmental conditions but it is unlikely in the wide scattering of the plots. This suspected virulence of the b strain is also confirmed by the result of the (b) series experiment where the b strain of Meria was the only one to give infection.

(ii) Another trend in the greenhouse experiments was also confirmed, namely that European larch are much more suspectible to Meria than Japanese larch, these being the only trees infected in each series of experiments, apart from the infection plots and a single leading shoot of one Japanese larch.

(iii) Peace and Holmes' observations regarding spread were confirmed, Meria will easily spread over short distances. It is not known whether the infection of the plots furthest from the inoculum source in the (a) series experiments occurred at the same time as the nearer plots but, judging by the appearance of the infected trees in each plot (one third to two thirds browning of needles, little shrivelling of

browned portion and needles still adhering to the shoot) it is possible that infection was simultaneous. The greatest distance of spread in isolated plots appears therefore to be six yards, regardless of wind direction - both diagonals were infected.

Within a continuous plot infection from a source again occurs easily but not, from the results obtained, very rapidly. This infection did not, in fact, spread to a neigh_bouring plot of European larch only two feet to one side of the infected plot.

The poor results to these field experiments are probably due to a combination of reasons. The inoculum used in 1958 was obtained from Meria cultures which had been isolated up to two years previously, giving a much reduced infective capacity (p. 91). There were many environmental difficulties mainly concerning the humidity and temperature under the incubation covers leading to non-infection of inoculation plots. Further experiments need to be designed with a greater distance between individual plots before further information regarding spread is available.

3. Laboratory larch culture experiments

To provide details of Meria infection within individual needles, and also to discover the method of infection, larch culture experiments were designed to

produce material for examination.

Larch culture methods - to determine the best culture method cut leading shoots from plantation larches were grown with their cut surfaces dipping in a sterile trial solution inside large boiling tubes sealed with a cotton wool plug. After the larch leader was inserted these were left undisturbed on the laboratory bench, and an estimate of their growth and health was made after three weeks.

Table 30: Trial solution and effect on growth and health

Solution	Growth and Health
Water	Moderate
0.1% Sucrose	Moderate
1% Sucrose	Good
10% Sucrose	Poor

Several of the shoots showed infection by saprophytic fungi, especially those in 10% sucrose solution. The growth and general health of the shoots in 1% sucrose appeared to be best, although there was slight mould infection, and this solution was used for future culture experiments.

When inoculum was being prepared for other inoculation experiments larch culture tubes were also

prepared. Leading shoots of European, Hybrid and Japanese larches were washed for five minutes under a cold running tap in an attempt to remove undesirable organisms. Using a, b and c strains of Meria both surfaces of all the needles on the shoots were then inoculated with the spore suspension using a sterile paint brush and working in the inoculation chamber. The cut end of the shoot was then placed beneath the surface of sterile 1% sucrose in a sterile boiling tube and the tubes were sealed and left on the laboratory bench. By inoculating replicate shoots sufficient material was available to enable removal of needles every few days for microscopic examination. Sample needles were removed at four, seven and ten days and those showing typical disease symptoms at three weeks, and embedded in wax for microtome sectioning.

Table 31: Plan of larch culture experiment and results.

Larch species	Meria strain	Infection	Remarks
EL	a	✓	Good on upper needles
	b	✓	do.
	c	-	Contamination by other fungi
HL	a	-	Sprouting of short shoot no disease symptoms
	b	✓	Scattered infected needles
	c	-	
JL	a	-	Contamination by other fungi
	b	✓	Good infection of several needles
	c	-	One possibly infected needle

Only four shoots showed any distinct disease symptoms after three weeks but four, seven and ten day samples of all shoots were embedded in wax and the reason for non-infection may be discovered by microscopic examination. Repeated inoculation of fresh specimens of the uninfected shoots failed to give infection but samples of these at four, seven and ten days were embedded and sectioned. No re-isolations of Meria were attempted from the infected needles.

After removal needles were treated as follows:

(i) Fixed in Carnoys fixative for twelve hours.

(ii) Embedding in wax blocks which were sectioned on a microtome, L.S and T. sections of each needle being cut;

(iii) Sections were stained and examined. Several staining techniques were tried. Aqueous Azure A (Huebschman 1952) and Fleming's triple stain were partially successful but a double staining technique using 1% aqueous safranin and picro-aniline blue gave the best results.

The observations confirm the results of previous workers (see Introduction). Meria is a facultative parasite, the needle cells being killed in advance of the hyphae. The spore germinates on the needle surface and produces a germ tube which enters the needle via the stomatal pore, where the humidity remains high.

The mycelium is intercellular and the hyphae have relatively thick walls. There are no haustoria. Prior to spore formation several hyphae grow towards a stoma and form a coiled mass beneath its pore. From this mass the conidiophores emerge through the stomata as aseptate club shaped hyphae later becoming one to three septate. Conidia are formed from each cell by the production of a 'bubble' from the sterigma near the end of the cell, as in culture (see Plates 11 and 16). Several nuclei pass into each conidium. Each sterigma produces a further conidium as soon as one is shed but it is not known how long this process continues.

PATHOGENICITY

In an attempt to devise a forecasting system or at least to specify the conditions under which Meria attack might occur it is important to study the pathogenicity of the fungus.

Mer (1895) suggested that under very wet conditions the attack would be very severe and that weak trees were more susceptible than healthy trees, although only young trees(up to two years) were attacked. The mycelium was not found in the branches and the fungus probably over-wintered in the needles as was shown by low incidence of attack when shed needles were removed and burnt in the autumn. Vuillemin (1905) confirmed attack of nursery trees only and suggested it occurred under humid conditions. Hiley (1921) suggested that sporulation also would only occur under humid conditions and noted the incidence of disease symptoms on top leading shoots of six and seven year old trees. Both Western larch and European larch were affected but not Japanese. Peace and Holmes noted that the fungus over-wintered in the needles but more probably those adhering to the tree and not those onthe ground. In the older trees it was likely that the shed infected needles, caught between the branches, provided the infection source for the following spring. As a result of their spread

experiments the most important pathogenic factors were (a) the age of the tree, as appreciable damage was only caused on one and two year old seedlings (b) the weather, mainly moisture content of the air, or more correctly the evaporation rate, and temperature, although infection could occur over a wide range of temperatures (0° - $25^{\circ}C$). There was no evidence that weak trees were more susceptible to attack than healthy trees. As a result of artificial infection experiments, when infection of Japanese larch proved impossible, Peace and Holmes decided to investigate the reasons for this resistance. To discover if resistance was purely mechanical a number of inoculations were made by clipping off the tips of needles with a pair of scissors dipped in spore suspension. Many successful inoculations were made on European larch but none on Japanese, indicating that resistance was largely physiological. Further experiments were made to test this hypothesis. Both boiling and cold water extracts of the different needles were made, the former sterilised by autoclaving and the latter by passage through a Berkfeld filter. Germination of spores and mycelial growth was similar on both extracts. In case the concentration of immunizing substance within the needle had been too dilute for effect on spore germination, a further experiment using expressed

juice from both types of needles was attempted. Uncertain mycelial growth occurred in both extracts but germination of spores in European larch extract occurred more rapidly than germination of spores in Japanese larch extract. This result could have been caused by decay of toxic substances under unnatural conditions, or it indicated that in nature on Japanese larch, by delay of penetration of the infection hypha under damp conditions, dry conditions would arise and cause death. From spread experiments Peace and Holmes concluded that for rapid infection to occur the disease source must be abundant and near to the uninfected plots when infection would occur in short steps from the origin.

Prichoda (1954) while confirming suggestions that the disease occurs under damp conditions and is prevented from spreading by dry conditions, mentions that it is more virulent on plants in poor soil than on plants in good soil.

Host range - No mention of Meria attack on other than European or Western larch is recorded prior to 1946 when Robak from Norway noted the symptoms on both Japanese and Hybrid larches. Orlos (1951) recorded the disease on Siberian or Asiatic larch and Batko (1955) records infection of both Japanese and Hybrid larches in Britain.

To summarise results from previous workers:

(i) Infection occurs under humid conditions and within a wide temperature range and is arrested by dry conditions.

(ii) Primary infection in the spring results from shed infected needles or those still adhering or trapped in the tree.

(iii) Infection occurs rapidly, by short steps, from a disease source.

(iv) Fructification will occur under humid conditions.

(v) Infection was confined to European and Western larch until 1946 when diseased Japanese and Hybrid larches were discovered.

(vi) The resistance of Japanese larch to Meria attack is apparently physiological.

From results already obtained these trends have been confirmed and given greater specificity and other trends have become apparent, which are as follows:

(a) Infection will occur only under very high humidity as the spores will not germinate unless relative humidity is above 95%. The temperature range for infection recorded by Peace and Holmes 0° - $25^{\circ}C$ has been confirmed by spore germination experiments.

(b) From artificial inoculation experiments it can be seen that European larch is more susceptible to Meria

attack than either Hybrid or Japanese larch and that the b strain of Meria is more virulent than either strain a or strain c. Much of the infection is confined to the leading shoots, especially in the taller trees, a point previously noted by Hiley (1921).

(c) Spread of Meria inoculum occurs at least six yards from an infected plot regardless of the wind direction and it will occur easily but not very rapidly, in continuous plots of similar trees.

In order to investigate the susceptibility of European larch to Meria attack, and the partial resistance of Japanese larch, a series of experiments following the lines suggested by Peace and Holmes, were performed.

Using a modification of a technique described by Turner (1953) an extract of larch needles was prepared. 50 gms. of larch needles were collected from six or seven year old trees and, after washing, were macerated in a Waring blendor with 50 ml. of water. The resulting suspension was then filtered, to remove the larger debris, and the filtrate centrifuged at a low speed for five minutes. The supernatant was sterlised by passing through a Seitz filter, and then transferred to a sterile burette.

One hundred and twenty 350 ml. flasks each

containing 50 ml. of the basic minerals found in Dox-yeast agar were prepared. To sixty of these was added sufficient sucrose to give a 2% solution and the whole batch was sterilised in the autoclave. To twenty four of the other flasks without sugar European larch extract was added, twelve receiving ten drops (≡ 5 ml.) of extract and twelve receiving 40 drops (≡ 20 ml.) of extract. Twenty four flasks with sugar were treated in a similar fashion. Japanese larch extract was added in the same way to a further forty-eight flasks. The remainder of flasks with and without sugar were controls. All extracts were added to the flasks in the inoculation chamber.

Inoculum was prepared by scraping the mycelium from the surface of Meria cultures grown on a plain agar plus sugar medium and homogenising in sterile water in the Waring blendor. 0.5 ml. of this suspension was then added to each flask using a sterile pipette. The flasks were then transferred to the shaker for three weeks, after which the mycelium was removed, dried and weighed.

See Table 32 overleaf and

PLATES 38 and 39

Table 32: The effect of different concentrations of larch needle extract on the growth of Meria in non-sugar and sugar solutions.

Sugar	Extract treatment		Mean dry weight of mycelium in gms.			
			a	b	c	d
No sugar	Control		.0020	.0008	.0011	.0012
	EL	10d	.0034	.0020	.0013	.0040
		40d	.0135	.0077	.0057	.0098
	JL	10d	.0054	.0012	.0021	.0036
		40d	.0089	.0038	.0021	.0125
+ Sugar	Control		.0432	.0249	.0177	.0493
	EL	10d	.0760	.0648	.0506	.0855
		40d	.1501	.0887	.1384	.1632
	JL	10d	.0771	.0578	.0315	.0851
		40d	.1028	.1042	.0628	.1780

The effect of larch extract on growth of Meria is greater in flasks containing sugar, and the following trends are apparent:

(i) In all flasks the addition of larch extract gives greater growth than in the control flask.

