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Olavi Laiho

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PAXILLUS INVOLUTUS AS A MYCORRHIZAL SYMBIONT OF FOREST TREES

OLAVI LAIHO

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and Forestry of the University of Helsinki, for public
criticism in Auditorium III in Porthania on
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HELSINKI 1970

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PREFACE

In the fall of 1965, in the neighborhood of *Paxillus involutus* sporophores I found mycorrhizae of the same color as the mycelium at the base of the sporophores. This aroused my interest in the species and led me to begin systematic studies the following fall. These studies were carried out at the Department of Silviculture, University of Helsinki, where I was stationed as a research assistant of the National Research Council for Agriculture and Forestry.

Professor PAAVO YLI-VAKKURI, D.For., Head of the Department of Silviculture, kindly made available the facilities of the Department and took an active interest in the progress of the work. My supervisor, Dr PEITSA MIKOLA, Professor of Forest Biology at the same Department, has followed the work from the very beginning with much valuable advice, particularly regarding the preparation of the manuscript. Similarly, the expert knowledge of Dr VEIKKO HINTIKKA, Docent of Botany, University of Helsinki, has been available during the whole work and he also read the manuscript. Statistical questions, in particular, were discussed with Mr PERTTI HARI, Lic. Phil., and the general lines of the work with Mr TAUNO U. KALLIO, Lic. For.

The field work was done by the author alone. Professor PENTTI J. VIRO, D.For., Head of the Soils Department of the Finnish Forest Research Institute, kindly permitted me to examine some of the forest fertilization plots made by the Department. In the laboratory I was assisted by Miss AINO PIISPANEN, especially in pure culture experiments and in preparing slides for microscopic study, and on some occasions also by my wife MARJA-LEENA, who is a nutritional chemist.

In typing help was received from Mrs IRJA THUSBERG. The originally Finnish manuscript was translated in collaboration with Mr KARL-JOHAN AHLSVED, B. For., and the translation was linguistically revised by Mrs JEAN MARGARET PERTTUNEN, B. Sc. (Hons.). The Society of Forestry in Finland kindly accepted the study for publication in Acta Forestalia Fennica.

I wish to express my sincere gratitude to all the persons and institutions mentioned above.

Helsinki, June 1970

Olavi Laiho

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1. INTRODUCTION

11. THE ECOLOGY OF *PAXILLUS INVOLUTUS*

Paxillus involutus (Batsch) Fr.[1] is a common mushroom both in Finland (Karsten 1879, Rautavaara 1947, Tuomikoski 1959) and in Scandinavia (Lange 1939, Cortin 1956). This probably also holds for the rest of the cool and temperate zones of Eurasia, e.g. Great Britain (Ramsbottom 1951), Central Europe (Moser 1956, Michael and Hennig 1958, Horak 1963, Lisiewska 1965), the Soviet Union (Karsten 1879, Shemakhanova 1967) and Japan (Imazeki and Hongo 1965). In North America, too, the range of this species probably covers the same climatic zones. It is known to be a common mushroom both in Canada (Groves 1962) and in the United States, as indicated by Atkinson (1901) and Krieger (1967) for the eastern, by Kauffman (1918) for the central, and by Trappe (1969) for the western parts of the country. It also grows in the parts of Greenland where birch is encountered (Lange 1957).

Although the genus *Paxillus* is well represented in the southern hemisphere (Singer 1962, Horak and Moser 1966), *P. involutus* may not belong to the natural flora of this region at all. Although the species is found, for example, in New Zealand (Gilmour 1958) and Argentina, to the latter country, at least, it has probably been introduced by man (Singer 1954, 1964 b).

In the tropical zone, *Paxillus involutus* has not been encountered at all (Singer 1964 b), which, of course, may be due to the fact that this area has not been studied extensively. In the arid zone, on the other hand, the species is found (e.g. Komirnaya and Fursayev 1953, Runov 1967), although, quite clearly, it is in the cool and temperate zones of the northern hemisphere that its occurrence is most frequent.

In the USSR, according to Zerova (1967), *Paxillus involutus* is encountered, for example, in the chernozem area, the soil of which is characterized by high contents of lime and humus and an almost neutral reaction. Generally speaking, it seems that the species does not avoid rich soils. In Central Europe, it is found in the richest forest sites (Buch and Kreisel 1957, Lisiewska 1965); likewise, its occurrence in gardens is well known (e.g. Cortin 1956, Michael and Hennig 1958, Kleijn 1962). On the other hand, it is also found on poor forest soils (Gerschler 1959, Witkamp 1959). Its occurrence on peat soils of different kinds must not be forgotten, either (Lange 1939, 1948, Kreisel 1954, Krieger 1967, Pachlewski 1967 a). Consequently, it may be concluded that *P. involutus* is more or less indifferent in its relations to the soil.

