STUDIES ON MYCORRHIZAL AND PSEUDOMYCORRHIZAL FUNGI

FROM AN EXPERIMENTAL AFFORESTATION AREA

Abstract of

Thesis submitted for the Degree of Ph.D.

by

Ida Levisohn

A B S T R A C T

The investigations here described were carried out in connection with afforestation experiments at Wareham Forest, Dorset.

The thesis consists of three papers. Some of the experiments and observations recorded in the first and second paper have already been published in a joint paper with Dr. M. C. Rayner, entitled: "THE MYCORRHIZAL HABIT IN RELATION TO FORESTRY. IV. Studies on mycorrhizal response in *Pinus* and other conifers". Forestry, vol.xv, 1941.

The first paper of the thesis deals with the mycelial and physiological characters of *Boletus bovinus* Linn. in pure culture, and with its growth reactions on such organic composts as are of interest in forestry practice. In connection with this, two types of mycorrhizal associations formed by *Boletus bovinus* with *Pinus* spp. at the experimental area, are described.

The second paper is concerned with the cultural behaviour of several forms and strains of Mycelium radicis atrovirens Melin, and with the description of various types and degrees of pseudomycorrhiza formation by this mycelium. Discussing the pathogenicity, attention is drawn to the fact that it is the proportion of pseudomycorrhizas and mycorrhizas formed by any individual tree which is of significance in respect to its general health.

The third paper of the thesis deals with experiments and observations connected with analysis of the factors responsible for the suppression of growth in the experimental area. The anomalous features in the humus constituent of the experimental soil were studied by the effect of addition of cellulosic materials to this soil. The results obtained support the hypothesis that changes in the biological activities of the soil through addition of certain organic products have a direct effect on tree growth.

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C O N T E N T S

INTRODUCTORY

The investigations here described form a side-line to investigations and forestry experiments at Wareham Forest, Dorset, carried out during a number of years by Dr. M. C. Rayner, and already placed on record in a number of publications (see Literature references Part I: 13, 14, 15, ~~13,~~ 16, 17).

The area belongs to the Forestry Commission of Great Britain. It consists of poor *Calluna* Heath of varying elevation derived from Bagshot Beds; the soil is highly leached and very poor in inorganic plant nutrients; the pH values vary from 4·7 to 4·9.

On the untreated soil in most parts of the area, seedlings and transplants of pine and other conifers suffer from high mortality and inhibition of growth often exhibiting symptoms of chronic starvation. Root growth is defective and formation of mycorrhizas suppressed or greatly inhibited. These deleterious effects on growth may be observed also in pot-cultures of transported soil.

The use of organic composts has provided complete amelioration of the deleterious effects on tree growth (see Literature references Part I: 15, 16), and experimental analysis of these treatments affords support for the hypothesis that such amelioration has been brought about mainly by modification of the nature of the humus decomposition through changes in the course and direction of biological activities.

The problem of tree growth here (at Wareham Forest) and in similar types of soil elsewhere is indeed a complex one inviting attack along several lines of investigation; among these are the mycological observations and experiments now presented. These include:-

I. Study of the fungus species responsible for mycorrhiza-formation in certain conifers at Wareham Forest.

II. Study of certain soil fungi forming pseudomycorrhizas with these conifers.

III. Study of the growth reactions induced by addition of cellulosic materials to Wareham Forest soil and to others of similar type.

I. A STUDY OF BOLETUS BOVINUS LINN.

CONTENTS OF PART I

1. INTRODUCTION

Independent investigations (11, 12, 13, 14, 15, 16, 17) have proved that the soil of the experimental area at Wareham Forest, Dorset, contains 'toxic' factors of biological origin which operate as growth inhibitors and lead to a condition of 'biological inertia' in the soil.

Greatly reduced fungal activity is one of the outstanding particularities of this soil as compared with non-toxic soils of similar type. There are present only a few fungus species concerned in the mycorrhizal association of pines and there is a notable absence of soil fungi deleterious to root growth. In general, fungus mycelium is relatively scarce and the 'peritrophic' flora often conspicuous about conifer roots in similar soils is lacking here (12, 17). Few species of the higher fungi have been recorded from the experimental area (17). Sporophores of *Boletus bovinus* appear regularly in the vicinity of young pines making healthy growth and now occur also on experimental seed plots and patches of Scots and Corsican pine as a result of compost treatment (17). *Thelephora terrestris*, *Clytocybe (Laccaria) laccata*, and *Lactarius rufus* are also present. The sporophores of the species mentioned have increased in number during the years of afforestation. Immediately outside the experimental area, sporophores of Boletus badius, B. erythopus, and Rhizopogon luteolus appear regularly.

Among mycelia directly associated with the roots of pine and other plants are found Mycelium radicis atrovirens which is relatively scarce and those forms of the vesicular-arbuscular group of soil fungi associated with Molinia caerulea and Agrostis setacea. Microscopic examination has also revealed the presence of Thamnidium spp., Mucor racemosus, M. hiemalis, and a few species of Penicillium and Trichoderma some of which are doubtful members of the true soil flora.

In beds inoculated with humus from established stands of pine, Mycelium radicis nigrostrigosum Hatch has been introduced and has appeared as an active mycorrhiza-former.

From observations made during several years, it seems that Boletus bovinus is the only fungus constantly associated with the species of pine grown in the experimental area. In view of the importance attached to the mycorrhizal habit and its significance in the particular soil in question, the present investigation was undertaken to isolate

Boletus bovinus and to study its behaviour in pure culture and as a mycorrhizal associate of certain species of pine at Wareham Forest.

2. HISTORICAL

As early as 1892, Frank (3) suspected *Boletus bovinus* of forming mycorrhizas but he did not name the tree associate concerned. Masui (8) reports pure culture experiments with seedlings of *P. densiflora* growing in large test-tubes on nutrient agar using the mycelium of *B. bovinus* as inoculum. In his cultures, the seedlings are overgrown and killed by the fungus. It seems very likely that the behaviour of *B. bovinus* as reported by Masui is attributable to the method and the experimental conditions thus created. In a few pure culture experiments set up by the writer with *P. silvestris*, the mycelium of *B. bovinus* used for inoculation rapidly covered the substrate within the experimental flasks but it did not overgrow or kill the seedlings. Melin (9) describing various mycelia extracted from pine mycorrhizas points out that the form called by him *Mycelium radicis silvestris α* is probably identical with *B. bovinus*. The fungus has been recorded by Hatch (4) to form mycorrhizal association with P. strobus. In order to prove that B. bovinus is involved in the mycorrhiza-formation of White pine, Hatch employed the pure culture method and succeeded in establishing an artificial association between the seedlings and the fungus. The mycelium designed for inoculation purposes was obtained from cultures isolated from sporophores of the species, also isolated from a mycorrhiza of P. strobus.

3. ISOLATION AND IDENTITY TESTS

a. Isolation from sporophores.

Isolations of B. bovinus were made from sporophores collected at the experimental area at Wareham Forest, October 1934, 1938, 1940, and 1942; also from young fruit-bodies from a pure stand of Scots pine in Surrey, September 1935 and 1940. The strains obtained from Wareham Forest and from Surrey are identical in every respect.

For the purpose of isolation, the tissue culture method was applied. Fragments of clean tissues from the inner part of a fresh young sporophore were transferred with aseptic precaution on nutrient agar. Isolations from fragments taken from pileus and stipe gave similar results.

During the course of this investigation the cultures from sporophores were used as a 'standard'.

b. Isolation from mycorrhizas.

The following isolations of a mycelium believed to be that of *Boletus bovinus* were made from mycorrhizas of Scots pine and Corsican pine.

Table I

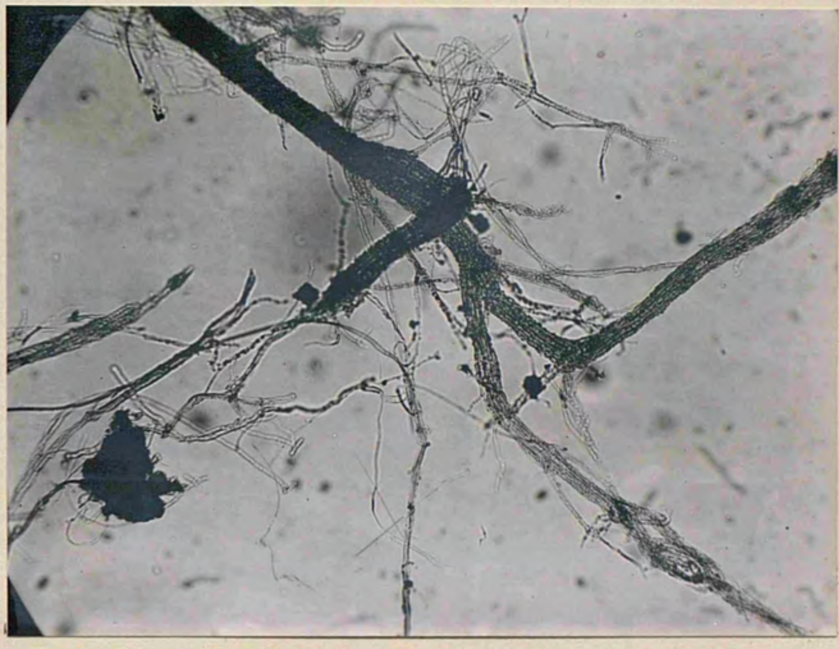
Isolations of *Boletus bovinus* from individual mycorrhizas of seedlings of Scots pine and Corsican pine growing in the Wareham Experimental area and in pot-cultures of soil transported from this area.

Species of pine	Isolate	Source	Date	Remarks
Scots pine	1a	Seed-plot, Wareham experimental area	Dec. 1935	Plot inoculated with humus from good stand.
" "	1b	Seed-patch, Wareham experimental area	Dec. 1938	Control: soil untreated.
" "	1c	" " "	Dec. 1939	Treated with hopwaste compost.
" "	1d	Pot-culture with transported soil	Dec. 1935	Control: soil untreated.
" "	1e	" " "	Jan. 1937	Treated with hopwaste compost.
Corsican pine	2a	Seed-plot, Wareham experimental area	Dec. 1935	Plot inoculated with humus from Corsica.
" "	2b	" " "	Dec. 1938	Control: soil untreated.
" "	2c	Pot-cultures with transported soil	Jan. 1937	Treated with hopwaste compost.

Cultures from these extractions are identical in all respects. The isolations were made from forked as well as from tuber mycorrhizas applying the following method:- After being washed and brushed thoroughly, and left in running water for several hours, the mycorrhizas are dipped in 0·1% mercuric chloride for about 15 seconds and again washed 3-5 times in sterilized water. In each case of isolation it is advisable to prepare a number of parallel sets varying the period of exposure to the sterilizing medium from 5-30 seconds. After sterilization, the mycorrhizas whole or cut into small pieces with a sterilized knife are plated on one of the usual culture media.

It was found during the course of this investigation that isolations of B. bovinus from mycorrhizas were only successful in autumn and winter (cf. Table I). This is not in accord with Melin's experience in Sweden (9). He was able to isolate Mycelium radicis silvestris α at all seasons, but found it more difficult to obtain pure cultures in winter than at other seasons, moulds and bacteria being more profuse in the cultures then.

PLATE I



Rhizomorph-like hyphal strands of Boletus bovinus from seed plot of Scots pine at Wareham; collected early spring. × 140.

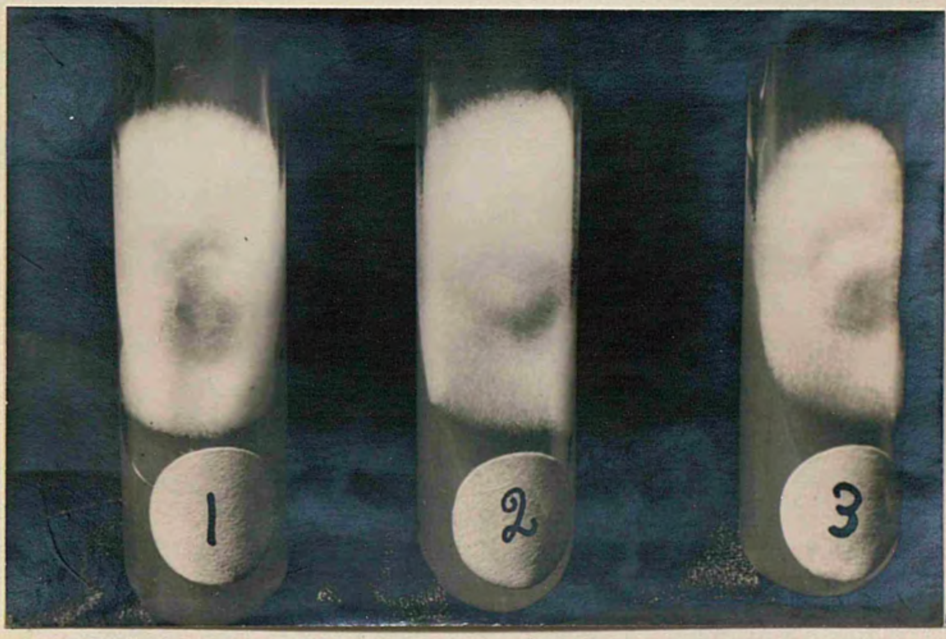
c. Isolation from rhizomorphs.

A third source of extraction of *Boletus bovinus* were the hyphal strands or rhizomorphs (Plate I) which are profusely distributed in the experimental soil at certain seasons and which show an organisation very similar to that observed in hyphal strands of other hymenomycetes (6, 7). The rhizomorphs of *B. bovinus* are of characteristic pinkish-brown colour; they vary considerably in diameter, their average diameter being 60-70 μ. Each rhizomorph is a well-developed hyphal strand consisting of a central cylinder formed by 1-3 hyphae, each 4-5 μ in diameter, enclosed within a sheath of slender hyphae each 2-3 μ in diameter. Clamp-connections have not been observed in the hyphal filaments forming these rhizomorphs. For the purpose of isolation, fresh rhizomorphs were washed for about 10 seconds in 0·1% HCl, rinsed in sterilized water and plated on nutrient agar. After about 5 days, characteristic colonies of *B. bovinus* developed.

Identity tests.

The isolates of *B. bovinus* from various sources resemble each other in respect to kind and rate of growth, colour, reactions to a number of culture media, and morphological and cytological details.

PLATE Ia



Boletus bovinus; cultures from three different sources:

1. from mycorrhiza of Scots pine seedling; experimental area.
2. from sporophore, experimental area.
3. from rhizomorph, experimental area.

On beerwort-agar, 18 days. Nat.size.