(ii) Forty drops of extract give greater growth than ten drops of extract except in strain b (+ sugar) flasks and strain c (- sugar) flasks.

(iii) In strain a and c (+ sugar) flasks forty drops of European larch extract give greater growth

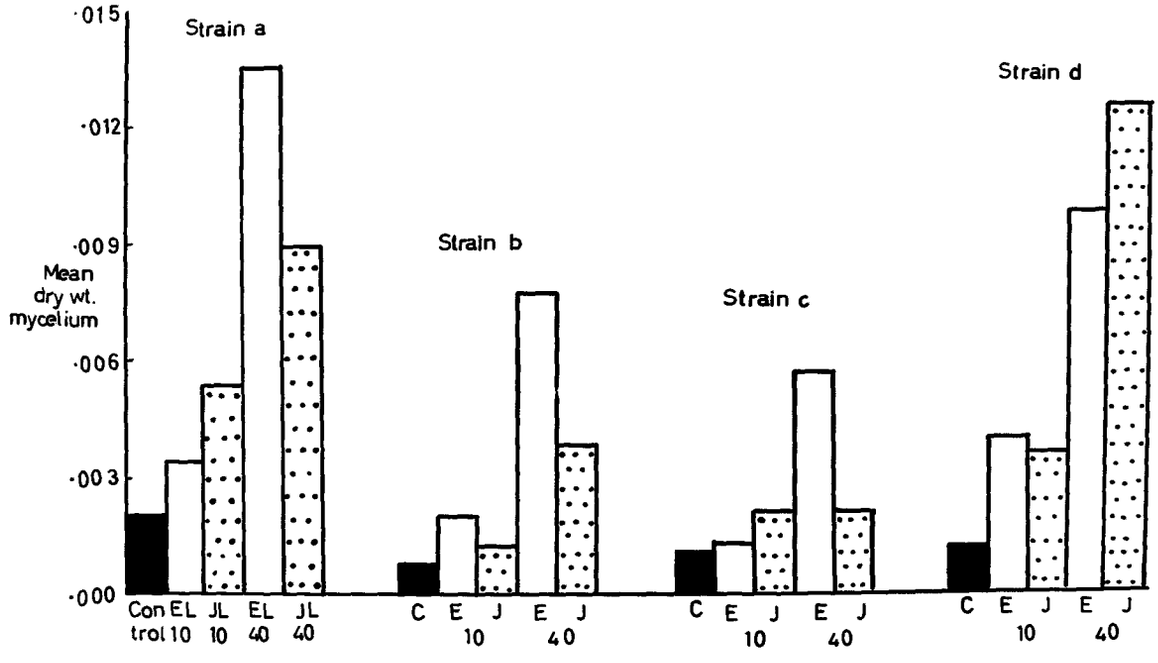


PLATE 38: The effect of different concentrations of larch needle extract on growth in non-sugar solutions.

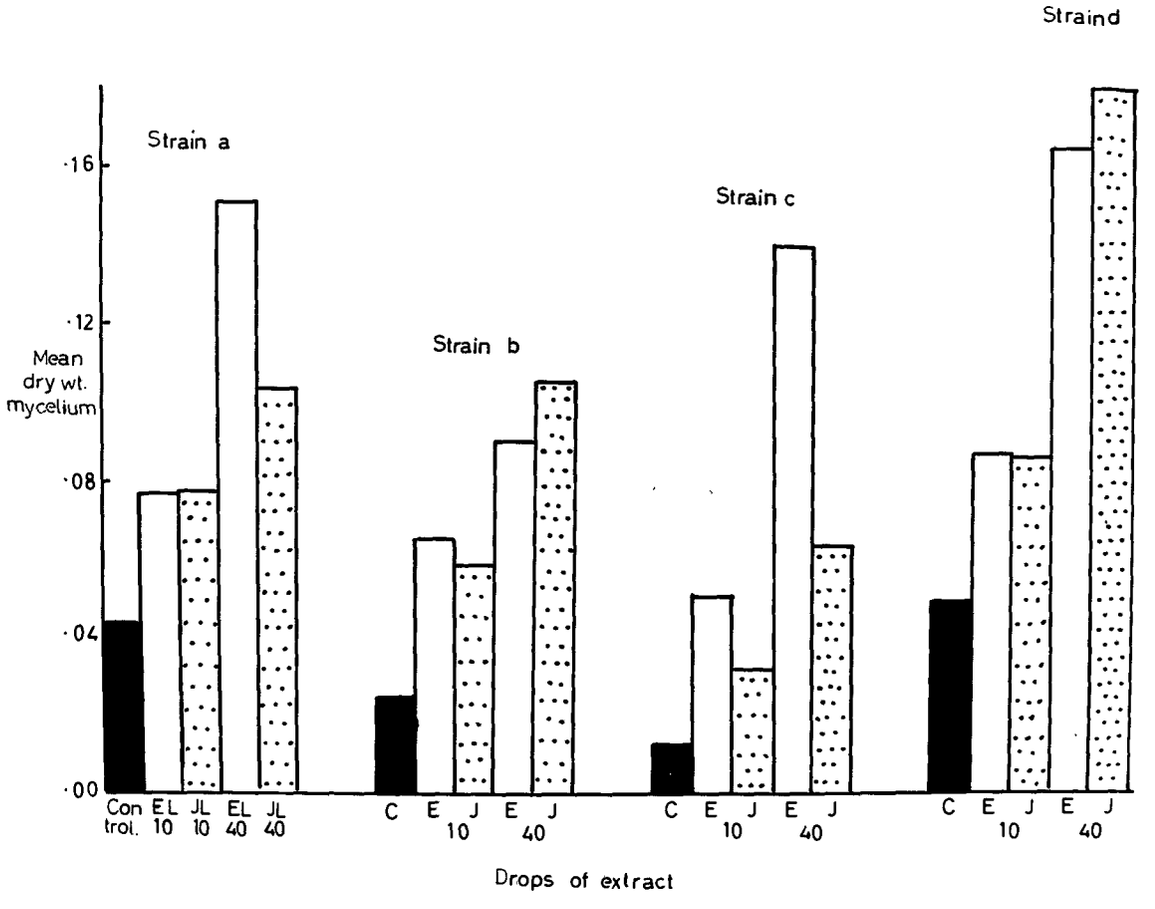


PLATE 39: The effect of different concentrations of larch needle extract on growth in sugar solutions.

than forty drops of Japanese larch extract. The position is reversed in strain band d flasks although the difference in growth is unlikely to be significant. However, in flasks without sugar, forty drops of European larch extract give greater growth than forty drops of Japanese larch extract in all flasks except those of the d strain.

These results indicate that there is a substance, or substances, present in larch needles that promotes the growth of Meria. This substance shows a greater concentration in European than in Japanese larch needles, or it is present in Japanese larch with an inhibiting substance that partially nullifies its effect on growth, or more especially in nature it inhibits or retards germination of Meria spores landing on the needle surface.

Slower germination of spores, leading to slower initial infection, followed by slower mycelial growth once within the tissues could account for the delay in the appearance of disease symptoms on Japanese larch when compared with European larch, if both trees are inoculated simultaneously.

It therefore seemed advisable to test the effect of larch extract on spore germination. Peace and Holmes had already found that, when using sap

expressed from Japanese larch needles, germination was delayed, but as water extracts had given a result in the growth experiments described above this technique was repeated.

A mineral and 2% sucrose agar was made but with the agar as a 5% instead of normal 3% solution, to allow for dilution. Prior to pouring the sterile agar into thin glass petri dishes ten or forty drops of needle extract were added to the test tubes, when the agar was almost solid, in case the substance in the needle was destroyed by heat. After thorough mixing the plates were poured and, after they had solidified, a spore smear was made in the usual manner. The plates were incubated for seventy-two hours at 20°C and then percentage germination was recorded.

See Table 33 (overleaf) and PLATE 40

The following trends are apparent:

(i) The addition of larch extract increases the percentage germination in all strains when compared with the control plates.

(ii) Strains b and c (forty drops) and strain b (ten drops) show a significant difference between germination percentage in European and Japanese larch extract. In all three treatments germination is

Table 33: Effect of larch extract on germination of Meria spores after seventy-two hours.

JL extract			EL extract		
Strain	Treatment	% germ.	Strain	Treatment	% germ.
a	Control	47	a	Control	53
	10 drops	53		10 drops	59
	40 drops	57		40 drops	62
b	Control	35	b	Control	30
	10 drops	43		10 drops	62
	40 drops	47		40 drops	59
c	Control	40	c	Control	43
	10 drops	51		10 drops	53
	40 drops	50		40 drops	65

much less in Japanese larch extract, although it is still greater than the control.

(iii) Strain a shows little difference in germination percentage under any treatment.

These results confirm those of the growth experiment. There is a substance present in larch needles which promotes spore germination and mycelial growth but the concentration of this substance is less in Japanese larch than European larch or its action is in some way inhibited in Japanese larch. The distinct difference in total germination percentage of the b strain treated with European and Japanese

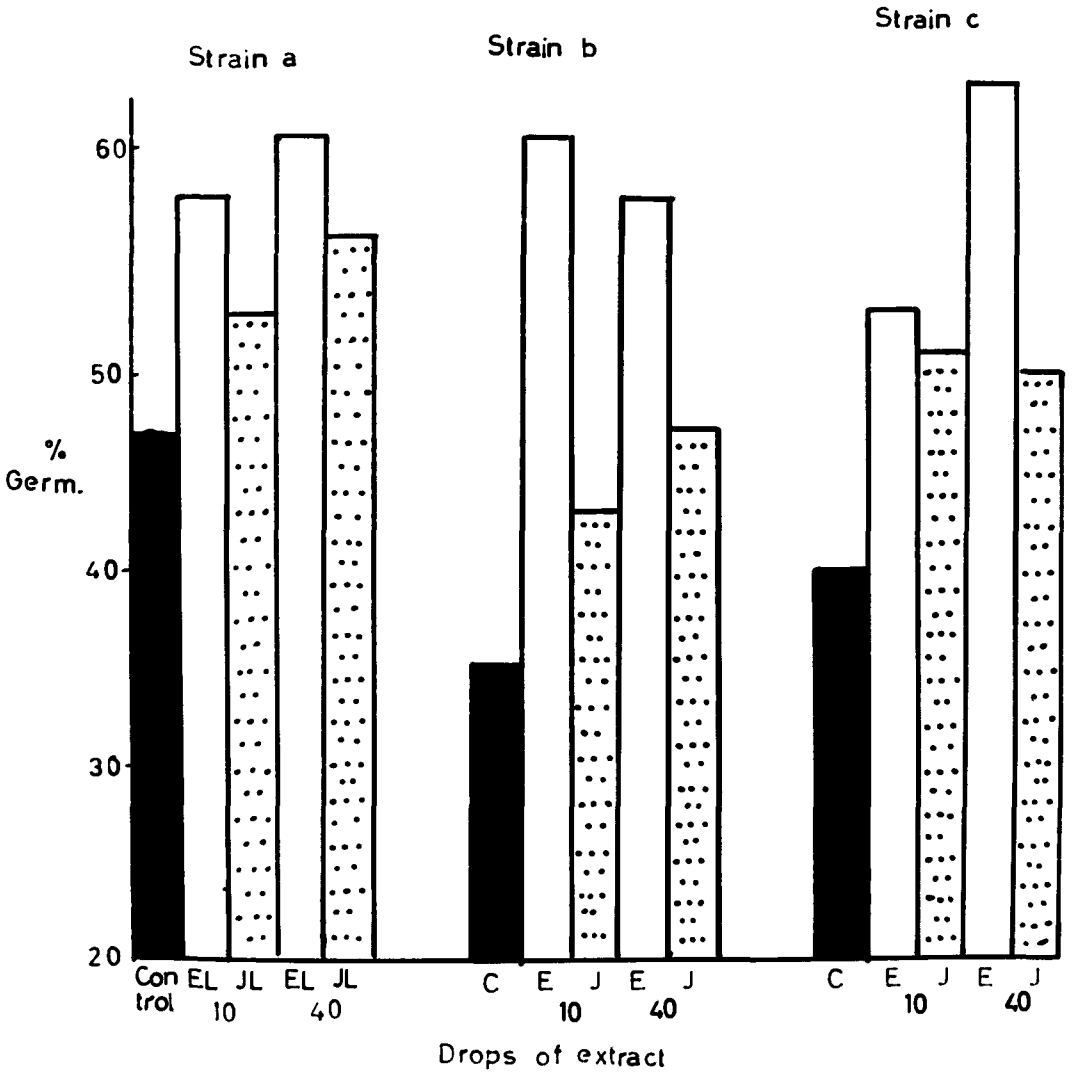


PLATE 40: The effect of larch extract on germination after 72 hours.