In its relations to the vegetation it is primarily a forest species. However different in character the above-mentioned site types were, in most cases trees were growing in the vicinity. Thus, *Paxillus involutus* occurs in forests of different kinds. Scandinavian scientists take the view that it favors birch to some extent at the expense of pine and spruce (e.g. Karsten 1879, Lange 1939, Cortin 1956). The species has a special tendency to grow on decaying wood, i.e. stumps and logs (Karsten 1879, Atkinson 1901, Kauffman 1918, Michael and Hennig 1958, Groves 1962, Singer 1964 b, Krieger 1967), sometimes even high above the ground (Chastukhin 1967). In general it grows in places where decomposing matter occurs in abundance, such as ant hills and heaps of logging waste (e.g. Karsten 1879, Kauffman 1918, Tuomikoski 1959).

Outside the forest *Paxillus involutus* is frequently found in sites such as grassy meadows, pastureland, open heaths, gardens, etc. (Atkinson 1901, Rautavaara 1947, Ramsbottom 1951, Singer 1954, Cortin 1956, Michael and Hennig 1958). Its occurrence in sites of these kinds, as well as on those men-

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[1] Fungal taxonomy according to Moser (1967 b)

tioned above, points to a saprophytic mode of nutrition. It has also been reported to fruit when experimentally isolated from tree roots (ROMELL 1939). Since, in addition, *Paxillus panuoides* and *P. atrotomentosus* are regarded as wood-decaying fungi, it is no wonder that *P. involutus* has been considered a saprophyte (e.g. SINGER 1946, RAUTAVAARA 1947, MICHAEL and HENNIG 1958).

On the other hand, the frequent fruiting of *Paxillus involutus* in the forest has led to the suspicion that it may be mycorrhizal. These suspicions are further strengthened by the fact that the species does not extend beyond either the arctic or the alpine tree limit, although in places it is encountered in abundance along them (LANGE 1946, 1957). According to ZEROVA (1967), *Paxillus involutus* does not fruit on the treeless steppes of the Ukraine. There is also the possibility that small tree seedlings growing on meadows and pastureland where *P. involutus* fruits have been overlooked. This suggestion, made by SCHWÖBEL (1956), is supported by the fact that the species »is never observed as occurring outside the general area of the ectotrophic forest unless introduced with foreign plant material in parks and plantations» (SINGER and MOSER 1965).

This more or less close connection with trees led PEYRONEL, as long ago as 1922, to conclude that *Paxillus involutus* was a mycorrhizal fungus. Subsequent observations have lent support to this conclusion. According to a list presented by TRAPPE (1962), there are at least twelve tree species which serve as hosts for this fungus.

These observations have not been accepted unreservedly, however. Thus, for instance, as recently as 1956, MOSER stated that »der mykorrhizenmässige Bindung von *Paxillus involutus* liegt heute noch in dunkeln». MELIN mentions *Paxillus* for the first time in his study of 1955, and even here, only as a genus that possibly includes mycorrhizal fungi.

Paxillus involutus has, indeed, been almost ignored by scientists working on mycorrhizae. It was only very recently isolated and studied by means of synthesis experiments, which confirmed that it is a mycorrhizal fungus. MOSER (1967 a) has synthesized it successfully with *Pinus cembra* as the higher partner. He considers the species a facultative symbiont. According to SHEMAKHANOVA (1967, p. 52), it also forms mycorrhizae in aseptic synthesis with *Pinus silvestris*. On the basis of the slow progress of the synthesis in comparison with *Suillus luteus*, she concludes that in nature *Paxillus involutus* forms mycorrhizae with pine only when no other ectomycorrhizal fungi are present.

In full agreement with the above research workers, others, too, have considered *Paxillus involutus* an exceptional mycorrhizal fungus in one way or another. According to ORLOŚ (1967) and PACHLEWSKI (1967 a), it is sometimes associated with trees and sometimes not, thus probably being a facultative symbiont. According to the trenching experiments carried out by ROMELL (1939), too, *P. involutus* can form sporophores when growing saprophytically. From its poor and capricious growth on litter, on the other hand, at least some kind of mycorrhizal association has been inferred (MIKOLA 1954 a, 1956). SINGER and MOSER (1965, p. 130) consider it a transitional form between ectotrophic and anectotrophic species, regarding it as capable, under special conditions, of functioning as an »ectotrophformer», generally in connection with obligate mycorrhizal fungi, while it is also capable of living independently, i.e. in stands where ectotrophs have never been found, or even far from any tree. HORAK (1963), too, considers the species a facultative symbiont and states that its »Leistung als aktiver Mykorrhizapilz ist fraglich, da es unter allen Holzarten zu finden ist».