Buller's method (2) of testing identical or related mycelia was applied to the following combinations:-

B. bovinus	(from	sporophore)	×	Mycelium	radicis	Scots	pine		(Table I, 1a)
"	"	"	×	"	"	"	"		(Table I, 1c)
"	"	"	×	"	"	Corsican	pine		(Table I, 2a)
"	"	"	×	"	"	"	"		(Table I, 2c)

The mycelia isolated from mycorrhizas and from rhizomorphs are identical with those extracted from sporophores of B. bovinus according to this test. (Pl. Ia)

4. CULTURAL AND MYCELIAL CHARACTERS

The initial platings were usually made on beer-wort agar*. This medium supports rapid growth of the mycelium. After 3-5 days of incubation, colourless hyphae are noticed to emerge from the inoculum. They gradually form a raised colony, white in colour (Plate II, fig. 1) flattening down after about 12 days of growth. Then the colour changes

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* 'Sweet wort' fresh from the brewery is diluted to specific gravity 1·04 and further diluted to three times the volume with water. 2% agar is added and the medium is sterilized by steaming (not autoclaving!) twice. The pH value of beer-wort agar ranges between 4·4 and 5·2.

PLATE II

Boletus bovinus: Type colonies on beerwort-agar; isolated from sporophore. Natural size.

Fig.1: 12 days' culture.

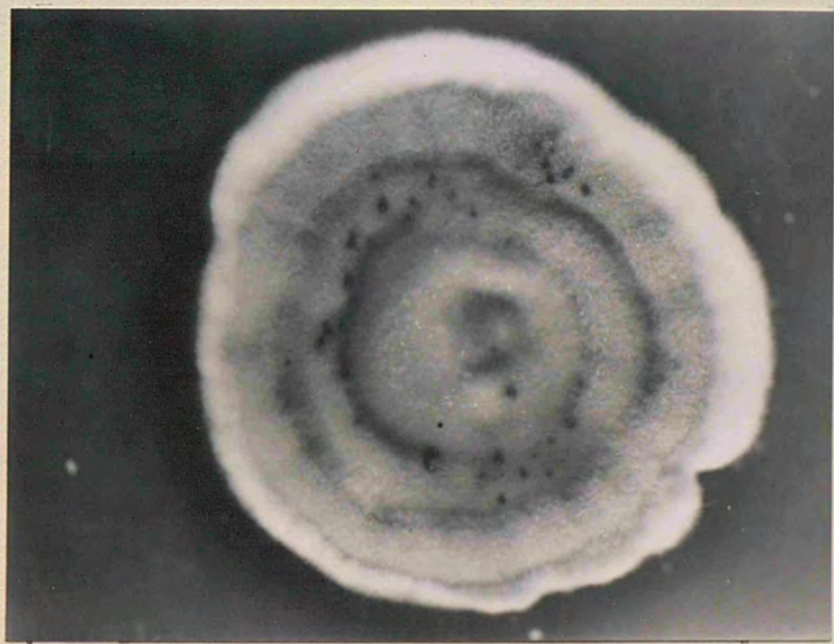

Fig.2: 30 days' culture.

to yellowish-pink. Simultaneously, zonation starts in petri-dishes and in culture flasks (Plate II, fig. 2). After 3 weeks, the mycelium begins to exude brown staling products indicating the time when subculturing is due. Monthly subculturing is the general practice.

The vigour of the fungus has been sustained by changes of media among which the following were used alternatively: beer-wort agar (cf. p. 10), 'glucose agar'*, malt agar, and in some cases steamed soil (cf. p. 19). But even when a change of 'diet' is given, pure cultures of B. bovinus loose the characteristic features of vigour after about 3 years.

The colonies of B. bovinus isolated in this country are of cotton-wool-like appearance, white when young, with a slightly pinkish tinge, becoming brown with age and exuding a dark-brown pigment. The hyphae are septate, varying in width from 2-6 μ, in extreme cases 10-12 μ, the substrate hyphae are of smaller diameter than the air hyphae. The width of the latter depends on the culture

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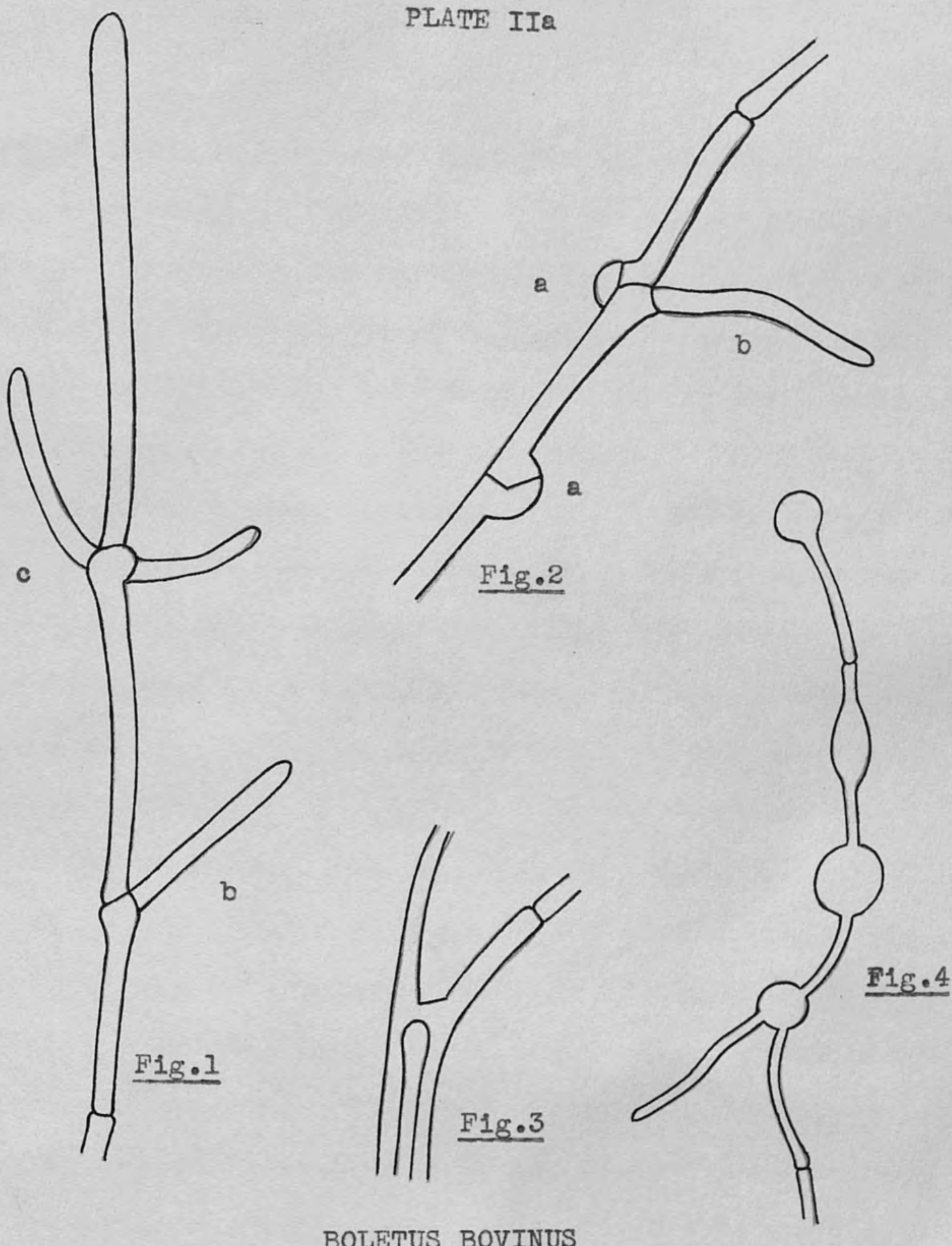
*	Distilled Water	1,000 c.c.
	Agar-agar	15·0 gm.
	Glucose	20·0 gm.
	$MgSO_4\ 7H_2O$	0·1 gm.
	$NH_4\ Cl$	0·5 gm.
	KH_2PO_4	1·0 gm.

PLATE IIa



BOLETUS BOVINUS

Figs.1,2: Air hyphae showing clamp-connections (a), simple branching (b), and 'paarige' branching (c).
Fig.3: Substrate hyphae showing hyphal fusion.
Fig.4: Substrate hyphae forming intercalary and terminal swellings.

All drawings were made from living material using a Leitz camera lucida. x 460.

medium used; it appears that certain carbohydrates, e.g. dextrose, induce formation of wider hyphae than do others. Clamp-connections are more or less numerous on aerial hyphae (Plate IIa, figs. 1,2) of wide diameter - approx. above 8 μ - independently of the nature of the substrate. They have not been observed on substrate hyphae. An effect of temperature on the formation of clamp-connections mentioned by Melin (9) has not been noticed. But it was noted that the English strains after being subcultured several times, show a decrease in the incidence of clamp-connections, these ceasing to be produced after a period of about twelve months.

Hyphal fusions are frequent in younger cultures Plate IIa, fig. 3). The substrate hyphae form often terminal and intercalary swellings of various shapes and sizes (Plate IIa, fig. 4); no true spores are formed. "Paarig" branching (9) occurs more or less frequently, its incidence decreasing with the age of the culture; in older cultures, the simple ("einfache") type of branching is dominant. Modifications of the "paarige" branching are not rare.

A constant feature of the mycelium of B. bovinus seems to be the presence of granules on substrate and

PLATE IIb

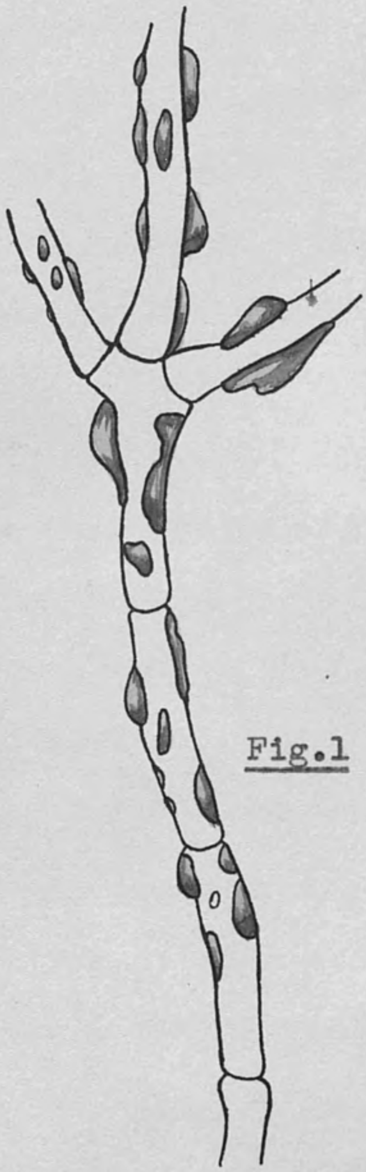


Fig.1

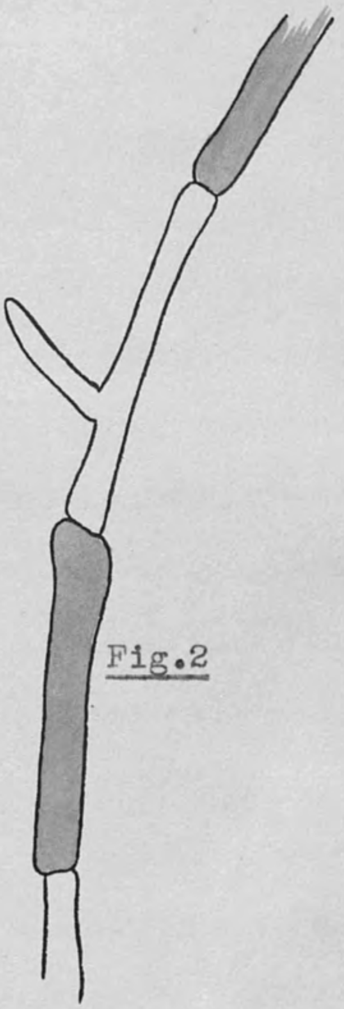


Fig.2

BOLETUS BOVINUS

Fig.1: Air hypha exuding large granules. From 2 months old culture on steamed soil from Wareham Forest.
Fig.2: 'Brown' air hypha. From 6 weeks old culture on malt-agar.

All drawings were made from living material using a Leitz camera lucida. × 460.

aerial hyphae (Plate IIb, fig. 1). They appear on mycelium growing in the soil and on all artificial culture media used during the present investigation. They have also been observed on mycelia of other species; e.g., B. luteus by the writer, and B. elegans by another investigator (7), and seem to be characteristic for the genus Boletus giving their hyphae a papillated appearance. The granules are hyaline, light-brown or dark-brown, the colour varying with the substrate and darkening with age. In certain media, e.g. in the soil of the experimental area at Wareham Forest, the hyphae exude masses of large granules which fuse, embedding the hyphae in a sheath of untidy appearance.

On all culture media used, but not in the natural soil, brown aerial hyphae of large diameter were observed (Plate IIb, fig. 2). These are identical with those described by Melin for cultures of Mycelium radicis abietis (9) and by How for B. elegans (7). The brown hyphae are usually unbranched and appear isolated. According to How (7), these hyphae are a general feature of the Boleti in culture.

The English strains of B. bovinus isolated by the writer are identical with the type of mycelium described by Melin and named by him Mycelium radicis silvestris α (9). Melin has classified his form M.r. silvestris α in two groups (9):-

Group a: forms with few if any clamp-connections; e.g. B. elegans, B. badius, B. granulatus.

Group b: forms with numerous clamp-connections; e.g. B. luteus.

With regard to Group b, Melin expresses the opinion that the mycelium of B. bovinus can probably be placed here. Observations in pure culture and in the natural soil medium from the field where clamp-connections are formed freely have confirmed Melin's view.

Hyphal strands.

On synthetic media, colonies of B. bovinus develop hyphal strands of an average diameter of 20 μ. They resemble those described by Melin (9) and are also similar to the type arising in old soil cultures (cf. p. 21) and to the rhizomorphs distributed in the soil of the experimental area (cf. p. 9). Physiologically, the hyphal strands produced in synthetic cultures appear to be different from the two latter types. Subculturing of hyphal strands from a colony of the ordinary beer-wort or malt agar medium has not been successful, while strands from the soil cultures mentioned as well as rhizomorphs from natural soil were successfully subcultured on various occasions. It appears that rhizomorphs function as a resting stage produced when the food supply begins to decrease and that they 'germinate' readily under certain conditions not yet fully known. Careful examination of the soil in the experimental area revealed that rhizomorphs were abundantly distributed, specially around roots of young pine seedlings, during March, and that, one month later, hardly any trace of them could be detected in the same area. This indicates that, in the field, the rhizomorphs germinate when root growth becomes active in the spring.

5. PHYSIOLOGICAL REACTIONS

Boletus bovinus does not differ from other Boletus spp. for which data exist in its reaction to the pH value of the substrate. The optimum pH for growth on beer-wort agar and malt agar lies between 4 and 5. Laboratory observations show that the mycelium grows well on all the common culture media on the acid side up to a pH of 6. It appears that in its natural habitat the fungus occurs on soils of similar range of pH. In this country, the writer has observed sporophores of B. bovinus on soils of pH ranging from 3·8 - 5.

Sources of Carbon.