larch extract could account for the delay in appearance of disease symptoms on Japanese larch on simultaneous inoculation of both trees, but as no record of percentage germination with time or maximum germination percentage were made this cannot be stated definitely. It is likely that a lower percentage germination coupled with slower mycelial growth inside the needles accounts for this delay.

A second experiment to determine the distribution of the promoting substance and its possible destruction by heat was carried out. The extraction technique previously employed (p. 154) was repeated, but after removal of larger needle debris from the suspension in the Waring blendor, centrifugation was commenced. The supernatant was divided into two portions; one was sterlised by passage through Seitz filter and added to the mineral plus sugar agar as described, while the other was added, in forty drop aliquots, to test tubes of non-sterile mineral plus sugar agar which were then sterilised in the autoclave and poured into petri dishes. The sediment from centrifugation was also divided into two portions, one was spread over the bottom of a sterile petri dish and a sterile cellophane disc placed on its surface, while the other was treated in a similar manner but then

re-sterilised in the autoclave. Spore smears were made, in all plates, of b and c strains and these were incubated for eighty-four hours at 20°C and then examined.

Table 34: The effect of liquid and solid larch extract on germination after 84 hours.

Strain of Neria	Liquid extract				Solid extract			
	EL		JL		EL		JL	
	Seitz filtered	Autoclaved	Seitz filtered	Autoclaved	Unsterile	Autoclaved	Unsterile	Autoclaved
b	28	25	20	21	16	10	19	19
c	19	21	12	15	0	0	0	0

The percentage germination in all experiments is low possibly due to the inoculum. In the b and the c strain with liquid extract there is little difference between any of the treatments in percentage germination. Unfortunately no germination was observed in any of the c strain solid extract plates. These results indicate that the promoting substance is not fully extracted in water, although germination is somewhat better in the b strain liquid extract plates than the solid extract plates, but this is probably due to the different techniques. The substance appears to

be thermostable as germination is hardly affected by autoclaving the extract.

These results give little further information as to the identity of the promoting substance but the fact that it is water soluble and not destroyed by heat and gives increased growth when both sugar and mineral salts are present in the medium, could indicate a vitamin or combination of vitamins. It is unlikely, however, that there is any difference in the vitamin constituents of European and Japanese larches to account for the differences in growth and germination given by these extracts. There is the possibility of an inhibiting factor in Japanese larch which in some way prevents either the assimilation or the enhancing effect on growth and germination of the promoting substance.

As increased growth of Meria is given with increasing concentrations of sucrose it is also possible that the promoting agent is this or another sugar; but again it is unlikely that there would be a sufficiently great difference in sucrose concentration or availability of other sugars in Japanese and European larches to cause the different results obtained. Strain a shows a preference for fructose rather than glucose and if this was more readily available in European than Japanese larch

it could account for different growth rate. However, strain d also shows a preference for fructose but the growth rate in European and Japanese larch extracts, with and without sugar, is very similar and also it would not explain the growth differences, in the different extracts, of strains b and c (without sugar) which show no preference for fructose.

Further experiments on the identification of the promoting or inhibiting substances were not undertaken.

These results do not give any explanation of why the disease was apparently confined to European and Western larch until 1946. The b strain of Meria, described by Peace and Holmes, is practically identical from cultural examination, with the current b strain isolates and this was the strain commonly isolated from nature in 1933. The b strain is still most commonly isolated in nature (see later section) and is obviously the most virulent. Some genetical and physiological change must have occurred within this strain, and within the a and c strains also, to enable infection of previously resistant larch species to occur, but this is not detectable in any simple morphological or physiological analysis.

Summary: An appreciation of the work of previous authors led to the confirmation of their results and investigations into various factors connected with the pathogenicity of Meria The following points are important in pathogenic studies of Meria :

(i) Infection will occur between 0° and 25°C but only when the relative humidity is over 95%. Infection is arrested under dry conditions by failure of germination of spores

(ii) Primary infection in the spring results from inoculum released by fungus in shed needles or those still adhering to or trapped in the tree. These spores are produced on the onset of warmer weather in the spring. Spores will be produced at humidities over 95% and at temperatures between 5°and 25°C and infection will occur by a germ tube growing through the stomatal pore.

(iii) The spread of inoculum occurs easily in continuous plots of similar trees and easily in separated plots of similar trees and probably between different tree species, although this has not been proved by field experiments but only by greenhouse inoculation.

(iv) European larch is more susceptible to Meria attack than either Hybrid or Japanese larches, and disease symptoms appear earlier on European than Hybrid or Japanese larch.

Extracts of both European and Japanese larch promote growth of Leria in liquid culture and also increase spore germination, but growth and germination in certain cases is retarded in Japanese larch extract. This accounts for the later appearance of disease symptoms on Japanese larch when the two species are inoculated simultaneously. The substance, or substances, responsible for the promotion of growth and spore germination is water soluble, thermostable, and is possibly a vitamin complex. The reasons for Japanese larch extract retarding growth and germination are not known, although some inhibiting factor, interfering with the assimilation or action of the promoting substance, may be present.

(v) The b strain of the fungus is more virulent than either the a or c strains as it can attack all three types of tree.

(vi) No explanation of the sudden appearance of Leria on previously resistant larch species is given by these experiments.

The significance of these findings will be discussed in detail later.

DISTRIBUTION OF DIFFERENT STRAINS OF MERIA

Peace and Holmes were the only workers to recognise distinct strains of Meria but they did not investigate the distribution of these strains in nature beyond noting that the b strain was isolated more often than the a strain.

In the present investigation infected material was received from seventeen nurseries scattered throughout England, Wales and Scotland (for exact locations see map p.176) together with several already established Meria cultures made at the Genetics nursery at Alice Holt, Surrey.

As many isolations as possible were made from the diseased material, using the method already described, and as soon as the cultures were established, single spore isolates were made. These were incubated for four weeks at 20°C and then examined to type the strain of Meria. Several nurseries sent material in 1955 and 1956 and isolates were made in consecutive years. Only a single nursery (Wykeham) sent material in 1957 and none was received in 1958.

See Tables 35 and 36 (overleaf)

Table 35: The distribution of Neria strains among different tree species for years 1954 - 1957.

Tree species	Nurseries sending inoculum	No. of infected specimens	Total isolations	Isolated strains a	b	c	d
European	12	14	38	15	16	7	0
Hybrid	6	7	19	7	4	7	1
Japanese	10	12	38	12	14	12	0
			Total	34	34	26	1

There is one extra specimen of the a strain from a Peace and Holmes culture lodged at Baarn in Holland.

From Table 35 the general conclusion is that strains a, b and c occur in approximately similar numbers and only one strain d culture has been obtained from a Hybrid larch. The smaller total number of Hybrid larch isolates is due to the infrequency of growth of this species. Strains a and b are isolated with equal frequency from both European and Japanese larch while hybrid larch isolates show equal number of a and c strains and rather less b.

Table 36: Details of the distribution of *Meria* strains among different trees and nurseries for years 1954 - 1957.

Nursery and location	Tree, isolate and year of isolation	Number and strain of isolates a	b	c	d	Total isolates per nursery
Alice Holt Surrey	EL culture sent 1954	3	1			4
St.Asaph Rhyl	EL isolated 1956		2			2
Bareagle Stranraer	EL culture sent 1954	3		1		
	EL isolated 1955	2				
	HL isolated 1955	1		2	1	
	JL culture sent 1954	3				
	JL isolated 1956	2	2	1		18
Benmore Argyll	HL isolated 1955	1	3	2		6
Castle-ton Dumfries shire	JL culture sent 1955	3		3		6
Brenin N.Wales	EL culture sent 1955	1	3	1		
	JL isolated 1955		3			8
Davidst-ow Cornwall	JL isolated 1956	1		1		2
Delamere Norwich	EL isolated 1955	3		1		4
Fleet Newton-Stewart Wigtown	EL culture sent 1954	3		1		
	EL isolated 1956		2			
	HL isolated 1955			2		

(continued)

Table 36 (continued)

Nursery and location	Tree, isolate and year of isolation	Number and strain of isolates				Total isolates per nursery
		a	b	c	d	
Fleet contd.	HL isolated 1956	2				
	JL culture sent 1954	3				
	JL isolated 1956		2	2		17
Glasfryn Caerns.	EL isolated 1956		1			
	HL isolated 1956			1		2
Glenfin-art Dunoon Argyll	EL isolated 1956		1	1		
	HL isolated 1956	1	1			
	JL isolated 1956		1	1		6
Glengap S.W Scot.	JL isolated 1956	2	3			5
Glentrool S.W Scot.	JL isolated 1956			1		1
Kielder Northum-berland	JL isolated 1955		3			3
Ledmore Perth.	EL isolated 1956		2			2
Millbuie Ross and Cromarty	EL isolated 1956		1	1		
	HL isolated 1956	1	1			
	JL isolated 1956		1	1		6
Mortimer	EL isolated 1956		3			3
Wykeham Yorks.	EL isolated 1957			1		1

1. Other isolates

EL possibly 1932 - strain a - obtained from Baarn from original Peace and Holmes culture.

2. Isolates from inoculation experiments -

Tree, isolate and year of isolation	Strain b	Strain c	Remarks
EL isolated 1957	10		Inoculated with a and b
EL " 1957		1	" " b
HL " 1957		2	" " b
JL " 1957		1	" " b
EL " 1958		1	" " c
HL " 1958		1	" " c

In an attempt to discover a relationship between the fungus strain and location of the nursery the data was analysed by using a map showing distribution of different Meria strains in relation to nursery location. (See MAP 1) The nurseries can roughly be divided into three groups: (i) Scottish nurseries; (ii) Welsh and West of England nurseries; (iii) East of England nurseries; the presence or absence of particular Meria strains on the different species of larch within these groups was recorded.