12. OBJECTIVES OF THE STUDY

The information on *Paxillus involutus* to be found in the literature is both deficient and contradictory, and based only on scattered observations made in connection with other investigations. This fact alone is a sufficient reason for a study of this species. On the other hand, it has been said that »there are no mycorrhizal fungi: there is only a mycorrhizal state» and that, in consequence, there is little point in studying the various species of mycorrhizal fungi (KELLEY 1950, p. 46). This opinion has met with sharp criticism (e.g. MELIN 1953, 1962), however, and the need to compare different species is presently emphasized in all mycorrhizal studies (SINGER 1963, BJÖRKMAN 1968, MIKOLA1970).

Paxillus involutus is more interesting than many other mycorrhizal fungi, because it is considered a facultative symbiont. Facultative symbionts are regarded as having reached an intermediate stage in the evolution from saprophytic fungi to specialized symbionts (MELIN 1925, p. 116, GARRETT 1956, p. 97, MEYER 1966). They are characterized by having features of both of these groups. The ability to use complex carbon compounds, a characteristic of saprophytes, has become weaker, but sporophore formation by saprophytic mycelium is nevertheless possible. Facultative symbionts form mycorrhizae, but their dependence on the higher partner is slight. Correspondingly, their »activity» and »virulence» (MELIN 1925, p. 94) are low, and they are easily supplanted by obligate symbionts. Furthermore, they are poorly specialized and their contribution to the growth of the host is considered to be small (e.g. MELIN 1925, DOMINIK 1966, MEYER 1966, MOSER 1967 a).

In addition to *Paxillus involutus*, there are many other species which are considered facultative symbionts. Examples are *Xerocomus subtomentosus* (MODESS 1941), *Laccaria amethystina* (MEYER 1963), *L. laccata* and *L. tetraspora* (SINGER and MOSER 1965), *Tricholoma fumosum* (NORKRANS 1950), *Schleroderma aurantium* (MODESS 1941), *Thelephora terrestris* (MIKOLA 1970) and *Cenococcum graniforme* (DOMINIK 1966). Information on their properties is limited and controversial, however. Thus MODESS (1941) did not find sporophores of *Xerocomus subtomentosus* in places where symbiosis was precluded. Moreover, *Cenococcum graniforme* »has not been convincingly documented as purely saprophytic in nature» (TRAPPE 1964).

Likewise, it has never been indicated by experiments that *Paxillus involutus* is a facultative symbiont (MOSER 1967 a). For this reason it was considered necessary in the present connection to focus special attention on those of its attributes which are expressly characteristic of facultative symbionts. The results of such a study were expected to provide further information about the fungi of this group, which are biologically interesting and practically important. Thus, answers were sought to the following questions:

Does *Paxillus involutus* form sporophores without mycorrhizal association?

Is *Paxillus involutus* able to decompose complex carbon compounds?

Is *Paxillus involutus* a specialized or nonspecialized mycorrhizal fungus?

How does the host respond to *Paxillus involutus* as compared to other ectomycorrhizal fungi?

In addition to these particular questions, attention was paid to many of the other problems which are usually given consideration in mycorrhizal studies. Several of these problems were approached from more than one angle, i.e., by different methods. This made it possible to discuss some methodologic questions, too.

The differentiation of various *Paxillus* species is not quite clear. For instance, there is disagreement as to whether *P. filamentosus* should be considered a distinct species or a variety of *P. involutus* (SINGER 1964 b, BRESINSKY and STANGL 1965). *P. filamentosus* is found at least in Europe (MOSER 1967 b) and possibly also in America (SINGER 1964 b). In Finland it is comparatively rare (TUOMIKOSKI 1950, SCHULMANN 1960). Because it is readily distinguishable from *P. involutus* (e.g. TUOMIKOSKI 1950, BRESINSKY and STANGL 1965, MOSER 1967 b), and differs from the latter in its occurrence as well, being clearly confined to sites where alder grows, the present study was restricted to *Paxillus involutus*. In certain comparisons, however, *P. filamentosus* will also be mentioned.

2. METHODS OF STUDY

From the methodologic viewpoint *Paxillus involutus* is in many respects a rewarding species to study. Its sporophores are easy to find and recognize. In addition, they are common and of at least relatively constant occurrence (Ramsbottom 1953, Tuomikoski 1959). From earlier studies it is known that pure cultures of this species can be isolated (e.g. Mikola 1955) and that it forms mycorrhizae in aseptic synthesis (Shemakhanova 1967). The preliminary experiments of the present study indicated that semiaseptic synthesis, too, is attended with success, and that the mycorrhizae are easier to recognize than those of most other species. Thus, the study could be focused on pure cultures and mycorrhizae of *Paxillus involutus*, as well as on sporophores.

Field observations on sporophores and mycorrhizae have often been considered of minor importance. According to Melin (1936, p. 1030), such observations should be made even though, as such, they do not lead to definitive conclusions, but rather to working hypotheses, which must be tested by experiments. In recent times, however, such observations have become more usual and greater significance has been attached to them; this is undoubtedly due to the advances in methodology that have taken place (e.g. Dominik 1966). In the present study, too, considerable attention has been given to observations on sporophores and mycorrhizae. A special reason for this is that ability to fruit without connection to a host, an important criterion of facultative symbiosis, can be established only in the field.