With regard to the sources of carbon utilized, among carbohydrates glucose and mannitol proved to be most acceptable, while galactose and xylose gave very poor results. Melin (10) states that glucose is the best source of carbon for the Boleti in general, an observation which was confirmed by How (7) for B. elegans. The relative amounts of growth on a standard nutrient agar with substitutions of different sugars are in the following order: Dextrose, Maltose, Mannitol, Levulose, Raffinose, Saccharose, Lactose, Galactose, Xylose. Mannite yields good results, glycerine proves to be no source for Carbon. Starch of oatmeal and maize is readily utilized.

It was of considerable interest to know the reaction of B. bovinus to cellulosic material, and accordingly, various forms of cellulose were tested, e.g. filter paper, cotton wool, 'pure' cellulose* supplied by the Chemistry Dept., Woolwich Arsenal and Xylinum cellulose (1)**

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*Prepared from cotton sliver by following treatments:-
1. Boiled with 5 parts of 4% caustic soda solution;
2. Well bleached with calcium hypochlorite;
3. Reboiled with 5 parts of 1% caustic soda solution;
4. Bleached mildly with sodium hypochlorite;
5. Soaked in dilute acetic acid;
6. Washed with distilled water;
7. Dried in vacuum.

[Note ** on next page]

In no case was the cellulosic material utilized by the fungus under the nutritive conditions provided (cf. p. 74).

Sources of Nitrogen.

No obvious difference in growth is observed with nitrates or ammonium salts as alternatives. Asparagine and nucleic acid give results not nearly so good as nitrates and ammonium salts, nucleic acid from the thymus being more effective than from yeast. Peptone and glycocoll are utilized very feebly. Tannin added in traces as pure acid or as an extract from the cortex of various tree species, is of no value; given in higher concentration it proved to be toxic.

Sources of Phosphate.

In synthetic media used during this investigation, phosphate was usually supplied in the form of KH_2PO_4 which is more effective to growth than the monohydrogen phosphate. The optimal concentration of KH_2PO_4 appears to be 0·1%. Researches to test the reaction to organic forms of phosphate,

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[Footnote ** from previous page]

** The cultures were prepared as follows:- Layers of *Xylinum* cellulose, approx. 5 mm. thick, were thoroughly washed in distilled water, placed in small petri-dishes, moistened with very dilute beer-wort or malt extract and then sterilized.

e.g. bone-meal, lecithin, are in progress.

A number of growth-promoting substances (the expression used in a wide sense) were tested. Among various substances supplied as additions to the ordinary culture media, a crude extract of fresh lentils (5) proved to be effective. It stimulates growth and is useful for isolation of *B. bovinus* from mycorrhizas.

6. GROWTH ON 'HUMUS' AND CERTAIN ORGANIC COMPOSTS

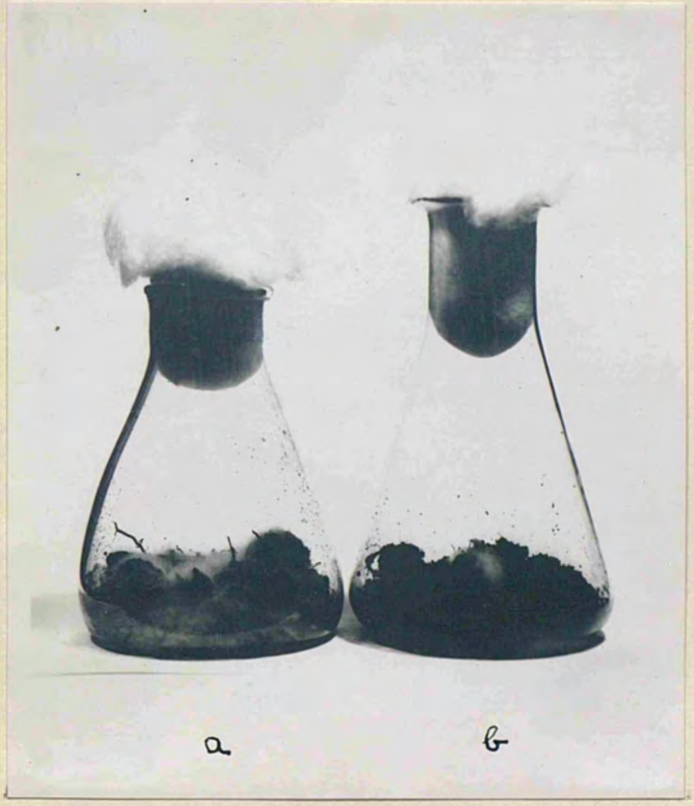
Growth on 'humus'.

During the course of these researches, a series of observations has been made with regard to growth of *Boletus bovinus* on certain organic soils. The general experience seems to be that, in the laboratory, the mycelia of hymenomycetes do not grow on 'humus' or grow poorly on it, an observation which has been confirmed repeatedly when experimenting with *B. bovinus*.

This behaviour is surprising as 'humus' is the natural medium in which the mycelium grows and from which it is isolated. Moreover, pure culture inoculation in the field (13, 14, 15, 16, 17) have shown that the mycelium of *B. bovinus* grown on synthetic media can be subcultured into the soil of the experimental area. The negative results in laboratory experiments indicate that the condition of the 'humus' samples when separated from the dynamic system in the field undergoes profound changes with regard to its biological qualities, which seems to account for the different behaviour of the mycelium in laboratory experiments.

Only after sterilization, organic soil does prove to be a satisfactory culture medium for B. bovinus. Among sterilizing agents the following were successfully used: alcohol, ether, formalin, toluol. Steaming at 100°C has also given good results. It has not been found necessary to leach the woodland soil after this 'partial sterilization' by steaming. Growth was equally good on steamed soil leached and not leached. In this respect, the experience of the writer differs from that of Melin (10), according to whom leaching of the soil after steaming is essential. "Auf Rohhumus, der durch Erhitzung auf 100°C oder mehr sterilisiert ist, entwicken sich die eigentlichen Mykorrhizapilze nur dann, wenn der Humus nach der Sterilisierung mit Wasser ausgewaschen wurde. Das Wachstum ist jedoch ziemlich schwach, und zwar wahrscheinlich aus dem Grunde, weil die beim Erhitzen gebildeten Giftstoffe durch das

PLATE III



Boletus bovinus: Cultures on a. Wareham soil steamed, and b. Wareham soil autoclaved; 1 month. ×2/3.

Note good growth on (a), poor growth on (b).

Auswaschen nur teilweise entfernt werden konnten." (10, p.46). Melin does not mention the period of sterilization he used, but apparently the time factor plays an essential rôle, since observations showed that steaming at 100°C for more than 40 minutes and autoclaving for more than 10 minutes affect growth of the mycelium adversely (Plate III), and it may be assumed therefore that Melin's statement probably refers to such treatments. It appears that the effect brought about by long-steaming and autoclaving for more than a short period (10 minutes) may be due not only to the production of toxic substances but also to the destruction or elimination of valuable food substances. It is well-known that autoclaving renders certain organic media unsuitable for the cultivation of higher fungi, but so far no critical research of this phenomenon has been carried out.

All attempts to grow the mycelium of *B. bovinus* on soil extracts have been unsuccessful. In the case of the experimental soil from Wareham Forest, addition of an extract of this to the ordinary culture medium definitely inhibited the growth of the mycelium (cf. toxicity, 12).

PLATE IV

<u>Boletus</u> <u>bovinus</u>: Colony on Wareham soil steamed with addition of beerwort. 2 months. Natural size.

Growth on humus with addition of nutrients.

Very effective are additions of the ordinary liquid culture media to steamed heath and woodland soils (Plate IV). They strongly stimulate growth of the mycelium which develops more freely on such substrate than on either of the constituent media alone. Whether soil and liquid medium are steamed together or separately, there is no difference in growth reaction. For convenience, they were usually steamed together. Soil (from the experimental area or similar soils) was placed in small Erlenmeyer flasks, the soil thoroughly moistened with dilute beer-wort (beer-wort used for the ordinary culture medium diluted to half its volume), steamed for about 20 minutes and inoculated with B. bovinus from a pure culture. The mycelium grew rapidly developing aerial hyphae of large diameter with clamp-connections the incidence of which was higher than on any other culture medium used, and producing a luxuriant growth of substrate hyphae which subsequently formed large hyphal strands. Diluted 'glucose' medium or malt when added to the soil, is nearly as effective as beer-wort. Addition of 2% glucose alone also promotes stimulation of growth.

PLATE V

Boletus bovinus: Colonies on 'treated' organic composts; 8 days. × 2.

Fig.1: On Hopwaste compost.

Fig.2: On Straw compost.

Growth of composts.

Since certain organic composts have brought about remarkable development of B. bovinus mycorrhizas on species of pine at the experimental area at Wareham (13, 14, 15, 16, 17), it was of special interest to study the growth of the fungus on the composts in question.*

The composts freshly made up as well as in various stages of decomposition were repeatedly tested as culture media for B. bovinus. Lowering the acidity from pH 6 and 5·4 to pH 4·8 (the average pH value of culture media used for B. bovinus) produced no effect; nor did sterilization by steaming give a positive result. Only when leached and afterwards steamed, did the 'old' composts tested support growth of the mycelium (Plate V, figs. 1, 2), the composts in an advanced stage of decomposition being more favourable to growth than in younger stages.

Other investigators (A. Morton, unpublished) have succeeded in growing the fungus on some of the organic composts in question, but not knowing the stage of the

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* Among the various organic composts applied in the field, two were mainly used during the course of these researches, one derived from hopwaste from breweries, the other from straw.

compost at which this was attained, I have not been able to repeat this experiment with a positive result; neither was I successful in repeating earlier experiments (unpublished, from Professor Raistrick's laboratories, School of Hygiene and Tropical Medicine) in which the mycelium grew on the composts with addition of sugar.

One has to bear in mind that during the decomposition of the composts various stages are gone through - each due to a particular group of organisms - which give rise to particular by-products that are made use of for the nutrition of the next group of organisms in the cycle of decomposition. B. bovinus is unable to use the raw product at the beginning; it is unable to utilise the final product; it may be able to use the products arising at one of the intermediate stages, but I have not been able to find that stage - if it exists.

There is the complicating fact that substances toxic to B. bovinus are produced during the decomposition; the presence of these makes it difficult to determine if, at some intermediate stage, there are produced nutritive substances that B. bovinus could use, because leaching may remove the latter as well as the former.

That there are potential nutrients for B. bovinus in the compost is suggested by it growing on compost that has been leached (which presumably removes toxins and nutritive by-products of microbiological origin) and then steamed (which presumably liberates a fresh lot of nutrients by chemical action, which can now be made use of by B. bovinus as the toxins have been removed by the previous leaching).

Growth on composted soil.

As it is believed on good evidence that certain composts bring about remarkable change in the soil bionomics through a profound influence on the micro-flora and its activity, a number of laboratory experiments were set up in order to study the growth reaction of B. bovinus on composted soil.

Samples of surface soil (4" deep) from beds of an area at Wareham Forest composted in 1933, were inoculated from a pure culture of the fungus at various periods during 1936 and 1937. The results were as follows:-

1. Wareham soil, untreated - no growth.
2. " " treated Hopwaste compost - growth fair, gradually decreasing.
3. " " " Straw compost - growth fair, gradually decreasing.

Parallel series with soil from pot-cultures composted with Hopwaste and Straw compost respectively gave similar results, on the whole the growth of B. bovinus being slightly better here than in the composted soil from the field, a result which is apparently due to the better leaching of the soil in pots.

When mixtures of soil from the Wareham area with the various composts were left for only 1-3 weeks and were then inoculated with the fungus, growth was poorer than in the above experiments.

7. MYCORRHIZAL ASSOCIATIONS WITH PINUS SPP.

In order to study the structural features of the mycorrhizal association formed by B. bovinus in Wareham soil, roots from Scots pine and Corsican pine from the field and also from pot-cultures with transported soil from the experimental area were examined at all seasons.

From observations stretching over a period of several years, it appears that there are two maxima of mycorrhiza development, one occurring in the early spring, the other from September to late November. During the summer months, mycorrhiza formation takes also place but is not

PLATE VI

Fig.1: Clusters of forked mycorrhizas of Corsican pine formed by B.bovinus; from composted bed, Wareham Forest. Nat. size.

Fig.1a: Forked mycorrhizas from fig.1, enlarged. × 8

Fig.2 Fig.2a

Figs. 2 and 2a:
.'Messy' mycorrhizas of Corsican pine formed by B.bovinus; from Wareham exptl. area.
Fig. 2a shows tuber formation. Nat. size.

profuse. Repeatedly, the writer has dug mycorrhizas from frozen soil and has used them for isolation of the fungus associate (cf. Table I).

For laboratory examination, whole root systems or pieces of roots from a surface layer of about 4" were washed in tap water, fixed in Doak's solution (50% - alcohol 90·0 c.c., commercial formalin 8·5 c.c., acetic acid 1·5 c.c.) and cut by hand. The sections were mounted in lactic-phenol, a method which elucidates clearly the morphological relations. The description of the mycorrhizal structure given below, apply to transverse sections.

In the soil from Wareham Forest, two types of association with B. bovinus can readily be distinguished in Scots and Corsican pine. The type here referred to as 'normal' appears in healthy seedlings and trees growing in treated soil. This 'normal' type is represented by simple and forked mycorrhizas, borne singly or in clusters, pale to golden-brown in colour (Plate VI, fig. 1). The other type, named 'messy', appears where soil conditions are unfavourable and accordingly the seedlings not vigorous. These mycorrhizas are also single or in clusters, tending to form tuberous complexes (Plate VI, fig. 2). They can be easily recognized without a lens by their pinkish-brown colour and the mycelial

PLATE VII

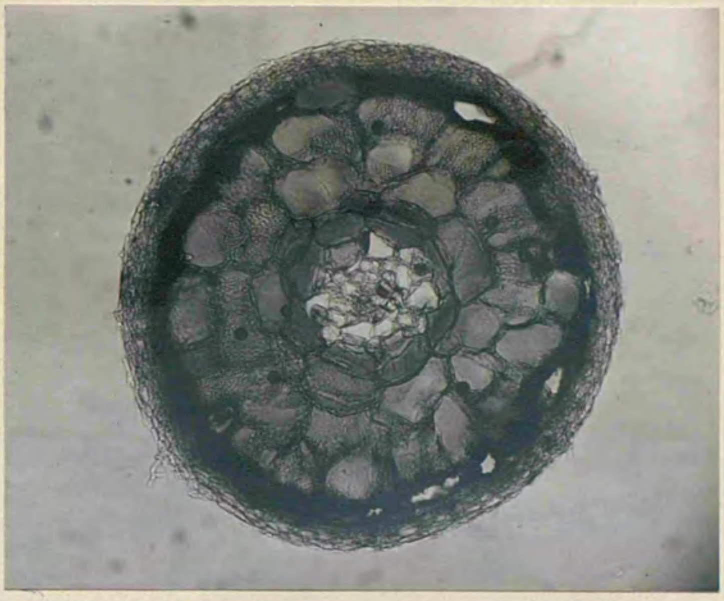

Fig.1: Transverse section of Boletus bovinus mycorrhiza, 'normal' type, of Scots pine, from Wareham soil treated with compost. × 165

Fig.2: Transverse section of Boletus bovinus mycorrhiza of Scots pine, from Wareham soil untreated, showing the 'messy' type of structure.

investment around them which gives them macroscopically the 'untidy' appearance responsible for the designation 'messy'. There are a number of intermediate stages between these two types, the more 'tidy' appearance always an indication of better soil condition.