Tables 37, 38 and 39.

Table 37: Distribution of Meria strains amongst different larch species in Scottish nurseries.

Nursery	Tree	Meria strain			
		a	b	c	d
Millbuie	EL		*	*	
	HL	*	*		
	JL		*	*	
Ledmore	EL		*		
Glenfinart	EL		*	*	
	HL	*	*		
	JL		*	*	
Kielder	JL		*		
Benmore	HL	*	*	*	
Bareagle	EL	*		*	
	HL	*		*	*
	JL	*	*	*	
Castleton	JL	*		*	
Glentrool	JL			*	
Benmore	HL	*	*	*	
Fleet	EL	*	*	*	
	HL	*		*	
	JL		*	*	
Glengap	JL	*	*		
SUMMARY:					
	EL	2	4	4	
	HL	5	3	3	1
	JL	3	6	6	
	Total:	10	13	13	1

Table 38: Distribution of Meria strains amongst different larch species in Welsh and West of England nurseries.

Nursery	Tree	Meria strain		
		a	b	c
St.Asaph	EL		*	
Glasfryn	EL		*	
	HL			*
Brenin	EL	*	*	*
	JL		*	
Mortimer	EL		*	
Davidstow	JL	*		*

SUMMARY:

EL	1	4	1
HL	0	0	1
JL	1	1	1
Total:	2	5	3

Table 39: Distribution of Meria strains amongst different larch species in East of England nurseries

Wykeham	EL			*
Delamere	EL	*		*
Alice Holt	EL	*	*	

SUMMARY:

EL	2	1	2

MAP showing distribution of Nurseries from which material and information has been received.

Table 40: Frequency of occurrence of Meria strains on different larch species.

Tree species	Strain of Meria		
	a	b	c
EL	5	9	7
HL	5	3	4
JL	4	7	7
Total	14	19	18

The following points are noticeable from examination of these tables.

(i) The total number of occurrences of a, b and c strains of Meria on the different larch species is very similar, although the b strain occurs approximately 25% more often than the a strain. This partially confirms the conclusions reached in an earlier section where it was suggested that the b strain of the fungus is the most virulent. The equal occurrence of b and c strains was not mentioned by Peace and Holmes, who only recognised the two strains a and b, but it is possible that c strain cultures could have been included under b strain for the sake of typing, as they resemble this strain rather than the a strain. It is unlikely that the c strain has arisen in the last twenty years as Peace and Holmes report many gradations in culture appearance between their a and b strains, but due to insufficient time, these

were not re-cultured as single spore isolates. From the photographs in the Peace and Holmes monograph it appears that some of these intermediate cultures contain sectors of the c strain, a feature common in preliminary isolations from diseased material before single spore sub-culturing, in which the c strain was identified. This does not however explain the incidence of a large number of c strain isolates in the present investigation. From an earlier section it can be seen that attempts at artificial inoculation using this strain gave very poor results, a fact incompatible with the high incidence of this strain in nature. However, an interesting observation was made in Meria cultures made by re-isolating the fungus from all three species of larch artificially inoculated with the b strain of Meria in 1957. In all three cases the re-isolated strain was the c strain. This indicates that a change of strain can occur while the fungus is within the host, a very important point in any appreciation of the identification or distribution of the different fungal strains. It is possible, therefore, that strain c is related to strain b - a point which will be discussed in detail later, and that a change from one strain to another, usually b to c, can occur within the larch needle. It has already been suggested (p. 63) that strains b and d

are related physiologically. This could mean that of the total of eighteen isolates of the c strain, the majority, as indicated by the results of the artificial inoculation experiments, could have been the b strain when infection occurred. In an attempt to test this hypothesis Table 36 was re-examined.

Table 41: Re-examination of results from Table 36.

Description of strain incidence	Number of nurseries
Nurseries in which all 3 strains occur	6
" " " only a strain occurs	0
" " " " b " "	4
" " " " c " "	2
" " " b " "	13
" " " b and c " "	7
" " " a and c only occur	3
" " " a and b " "	1
" " " b and c " "	1
Total nurseries sending material	18

Several further points are now evident.

(a) Out of the eighteen nurseries from which Meria cultures were obtained thirteen showed the presence of the b strain and four showed the b strain only. This is further evidence of the virulence of the b strain of Meria as the c strain only occurred alone

in two nurseries and the a strain was never found alone.

(b) The b and c strains occur together in seven of the nurseries but in six of these it is together with the a strain, only a single nursery, Glasfryn, showing b and c strains alone. This confirms the fact that strain changes can occur between the b and c strains.

(c) The three occurrences of a and c strains alone also indicate that strain changes between these two strains may occur within the needle especially as in six examples out of eighteen the a, b and c strains occur together. This relationship between the a and c strains has already been reported (p.105) when a strain colonies appeared within c strain colonies in a c strain spore germination plate left for forty-eight hours in a refrigerator and then incubated at 20°C. It is obvious that the different strains of Meria must be related to each other and it now appears from both experimental and analytical evidence that changes from one strain to another can occur while the fungus is in its parasitic phase. However, as already mentioned, Meria remained extremely stable in culture apart from the instance mentioned above and certain other phenomena already reported (p.63). This whole question will be discussed later.

(ii) Both European and Japanese larch show

approximately twice as many occurrences of b strain infection as compared with a strain infection. It may be possible in the next section of the work to obtain further confirmation of the suggestion that the b strain of Meria is the most virulent by examining nursery data concerning the actual date of appearance of disease symptoms.

(iii) Of the nurseries showing all tree species infected Millbuie and Glenfinart show b as the most infective strain with little a strain infection, while Bareagle and Fleet show a and c strains as the most infective with some b strain infection.

(iv) There is no pattern of distribution of the different Meria strains either on particular larch species, except for traits mentioned in (i) and (ii), or within definite areas of England, Scotland and Wales. One interesting point, however, is the occurrence of strain b on all European larch from all the nurseries in the Welsh and West of England group. Owing to the close proximity of three of the nurseries, St. Asaph, Glasfryn and Coed-y-Brenin it is a possibility that infection arose from a common source, possibly a plantation of older trees, or by a series of steps from nursery to nursery.

Although more data from other nurseries is available and will be examined in the next section,

no infected specimens were received and this data cannot be used here.

(v) From the identification of re-isolations of Meria made from artificially inoculated larch trees, it can be seen that several changes of strain have occurred by passage of Meria through the host. In the first 1957 experiment when ten re-isolates of the b strain were identified from European larch, four of these were from trees sprayed with the a strain, the other six isolates being from trees inoculated with the b strain. This confirms conclusions reached in (i) of a connection between a and b strains of Meria.

To help in the explanation of the occurrence of the cultural strains of Meria the number of different strains identified from each single isolate was examined.

Table 42: Occurrence of different strains of Meria from single isolations from nature.

Tree species	1 strain isolated	2 strains isolated	3 strains isolated
EL	7	6	1
HL	3	2	1
JL	5	6	2
Total	15	14	4

Out of thirty-three isolates from diseased material fifteen gave a single strain on monosporus culture, fourteen gave two strains and only four showed three strains. Isolates from the twelve nurseries where only a single tree species was grown or infected with Meria gave seven mixed cultures that produced two or more strains on monosporus culture.

These results indicate that mixed strain infections are as common in nature as single strain infections and they occur with equal frequency in nurseries where a single tree species or two or three tree species are infected.

SUMMARY:

No previous work has been done on the distribution of the different strains of Meria apart from a comment of Peace and Holmes that isolations from nature more commonly gave the b strain of the fungus than the a strain. Infected material sent from eighteen nurseries throughout the country was used as a source of fungus isolates and the strain or strains of each isolate was determined. The results were then examined with regard to nursery location. There is no apparent pattern of distribution of strains of Meria throughout the country apart from a tendency for all European larch in the Western region (including Wales) to be infected with the b strain of Meria.

The b strain of Meria was more commonly isolated than the a strain although the c strain was found as commonly as the b strain and various reasons for this occurrence were examined.

FORESTRY COMMISSION DATA AND DISEASE FORECASTING

INTRODUCTION

The previous work regarding conditions necessary for infection and spread of Meria has already been summarised (p. 150). As results tend to be slight it was decided to investigate these phenomena more fully. The investigation was divided into two parts, the first concerned with the analysis of Meria attack in particular nurseries over a period of four years, and the second using data from the first together with extra information from the Meteorological office, was an attempt to devise a forecasting system for Meria similar to the Beaumont system for Potato blight (Phytophthora infestans).

Part 1 - Analysis of Meria attack in certain larch nurseries

In conjunction with the Forest Pathologist (Mr. T. R. Peace), a simple form was designed so that information regarding Meria attacks could be recorded by Foresters at the nurseries used in analysis studies. The Forester was asked to supply information regarding the presence or absence of Meria attack and, if present, on which species of larch, and the date of appearance; the severity of attack, whether severe, moderate or slight; the subsequent behaviour of the

disease on the infected trees; brief notes about weather conditions, especially any prolonged wet or dry periods and any other information that could possibly be of use in the investigation. These forms were sent annually to the various larch nurseries throughout the country and were returned, either at the end of August or late September. Negative results, no obvious disease attack or a particular species of larch not grown were also recorded. Not all the nurseries circulated returned the completed forms and some of the others for only one or two years, and unfortunately some of the nurseries that sent infected material did not send completed reports. The map on page 176 shows the location of the nurseries and presence or absence of diseased material and completed reports.

The severity of attack is probably the criterion which would involve the most difficulty in estimation by the Forester as no distinct rules were given as an aid. A severe attack is likely to mean most of the trees in a particular plot showing sufficient infected needles to be obvious to the observer on a cursory examination of the plot. The density of planting may influence this estimation but no information is available on this point. A slight infection means that only a few, about 20%, of the

trees are infected and that Meria attack can only be verified by a close examination of the individual trees.

Certain nurseries supplied information regarding the seed source of the trees and other details regarding their histories, but without recourse to Forestry Commission data scattered over the country it was not possible to obtain the missing data about the other nurseries. Peace and Holmes have pointed out that source of seed had no effect on attack in nature or by artificial inoculation, but Langner (1951) mentioned that among Hybrid larch susceptibility to Meria attack was more marked when Japanese larch was used as the male parent, than when it was used as the female parent. This particular correlation between seed source and Meria attack was not analysed in the present investigation.

The information from the nurseries is recorded by arranging the nurseries geographically from the north of Scotland to the west of England.

Table 43: (overleaf)

Table 43: Data from larch nurseries for years 1955-1958 showing first noticeable infection dates and severity of attack.