For this purpose, certain treeless plots were isolated from living tree roots and kept under observation in order to find out whether *Paxillus involutus* would fruit in them or not. With the same goal in mind, the occurrence of sporophores of this species was studied on a variety of sites. An additional aim of these observations was to map the distribution of the species. Theoretically, it would have been more correct to map its mycelia and mycorrhizae, but in practice this would be impossible or at least extremely laborious. Counting the sporophores and determining their weight, on the other hand, can readily be done. These studies were performed both as transect surveys and on permanent sample plots.

Pure culture experiments were primarily aimed at ascertaining the ability of *Paxillus involutus* to utilize various carbon compounds as sources of energy. From the results of such experiments one can estimate whether it is possible for the species to live independently in the soil. In these experiments both simple and complex carbon compounds as well as humus substances were used.

Many other physiologic attributes of *P. involutus* were also studied in pure culture experiments, and the results were compared with corresponding data for other species of fungi. Such investigations have been made in particular by Melin and his school, and the procedures developed by these workers have been of great value in the present study.

A special feature of the root studies reported here is that mycorrhizae formed by *Paxillus involutus* were identified in the field. These identifications were made, for instance, from roots sampled from the permanent sample plots already mentioned. Thus it was possible to gain an idea of the numerical relations between the mycorrhizae and the sporophores formed by *P. involutus*.

Mycorrhizal studies were also performed using synthesis experiments. The aim here was to obtain information on the hosts of *Paxillus involutus*, to acquire material on which to base a description of the mycorrhizae formed by this species, and to solve certain problems connected with their formation. Seedlings inoculated with *P. involutus* were also grown under semiaseptic conditions and planted out in the field. The development of these seedlings was compared with that of control seedlings, and at the same time the survival of the *Paxillus* mycorrhizae was followed. The results revealed the ability of this particular species to compete with other mycorrhizal fungi on various sites, as well as providing information on its symbiotic efficiency.

3. OCCURRENCE OF *PAXILLUS INVOLUTUS* SPOROPHORES

31. STUDY AREAS AND CLIMATIC CONDITIONS

Field investigations were mainly performed in two localities: namely, the communes of Suomusjärvi and Juupajoki. However, supplementary observations were made in many other regions. Suomusjärvi, the author's home commune, was chosen as the main study area. This was a suitable choice in several other respects; the Finnish Meteorological Office has a weather station at Suomusjärvi, and the short (80 km) distance to Helsinki made rapid access to the laboratory possible when needed. In the Juupajoki area, field studies were chiefly performed in the vicinity of the Forestry Training Station of the University of Helsinki. The study areas are both typical of the forest land of southern Finland, although the Juupajoki area, being located in a relatively high (140—190 m above sea level) watershed area, has a rather infertile appearance. The Suomusjärvi area lies at an altitude of 50—130 m above sea level.

The average duration of the growing season (5° C —5° C) is 172 days at Suomusjärvi (Kolkki 1966). The warmest month is July, with a mean temperature of 17° C (Table 1).

Most of the field observations were made in 1968 and 1969. In the former year, the summer was quite normal. The fall, on the other hand, was colder and shorter than average; in September there were relatively hard night frosts and the permanent snow cover appeared in October. The summer of 1969 was exceptionally sunny and warm, although this is not evident from the mean temperatures because of the relative coolness of the nights.

Precipitation tends to increase toward late summer (Helimäki 1967). In addition, the saturation deficit is high in the early summer, the months of June and July being relatively dry. Normally, there is no real dry spell, but in 1969 the soil became exceptionally dry because the period from July through August was sunny and almost without rain. This had a very adverse effect on the mushroom crop, as will be seen later in this paper.

Table 1. Data on the weather conditions at Suomusjärvi weather station (60° 24′ N, 23° 46′ E).

Year	Month								Whole year
	IV	V	VI	VII	VIII	IX	X	XI	
	Mean temperature, °C								
1968	3.8	7.1	16.0	14.7	15.6	9.8	1.9	−1.7	3.7
1969	3.0	8.6	15.7	16.4	15.9	9.3	4.9	−0.3	3.3
1931−1960	2.4	9.3	14.0	17.1	15.4	10.3	5.0	0.7	4.5
	Precipitation, mm								
1968	49	62	37	45	92	126	89	50	705
1969	62	21	19	46	57	118	46	145	634
1931−1960	35	37	45	72	77	70	67	61	628

32. METHODS AND MATERIAL

321. Transect surveys

In order to obtain a statistically representative picture of the abundance of sporophores in an area which can not be studied in its entirety, either systematic or random sampling must be employed. In forest mensuration, which the present task resembles in many ways, these two sampling methods give almost identical results (Nyyssönen, Kilkki and Mikkola 1967). It is solely for practical reasons that systematic sampling is usually employed. In the present work the systematic sampling method decided upon was the transect survey.