Microscopic examination.

a. 'Normal' type. (Plate VII, fig. 1). Width of mantle between 10 and 30 μ, with smooth outer surface. Filaments of the mantle fairly loosely interwoven; mantle of more or less pronounced brick-shaped structure. Few occasional hyphae radiating from the mantle; in rare cases they show clamp-connections. Generally, the mycelium of B. bovinus - probably like other mycorrhiza-formers - does not show clamp-connections in the stage when forming the mycorrhizal mantle. The typical tannin-layer, one-celled in depth, is always present consisting of cells filled with tannin and of a varying number of empty cells with thickened brown walls. 'Granular' cells are either incorporated in the tannin layer or occur within the underlying cell layer. The Hartig net extends throughout the entire outer cortex without reaching the central cylinder. Intracellular infection of the haustorial type has not been observed in material from the experimental area. Only in very rare cases was haustorial infection detected in roots from pot-experiments in soil from Wareham, and it may be assumed that the fungus responsible for this intracellular infection has grown into the experimental soil by contact infection.

b. 'Messy' type. (Plate VII, fig. 2). A conspicuous mycelial investment is surrounded with the roots. The hyphae form exudates of brown colour, and are often embedded in a sheath of these exudates which are of oily nature (cf. p. 21). The mantle varies in width from 60 μ - 300 μ; adjacent mantles often coalesce and thus forming tuber-mycorrhizas. Mantles of smaller diameter show a number of 'messy' hyphae radiating from the surface. Clamp-connections have not been observed here. The margin of the mantle is rough and of brown colour; the mantle itself consists of rather loosely-woven hyphae which run in a direction transverse to the root axis. Mantles of very large diameter show a pseudoparenchymatous structure, the margin is smooth, radiating hyphae are not frequent. No true tannin layer has been observed, casual tannin cells only are present. There is always a conspicuous disintegration of the 1-2 outer cortical cell layers and it is not rare to find isolated cells embedded in the mantle.

The Hartig net extends irregularly throughout the whole cortex. All cortical cells show more or less thickened walls and frequently have granular contents, hyaline or brown in colour, or are filled with a pale to dark-brown substance, the identity of which is unknown.

From examination of root material from other areas than the experimental, it appears that generally, where soil conditions are unbalanced this disturbance may be indicated by the particular structure of the mycorrhizas here described under the designation 'messy'. It seems likely that not only associations formed by the Boleti but also mycorrhizas formed by other fungal species, may reflect soil conditions in a similar modification of structure.

8. SUMMARY

1. The fungus Boletus bovinus has been isolated from sporophors and rhizomorphs occurring naturally in the experimental area, and from mycorrhizas of Pinus spp. formed by its association. Identity tests applied to the isolates from these sources have shown positive results.

2. Cultural and mycelial characters of the fungus are described. The English strain of B. bovinus agrees in all important respects with that isolated in Sweden by Melin.
3. In the natural soil, rhizomorphs appear to represent a resting stage of the mycelium.
4. Experiments have proved that on artificial media B. bovinus does not differ from those Boletus spp. for which data exist in its reaction to pH values and its utilization of various sources of C, N, and P.

   Cellulosic material is not attacked by the fungus in synthetic media.
5. Through 'partial' sterilization (steaming), heath and woodland soil is turned into a favourable medium for growing B. bovinus; autoclaving has proved to be not so effective as steaming.
6. After special treatment certain organic composts have produced satisfactory growth of B. bovinus.
7. Detailed descriptions are given of the structural features of the mycorrhizal associations formed by the fungus with Scots and Corsican pine in the experimental soil at Wareham Forest.

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II. A STUDY OF MYCELIUM RADICIS ATROVIRENS MELIN

CONTENTS OF PART II

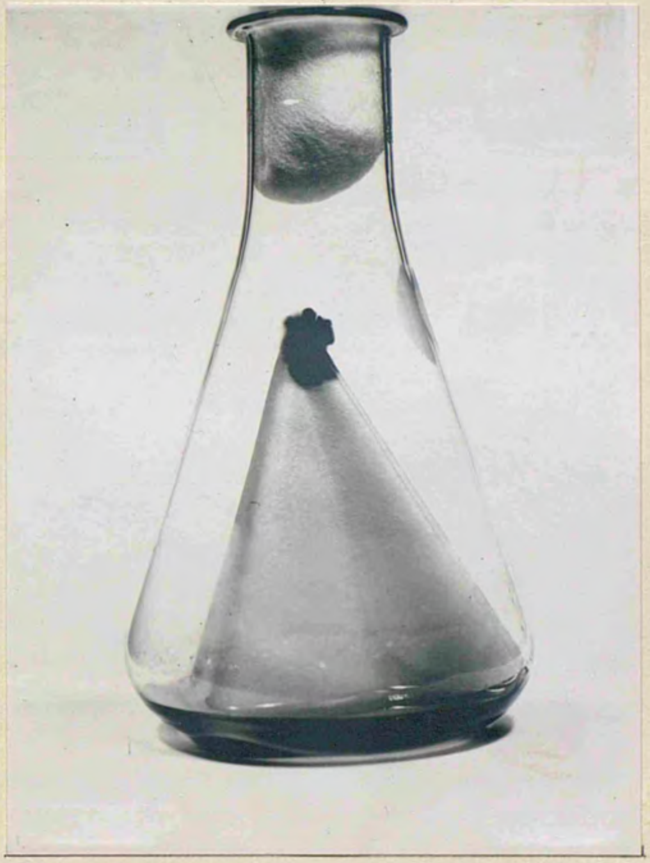
1. DISTRIBUTION

A dark mycelium identical with that described by Melin (2) under the name of *Mycelium radicis atrovirens*, common in forest soils in U.S.A. and in Europe, has been repeatedly extracted from roots of various pine species from the experimental area at Wareham.

Hatch (1), like Melin, observed that *M.r.atrovirens* is a very common soil organism, widely distributed in all types of forest soils. The writer confirms this fact for Great Britain where the mycelium associated with pine, fir, spruce, larch, and beech roots is present in all woodland and heath soils examined hitherto. It was found in soils from Wareham Forest, the New Forest, Hope Forest, from the Yorkshire Moors, etc.

While infections by *M.r.atrovirens* of pine and other tree species are very common in woodland soils, they are very rare in nursery soils. When an examination of beech root material from 23 nurseries belonging to the Forestry Commission of Great Britain was carried out in 1938, attack by *M.r.atrovirens* was observed in material from one nursery only. There was no trace of this pseudomycorrhiza-former

PLATE I



Mycelium radicis atrovirens P.contorta:
Colony on filter paper dipping into a
nutrient solution. 2 weeks. $\times \frac{3}{4}$.

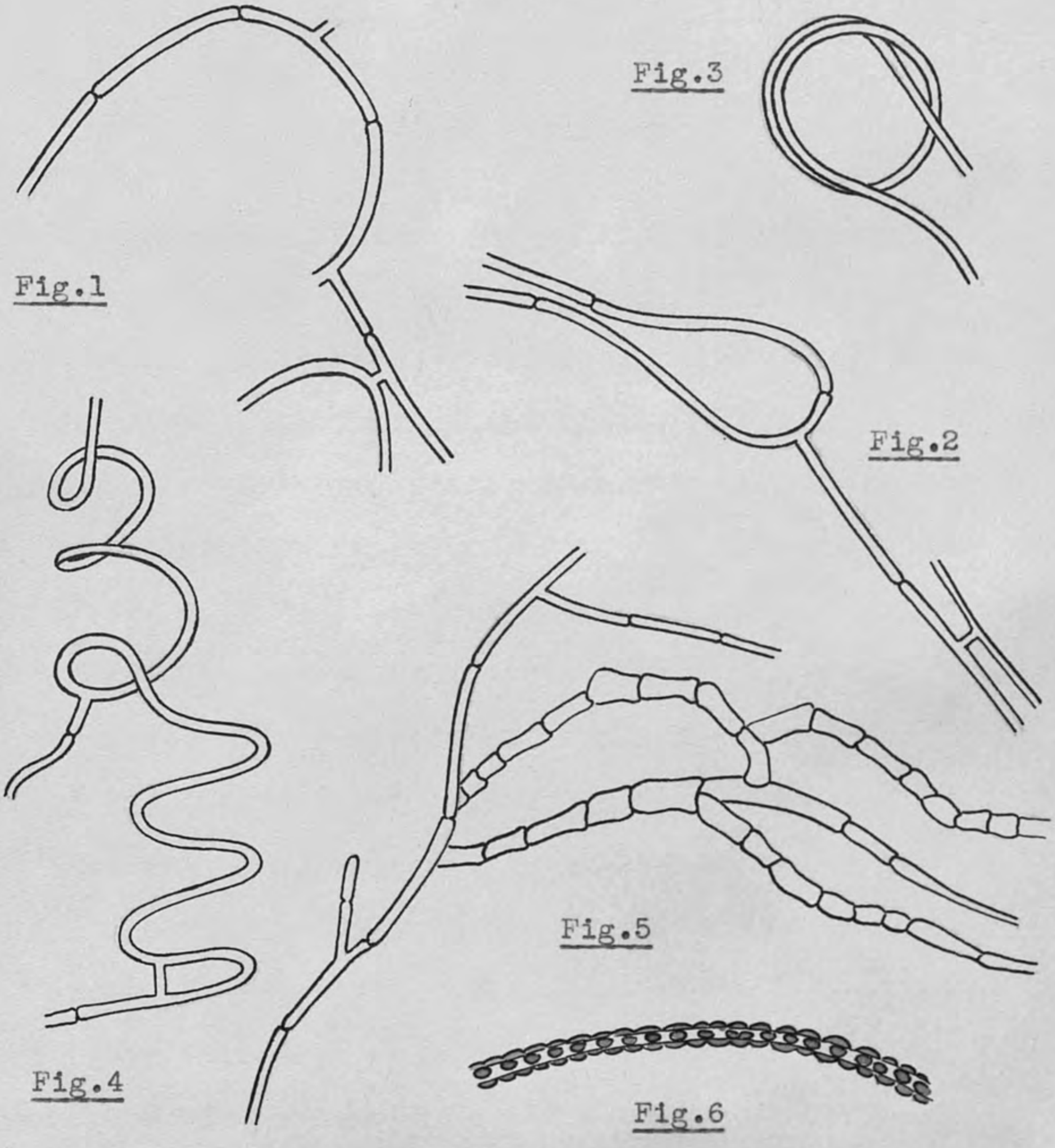
in any of the other nursery soils. (The expression 'pseudo-mycorrhiza' is used here for a root association regularly formed under certain conditions with a soil fungus and showing evidence of parasitism on part of the fungus.)

2. ISOLATION AND GENERAL CULTURAL REACTIONS

For the purpose of isolation, the technique described for the extraction of *Boletus bovinus* from mycorrhizas (cf. *Boletus bovinus*, p. 8) was used. Extractions were carried out at various seasons with the same ease and were repeated during the course of the investigation using pseudomycorrhizas from seedlings and from mature trees.

Approximately one week after planting, a rapidly-growing mycelium of greyish colour emerges from the root tissues and shows vigorous growth for several weeks. Sub-culturing is due after 2-3 months. The fungus retains its vigour of growth on artificial media for several years; there is no need for change of 'diet' (cf. *Boletus bovinus*, p. 11). For physiological studies, the mycelium was grown on filterpaper pyramids dipping in liquid culture medium (Plate I). As standard culture media were used 2% malt agar, beer-wort agar (cf. *Boletus bovinus*, p. 10) and 'glucose' agar (cf. *B. bovinus*, p. 11).

PLATE II



<u>Mycelium radicis atrovirens</u>

Figs. 1,2,3,4: Air hyphae showing hyphal fusions, coils, and spirals. From 5 weeks old culture on beerwort-agar.
Fig.5: Substrate hyphae forming chlamidosporae. From 2 months old culture on malt-agar.
Fig.6: Air hyphae with secretions. From 2 months old culture.

All drawings were made from living material using a Leitz camera lucida. × 460.

3. MYCELIAL CHARACTERS

On the common culture media, the air hyphae of Mycelium radicis atrovirens P. silvestris* show luxuriant development. The young colonies are light-grey to green in colour, later dark brown and black. The air hyphae are regularly septated; their diameter ranges between 1-3 μ; the average length of the cells (grown on beer-wort agar) is 40 μ. No clamp-connections have been observed. Hyphal fusions, coils and spirals (Plate II) are frequently formed. The substrate hyphae are differentiated into 'Lang-' and 'Kurzhyphen' (2), the former 3·5-4 μ in diameter, with thin walls and frequently forming spirals; the latter 1·5-2·5 μ producing chlamidosporae which - especially on poor media - develop into sclerotia (Plate II). Hyphal strands are formed by both air and substrate hyphae. These strands are also frequent in the natural soils. The strands vary in diameter, the average being 20-35 μ, extreme cases 250 μ. On culture media more or less exhausted, the strands are very dark in colour and brittle. They represent big amorphous sclerotia noticeable without a lens. They consist of closely-packed dark brown hyphae, frequently septated, with thick cell walls. When subcultured,

* The mycelia here under observation, were designated according to Melin as Mycelium radicis atrovirens with addition of the generic and species name of the vascular plant from which the mycelia in question were extracted.

even from 10-12 months old colonies, they 'germinate' readily. Hyphal strands of large diameter have not been observed in the field. It appears also that formation of coils and spirals is less abundant in the natural soil than on artificial culture media.

4. FORMS AND STRAINS

Forms

Melin (2) distinguishes two forms of Mycelium radicis atrovirens, α and β, apparently identical or similar for pine and spruce. The β form differs from the α form in the following respects:- 1. It changes the colour of the common culture media to reddish-brown. 2. It does not form sclerotia. Melin holds that only the α form is a cellulose destroyer. He believes both forms to belong to the ascomycetes. In one special case, in a synthetic culture with spruce and M.r.atrovirens β, he succeeded in obtaining peritecia belonging to Chaetomium Kunzeanum. The α form is very common, and in Sweden (2) the β form seems also to be widely distributed. In this country the writer has not yet met the β form. Only on one occasion it was isolated here from Sitka spruce grown in soil from a

Sitka spruce stand from Vancouver Island. This mycelium is available in pure culture (Plate III). It agrees with Melin's β form.