Nursery	1955			1956			1957			1958			Notes
	EL	HL	JL	EL	HL	JL	EL	HL	JL	EL	HL	JL	
Millbuie	-	-	-	*20/6	28/7	28/7	*19/7	NG	19/7	No	records	19	57 other nurseries infected
Newtown	-	-	-	-	-	-	6/9	-	-	15/8	-	/	
Tulliallan	-	-	-	-	-	-	-	-	-	-	-	-	
Ledmore	-	-	-	*6/7	NG	-	10/7	-	-	15/7	-	-	
Glenfinart	-	-	-	*15/8	15/8	15/8	26/7	-	-	-	-	-	
Benmore	/	/	/	NG	NG	-	NG	NG	-	-	-	-	
Bareagle	*	x	/	x27/8	13/8	13/8	NG	NG	4/8	NG	NG	7/8	
Fleet	*	/	x	*22/5	28/7	28/7	-	-	15/7	-	-	-	
Widehaugh	-	-	-	x30/5	-	-	-	-	11/10	-	8/8	-	
Wykeham	NG	-	-	NG	NG	-	20/8	-	-	-	17/7	-	
Willingham	-	-	-	-	-	-	-	-	-	-	-	-	
Coed-y-Brenin	/	-	/	-	-	-	-	-	-	NG	NG	NG	
Shobdon	-	NG	-	15/8	NG	-	13/8	-	-	-	-	-	
Tintern	-	NG	-	-	NG	-	NG	NG	-	NG	NG	20/8	
Tair Onen	NG	NG	-	-	-	-	-	-	-	-	-	-	
Delamere	/	NG	-	-	NG	-	NG	-	-	-	-	-	
Ampthill	-	NG	-	-	NG	-	-	NG	-	*15/7	x10/8	-	
Davidstow	NG	NG	x	-	-	15/8	NG	NG	x26/8	NG	NG	*15/6	Moved seedlings were infected.

* Severe attack and date. x Moderate attack and date / slight attack NG - not grown.
- not attacked

Table 44: Summary of results.

(a) Total infection in all years

Larch species	Total reported attacks	Severity of attack		
		Severe	Moder-ate	Slight
EL	21	8	4	9
HL	10	-	4	6
JL	19	1	3	15
TOTAL	50	9	11	30

Table 45: (b) Infection totals in particular years.

1955

EL	5	2	-	3
HL	3	-	1	2
JL	5	-	2	3

1956

EL	7	4	2	1
HL	4	-	2	2
JL	5	-	-	5

1957

EL	6	1	1	4
HL	0	-	-	-
JL	5	-	1	4

1958

EL	3	1	1	1
HL	3	-	1	2
JL	4	1	-	3

The following trends can be deduced from the data:

(i) Most attacks occur on European larch while Hybrid larch shows the least. This is partly due to the scarcity of Hybrid larch nursery stock as indicated by the few nurseries that actually grow this species. This, however, confirms the conclusion that European larch is the most susceptible species of the three especially when it is also noted that out of twenty-one records of the disease on this species eight of these indicate severe attacks. No severe attacks are recorded on Hybrid larch and only one on Japanese larch.

(ii) Most disease attacks occur in the eight Scottish nurseries. Out of a total of fifty recorded attacks, thirty-two occur in Scottish nurseries and thirteen of these occur in 1956. There may be several reasons for this particular distribution. Firstly the weather conditions in Scotland may be more conducive to infection, i.e. the humidity remains sufficiently high for long enough to allow good germination and infection, or more probably infection sources are more frequent.

Of the eight Scottish nurseries from which records have been received, four report disease

occurrence for at least two consecutive years and one, Bareagle, showed infected larch for all four years of the investigation. Ledmore, where European larch suffered a severe attack in 1956, showed moderate attacks in both 1957 and 1958 on this species and it can be seen from the further comments supplied by the foresters of this and other nurseries, that infection occurs from year to year by contamination of the nurseries with infected needles which act as an inoculum source in the spring or, infected needles still adhering to the tree provide inoculum in the spring after transplanting the seedlings. Once inoculum is available the disease easily spreads among the different larch species as in 1956, four out of six nurseries that grew the three species of larch reported attack on all three species, while the other two were entirely free from the disease. Only a single Scottish nursery, Tulliallan, remained entirely free from the disease for the four year period of the investigation. The occurrence of thirteen recorded attacks in 1956 is no doubt due to the weather conditions at the time and the fact that three of the nurseries reported infection in 1955, all on all three larch species.

(iii) All the severe attacks are reported from Scotland apart from one on European larch in 1958

from Ampthill and one at Davidstow on Japanese larch in the same year. In fact few of the Welsh or English nurseries show much disease and in those in which it does occur attack is usually only slight and only very occasionally moderate or severe. Referring to the results of the previous section it can be seen that this is not in fact correlated with the incidence of a particular strain of Meria. The Scottish nurseries show approximately equal incidence of a, b and c strains (10, 13 and 13) as do the Welsh and English nurseries (4, 6 and 5). It is interesting to note however, that the only isolate of the d strain was obtained from material from a Scottish nursery - Bareagle.

(iv) Although the total number of attacks reported on Japanese larch (nineteen) is similar to the total number reported on European larch (twentyone) only one is a severe attack, while fifteen are slight attacks. This confirms the conclusion that Japanese larch is the most resistant species to Meria attack. Again referring to the previous section this is not correlated with the incidence of any particular strain of Meria as out of a total of eighteen single spore isolates of Meria from infected Japanese larch needles four were strain a, seven were strain b and seven were strain c. This helps to confirm the

conclusion that the b strain of the fungus is the most virulent as approximately half of the isolates from Japanese larch were of this strain. Although seven isolates of the c strain were also identified, the possible change in strain between b and c within the needle, explained in the last section, could account for this fact.

(v) Of the seven nurseries that report attack of European larch together with either Hybrid or Japanese or both and give infection dates, four show that disease symptoms occurred on European larch prior to their appearance on the other species, two note appearance of symptoms at the same time on all species and only a single nursery, Fleet, records the appearance of disease symptoms on European larch after symptoms on the other two species. This indicates firstly that European larch is more susceptible to Meria attack and that secondly the diseased European larch may act as an inoculum source for infection of the other larch species. This second indication is confirmed by the Forester's reports from Bareagle and Fleet in 1956 where the other species of larch were grown in beds alongside the European larch and infection of European larch and Japanese larch occurred approximately two weeks and two months from the first noticeable symptoms

occurring on the European larch. On the other hand it must be pointed out that in one nursery at least, Widehaugh, Japanese larch remained uninfected in a bed next to European larch showing a moderate Meria attack.

In one of the nurseries, Millbuie 1957, where attacks occur simultaneously on European and Japanese larch, infected material was already present as all three species of larch were infected in the previous year and it is possible that infection of the two species occurred simultaneously also.

(vi) Of the ten recorded instances of attack of European larch together with attacks on one or both of the other species eight show a more severe attack on European larch than either of the other two larches, further evidence that European larch is the most susceptible of the three species.

(vii) Only a single nursery, Davidstow, Cornwall, reports infection of Japanese larch when the other species of larch were not grown. This infection was moderate in 1955 and 1957 but severe in 1958, the only recorded instance of a severe attack on Japanese larch. Isolation of Meria from infected material received in 1956, when the attack was slight, gave one a and one c strain culture of the fungus. More information

about the behaviour of this disease within this nursery is available. Previous years infected seedlings that have been planted out appear to supply the inoculum source for attack of the current year's seedlings although this attack does not become serious until the seedlings are transplanted after one year. In one instance seedlings were transplanted fifty yards from an infected plot of transplants and still became infected later in the season. The Forester also notes that a system of vertical lath sheltering to protect the seedlings from exposure, decreases the incidence of disease from severe to slight, a fact also reported in one instance by Peace and Holmes, who also reported that certain types of sheltering increased Meria attack by lowering the evaporation rate.

Bareagle in 1957 and 1958 also shows infection of Japanese larch in the absence of both European and Hybrid larches, but in 1955 and 1956 all three species were grown and all were attacked, giving a large infection source from year to year. At Fleet in 1957 Japanese larch only was attacked but this time both European and Hybrid larch were present. In 1955 and 1956 in this nursery all three species were also grown and all attacked by Meria, but unfortunately insufficient information as regards the positioning

of the different larch species in 1957 is available and no apparent reason for non-infection of the other two species can be deduced. This nursery shows no disease at all in 1958. It is interesting to note that in both Bareagle and Fleet nurseries severe attacks had previously occurred and that the b strain of Meria had been isolated from diseased material sent from these nurseries.

(viii) Of the four nurseries, Bareagle, Fleet, Glefinart and Millbuie in which severe Meria attacks have been recorded, and which grow all three species of larch and from which material has been received, three show the presence of the b strain of Meria on all three larch species. Also in these nurseries b strains of Meria have been isolated from the tree species, usually European larch, on which the primary attack occurred. This is a further indication that the b strain of the fungus is the most virulent. Fleet gives b strain isolates from only European and Japanese infected material. The only other nursery sending diseased material and from which a severe attack has been recorded, Davidstow, only gives a and c strain Meria isolates as already mentioned but these isolations were made in 1956 when the attack was only slight.

(ix) Fifteen nurseries record infection by Meria at some time and out of these, eleven show infection for more than one year, six show infection for two consecutive years, three show infection for three consecutive years, and two, Bareagle and Davidstow, show infection for four consecutive years. These facts indicate that once infection has occurred within a nursery it is extremely likely that it will reappear in subsequent years unless drastic steps are taken to eradicate the inoculum source of the current year. This may mean the collection of all fallen infected needles for burning as suggested by Mer (1895), or the removal of the infected seedlings to a new bed where, as transplants, they may not retain the infection. This new bed must be sufficiently far from the previous or any other likely infection sources to prevent passage of spores. Even the planting of Japanese larch in areas previously bearing infected European larch would not prevent infection, especially if the European larch had been infected with the b strain of Meria.

The best method of eradication is undoubtedly non growth of any larch seedlings in the year following a severe attack. Although the fungus

overwinters in the fallen needles and produces conidia in the following spring, it is extremely unlikely that these diseased needles could act as an infection source in subsequent years. Slight attacks of the disease will easily disappear in the following year without recourse to drastic measures but once severe attacks become commonplace the disease is difficult to eradicate. Once infection has become established within a nursery in one particular season it is possible to control it by using the spraying methods devised by Peace and Holmes. Unfortunately these are expensive and not always satisfactory. Firstly, new growth is continually appearing which is free from the chemical coating, and secondly no indication is as yet available of the most suitable time to spray. The devising of a forecasting system to indicate suitable spraying times is the object of the next section of the work.

PART 2 - DISEASE FORECASTING

Forecasting possible attacks of plant disease with any accuracy is a fairly recent technique in this country. Large (1953) in conjunction with the Agricultural Meteorology Branch of the Meteorological Office has devised a successful system for forecasting attacks of Potato Blight. Previous work on the disease indicated that a forty-eight hour period during which the temperature in a Stevenson screen did not fall below 50°F (10°C) and the relative humidity did not fall below 75% was a good indicator of a blight outbreak some seven to twenty one days later. These criteria were used in a country wide survey of the incidence of the disease although it was realised that

(i) The criteria assume a constant relationship between the screen climate and the climate within the crop - an assumption which is by no means valid throughout the life of the crop or during wet or very dry spells.

(ii) The weather reporting stations were not all situated in areas where potatoes were grown.

However with certain refinements, due to observations over a period of years, the above criteria give a very good indication of the onset of potato blight and on receiving a blight warning the potato farmer can spray his crop with the appropriate fungicide.

After success with potato blight a further system was devised to forecast attacks of Apple Scab (Venturia inequalis) and various groups of infection criteria have been devised (Smith(1962) Preece and Smith (1961)).

It was decided to attempt to devise a forecasting system for Meria using the information from the nurseries in conjunction with weather data supplied by the Meteorological Office. The weather data thought to be necessary in devising a forecasting system were -

1. Daily maximum and minimum temperature readings.
2. Daily humidity readings, preferably several throughout the twenty-four hour period.
3. Daily rainfall.
4. Information as to wind strength and direction.