In investigations into the occurrence of mushrooms the width of the survey strips employed has sometimes been as little as two meters (Rautavaara 1947), but in the present study, which was concerned with a single, readily visible species, broader strips were considered better. As on most sites the sporophores of *Paxillus involutus* could be seen for a distance of five meters from the middle of the strips, a strip width of ten meters was adopted.

The largest area covered by such a transect survey, 10 × 10 km², was in the Suomusjärvi area. The distance between the survey strips was one kilometer. But even in an area of this magnitude, the data collected were not sufficient to include all uncommon site types, and these had to be studied separately. Such sites were searched for independently at Suomusjärvi and in several other localities as well. For this purpose, help was received from the personnel of Korkeakoski Forest District (State Board of Forestry), in the forests of which such measures as prescribed burning, draining, scarification and forest road construction have been undertaken on a large scale. In the District Office a map was studied on which the localities where such work had been carried out were indicated, and of these the ones which would probably best supplement the data were chosen for closer examination. Further, the Soils Department of the Finnish Forest Research Institute furnished a list of the fertilization trials being carried out by the Department. On the basis of this list, five large, replicated fertilization trials were made use of for this study.

The studies were carried out in 1966—69, but mostly during the last two years. Usually, each place was visited only once, but some of them in all four years. Small areas were studied in their entirety and larger ones with transect surveys. For the forest-covered areas the following data were recorded: forest site type, age of stand, tree species and stand density as well as cuttings and other silvicultural measures carried out and the estimated dates of their performance. These studies were made in the period August through October, most of them, however, in September, when the number of the sporophores of *Paxillus involutus* reaches a peak in the areas in question (Fig. 4). At this time of year the sporophores are easy to observe, too, because the grasses and herbs have already withered, while the leaves still remain on the trees. It can be estimated that the numbers counted each year represent 40—60 per cent of the sporophores of *P. involutus* formed in the areas studied.

322. Permanent sample plots

Permanent sample plots were used primarily to find out the total annual number of sporophores of *Paxillus involutus* under some tree stands. For the sake of comparison, other large-sized mushrooms in the same sample plots were also noted.

Such a count can only be made if the sporophores are picked. Picking was done in full awareness that it may be harmful to the mycelia of the fungi in question. To ascertain the best time interval between pickings the cycle of the sporophores of *P. involutus* was studied. It was established that in the summer they begin to deteriorate on an average two weeks after emerging from the soil. Then, riddled with insect larvae, from which *Bolidophila hybrida* in particular has been reared (Eisfelder 1954), they collapse in a few days, often forming a yellow mass caused by the mold *Hypomyces chrysospermus* (Ramsbottom 1953). In the fall the length of this cycle was three weeks and destruction, too, was slower. Traces of the sporophores could still be seen in the spring after the snow had melted.

Ten days was chosen as the time interval between the picking dates. For *Paxillus involutus* this was short enough, although every now and then it was observed that some of its sporophores, which on a certain date were too young, had disappeared by the next picking. Presumably they had been eaten by birds. The sporophores of many other fungi are known to live only a few days or even hours. Large-sized mushrooms, however, usually have a lifetime of ten days or more (Rautavaara 1947, Lange 1948). Among the exceptions are the sporophores of certain *Russula* species, which were badly damaged by the time of the next picking. In the present work, however, such specimens were also collected and, therefore, the long time interval used did not lead to a great underestimation of the mushroom yield.

The occurrence of sporophores was studied in the permanent sample plots during the growing seasons of 1968 and 1969. The former was quite a normal mushroom year, although in the fall, because of hard night frosts, the usual rich crop did not appear. In 1969, the growing season was dry and the mushroom crop exceptionally small. *Paxillus involutus*, however, fruited abundantly.

Small sample plots (1 are or even 1 m²) are used in fungal studies (LANGE 1948, MOSER 1949), but they must be used in numbers in order to cover the variation. In the data presented by LISIEWSKA (1965), for instance, only 30—53 per cent of the species encountered in 4-are squares were found in each of the 1-are plots. Therefore, large samples, e.g. 0.5 ha, are recommended (MICHAEL and HENNIG 1967).

The average size of the permanent sample plots of the present study was 0.37 ha and the total area covered 8.13 ha. The examination of an area of this size was only made possible by disregarding small sporophores. Furthermore, plots in grassy sites and places which were difficult to study were smaller than those located on poor upland sites.

Most of the plots were chosen in pairs, one being in a closed tree stand and the other in a cutover area in its immediate vicinity. In six of these plots (2 c, 5—7 o, 9—10 d; Table 2) *Paxillus involutus* was known to fruit, whereas for other species, all the 22 plots were chosen at random.