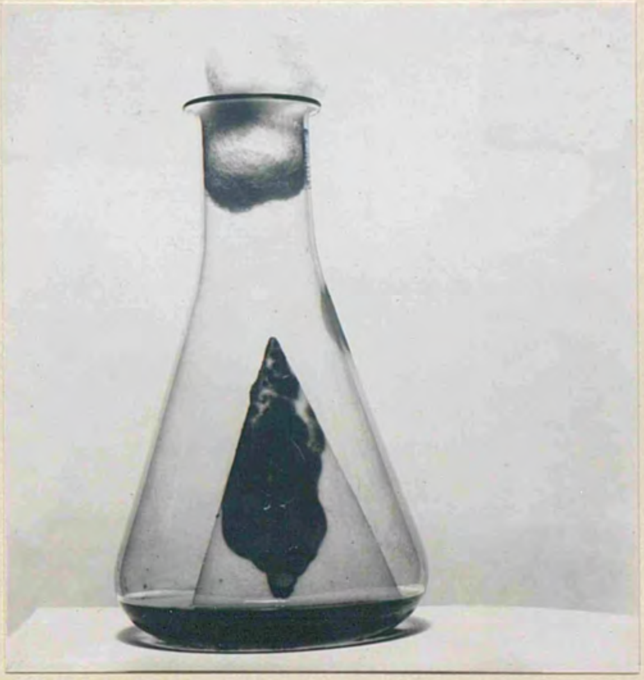
A dark-coloured mycelium has been repeatedly isolated from Scots pine seedlings in a humus-inoculated seedbed at the Wareham experimental area (Plate IV).

It does not agree with all the characteristic features of M.r.atrovirens as described, but is more similar to this fungus than to any other dark root mycelium hitherto observed. Buller's test has given doubtful results so far. The mycelium has been grouped provisionally with M.r.atrovirens and named Mycelium radicis atrovirens x. It has been extracted from Scots pine only.

The rootlets from which it was isolated were black in colour, small in diameter and tapering towards the tip. They had the typical appearance of pseudomycorrhizas.

There is no difficulty in isolating this mycelium, as is the case in the recognized strains of M.r.atrovirens and in contrast with Rhizoctonia spp. The fungus grows well on the common culture media without changing the colour of the substrate. The rate of growth is similar to that of M.r.atrovirens. The colonies are greyish, compact, crusty, slightly 'pleated', reminiscent of a lichenes thallus. The margin of the colony is scaly, and of a

PLATE IV



Mycelium radicis atrovirens x:
Colony on filter paper dipping
into beerwort; 1 month. × $\frac{3}{4}$.

light grey colour. Colonies of medium age exude droplets hyaline to yellowish-brown in colour, partly oily in nature. Old colonies are brown to black in colour. The hyphae, 2–3 μ in diameter* form strands of about 10 μ diameter; coils and spirals, frequent features of M.r.atrovirens and Rhizoctonia, are very rare. Like the strains of M.r.atrovirens recorded, the substrate hyphae form chains of chlamidospores, hyaline to brownish in colour. True sclerotia have not been observed. On a number of culture media, substrate and aerial hyphae show secretions which consist of masses of granules. These are hyaline, light-brown or dark-brown. They are found in greater abundance on cultures of M.r.atrovirens than on cultures of any other dark root fungi hitherto examined.

Strains.

The strains of M.r.atrovirens dealt with in this paper and now available in pure culture (Table I), were extracted from seedlings of Pinus spp. from Wareham Forest and from pot-cultures with transported soil from this area. They derive from the following host plants: P. silvestris, P. nigra, P. strobus, and P. contorta.

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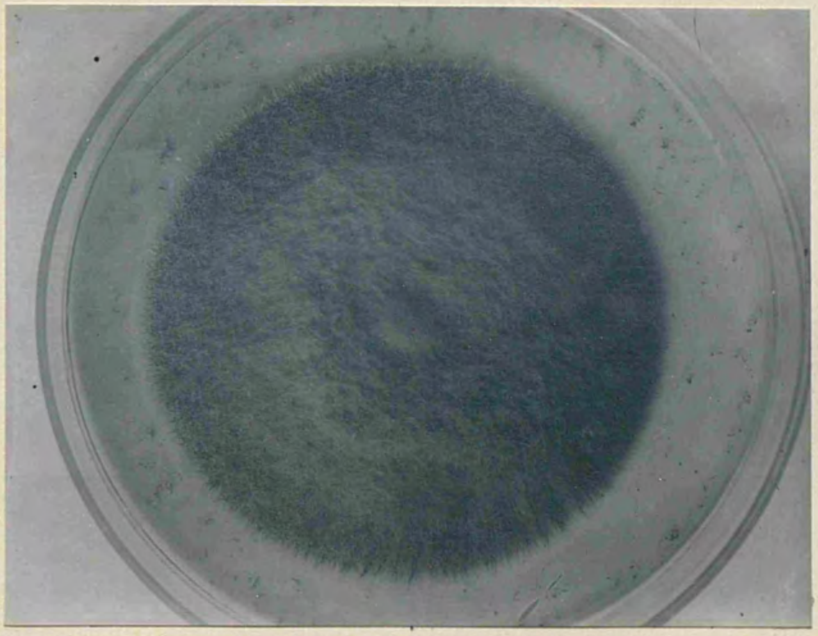
* The hyphae of Rhizoctonia are 3–4 μ in diameter.

PLATE V



Mycelium radicis atrovirens P.strobus:

Colony on beerwort-agar; 1 month.

Natural size.

TABLE I

Data concerning strains of Mycelium radicis atrovirens isolated from Pinus spp. growing at Wareham Forest. (Cultures on beer-wort agar).

Host	Diameter growth of colony 3 weeks old	Type of colony	Colour of hyphae			Diameter of air-hyphae
			young	medium	old	
Scots pine	5 cm.	aerial	grey-green	dark brown to black	dark brown to black	2-3 μ
Corsican pine	2·5 cm.	velvety, compact	grey-green	dark green to black	dark green to black	1-3 μ
White pine	4 cm.	aerial, feathery	grey	grey to brown	dark grey to brown	2-3 μ
Lodgepole pine	3·5 cm.	velvety, compact	grey-green	dark green to black	dark green to black	2-3 μ

The strains extracted from these species of pine are similar with regard to a number of morphological and cytological details and have yielded positive results using Buller's test for identical or related mycelia. They differ in respect to the type of colony formed on synthetic media, e.g. they form 'velvety' colonies in the case of the mycelium extracted from Corsican pine; 'feathery' colonies in the case of M.r. atrovirens P. strobus (cf. Table I and Plate V). All strains form hyphal strands as described. They are more abundant and of wider diameter in extractions from Scots pine and White pine than in those from Corsican pine and P. contorta. The strains of M.r.atrovirens extracted from Pinus species from the experimental area and from other districts have been compared with M.r.atrovirens P. silvestris from Sweden (kindly supplied by Professor E. Melin), also with M.r. atrovirens isolated from Sitka Spruce and Norway Spruce in this country. They are all similar in respect to the characteristic features as described.

5. GROWTH ON 'HUMUS'

Repeated attempts have been made to grow the mycelium of M.r.atroviens on organic soil. The result was negative in all cases where the soil was not 'treated'. Steaming and other forms of partial sterilization; e.g. with toluol, ether or formalin, convert the soil into a satisfactory culture medium. Cf. Table II for the vigour of growth on 3 soils of similar geological origin, one from the poor heathland at Wareham, one from a stand of pine in Surrey, on soil carrying Calluna heath, and one from the New Forest, supporting woodland.

Table II

Growth of M.r.atrovirens mycelia on soils from various localities; Soils were steamed once for 20 minutes.

Organism	Wareham soil	Surrey soil	New Forest soil
M.r.a. P.silvestris	+ +	+ + +	+ + +
" " nigra	+	+	+
" " strobus	+	+ +	+ +
" " contorta	+	+	+

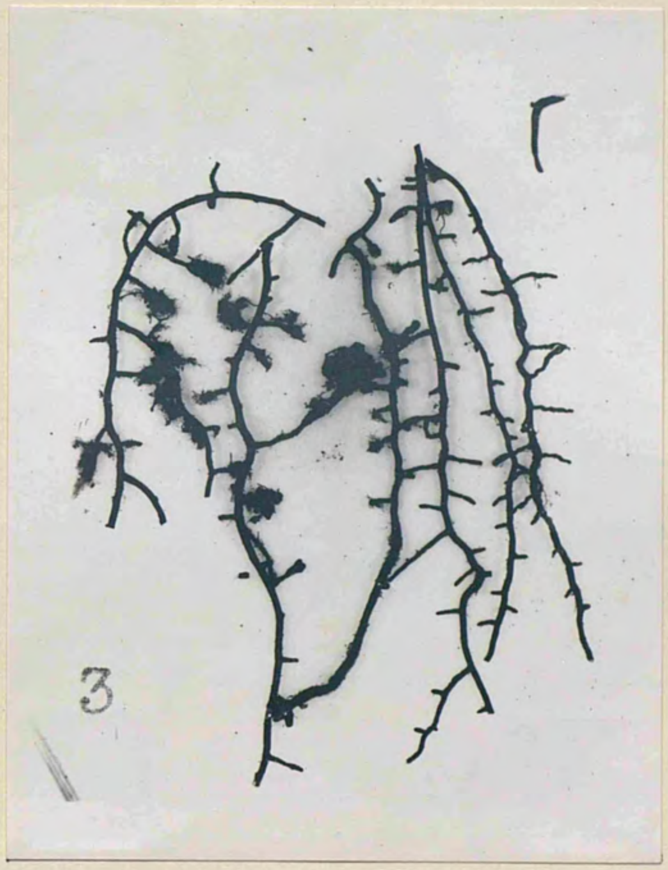
+ growth fair
+ + " good
+ + + " very good

The modus of hyphal growth in the soil cultures is in some details different from that on synthetic media. On the steamed soil, the mycelium forms hyphal strands very rarely and the incidence of the typical spirals and coils is greatly reduced. Hyphal fusions are formed freely.

6. FORMATION OF PSEUDOMYCORRHIZAS

Microscopic examination of soil samples from stands of pine and other conifers commonly show fragments of dark brown-coloured hyphae of M.r.atrovirens among the soil particles and connected with the contained decaying organic

PLATE VI



Pseudomycorrhizas of Scots pine formed by *Mycelium radicis atrovirens* α; from exptl. area at Wareham Forest. Nat.Size.

Note primary attack on simple short roots and secondary attack on preformed forked mycorrhizas.

material. In such samples, the roots are usually more or less infected by the mycelium which forms very characteristic pseudomycorrhizas (Plate VI). They are easily identified, frequently by their macroscopic characters. They differ from the other two types of associations with dark-coloured mycelia hitherto encountered (one formed by *Mycelium radicis nigrostrigosum*, the other by *Rhizoctonia* sp.) in respect to the following features: Pseudomycorrhizas formed by *M.r.atrovirens* are slender, sometimes threadlike, frequently tapering towards the tip, and often slightly 'beaded'. They do not show the stiffly projecting hyphae characteristic of *M.r.nigrostrigosum* mycorrhizas, nor do they have the 'woolly' appearance by which pseudomycorrhizas formed by *Rhizoctonia* are readily recognized. The diameter of those formed by *M.r.atrovirens* is less than those of any other example of pseudomycorrhizas observed or of any true mycorrhiza of comparable age. Examination of the attacked short roots shows great variation with regard to localization and stages of infection. Black tips, black regions, or rootlets entirely black may be observed. In all, the type or stage of infection, as seen in section, may be similar.

It appears that the mycelium of M.r.atrovirens is capable of attacking the roots directly as well as attacking pre-existing true mycorrhizas.

The primary mode of attack was studied in a series of pot-cultures in which Scots pine seedlings were grown in sand. These pot-experiments were carried out under relatively sterile conditions: Pots and sand were steamed 3 times at 100°C, plunged in gravel thoroughly washed with potassium permanganate and sown with seed superfically sterilized. Four weeks from sowing, at the end of May 1941, these pots were inoculated with mycelium of M.r.atrovirens from a pure culture and examined at monthly intervals for about 15 months. In September all pots showed few attacks by M.r.atrovirens. The proportion of roots attacked did not increase during the time under investigation. The material from the above cultures in common with root samples of Scots pine and other pine species examined from various localities exhibit the following stages of infection, each characteristic stage separately or combined with one or more of the others:

1. Intercellular infection: Dark brown to black mycelium of M.r.atrovirens passing in or throughout the cortical

PLATE VII

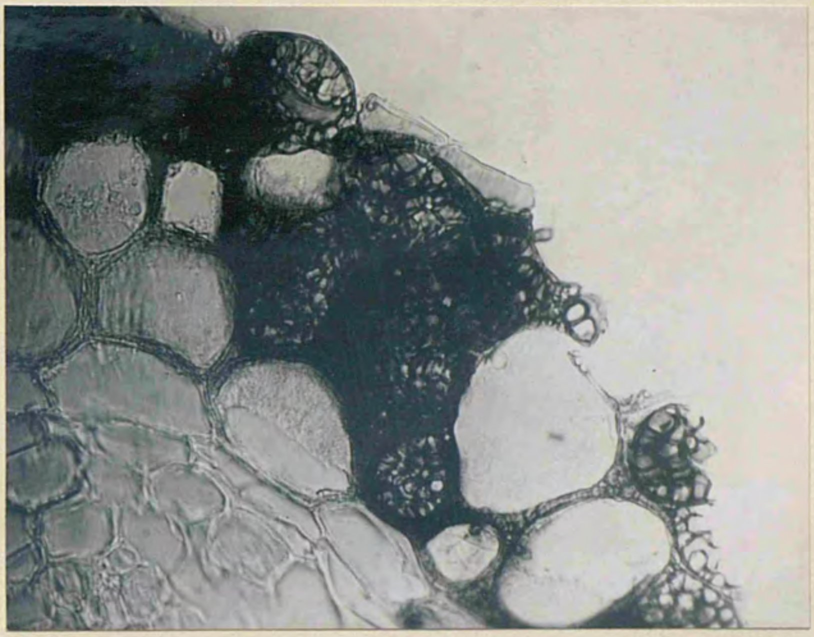


Fig.1: Sclerotia and intercellular net in cortex of short root of Scots pine formed by M.r.atrovirens (transverse section). X 400

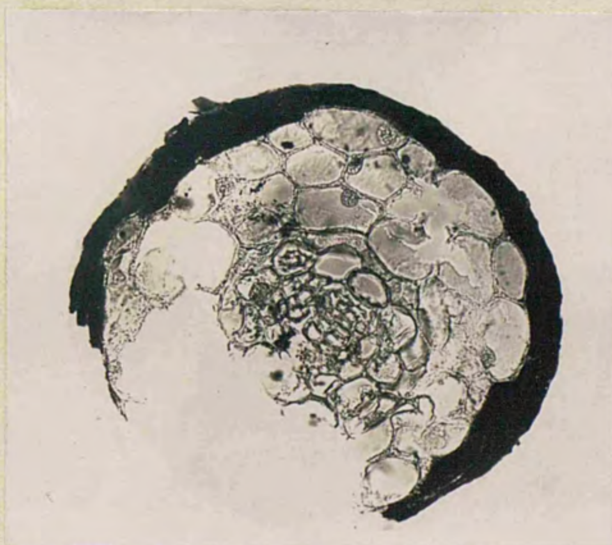


Fig.2: Transverse section of pseudomycorrhiza of Scots pine formed by M.r.atrovirens. x 230

tissues; the hyphae tending to become hyaline, specially in the inner part of the cortex.