The Meteorological Office intimated that most of this information was available for extraction. The grid reference location of each of the nurseries that had been circulated with the printed forms was sent to the Meteorological Office who supplied details of the nearest climatic (giving temperature, sunshine and rainfall) and synoptic (giving wind and humidity) weather stations, including their heights above sea level. These were necessary in order to make adjustments to the temperature figures if this height

differed appreciably from that of the nursery. The extraction of the vast quantity of data for the original criteria above would have proved impossible and it was decided to modify these criteria. Humidity and temperature were considered to be the most important variable phenomena of use in devising a forecasting system for Meria, and only these were to be extracted for the six monthly period, April to September in the year of attack. This meant using data from the synoptic weather stations, many of which were up to twenty-five miles from the actual nurseries. It is probable that the conditions here bear little resemblance to those in the nursery. However, six nurseries were chosen for weather data analysis sufficiently close to their synoptic stations to indicate that the weather conditions would be similar to those of the station. Further difficulty arose when four of these reported no Meria infection. Eventually seven nurseries were chosen for a trial run with the 1956 weather data extracted from the Meteorological Office records. Four of these nurseries were Scottish and three English and they were chosen for several reasons -

(a) Proximity to a synoptic station
(b) The type of disease records available
(c) Their distribution throughout the British Isles.

Table 46: Nurseries chosen for preliminary examination.

Nursery and location	Nearest synoptic stn. distance(miles) Difference in h.a.s.	Infection date		Strain of Meria isolated	
		1956	1957	1956	1957
Millbuie Ross and Cromarty	Tarbatness 22m. 580'	EL 20/6 HL) 28/7 JL)	19/7 NG 19/7	1b 1c 1a 1c 1b 1c	-
Glenfinart Dunoon Argyll	Renfrew 25m. 20'	EL) HL) 15/8 JL)	26/7 - -	1b 1c 1a 1c 1b 1c	-
Bareagle Stranraer	West Freugh 3m. 0'	EL 27/8 HL) JL) 13/8	NG NG 4/8	-	-
Fleet Wigtown	West Freugh 28m. 50'	EL 22/5 HL) JL) 28/7	- 15/7	2b 2a 2b 2c	-
Widehaugh Northumberland	Tynemouth 25m. 150'	EL 30/5 HL - JL -	- - 11/10	No material sent	
Shobdon Shropshire	Ross-on-Wye 28m. 537'	EL 15/8 HL NG JL -	13/8 - -	No material sent	
Davidstow Cornwall	St.Mawgan 22m. 613'	EL) HL) NG JL 15/8	NG NG 26/8	1a 1c	-

Table 47: Further nurseries for second year (1957) investigation.

Newton Morayshire	Lossiemouth 6m. 83'	EL 6/9 HL - JL -		No material sent
Ledmore Perthshire	Leuchars 27m. 317'	EL 10/7 HL - JL -		1c - -
Wykeham Yorks.	Driffield 17m. 413'	EL 20/8 HL - JL -		No material sent

Before attempting to plot the available data exact weather criteria for initial Meria infection would have to be postulated. From cultural studies of the fungus and artificial inoculation experiments in the greenhouse it is obvious that relative humidity is the most important factor, controlling spore germination, which will only occur at relative humidities of over 95% (see p.103). Therefore, in nature, it is assumed that the spore will not germinate on the needle surface unless the humidity is above this percentage. The spore will germinate over a range of temperatures between 0°and 25°C but as germination is very slow at temperatures below 10°C (50°F) - only 5% of the spores of the a strain having germinated after thirty-six hours at this temperature while b and c strain germination had not even commenced - it was decided to use 10°C as the lowest temperature reading. Consequently the criteria postulated for the occurrence of a Meria attack are a Stevenson screen relative humidity over 90%, as Yarwood (1939) has said that the humidity in the immediate vicinity of the leaf is greater than in the surrounding air due to the lower leaf temperature, and a temperature above 10°C continuously for forty-eight hours.

It was decided to plot the data in two different ways in order to obtain maximum interpretation of

results. Firstly the data was plotted as a typical Potato Blight warning and record table using the above criteria as the critical period and with two near critical periods. As humidity was the most important criterion this was considered as a standard and the temperature requirements were altered. The first near critical period was humidity of over 90% and temperature over 10°C for twenty-four hours (as at fairly high temperatures 20° and 25°C germination could easily occur in this time) and the second was humidity of over 90% but temperature under 10°C for forty-eight hours, as it is possible for germination to occur slowly under this temperature.

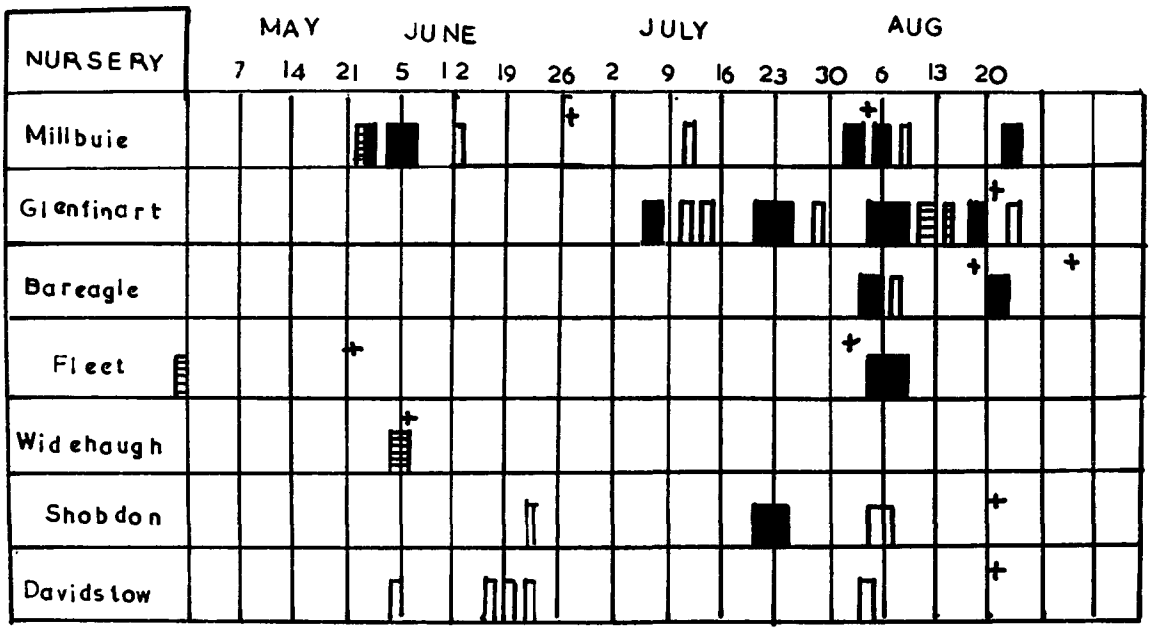
The second method of plotting consists of a table recording the number of days on which particular weather conditions occur in the month before the infection is first reported. This is more detailed than the first method and acts as a check to the criterion of the critical period and it will also indicate if other criteria can be accepted to describe a critical period.

The seven selected sets of data were then plotted by both methods.

METHOD 1 - overleaf.

METHOD 1: MERIA FORECASTING 1956

1956



▮ Critical period - 48 hours humidity over 90% temp. over 10°C.

▯ Near critical - 24 hours " " " " " "

▯ period - 48 hours " " " " below 10°C for more than 12 hours.

+ First reported infection.

METHOD 2: MERIA FORECASTING 1956

Nursery	No. of days of 90% R.H Weeks before reported infection		No. of days of 70% R.H Weeks before reported infection		Temperature for 30 days before reported infection		
	Week 2	Week 3	Week 2	Week 3	Below 10°C	Above for 12hrs.	Above 10°C
Millbuie EL	1	3	3	3	-	20	10
HL & JL	-	1	3	-	-	4	26
Glenfinart EL,HL,JL	4	1	4	1	-	5	25
Bareagle HL & JL	3	-	2	2	-	10	20
EL	2	-	2	3	-	16	14
Fleet EL	-	1	3	2	4	26	-
HL & JL	-	-	1	2	-	6	24
Widehaugh EL	-	-	1	1	1	27	2
Shobdon EL	1	-	2	-	-	6	24
Davidstow JL	1	-	3	3	-	-	30

From the disease record and warning table four reported outbreaks occur after critical periods and four occur after near critical periods while two, Widehaugh and Fleet infection of Hybrid and Japanese larch show no connection with plotted data. Of the

four attacks after the critical period one, Killbuie European larch occurs three weeks after the incidence of the critical period while the other three (Glenfinart, all species, Bareagle Hybrid and Japanese and Bareagle European larch) occur two weeks after the critical period.

Examining the details of the Killbuie European larch attack it is seen that there are three days when the humidity is continuously over 90% three weeks prior to reported infection; of the thirty days temperature readings twenty of these show temperatures below 10°C at night. In contrast the details of Glenfinart infection (on all trees) show four days with continuous 90% humidity two weeks prior to infection and a total of twenty five out of thirty days when the temperature is above 10°C for twenty-four hours. This indicates that if the humidity conditions are fulfilled it is the temperature that then controls the time of the development of disease symptoms. The occurrence of several days with humidities over 70% close to the critical period will obviously prevent desiccation and enable the germ tube to enter the stomatal pore. These days occur in both cited examples.

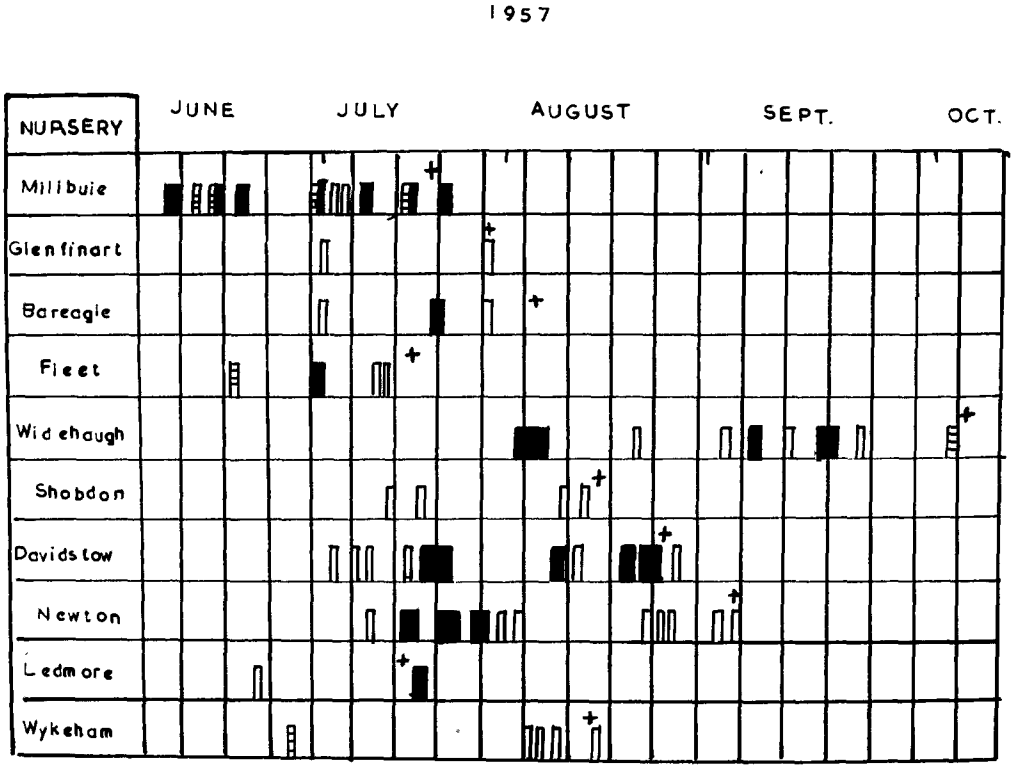
It would appear from this preliminary examination of the data that the criteria chosen to estimate the

occurrence of a Lerio attack are in fact valid in the majority of cases, in spite of the distances from the synoptic stations to the nurseries.