The plots were all located close to each other and at a distance of about three kilometers from the weather station. The dominant tree species was *Pinus silvestris* L., *Picea abies* (L.) KARST. or *Betula pubescens* EHRH. but in almost all plots other tree species were admixed. Attention is especially called to the fact that no sample plot located in a cutover area was completely treeless, because seedlings were always found and in some cases, scattered seedbearers, too. In addition, roots of the trees surrounding the cutover areas were present in their outer parts. The mineral soil was either outwash sand, fine sand or sandy till. In the sample plots with closed tree stands the ground vegetation was dominated by mosses, especially by *Pleurozium schreberi* (WILLD.) MITT., whereas in the cutover areas grasses and herbs were abundant. The humus layer consisted of mor, being typical raw humus in many closed stands, but having features of mull in the cutover areas. Table 2 gives more detailed information on these sample plots, whereas Figs. 1 and 2 give an idea of their external appearance.

33. FACTORS AFFECTING THE OCCURRENCE OF SPOROPHORES

331. Trees

The areas examined in the present study included both treeless and forested sites, the latter being represented by sparsely as well as heavily stocked areas. The corresponding occurrence of *Paxillus involutus* sporophores is presented in Table 3.

On treeless peatland no sporophores of this species were ever found. Their absence, however, can not be confidently attributed to the absence of trees, but there is always the possibility that the same factors which prevent tree growth also check sporophore formation.

Difficulties of these kinds do not arise when the tree stand is cut from a certain area because, under Finnish conditions, treeless cutover areas become reforested quite soon. A great number of such cutover areas were examined, but no sporophores of *Paxillus involutus* were found in them. There were also four areas that had been cleared: the ground surface had been broken because of draining and stump removal. As shown on p. 18 these measures increase the fruiting of *P. involutus*. In all four instances, however, its sporophores were absent from the middle of the clearings, although they were abundant along the forest edge.

On arable land sporophores of *Paxillus involutus* were found on five occasions. One was on a grassy sod, and the others in fields plowed in the fall and tilled in the spring. All the sporophores were within 1—10 m of the forest edge. By digging it could be established that in each case birch roots had grown

Table 2. Information on the permanent sample plots. Suomusjärvi 1968.

Code[1]	Area, ares	Treatments		Tree stand							
		Years since cutting	Scarification	Volume, m³/ha	Per cent of volume				Dominant age, years	Depth, cm	pH[2]
					Pine	Spruce	Birch	Others			
1 c_1	26	5	—	102	△	△	94	6	50	3.0	5.2
1 c_2	13	5	—	124	△	66	34	△	50	2.9	4.8
1 o	3	5	—	19	10	28	59	3	5	2.4	4.9
2 c	57	5	—	194	71	13	16	0	50	2.9	4.4
3 c_1	45	4	—	167	48	51	1	△	90	3.8	4.2
3 c_2	43	25	—	124	1	97	2	△	90	4.0	4.2
3 o	14	8	—	7	42	4	52	2	12	3.7	4.7
4 c	54	4	—	234	52	44	4	△	90	3.6	4.0
4 o_1	35	1	—	59	91	9	△	△	110	4.1	4.3
4 o_2	12	8	—	3	26	10	41	23	12	3.6	4.5
5 c	150	10	—	203	59	40	1	0	110	3.1	4.3
5 o	31	5	+	33	58	25	17	△	10	2.8	4.6
6 c	18	6	—	140	100	△	△	△	70	4.3	4.0
6 o	20	6	+	17	94	0	5	1	10	3.6	4.2
7 c	49	15	—	96	100	△	△	△	70	2.5	4.4
7 o	31	5	+	27	92	2	6	△	10	2.4	4.5
8 c	104	15	—	128	100	0	0	0	80	3.4	4.2
8 o	84	2	+	17	100	△	△	△	1	3.3	4.4
9 c	5	20	—	23	100	△	△	△	120	100 +	4.0
9 d	3	9	—	37	96	0	4	0	8	100 +	3.8
10 c	13	20	—	12	100	0	0	0	120	100 +	3.7
10 d	3	9	—	19	98	0	2	0	8	100 +	3.9

[1] c Closed stand
d Ditch bank, width 3 m
o Cutover area

[2] Soil analyses by Satoturve Oy

under the trap ditch along the forest edge. These roots regularly bore mycorrhizae formed by *P. involutus* (they were identified by the procedure described on p. 51).

According to many investigations (Thesleff 1919, Lange 1944, Moser 1949, Hintikka 1960 a), *P. involutus* is not one of the species favoring burned sites. In the present study its sporophores were not found at all in areas where prescribed burning had destroyed all possible hosts. In places where mature trees had survived or seedlings had reappeared after burning, the species was observed to fruit. On campfire sites in the forest, even when quite recently burned, it sometimes fruited in abundance. Thus, its complete absence from burned-over treeless areas can not be due to the changes in the soil caused by burning, although these are great (Uggla 1958, Viro 1969).