2. Intracellular infection: Mycelium of *M.r.atrovirens* growing within the cell lumena, often exhibiting pronounced haustoria. Colour of the mycelium similar to that described in (1).
3. Formation of sclerotia (Pl. VII, fig.1): The intracellular hyphae swell to a diameter of 6-8 μ, become dark brown to black in colour and become associated to form definite sclerotia. They have never been observed on the surface of the root (in contrast with sclerotia formed by *Rhizoctonia silvestris*). They may be distributed throughout the cortex, in cells of the endodermis, and in rare cases within the central cylinder.
4. Formation of 'mantle' (Pl. VII, fig.2): Mycelium of *M.r.atrovirens* forms a compact, crusty sheath around the cortex consisting of one, two, or more layers of hyphae, the whole 'mantle' having a width of 10-40 μ. The hyphae are closely interwoven and run in a longitudinal direction.
5. Formation of intercellular 'net' (Pl. VII, fig.2): This is either of dark brown colour or hyaline, always

irregular in depth. The hyaline net is easily distinguished from the hyaline net of a true mycorrhiza former by its peculiar structure which is not well outlined and not clearly defined. The conditions that determine the colour of the 'net' are at present under investigation.

Absence or reduction in number of root hairs is generally connected with infections by M. r. atrovirens.

Comparative study of the root tissues in mycorrhizas and pseudomycorrhizas formed by M. r. atrovirens have shown that the cortical tissues of the latter are generally dark, compressed, with thickened cell walls. It appears that the cell lumen is considerably reduced, specially in the case of intracellular infection, with little content. A pronounced tannin layer is rarely encountered.

Secondary attacks. (Pl. VI[illegible])

While there is little difficulty in recognizing pseudomycorrhizas formed by primary attack of M. r. atrovirens on young roots, attacks on pre-existing mycorrhizas - a combination quite common in nature - cannot always be identified with the same ease.

The mycelium of the pseudomycorrhizal constituent forms a secondary sheath on a pre-existing mantle built by the mycorrhizal associate. The primary mantle is gradually absorbed according to the degree and stage of the attack by M.r.atrovirens.

In the case of a mixed infection formed by Boletus sp. and M.r.atrovirens, the analysis is rendered comparatively easy, not because of the colour contrast of the two mantles in question (as the mycelium of M.r.atrovirens tends to become pale to hyaline when growing in close contact with the tissues of the host) but in respect to difference in structural features. The mantle of the Boletus mycorrhiza is spongy in structure consisting of either fine or brick-shaped 'cells', while that formed by M.r.atrovirens is more compact, the 'cells' constituting the mantle resembling those of the free mycelium outside the association. In cases where the mantle formed by the true mycorrhiza-former is entirely absorbed by the superimposed pseudomycorrhiza-former, the analysis is more difficult, the structure of the net not always being a safe diagnostic feature.

The presence of both, M.r.nigrostrigosum the jet-black mycorrhiza-former, and of M.r.atrovirens in the same association is difficult to distinguish although it may be sometimes inferred from the presence of both fungi in connection with the mantle that both mycelia are involved in the association. On tuber mycorrhizas, attacks of M.r.atrovirens have hardly been observed.

The cultural experiments of Melin (2) show a pronounced deleterious effect of the mycelium. Melin gives a detailed description of the type of infection produced by the fungus in these synthetic cultures: infection of roots, hypocotyledon, and needles. He holds that (in nature as well as in culture) the attack starts from the younger parts of the long roots by hyphae growing within the root hairs, and that the infection spreads from there into the short roots.

With regard to the pathogenicity of M.r.atrovirens to seedlings and young trees of Pinus spp., and probably also to those of other genera, the present state of knowledge can be summarized as follows:- In cases where the short roots are transformed directly into pseudomycorrhizas, the health and vigour of the attacked host is correspondingly influenced. The plant tends to be smaller in size and chlorotic in colour. This is a common phenomenon, frequently observed in natural seedlings as well as in plants from experimental pot-cultures. Casual infection by M.r.atrovirens has no effect on growth and vigour; it is the proportion of pseudomycorrhizas to true mycorrhizas that is significant to general health. Under certain soil conditions, e.g. at Wareham Forest, where mycorrhizal development is defective, the replacement of even a small proportion of non-mycorrhizal short roots by pseudomycorrhizas may lead to effects fatal to the host, while the presence of M.r.atrovirens attacks on root systems well furnished with normal mycorrhizas; e.g. in seedlings from the Wareham area that has received compost treatment, does not appear to affect the health of the plants.

7. SUMMARY

1. A dark soil fungus, named by Melin *Mycelium radicis atrovirens*, is widely distributed in heath and woodland soils of this country.
2. It has been isolated from various species of pine and spruce.
3. Only the α form of *M.r.atrovirens* appears to be common in Great Britain.

4. A dark mycelium very similar to the α form of M.r.atrovirens, extracted from Scots pine, is described.
5. Strains isolated from various species of pine are compared.
6. Steamed 'humus' provides a satisfactory medium for the growth of the strains of M.r.atrovirens under observation.
7. A detailed description is given of pseudomycorrhizas of various types and stages formed by M.r.atrovirens with pine roots.
8. The pathogenicity of M.r.atrovirens to pines and other conifers is discussed.

8. LITERATURE REFERENCES

(1) Hatch, A. B.: 'A Jet-Black Mycelium Forming Ectotrophic Mycorrhizae', Svensk Botanisk Tidskrift, Bd. 28, H. 3, 1934, pp.369-83.

(2) Melin, E.: 'Experimentelle Untersuchungen über die Konstitution und Ökologie der Mykorrhizen von Pinus silvestris L.und Picea Abies (L.) Karst', Mykol. Unters. u. Ber. von R. Falck, ii, 1923, Cassel, pp.73-331.

III. STUDY OF THE EFFECTS PRODUCED BY ADDITION OF CELLULOSIC MATERIALS TO THE WAREHAM FOREST SOIL AND TO OTHERS OF SIMILAR TYPE

CONTENTS OF PART III

page

STUDY OF THE EFFECTS PRODUCED BY ADDITION OF CELLULOSIC MATERIALS TO THE WAREHAM SOIL AND TO OTHERS OF SIMILAR TYPE.

1. INTRODUCTION

Experiment has shown that poverty in mineral nutrients is not the only factor concerned in the suppression of growth at Wareham Forest; the failure to grow trees on the area seems to be essentially associated with anomalous features in the humus constituent leading ultimately to the production of substances unfavourable to normal growth of trees (3).

For the purpose of the experiments about to be described, it was assumed that the percentage of such an organic constituent as cellulose, in Wareham soil and in other soils of similar type but favourable to growth, might be one variable responsible for the marked dissimilarities in growth.

The following studies were designed to test this hypothesis in respect to raw cellulose using the growth reactions of Scots pine seedlings as an index of the effects brought about by addition of this substance.

2. EXPERIMENTAL

(A) Addition of filterpaper and 'pure cellulose'.

The experiments were begun in 1935 and carried out in pot-cultures of Scots pine with comparable soils of three localities: (a) from Wareham Forest, (b) from Oxshott (Surrey), and (c) from a part of the New Forest. In Wareham soil factors are inimical to pine growth; in the soils from Surrey and the New Forest, pines grow well. All three soils are podsols on Bagshot Beds, Oxshott like Wareham carrying Calluna heath, and the New Forest soil supporting woodland. The range of the pH values of the three soils is identical; namely 4·7 - 4·9.

Varying amounts of cellulose - 1%, 2%, 3%, 5%, 10% - were added to each of these soils at sowing, the percentage values being based on the air dry-weights of the soils. The material was used in the form of finely-chopped filter-paper (product No.1, W. and R. Balston Ltd.) or as 'pure cellulose'* treated in the same way. I am indebted to the Chemistry Dept., Woolwich Arsenal, for supplying me with this 'pure cellulose'.

At the end of the first season's growth, additions of cellulose had produced definite effects on the pine seedlings,

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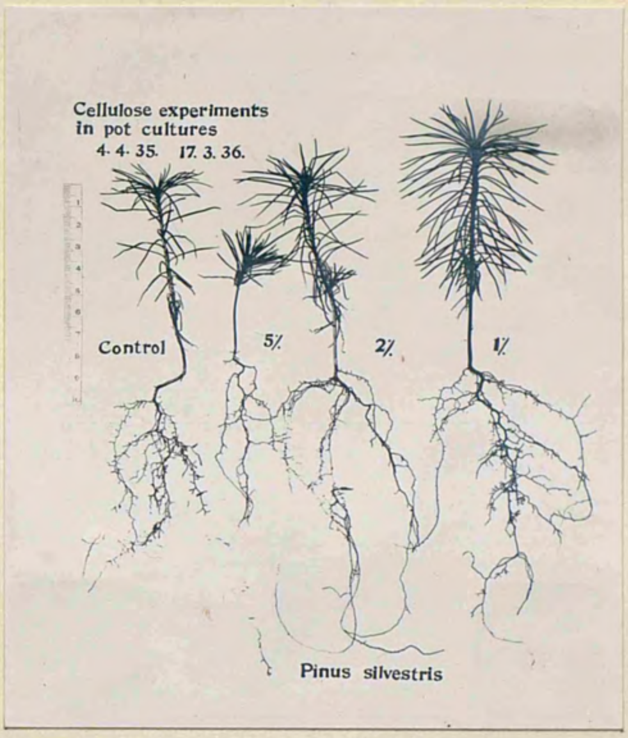
* Prepared from cotton sliver by the following treatment:-

1. Boiled with 5 parts of 4 per cent caustic soda solution.
2. Well bleached with calcium hypochlorite.
3. Reboiled with 5 parts of 1 per cent caustic soda solution.
4. Bleached mildly with sodium hypochlorite.
5. Soaked in dilute acetic acid.
6. Washed with distilled water.
7. Dried in a vacuum.

PLATE Ia



Representative seedlings of Scots pine from pot-cultures in Oxhsott soil to which various amounts of cellulose in the form of chopped filter paper have been added (cf. Pl.Ib).

PLATE Ib

Seedlings of Scots pine in their 1st season of growth in New Forest soil (fig.1), Oxshott soil (fig.2), and Wareham soil (fig.3) to which various amounts of cellulose in the form of chopped filter paper have been added.

Sown: 4.4.36; photographed 4.11.36. x1/7.

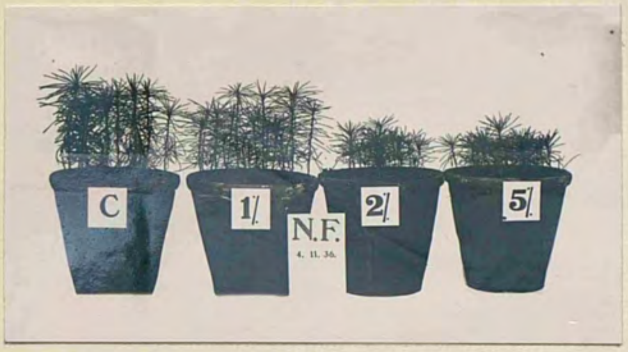


Fig.1: New Forest soil
Note that more than 1% cellulose has a deleterious effect on growth.

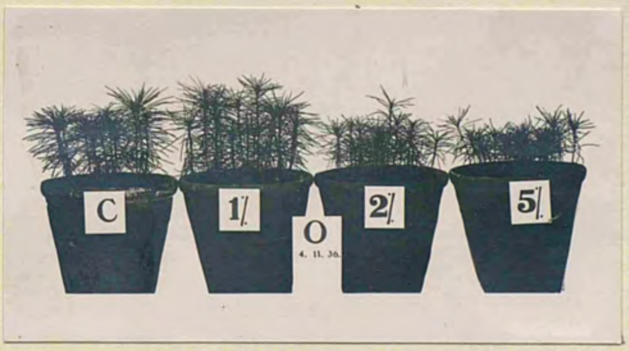


Fig.2: Oxshott soil
Note that 1% cellulose has a slightly beneficial effect, but that more than this retards growth.

Fig.3: Wareham soil
Note that 1% cellulose has a slightly beneficial effect, and that addition of as much as 5% gives about as 'good' growth as controls.

the symptoms induced being identical in the three soils under consideration. The addition of 1% cellulose acted, on the whole, as a stimulant; it slightly improved root and shoot growth and gave an impetus to root development in respect to increase of branching and mycorrhiza-formation (Pl. Ia). Higher percentages of cellulose depressed root and shoot growth, yielding smaller seedlings with purplish foliage and inhibiting mycorrhiza-formation (cf. Chapter C) (Pl. Ib, figs. 1, 2, 3; Pl. Ia); above 1% the deleterious effects increasing with the amount of cellulose used. The beneficial effect of 1% is more marked in Oxshott soil than in that from the New Forest and is least noticeable in Wareham soil; the deleterious effects of larger amounts of cellulose is also least marked in the latter soil, an addition up to 5% causing no noticeable retardation in growth (Pl. Ib, fig.3). Comparison of the plants in the three soils receiving 5% cellulose shows that under this treatment growth in Wareham soil is relatively better than in either of the two other soils in which Scots pine normally grows well (Pl. II, fig.1).

The deleterious cellulose effect is most marked at the end of the first season's growth. From the beginning of the second season onwards, the retarded seedlings exhibit improved growth without, however, reaching the level of the controls. Correspondingly, the beneficial effect of addition of 1% cellulose gradually disappears after the first season of growth. These changes in growth reaction are accompanied by (or correlated with) more rapid decomposition of the cellulose.

During the course of the experimental researches, a rough survey of the rate of filterpaper decomposition in the three soils was made. Of the two fertile soils, that from the New Forest brings about the more rapid destruction, the whole of the cellulose material (2%) being decomposed in 7 months. This process took 8 months in Oxshott soil, while the complete disappearance of the same amount of cellulose in Wareham soil required 12 months. That the cellulose is decomposed relatively slowly in Wareham soil has been considered to be due not to the absence from this soil of some specific cellulose-splitting organisms but rather to the inhibition of their acitivities (3). For 'pure cellulose' (2%), the rate of decomposition was 11 months in New Forest soil, 14 months in Oxshott soil, and 20 months in Wareham soil. Observations made on Wareham soil and other poor soils; e.g. from Hope Forest, and on more fertile soils of the same raw humus type suggest a direct correlation between the rate of cellulose decomposition and the degree of soil fertility indicated by the growth reactions of the pine seedlings.

That the rate of cellulose destruction in Wareham soil can be accelerated has been shown in recent researches by other investigators (W. Neilson-Jones and A. Morton, unpublished). During the course of the investigation here recorded it was repeatedly observed that the rate of cellulose decomposition in Wareham soil increased twofold and more by adding certain organic composts to it.

The immediate cause of the deleterious effects induced by additions of cellulose are still obscure. It is frequently assumed that such effects are due only to nitrogen starvation following increased competition for available nitrogen by cellulose-splitting organisms. This would explain the increasingly deleterious results brought about by percentages above 1% but not the beneficial effects produced by the smaller amount.