The failure of the two nurseries Widehaugh and Fleet to fit in with the critical period criterion may be due to several reasons. Firstly the distance from the synoptic station may be exerting an effect, although Fleet European larch infection fits in with the critical period, and secondly the visible signs of infection may have occurred sometime before they were in fact reported.

This latter criticism must be considered in the examination of all data collected from the nurseries, as very few specific instructions were given to the Foresters about examination of diseased plots and little specific information was received on the forms. The effect of the large distance from the synoptic station on the weather in the nursery was difficult to evaluate, and so it was decided to repeat weather data analysis for the 1957 growing season using the same nurseries and adding three others. This would also help to verify the connection between overall temperature and time taken to develop disease symptoms.

METHOD 1: MERIA FORECASTING 1957.



▮ Critical period

▯ Near critical period

+ First reported infection.

METHOD 2: MERIA FORECASTING 1957

Nursery	No. of days of 90% R.H Weeks before reported infection		No. of days of 70% R.H Weeks before reported infection		Temperature for 30 days before reported infection		
	Week 2	Week 3	Week 2	Week 3	Below 10°C	Above for 12hrs.	Above 10°C
Millbuie	2	3	3	3	-	11	19
Glenfinart	-	-	2	2	-	5	25
Bareagle	2	-	3	2	-	2	28
Fleet	2	-	1	-	-	9	21
Widehaugh	1	2	2	3	-	18	12
Shobdon	-	-	-	1	-	2	28
Davidstow	3	-	3	2	-	1	29
Newton	2	-	2	1	-	16	14
Ledmore	-	1	2	-	-	12	18
Wykeham	2	-	2	-	-	4	26

From the disease record table five reported outbreaks occur after critical periods and three after near critical periods with two, Glenfinart and Shobdon showing no correlation with criteria employed. On reference to method 2 table it is seen that four of five critical period outbreaks are reported two weeks after the critical period and one, Widehaugh, three weeks after the critical period. This verifies the temperature effect noted in the previous years' analysis as the former nurseries show 19, 28, 21 and

29 days with the temperature above 10°C continuously, while Widehaugh shows only twelve days above 10°C continuously.

This second analysis again verifies the validity of the weather criteria chosen to estimate Meria attack and the results of two years' analysis are summarised below:

Table 48: SUMMARY OF TWO YEARS' RESULTS OF MERIA ATTACK AND FORECAST

Year	Infection after critical period	Infection after near critical period	Not analysable by criteria used	Total
1956	Millbuie EL Glenfinart Bareagle HL JL Bareagle EL	Millbuie HL JL Fleet EL Shobdon EL Davidstow JL	Fleet HL & JL Widehaugh	10
1957	Millbuie EL JL Bareagle JL Fleet JL Widehaugh JL Davidstow JL	Newton EL Ledmore EL Wykeham EL	Glenfinart EL Shobdon EL	10
Total	9	7	4	20

Out of twenty reported Leria attacks nine occurred after critical periods, seven after near

critical periods and only four did not fit into the criteria used. This could be due to the differences in weather conditions between the nursery and the synoptic station, especially as no ecological details of nursery siting are known, or delay in reporting incidence of the disease. The former reason is more important as there can be fairly large humidity fluctuations within a small area.

A more specific statement of weather criteria necessary for Meria attack can be made by further analysis of the data, considering length of time between critical and near critical period and appearance of disease symptoms and the temperature before the reported attack.

Table 48: (overleaf)

Table 49: Further analysis of weather data showing connection between temperature and disease incidence.

Time between critical period and reported disease	Nursery and tree species	Number of days		Number of days near critical period with R.H 70%
		Temp. over 10° for 24 hours	Temp. over 10° for 12 hours	
3 weeks	1956 Millbuie EL	4	17	3
	Millbuie HL JL	18	3	2
	Fleet EL	-	21	2
	1957 Widehaugh JL	10	11	3
	Ledmore EL	12	9	-
2 weeks	1956 Glenfinart EL HL JL	14	-	4
	Bareagle HL JL	7	7	2
	Bareagle EL	6	8	2
	Shobdon EL	9	5	2
	Davidstow JL	14	-	2
	1957 Millbuie EL JL	11	3	3
	Bareagle JL	14	-	3
	Davidstow JL	14	-	3
	Newton EL	3	11	2
	Wykeham EL	11	1	2

If the humidity requirements for germination of Meria spores are fulfilled then it is the temperature that is the limiting factor in time of development of Meria symptoms. If the temperature is above 10°C

continuously for most of the month the infection is visible after two weeks but if the temperature is below 10°C for more than half the month prior to visible infection, the symptoms take three weeks to develop.

This enables the postulation of a more specific set of weather criteria for the estimation of Meria attack. A Meria outbreak is likely to occur two to three weeks after a forty-eight hour period whenthe temperature in the nursery does not fall below 10°C and the relative humidity does not fall below 90%. The outbreak will be three weeks after this period if the majority of the average daily temperatures are below 10°C but will occur two weeks after this period if the average daily temperatures are above 10°C.

There appears to be no distinct correlation between date of appearance of symptoms and location of nursery (see previous section) as most outbreaks occur in late July, August or September. This may be due to unfavourable weather conditions, probably too low a temperature in the early part of the year, as there are only two records in May, Fleet European larch 1956, Widehaugh European larch 1956 and one in June, Millbuie European larch 1956. Another possibility is that primary infection does occur

and the reported outbreaks are instances of a larger secondary infection from the primary inoculum released.

D I S C U S S I O N

The fungus

Four distinct cultural strains of Meria have been recognised, designated a, b, c and d, and these can be identified by various cultural characteristics. Peace and Holmes described two strains, a and b, which correspond to the present a and b strains, and also mentioned the occurrence of a further sparsely sporing variant with a white fluffy mycelium corresponding to the current d strain.

All the strains remain stable during frequent sub-culturing under normal conditions, but under certain circumstances strain changes occur. Growth for long periods at 10°C caused a strain cultures to take on c strain characteristics and c strain cultures to show b strain characteristics. However, all these cultures reverted to normal type when sub-cultured and grown at 20°C. In the pH experiment permanent strain changes occurred at pH 9.2, when a d strain changed to a b strain and a c strain changed to an a strain. Further permanent strain changes occurred in the passage of the fungus through the needle. In several artificialinoculation experiments using spore suspensions of the b strain of Meria, the c strain was re-isolated from the

infected needle, and storage of spores of c strain at 0°C for forty-eight hours prior to germination led to the formation of a strain colonies.

To explain these changes it is necessary to regard Meria as a hetrocaryotic fungus, in common with many other fungi imperfecti. The cells are multinucleate, although the number of nuclei within each cell varies from one to ten, and numerous anastomoses occur. Each nucleus probably carries the characteristics of one strain and the phenotype will depend upon the relative proportion of particular strain nuclei within the mycelium. Under certain conditions this proportion can be altered to produce impermanent strain changes (effect of 10°C mentioned above) or permanent strains changes (effect of a high pH or passage through the needle). The frequent anastamoses occurring in culture probably account for the stability of the strains at 20°C as each hypha will have a similar nuclear content to those formed from the original monosporus culture. These anastamoses are less frequent within the needle and this could account for the strain change by altering the particular nuclear proportion.

The spores of Meria are multinucleate, several

nuclei passing into each conidium from the sterigma. However, observation of spores during formation and spores prior to germination shows a reduction in number of nuclei from the former to the latter state. It was not possible to observe whether this reduction in the number of spores was caused by nuclear fusion prior to germination or by nuclear disintegration. Nuclear fusion, giving heterozygous diploids, has been observed in Aspergillus niger (Pontecorvo et al. 1953) and Burges (1955) has suggested that the variability and adaptive capacity of such a mechanism is probably intermediate between gene mutation and sexual reproduction. It is unlikely that this occurs in Meria as little variation appears to have occurred within the fungus since the original cultural work in 1933. Three of the present strains were described by Peace and Holmes and the c strain was probably missed only because the epidemiological studies were the prime object of the work. If the nuclear degeneration hypothesis is correct Mitotic division will occur in the primordial cell at spore formation and the daughter nuclei will pass into the spore. Some of these will degenerate and, on germination, the hyphae produced may in fact be of one or more different strains As the culture cannot be attributed to a

particular strain for at least fourteen days after germination, sufficient hyphal anastamoses will have occurred to enable the hyphae to regain their original nuclear complement and strain pheontype.

Isolations from natural infections of Meria from all tree species reveal the equal occurrence, in any one nursery, of single strain or mixed strain infections regardless of the number of different tree species infected. This indicates that the infection can be caused by a mixture of strains or by a single strain of Meria, depending on the available inoculum, or that in approximately 50% of the infections a strain change has occurred within the needle. It is likely that both of these phenomena have occurred. There is also little difference between the frequency of occurrence of a, b or c strains of Meria in nature indicating that the differences between the strains have limited pathological significance. However, it is obvious from the artificial inoculation experiments and certain nursery data that the b strain is the most virulent of the three. Symptoms of needle cast on trees inoculated with the b strain occur prior to symptoms on trees inoculated with the other strains, and several re-isolations from these infected needles reveal only the c strain of Meria. This

strain change from b to c is also shown in the examination of nursery data and indicates that some of the c strain isolations from nature were derived from infections caused by b strain inoculum.

What therefore is the significance and relationship between the different strains of Meria? From cultural studies there appears to be a physiological relationship between strains a and c and strains b and d respectively. Strains a and c have similar pH optima, do not require choline chloride for growth and, when cultured with Botrytis allii have no effect on its growth. Strains b and d have similar pH optima, have a partial requirement for choline chloride and when cultured with Botrytis allii prevent its growth. During the experiment on effect of pH on growth a strain change from d to b occurred, and during a germination experiment a strain change from c to a occurred. However, artificial inoculation experiments and nursery data indicate strain b is also related to strain c and strain a to strain b. All the strains within the species Meria laricis must be related, but it is possible that certain strains are more nearly related than others. Hansen (1938 and 1942) and Hansen and Snyder (1943) working on the problem

of heterocaryosis in the Fungi Imperfecti suggested that many of these show the 'dual phenomenon'. Within each species of heterocaryotic fungus there are two distinct homocaryotic genotopes, the M type with much mycelium and few conidia and the C type with little mycelium and many conidia. A great number of intermediates are possible amongst the heterocaryons (M and C) as each can contain different proportions of the M and C nuclei. The M type is very stable in culture and never reverted to the C type and both types remained true after passage through the host, indicating that M is a true mutant of C. Is it possible to apply this hypothesis to Meria? Examination of the data certainly reveals the M type (strain d) and possibly the C type (strain c); it could be postulated that strains a and b are heterocaryons of M and C nuclei. Strains c and d remain stable in culture, except under abnormal conditions, and only strains a and b, the heterocaryons, change in passage through the needle while c strain remained unaltered.