From the results obtained it can be concluded that *Paxillus involutus* only fruits when within the reach of tree roots. The need for host plants, however, is not great. In a *Sphagnum fuscum* swamp, for instance, which had been used for winning of peat moss and carried scattered and stunted pine and birch seedlings of 10—30 cm height and in which almost no other vegetation occurred, small-sized *P. involutus* sporophores were found in

Humus layer						Vegetation					
Loss on ignition, %	Total nitrogen, %	C/N	Exchangeable			Site type [3]	Lichens	*Sphagnum* spp.	Other mosses	Herbs, grasses	Dwarf-shrubs
			K	P	Ca		Coverage, %				
			mg/l								
40	0.54	74	80	10	237	OMT	0	0	7	76	7
51	0.58	88	103	11	124	OMT	0	0	37	28	10
52	0.74	70	95	12	337	OMT	0	0	11	83	4
70	0.64	109	57	12	212	OMT	0	0	85	14	9
81	0.67	121	73	10	156	MT	0	5	68	14	37
65	0.68	96	48	10	200	MT	1	0	88	13	14
68	0.73	93	79	11	362	MT	0	0	16	76	11
82	0.67	122	62	10	156	MT	8	0	67	15	26
89	0.71	125	63	11	275	MT	7	0	56	11	34
72	0.58	124	100	10	287	MT	0	0	14	61	20
70	0.52	135	78	10	225	MT	0	3	76	30	15
47	0.48	98	84	11	206	MT	0	1	26	77	7
89	0.61	146	50	9	118	MT	0	0	68	14	36
71	0.66	108	53	11	250	MT	0	0	32	51	19
87	0.58	150	58	10	135	VT	14	0	74	1	27
70	0.56	125	57	9	218	VT	10	0	48	21	24
71	0.55	129	56	8	128	VT	4	0	86	1	24
72	0.67	107	52	7	181	VT	2	0	58	28	14
98	0.73	134	30	6	108	IR oj	0	36	5	12	67
99	1.02	97	10	6	89	IR mu	0	11	11	37	21
99	0.51	194	31	7	56	IR oj	3	57	0	12	39
98	1.21	81	24	6	74	IR mu	2	16	17	29	13

[3] Upland forest sites according to CAJANDER (1949). CT = *Calluna* type, poor for pine; VT = *Vaccinium* type, fair for pine; MT = *Myrtillus* type, fair for spruce, fertile for pine; OMT = *Oxalis-Myrtillus* type, fertile for spruce, exceptionally fertile for pine; *Lehto* = a group of very rich forest sites, all exceptionally fertile for spruce.

Forest sites on peatland according to HEIKURAINEN (1964). IR = dwarf-shrub pine swamp, relatively infertile; oj = newly or inefficiently drained peatland; mu = drained peatland, the vegetation of which has changed towards upland vegetation.

numbers of about 100 per hectare. In stands formed by slightly larger seedlings the numbers of these sporophores were sometimes extremely high, in fact much greater than in closed stands. Thus, the conclusion can be drawn that, when certain minimum requirements are fullfilled, the fruiting of this fungus is influenced by factors other than the number of trees present. The tree species, too, seems to be of minor importance. *Paxillus involutus* was observed to fruit in pure stands of all the main tree species occurring in Finland, viz. Scots pine, Norway spruce, common birch, and white birch.

332. Site

The relations between the occurrence of sporophores of *Paxillus involutus* and the quality of the soil were studied on the basis of data systematically collected in the Suomusjärvi area (Table 4). The tree stands were divided into two categories: namely, closed stands and other stands. The term closed stands is used throughout this study to denote both thinned stands and stands not treated by cutting. The concept covers stands in all stages of development from crown closure to regeneration. Stands in which fer-

Fig. 1. Sample plots 5 o and 5 c, *Myrtillus* type. In the cutover area 946, and in the closed stand (in the background) two *Paxillus involutus* sporophores per hectare in 1968. The frequency of corresponding mycorrhizae 12.0 and 0.9 %, respectively, of the number of short roots.

Fig. 2. Sample plot 8 c, *Vaccinium* type. Two *Paxillus involutus* sporophores per hectare in 1968. The frequency of corresponding mycorrhizae 0.1 % of the number of short roots.

tilization or scarification had been carried out, however, were not included. The term other stands here primarily includes regeneration areas of different kinds.

Within the category of closed stands sporophores of *Paxillus involutus* were encountered in all except the poorest forest site types. The results are consistent in that the better the site, the greater was the number of sporophores. Maximum figures were obtained in the very richest forest site types of the Finnish classification, referred to in Table 4 by their Finnish name, *lehto*. In the other study areas, too, finding these sporophores was easy in forests of these site types. On many occasions the neighboring sites representing the forest class dry and dryish land were also studied. On these, few if any sporophores were found. Because of the method of sampling employed, the differences in sporophore densities shown in Table 4 were not tested with regard to their significance. From the magnitude of the differences, however, and in view of the consistency of the trends shown, it may be concluded (cf. NYYSSÖNEN, KILKKI and MIKKOLA 1967) that the number of *Paxillus involutus* sporophores in closed stands clearly increases with the quality of the site.