In order to test the hypothesis that nitrogen starvation causes the growth depression, a series of pot-cultures was set up with addition of nitrogen as well as cellulose.

PLATE II

Fig.1: Seedlings of Scots pine in their first season of growth in New Forest soil, Wareham soil, and Oxshott soil, all receiving 5% cellulose.
Sown 4.4.36; photogr. 4.11.36.
×1/5

Fig.2: Seedlings of Scots pine in their 1st season of growth in Oxshott soil
1.Control, 2. + Nitrate,
3. + 5% cellulose,
4. + 5% " + Nitrate.
Sown 4.4.39; photographed 5,11.39.
×1/7

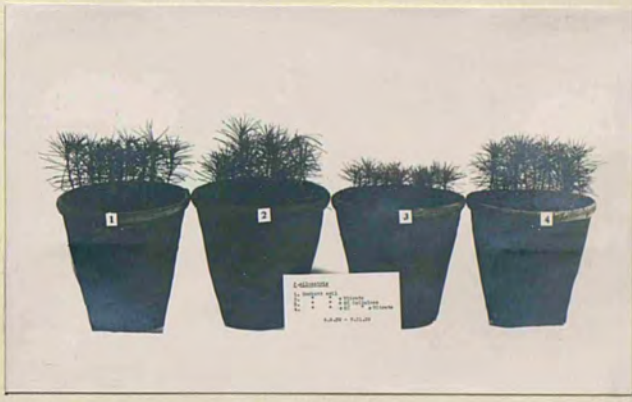


Fig.3: Seedlings of Scots pine in their first season of growth in Wareham soil to which 5% cellulose, 25% C5 (compost from hopwaste), and C5 and cellulose have been added.
Sown 18.4.40; photographed 4.9.40.
×1/5

Nitrogen was added in the form of $NaNO_3$ to the amount of 1%, the percentage being based on the dry-weight of the soil. The nitrate was given as a solution in rain water over a period of six weeks starting at the end of May, and supplied in 12 doses. The series was set up as follows:

1.	Oxshott soil,	Control	6 pots
2.	" "	+ 1% $NaNO_3$	"
3.	" "	+ 5% cellulose	"
4.	" "	+ 1% $NaNO_3$ + 5% cellulose	"

At the end of the first season of growth, series (2) showed no improvement, series (3) exhibited the deleterious effect as usual, but in series (4) this effect was entirely overcome by the addition of $NaNO_3$. (Pl. II, fig.2).

A similar effect was produced by addition of a certain organic compost.* Pot-cultures in composted Wareham soil receiving 5% cellulose produced nearly as fine seedlings as composted Wareham soil alone (Pl. II, fig.3). It was assumed that the amount of available nitrogen in the compost might be responsible for the counteracting effect in the same way as in the experiments with addition of nitrate. In order to estimate the rôle of nitrogen in the compost effect on the cellulose cultures, experiments were set up with leached compost added to the three soils dealt with

* This particular organic compost is one of the composts made under the direction of Dr. M. C. Rayner at Bedford College for Women, University of London, Regent's Park. It derived from hopwaste from a brewery and was composted with dried blood.

in this paper. The compost was washed out three times and kept in running water for one hour before addition to the soils. Table III shows the average height of shoots - based on measurement of 30 seedlings - at the end of the first season of growth.

Table III

Cellulose - compost - experiments

P. silvestris pot-cultures

Sown: 23.4.1940
Examined: 4.12.1940

Average height of shoot after 7 months' growth

	Wareham soil	Oxshott soil	New Forest soil
Control (untreated	5 cm.	7 cm.	7·5 cm.
+ C5* compost	8·5 "	10·5 "	9·5 "
+ C5 compost leached	7 "	8 "	8 "
+ 5% cellulose	4·5 "	4·5 "	4 "
+ C5 + 5% cellulose	8 "	10 "	9 "
+ C5 leached + 5% cell	5·5 "	5·5 "	5 "

The results of the cellulose-compost experiments can be summed up as follows:

* C5 compost derives from hopwaste.

1. Growth of the seedlings is best in New Forest soil with regard to height and colour (cf. rate of cellulose decomposition in the three soils in direct proportion to growth reaction).
2. There is a compost effect not only in the poor soil (Wareham) but also in the comparatively good soils (Oxshott and the New Forest).

   This compost effect is most marked in the poor soil, decreasing in the better soils, and having least effect in the best soil.
3. The effects produced by the leached compost are of the same order as those of the ordinary compost.
4. Addition of cellulose produces the greatest effect in the best (N.F. soil) and is hardly effective in the poor soil (W.), (cf. p. 53).
5. The compost counteracts the inhibition of growth produced by the addition of cellulose in the three soils similarly.
6. With regard to growth of the seedlings, leached compost is little effective in the cellulose treated soils. The colour of the seedlings is as bad as in the cultures treated with cellulose only.

The theory of nitrogen starvation may explain the deleterious effects of additions of higher percentages of cellulose, but it does not elucidate the observed fact that addition of 1% cellulose improves growth during the first season in all three soils; i.e., that there is a threshold value at which depression replaces stimulation of growth. It seems possible that some by-product or products of the decomposition of cellulose may act as a stimulant when present in small amounts, but that accumulation of the same substance following the addition of larger quantities of cellulose, may be progressively deleterious; the other suggestion being that the depressant result is due not so much to higher concentration of the stimulant but to the overpowering effect of nitrogen depletion associated with the larger doses of cellulose.

In order to test the effect of smaller amounts, treatments with various quantities of cellulose were given to Scots pine seedlings in pot-cultures started in spring 1939. 1%, 0·5%, and 0·1% of chopped filterpaper were added to Oxshott soil. As in previous experiments, the seedlings in pots treated with 1% cellulose were slightly better than the controls; smaller quantities - 0·5% and 0·1% - were not beneficial and produced seedlings on the level of the controls. There was no differential effect noticeable between the pots receiving 0·5% and those with 0·1% cellulose.

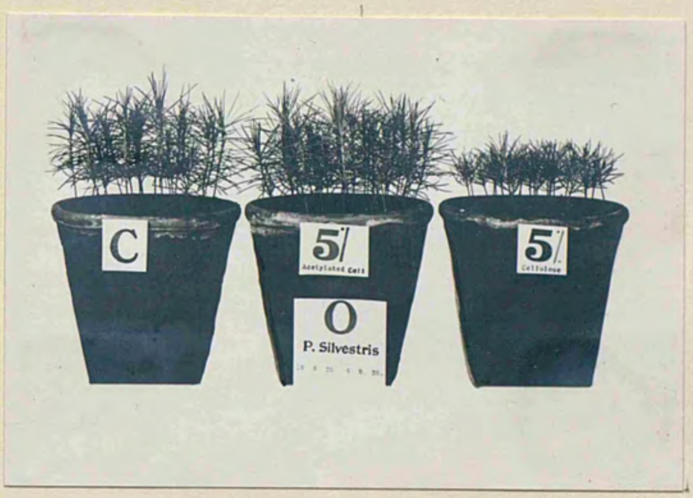
If, as is possible, some by-product of cellulose decomposition acts as a stimulant to growth, it must be assumed from the above experiments, that the substance in question is only effective when present in a certain quantity.

B. ADDITION OF ACETYLATED CELLULOSE

In order to discriminate between physical and other effects brought about by the addition of cellulose to these organic soils, two series of pot-cultures with Scots pine were grown, one series receiving 1% and 5% additions of cotton-cellulose, the other 1% and 5% of the same material acetylated. The cellulose for these experiments was kindly provided by Dr. A. C. Thaysen, Chemical Research Laboratory, Teddington.

The untreated cotton-cellulose used in the 1938 sowing in Oxshott soil was completely decomposed by the end of the year; the acetylated material was still unattacked after 3 years. Effects on the growth of seedlings are brought about only by the former material and are identical

PLATE III



Seedlings of Scots pine in their first season of growth in Oxshott soil: Control, + 5% acetylated cotton-cellulose, + 5% untreated cotton-cellulose.

Sown: 18.4.38; photographed: 4.8.38. x 1/5.

Note that the cellulose effect is brought about only by the untreated cotton-cellulose.

with those produced by the cellulosic materials used in the earlier experiments. The pots supplied with acetylated cellulose show hardly any differential effects as between controls and treated pots (cf. Pl. III, ~~fig. 1~~).

The average height of shoots estimated from measurements of 40 seedlings after the second year of growth was as follows:

Oxshott soil (control)	10 cm.
" " + 5% cotton-cellulose	6 "
" " + 5% acetylated cotton-cellulose	9·5 "

From microscopic examination of the two forms of cellulose taken from the soil of the experimental pots during 2 years, it is evident that acetylated cellulose is immune to attack by the ordinary cellulose-splitting soil organisms. It is also evident that the fibres of acetylated cellulose are not invaded by the soil fungi which readily invade the fibres of the untreated cotton-cellulose.

C. <u>GROWTH OF CERTAIN SOIL FUNGI ON CELLULOSIC MATERIALS</u>

Among the soil fungi that invade the fibres of cotton-cellulose without destroying them, two are readily distinguished by the colour and diameter of the hyphae and mode of branching of the mycelium. They have been isolated by the writer and were available in pure culture for the observations and experiments described below. One of them, *Mycelium radicis atrovirens* common in organic soils and widely distributed in Europe and America, has been the subject of intensive study by Melin (2) and other workers (cf. Part II of this thesis). The other, a form identical with that named *Rhizoctonia silvestris* by Melin (2), is seemingly comparatively rare in nature, and, in the case of the three soils now under observation, is present only in that from Oxshott.

The observations on the two fungi referred to are as follows. Fibres of cotton wool, filterpaper, and untreated cotton-cellulose imbedded in the experimental soils show invasion by the hyphae of *Mycelium radicis atrovirens* in all three soils, and by this mycelium and also that of *Rhizoctonia silvestris* in Oxshott soil. The central lumena of the acetylated fibres in the comparative series are absolutely free from any hyphal infection.

Experiments were carried out to test the resistance of acetylated cellulose from Teddington to mycelial invasion of the two fungi under conditions very favourable to their active growth. Pyramids and cones of cellulosic material,

PLATE IV

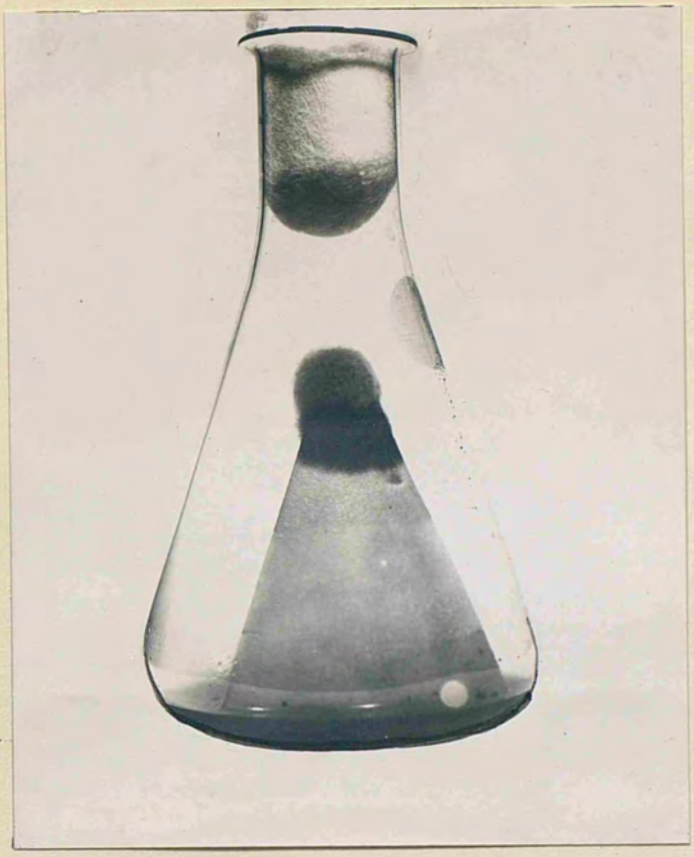


Fig.1:

Mycelium radicis atrovirens

growing on filter paper dipping into a nutrient solution.

Culture 2 months old.

× ½

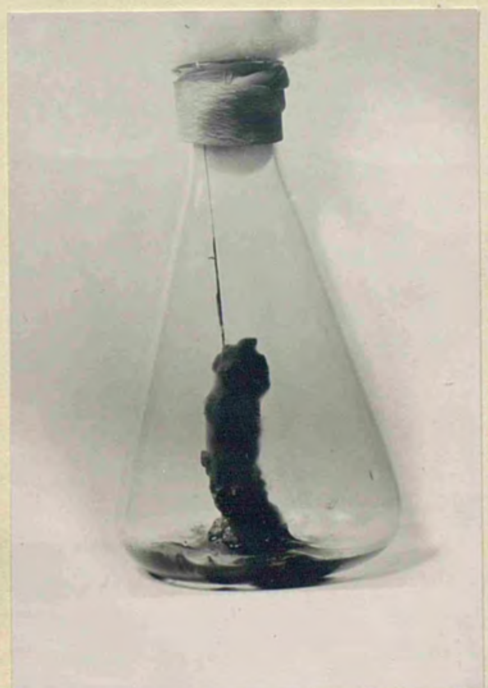


Fig.2:

Mycelium radicis atrovirens

growing on acetylated cotton cellulose dipping into a nutrient solution.

Culture 2 months old.

× ½

PLATE V

Mycelium radicis atrovirens

invading a fibre of filter paper

dipping into a nutrient solution

(cf. Plate IV,fig.1). x 1000

The mycelium is forming sclerotia
inside the central lumen of the fibre.

PLATE Va

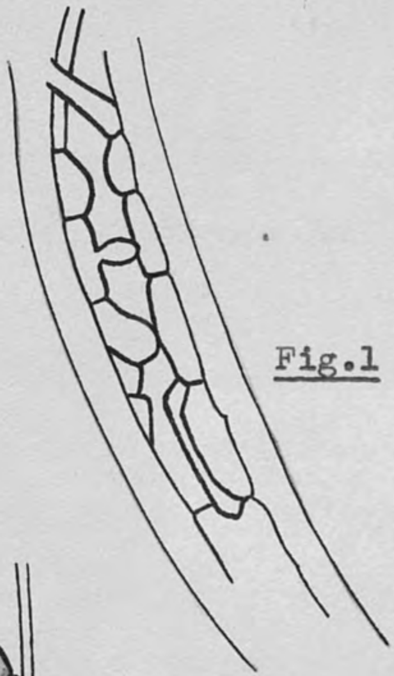


Fig.1

Fig.2

Fig.3

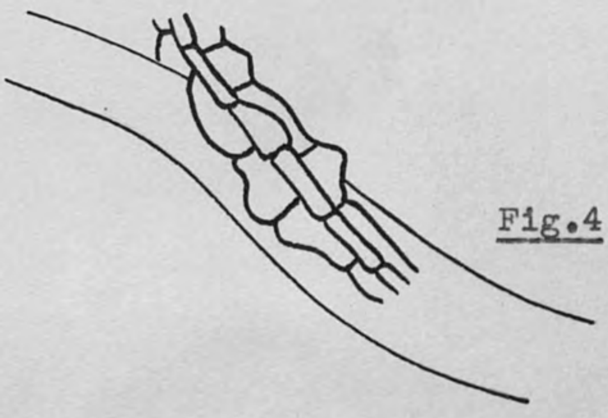


Fig.4

Mycelium radicis atrovirens. From colony on filter paper dipping into a nutrient solution.
Drawings made with an Abbé camera lucida. x750.