Jinks (1952) found that simultaneous inoculation of a liquid medium with spores of two homocaryons produced a greater dry weight of mycelium than either homocaryon alone in 80% of the experiments and suggested that this improved growth was due to heterocaryon formation. This experiment was repeated using

mycelial suspensions of the different strains of Meria in order to test the dual phenomenon hypothesis, but the results were inconclusive as all mixed strain cultures, including the postulated heterocaryons, produced better growth than either of their single strains. Growth in plate cultures using discs of different strain mycelium placed close together was examined, but no hyphal fusion between the different strain mycelia could be observed although it was found that these fusions would occur between homocaryons (Christensen et al.1947). It is therefore unlikely that the dual phenomenon can be applied to Meria.

It would therefore appear that these strains are naturally occurring variants within the species Meria laricis which is a heterocaryotic fungus. They occur both in culture and in nature where three are found in approximately equal numbers, showing little pathological significance (although the b strain is more virulent). The strains are interchangeable under certain cultural conditions and also during passage through the host. The phenotype of each strain could arise as follows: If a single nucleus carries a single strain characteristic the relative numbers of different nuclei present in the mycelium would account for a particular strain phenotype. This phenotype will remain stable under normal cultural conditions owing to hyphal anastomosis.

However, under extreme cultural conditions, a high pH or prolonged exposure to low temperature, an alteration of the relative nuclear numbers within the hyphae can occur. The nuclei exerting strain dominance may be adversely affected by the conditions and fail to divide or possibly degenerate. Nuclei of a different strain, that can withstand the adverse conditions, will continue to divide and very soon the influence of these nuclei will supersede that of the original nuclei. When the mycelium is cultured again, under normal conditions, these other nuclei will continue to exert their dominance and hence a permanent strain change occurs. This system could also account for the temporary strain changes observed. Under less extreme cultural conditions the original strain nuclei cease to exert their influence, and another group/strain of nuclei influence the phenotype, causing a different morphological appearance, but without an increase in their number. On re-culturing under normal conditions the original s train nuclei re-exert their dominant influence and the culture reverts to the original phenotype. Why then is there not an unlimited number of variants? It is probable that there are only a limited number of nuclear mixtures which give vigorous growth and sporulation, both in culture and within the host, and these will therefore remain dominant. In fact, under ideal cultural conditions,

other variants are found, although these can usually be attached to a particular strain, but these normally revert to a more distinct phenotype after severl sub-culturings. Once in monosporous culture the mycelium remained morphologically constant, i.e. no sectoring was observed, and therefore probably no mutations occurred. Another possible explanation of the phenotyptic behaviour of Meria is the mitotic recombination theory (Roper 1952) leading to the parasexual cycle (Pontecorvo 1956) which has been observed in a few fungi imperfecti. These workers have found that within heterocaryotic mycelia some of the unlike haploid nuclei fuse, a mitotic crossover occurs and on haploidisation different characteristics are found in the haploid nuclei. However, observations of this type of crossover are not possible in Meria owing to the impossibility of isolating and typing uninucleate hyphal segments.

Cytoplasmic inheritance (Fincham and Day 1963) must also be considered as a third explanation but again no evidence for this phenomenon was observed during the current investigation. In fact, the observed behaviour of the fungus both in culture and in nature can best be explained by the conventional heterocaryosis theory outlined at first.

The taxanomic position of Meria, according to previous workers, has already been discussed in the

introduction and little further can be added. Meria is an imperfect fungus, the spores being produced asexually, and using the classification of Mason (1937) and Wakefield and Bisby (1941) of slime spored and dry spored groups, it must be placed in the slime spored group. C strain cultures prior to conidial formation are dry but as soon as conidial formation commences the surface of the mycelium becomes wet and slimy. There appears to be little reason therefore for altering the classification given by Clements and Shear (1934) - Order: Moniliales, Family: Moniliaceae - Macronemeae. Meria laricis.

The disease

It is apparent, from the introduction, that needle cast was more or less confined to European larch and Western larch until 1946 when Robak (1946) reported its occurrence on Hybrid and Japanese larch in Norway. In Britain outbreaks on these species were not reported until 1954 (Batko) but no explanation for sudden appearance of disease on these previously immune species is given. It is possible, however, that these species were attacked in Britain prior to 1954, but the attacks were very slight and not immediately noticeable. The weather conditions during 1954 may have been such that an obvious infection could easily be seen.

It is obvious both from artificial inoculation experiments and reported disease data that European larch is the most susceptible larch species, Japanese larch is the most resistant and Hybrid larch has an intermediate susceptibility, varying with conditions and parentage. The only reported severe attacks of Meria occur on European larch, with one exception, it has the most reported disease attacks and where this species is grown together with other species the attack on European larch is more severe.

Disease symptoms appear earlier on European larch than the other two species if all three are inoculated simultaneously, a fact also confirmed by the nursery data, and it is also evident that in nurseries growing other species beside European larch Meria attack occurs by the European larch acting as an inoculum source.

Experiments to investigate the nature of the difference in susceptibility between the larch species indicate that both mycelial growth and germination of Meria spores is affected by an extract of larch needles. Mycelial growth and germination of spores is enhanced when this extract is added to non-nutrient media. However, this enhancing effect is greater when using European larch extract than using Japanese larch extract, and this could account for the resistance of Japanese larch. Spore germination is promoted in European larch

by exosmosis of substances onto the needle surface and subsequent mycelial growth inside the needle is also increased. The disease symptoms in this species will therefore appear fairly rapidly, provided that the other conditions for infection and growth are at their optima, and infection is evident on European before Japanese larch. The delay in appearance of disease symptoms on Japanese larch may well be due to the smaller effect that this species has on promoting germination and mycelial growth of the pathogen. The identity of the substance or substances having this effect on Meria is not known, except that it appears to be water soluble and thermostable, but it is possible that it is not present in Japanese larch in such a large concentration as in European larch, or that it is found in Japanese larch together with an inhibitor which partially nullifies its effect. This explanation accords with the balance hypothesis of parasitism (Lewis 1953) where resistance or susceptibility of a host can be explained by the fact that there are substances amongst the host's metabolites available to the parasite that may favour or hinder the parasites growth and that the kinds and concentrations of these metabolites may vary as conditions alter. It is possible therefore that an inhibiting metabolite, possibly a vitamin or vitamin complex, is present in Japanese larch cytoplasm

which affects germination and growth of Meria and delays the appearance of disease symptoms. This metabolite may also limit the severity of the disease attack as there is only a single recorded severe attack on this species, the majority being slight attacks. However, the balance hypothesis does not explain the confinement of Meria attack to European and Western larch until 1946. It is unlikely that changes have occurred within Hybrid and Japanese larch within the short period from 1933 (Peace and Holmes) and much more likely that physiological changes have occurred within the fungus, to give greater virulence, enabling infection of the previously resistant larch species to occur. It is a combination of these changes and their relation to the balance hypothesis that accounts for the present infective ability of Meria.

It has already been pointed out that the different strains of Meria have little pathological significance apart from the fact that the b strain is certainly the most virulent. It is not possible to correlate a particular strain with a particular larch species or even a particular geographical area and it is probable that most infections are a mixture of strains and probably emanate from mixed inoculum sources. This is obvious as passage through the tree can affect the fungal strain.

Strain b has changed to strain c during passage through the needle, indicating a relationship already shown during cultural work, and from nursery data and isolations from nature, strains c and a are also interchangeable during passage through the host. Peace and Holmes mentioned that the b strain was most commonly isolated from nature, but some of these cultures are probably heterocaryotic containing a mixture of b and c nuclei. Their results may be considered to be in accordance with the findings in the current work that b and c strains taken together preponderate over the other strains isolated.

No explanation for the greater virulence of the b strain is obvious from the cultural work on the fungus, and it may be that the balance hypothesis used to explain the susceptibility of European larch to Meria attack also offers an explanation for this phenomenon.

PATHOGENICITY AND EPIDEMIOLOGY

Various conditions must be fulfilled before Meria attack can occur. From artificial inoculation experiments and studies on spore germination the two most important are humidity and temperature. The spores only germinate at a relative humidity above 95% and this is obviously the limiting factor under natural

conditions; a period of at least forty-eight hours of this percentage humidity is necessary as the spores germinate very slowly. If the humidity conditions are fulfilled then the temperature becomes the limiting factor but only in connection with the date of appearance of the disease symptoms as germination of spores can occur at all temperatures between 0° - $25^{\circ}C$. A Meria outbreak will occur two weeks aft r a forty-eight hour period when the humidity is above 90% provided that the temperature does not drop below $10^{\circ}C$ during this period. If the temperature drops below $10^{\circ}C$ for more than half the period after the infection date disease symptoms are not visible for three weeks.

Spores are formed also under high humidity conditions and between temperatures of 10° - $25^{\circ}C$. As the fungus grows slowly at $0^{\circ}C$ it can possibly withstand frost, survive the winter in the shed needles on the ground and those still adhering to or trapped in the tree. On the onset of warmer weather, together with the correct humidity requirements spores are produced and released.

No observations have been made on the method of spore release by any of the previous workers but it is possible that one spore is pushed off the sterigma by the formation of a second spore underneath. Rain splash could also account for spore release both from

the shed needles and the living infected needles occurring later in the season.

The spores are dispersed by the wind and infection of uninfected trees can occur up to 50 yards from an infected plot. It is likely, however, that as Meria is a slimey spored fungus the initial infection from a shed needle on the ground will be restricted to a much shorter range as the spores will tend to clump together and stick to the needle surface giving a heavier spore mass to be moved.

The spores will germinate on reaching a larch needle surface, provided the temperature and humidity conditions are fulfilled, and the germ tube will enter through the stomatal pore. The mycelium grows intercellularly and disease symptoms will eventually become visible, the time lapse depending on the temperature. Spores produced from the living needles have a better chance of further dispersal as they are already up to two feet from the ground, as usually the top shoots of the trees are infected.

It has been possible to devise a forecasting system to anticipate Meria attack, based on the Beaumont system for Potato blight. The critical period, when infection is likely to occur if inoculum is present in the air, is a Stevenson screen humidity of over 90% and a

temperature over 10°C, continuously for forty-eight hours, and there are two near critical periods with the same humidity criterion but different temperature requirements. Analysis of twenty reported Meria attacks using this system showed that sixteen occurred after critical or near critical periods in spite of the possible discrepancies betweenthe weather conditions at the nursery and the synoptic weather stations from which the weather data was obtained. It is possible that with continuous annual records of disease behaviour from the nurseries this system could be made more specific.

The spraying techniques devised by Peace and Holmes can now be used only at the onset of critical or near crutical periods to prevent the spread of the disease.

Owing to the distance that Meria infection has been reported to travel from infected to uninfected beds, the prophylactic measure of transplanting previously infected seedlings some distance away from uninfected beds is of doubtful value unless the beds are at least 100 yards apart. It is impracticable to burn the shed infected needles, but care must be taken during transplanting to remove all needles lodged in the branches as these will supply an inoculum source in the following spring.

Practically nothing is known of the infection

of larches other than seedlings. Cursory examination of older larch trees, scattered throughout the country, in or out of plantations often reveal Meria infection. This is only visible on fairly careful shoot examination and in no way affects the health of the tree. However, these infected trees must act as inoculum sources for nursery infection and it is obviously necessary to investigate this effect in relation to specific nurseries before the final pattern of the epidemiology of Meria can be ascertained.

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