Fig.1: Hyphae inside central lumen of fibre, forming a 'net'. Early stage.
Fig.2: Ditto; advanced stage.
Fig.3: Sclerotia inside central lumen of fibre, ~~forming~~
Fig.4: Beginning of Sclerotia formation on surface of fibre.

filterpaper, untreated cotton-cellulose and acetylated cotton-cellulose, were placed in Erlenmeyer flasks in such a way that they were constantly moistened by capillary rise from the nutrient in which they were dipped (Pl. IV, figs. 1, 2). The flasks were then sterilized and the pyramid or cone inoculated from a pure culture of one or other of the fungi. In all experimental flasks, the mycelia grew well, covering the cones with a dense layer; in all except those for which acetylated cotton-cellulose was used, the fibres were invaded by mycelium (Pl. V): The invading mycelia form resting sclerotia and net-like fusions within the fibres of the various forms of untreated cellulose, although no evidence of decomposition was observed in any such culture. (Plate Va)

It is suggested provisionally that mechanical changes in the fibres - swelling of the walls with reduction of the lumena, or the decreased capacity for imbibition* - may possibly be responsible for the observed resistance to invasion by hyphae in the case of acetylated cellulose.

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* Experiments were carried out which indicated that acetylated cotton-cellulose has a lower rate of water absorption than ordinary cotton-cellulose.

PLATE VI

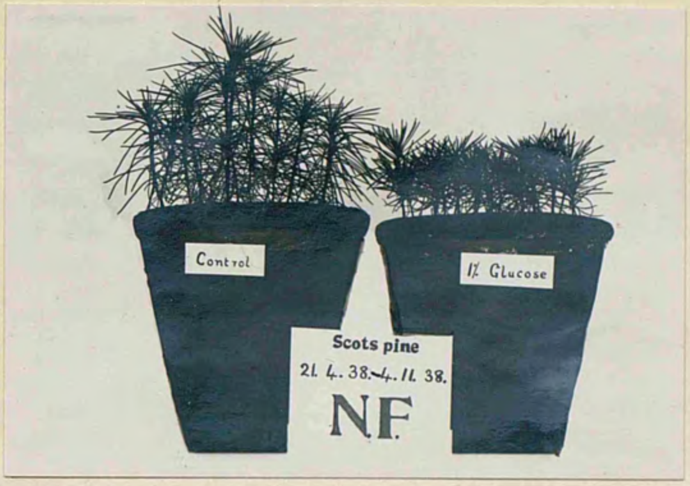

Fig.1: Seedlings of Scots pine in their 1st season of growth in New Forest soil without (Control) and with addition of 1% glucose. × ¼.

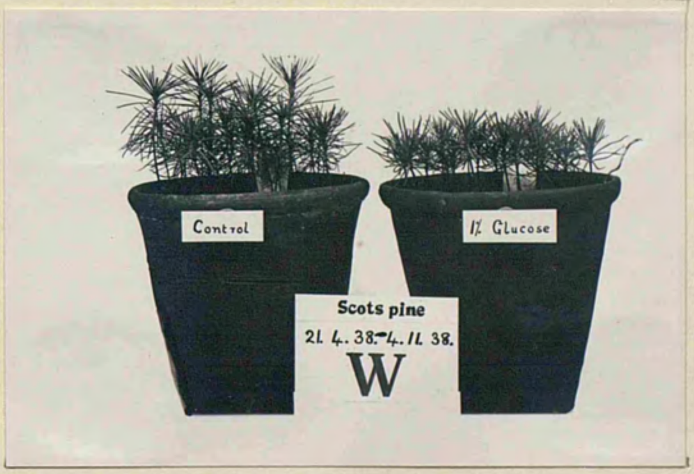

Fig.2: Seedlings of Scots pine in their 1st season of growth in Wareham soil without (Control) and with addition of 1% glucose. × ¼.

D. ADDITION OF GLUCOSE

The assumption that during the course of cellulose-decomposition in soils sugars are formed, led to a study of two series of pot-cultures of pine in Wareham soil and New Forest soil respectively that received a solution of glucose in their first growing season. The total amount of sugar given per pot was in one series 1 gm., in the other 5 gm. supplied in 12 doses over a period of 4 weeks. (1 gm. per pot is about 1% of the dry weight of the soil). Each dose of sugar was given as a solution in 20 c.c. of rain water. The treatment started in June. By the middle of September, the difference between the controls and the sugar-treated cultures was already noticeable and increased up to the beginning of November. In both soils, the seedlings receiving glucose, whether 1 gm. or 5 gm. per pot, showed retarded growth and developed unhealthy symptoms, the effects being more conspicuous in the better (New Forest) soil (Pl. VI, figs. 1, 2). The results are shown in the following table in which are given the average height of shoot estimated from measurements of 40 seedlings after their first season of growth:

	Wareham soil	N.F. soil
No glucose (control)	6 cm.	9 cm.
1% glucose	4 "	4·5 "
5% "	4 "	4·5 "

In the first and second season of growth, there is no difference noticeable between the series having received 1% glucose or 5% glucose. Only in May 1939, that is in the third season of growth, the series with 1% glucose in both Wareham and New Forest soil began to develop healthier seedlings than those treated with 5% glucose, the plants in New Forest soil still exhibiting the difference between the two series more conspicuously.

In the third season of growth, the average height of shoots were as follows:

	Wareham soil	N.F. soil
No glucose (control)	7·5 cm.	12·5 cm.
1% glucose	7·5 "	12·5 "
5% "	5·0 "	6·0 "

Thus, by the third season after application, the deleterious effect of addition of 1% glucose had disappeared while the addition of 5% sugar was still producing a pronounced inhibition of growth.

Comparison between pot-cultures treated with the higher percentages of cellulose and glucose respectively shows that the growth reactions of these two experimental series are identical.

In both cases the results may be interpreted as due to nitrogen starvation, due in one case to utilization of nitrogen by cellulose-splitting organisms and in the other to competition by the numerous organisms that can use glucose. (cf. Chap. B.1).

3. OBSERVATIONS ON MYCORRHIZAL ASSOCIATIONS IN THE CELLULOSE- AND GLUCOSE-TREATED CULTURES

It was of special interest to study the influence of additions of cellulose and sugar upon the associations of soil and mycorrhizal fungi with roots of pine seedlings in the various cultures.

During the course of the investigation, the roots of the experimental seedlings were examined repeatedly with regard to their mycorrhizal equipment, leading to the following observations:-

1. Growth improvement in the seedlings treated 1% cellulose is accompanied by an increase in the amount of what are considered to be 'normal' mycorrhizas. This applies to the three soils under observation. In the case of Wareham soil, the 'normal' mycorrhizas are hymenomycete associations (formed by _Boletus bovinus_); in Oxshott and New Forest soil associations formed by other hymenomycete mycelia and by _Mycelium radicis nigrostrigosum_.
2. The depression of growth in the pot-cultures receiving percentages above 1% is accompanied by reduction in the number of 'normal' mycorrhizas as described, and

by other features which are considered to be undesirable; e.g. by profuse development of mycelia on the ball and increase in the number of pseudomycorrhizas. In Wareham soil, cultures treated with higher percentages of cellulose produced root systems rather heavily attacked by Mycelium radicis atrovirens, while the controls were hardly affected. In Oxshott soil, additions of more than 1% cellulose brought about severe attacks of Rhizoctonia silvestris; in New Forest soil, pseudomycorrhizas formed by an unidentified dark mycelium developed profusely under these conditions.

3. After the second season of growth, when the experimental seedlings are getting away, the attacks of the deleterious root fungi gradually decrease and the root systems of controls and treated plants become more and more similar.
4. Mycorrhizal examination of glucose-treated seedlings have led to observations similar to those on cellulose-treated plants.

4. OBSERVATIONS ON THE EFFECT OF CELLULOSE IN 'PARTIALLY STERILIZED' SOIL

In an endeavour to determine whether under special conditions the deleterious effect of addition of cellulose or glucose to soil on the growth of pine seedlings can be a direct effect of these substances on the pines instead of - as it usually is - an indirect effect through changes brought on the mycorrhizal equipment of the plants, the following experiments were devised: Scots pine seedlings were grown in Oxshott soil steamed thrice for 20 minutes (the soil was leached after each steaming) and mixed with either 1% or 5% cellulose (sterilized filterpaper). A duplicate series of experiments was set up, using in one case untreated cotton-cellulose, in the other case acetylated cotton-cellulose. Thus 5 different treatments were applied:

1. Oxshott soil 'partially sterilized' (control)
2. " " " " + 1% cotton-cellulose
3. " " " " + 5% "
4. " " " " + 1% acetylated cotton-cellulose
5. " " " " + 5% " "

Identical treatments were given to cultures in unsterilized soil. Each series of the sterilized soil experiments, comprising each 6 pots, was kept in sterile gravel in a zinc bath. The 'sterilized' pot-cultures were grown in a roof shelter house which was relatively well protected from casual infection by mycorrhizal fungi. Throughout the experiment, autoclaved rainwater was used for watering.

During the course of two years' growth, no difference was observed in the growth reactions of plants in unsterilized soil and in those grown in 'partially sterilized' soil. In both series, the cellulose effect, beneficial in the case of addition of 1%, deleterious in that of 5%, is brought about only by the untreated cotton-cellulose.

The root systems of the experimental seedlings were examined repeatedly: there was no fungal association observed in any of the roots from 'partially sterilized' soil.

The result of the above experiments leads to the conclusion that, under special cultural conditions, the change brought about by soil treatment with cellulose _can_ influence the growth reaction of the pine _directly_, but as these 'special' conditions do not occur in nature, this conclusion has no general value.

The rate of cellulose decomposition in the 'partially sterilized' soil is approximately the same as in the unsterilized soil; but the two soils are not comparable with regard to micro-flora and chemical constitution, and though the cellulose effect on plant growth is the same in both soils, the causes may not be identical.

Thus, one can assume either that some forms of cellulose-splitting organisms are not affected by steaming and are still active in the 'partially sterilized' soil, or that air-borne cellulose-splitting micro-organisms (bacteria or fungi or both) are involved in the disintegration of the cellulosic material, or that both these reactions occur. These organisms may be favoured in their growth by substances liberated by the steaming of the soil and under these conditions may be as effective with regard to the decomposition of the cellulose material as are the micro-organisms in the unsterilized soil - the most effective of which, under the conditions provided by unsterilized soil, may be those killed by the sterilization treatment.

The following series were set up as an attempt to analyse further the flora concerned in the cellulose destruction which takes place in the soil:

1.	Wareham soil	unsterilized (control)		+	cellulose
2.	"	"	steamed once	+	"
3.	"	"	" thrice	+	"
4.	"	"	autoclaved	+	"

The four sets were run in duplicate, one series being kept 'sterile', the other exposed to air infection. The cellulose was added in the form of filterpaper absorption blocks, each of which weighs about 1·1 gm. After 7 months, no cellulose decomposition has occurred in any of the experimental series. Further observations could not be made as the experiments were destroyed by enemy action.

5. SUMMARY

1. Growth reactions induced by addition of raw cellulose have been tested in pot-cultures of Scots pine.
2. The investigations here described support the hypothesis that changes in the biological activities of the soil brought about by additions of certain organic materials are directly responsible for effects on plant growth.
3. The experiments show that the addition of 1% cellulose has a slightly beneficial effect on growth, whilst higher percentages are deleterious when added to raw humus soils. Smaller quantities - below 1% - produce no effect.
4. Supplies of nitrate to the cellulose-treated cultures counteract the inhibiting effect, thus suggesting that

nitrogen starvation may be the cause of growth depression produced by addition of higher percentages of cellulose.

5. Glucose added to the three soils under observation, induces growth reactions similar to those produced in the cultures supplied with larger amounts of cellulose.
6. Examination of the root systems of the experimental seedlings shows a definite relationship between health and vigour of the plants and their mycorrhizal and pseudomycorrhizal equipment.

6. APPENDIX

The fact that the rate of cellulose destruction in steamed soil is approximately identical with that in untreated soil with its additional flora of soil and root fungi makes the rôle of the latter as obligate cellulose destroyers in the soil rather doubtful.

During the course of this investigation, experimental series were set up to test in pure culture work whether the mycorrhizal and pseudomycorrhizal mycelia can utilize cellulose.

Filter pyramids were placed in (a) Melin's 'glucose' medium (Glucose 20 g., $MgSO_4.H_2O$ 0·1 g., NH_4Cl 0·5 g.,

KH_2PO_4 1·0 g., Agar 15 g., H_2O 1000 c.c.); (b) in the same medium without glucose; and (c) in the same medium without nitrogen, and inoculated with the following root fungi: B.bovinus, M. radicis nigrostrigosum, M.r.atrovirens, and Rhizoctonia silvestris. The two mycorrhiza-formers hardly developed any growth on the medium without glucose and without nitrogen, whereas the pseudomycorrhiza-formers formed a definite layer of mycelium in the cultures without glucose; in the absence of nitrogen, their growth was extremely poor, aerial growth being very limited.

There was no effect on the cellulose noticeable in either of the cultures under observation.

Comparison with control material, cellulose kept under the same cultural conditions but not inoculated with mycelium, showed that the elasticity (tensile strength) of the cellulose was not altered by the fungal growth.

In further experiments, culture media as applied for cellulose-destroying organisms and various forms of cellulose, i.e. hydrocellulose and 'Xylinum' cellulose were used. The latter is a cellulose medium produced by Acetobacter xylinum (1), and other members of the Xylinum group of the acetic acid bacteria and which permits rapid identification of cellulose-destroying organisms which liquefy the medium as soon as 48 hours after inoculation.

The two mycorrhiza-formers referred to, B.bovinus and M.r.nigrostrigosum proved to be unable to attack cellulose in either of the forms and conditions in which it was offered.

The pseudomycorrhiza-formers behaved similarly in all cases but one where 'Xylinum' cellulose was treated with small amounts of a usual culture medium (beer-wort or malt)*. In this case, the Xylinum cellulose was readily attacked by M.r.atrovirens and Rhizoctonia sp. which grew rapidly liquefying the 'Xylinum' layers remarkably quickly.

From the results of the above experiments it seems evident that the two mycorrhiza-formers under consideration are not capable of splitting cellulose whereas the two pseudomycorrhiza-formers referred to are able to utilize certain cellulosic material.

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* The cultures were prepared as follows: Layers of 'Xylinum' cellulose, approximately 5 mm. thick, were thoroughly washed in distilled water, placed in small petri-dishes, moistened with very diluted beer-wort or malt extract and sterilized.

7. LITERATURE REFERENCES

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