Table 3. The occurrence of *Paxillus involutus* sporophores according to transect surveys.

Site	Survey strip, km	Number of sporophores per hectare
Treeless peatland	2.54	0
Treeless cutover areas	3.06	0
Arable land	15.20	1
Burned-over cut areas	10.08	4
Sparsely stocked cutover areas	25.76	38
Sparsely stocked scarified cutover areas	10.64	72
Roadsides and ditch banks with trees [1]	55.71	104
Closed stands	147.09	6

[1] Width 5 m from ditch

In the category of other stands *Paxillus involutus* sporophores were found in abundance on all sites, i.e. on poor mineral soils, too. For instance, in cutover areas in forest of *Calluna* type they were repeatedly encountered, and along roads crossing such forests they sometimes occurred in great abundance. Most of them were found on ditch spoil banks, while some grew between the ditch and the road surface, in sand almost free from humus.

Table 4. The occurrence of *Paxillus involutus* sporophores on stocked sites in the Suomusjärvi area (10 × 10 km²) in September 1968 according to a transect survey.

Stand type	Site							
	Rocky ground	Peatland	CT	VT	MT	OMT	*Lehto*	All sites
	Length of strip, km							
Closed stands	4.46	6.89	3.10	11.59	16.20	8.00	1.40	51.64
Other stands	0.63	1.43	0.84	4.69	9.00	2.97	0.70	20.26
All stands	5.09	8.32	3.94	16.28	25.20	10.97	2.10	71.90
	Number of sporophores per hectare							
Closed stands	14.8	4.5	0	4.0	4.7	6.8	15.7	5.7
Other stands	30.2	50.3	22.6	19.2	24.0	29.6	57.1	26.9
All stands	16.7	12.4	4.8	8.4	11.6	12.9	29.5	11.7

Their occurrence on arable land, burned soil and peat also indicates the indifference of the species to many edaphic factors. This is also supported by its growing on rocky ground, which is an extremely poor site for trees, at times drying up severely. Sporophores of *P. involutus* were found in abundance, however, although they were small-sized.

These observations show that the fungus species in question prefers good sites. Occasionally, however, it also fruits on poor sites, and may even do so abundantly. Certain silvicultural measures, particularly, appear to stimulate sporophore formation.

333. Certain silvicultural measures

3331. Fertilization

The relatively frequent occurrence (23 per hectare) of *Paxillus involutus* sporophores in certain areas treated with fertilizers strongly suggested that forest fertilization influences their appearance. To verify this and to obtain a more detailed picture of the situation, the occurrence of these sporophores was studied in five large fertilization experiments.

In two of these, at Liesi and Vatinsuo, the numbers of *Paxillus involutus* sporophores per experimental plot were submitted to three-way analysis of variance, applying the variant log (x + 1) instead of x (cf. Snedecor and Cochran 1967). Table 5 indicates the densities actually observed. It also gives the F-values obtained. They show that nitrogen explained the occurrence of these sporophores to a highly significant degree. Phosphorus had no influence either way, and the same was true of potassium.

Closer examination revealed that *P. involutus* sporophores only grew in plots to which nitrogen had been applied. In 1968, they occurred in 17 out of the 324 plots treated with nitrogen, while all of the 108 untreated plots were empty. If the probability that these sporophores occur in a certain plot is $\frac{17}{432}$, the probability that all of the 108 plots mentioned remain empty is $\left(1-\frac{17}{432}\right)^{108}$ ~ 0.013. In 1969, *P. involutus* fruited in 74 plots treated with nitrogen, and only in these. The corresponding probability is extremely low ($\sim 2\cdot10^{-9}$).

In the other experimental series, too, nitrogen application considerably increased the appearance of these sporophores (Table 6). The more nitrogen had been applied, the more common they were. In the experiment carried out at Pikku Patasalo, the frequency of these sporophores was $\frac{1}{7}$ in the unfertilized control plots, $\frac{2}{21}$ in the N_{80} plots, $\frac{6}{21}$ in the N_{160} plots and $\frac{10}{21}$ in the N_{240} plots, the average thus being $\frac{19}{70}$. When the probabilities are calculated from the binomial distribution as above, there is only a 3.6 % risk in

Table 5. The occurrence of *Paxillus involutus* sporophores in two dense mature spruce stands in September 1969. Fertilization with urea, Kotka phosphate[1] and KCl, at the same final rate but in one (1964, a), two (1964 – 1966, b) or three (1964 – 1966 – 1968, c) portions. Randomized circular plots, radius 6 m.

Nutrients added, kg/ha		P								
		0			61 a			61 b		
		K								
		0	166 a	166 b	0	166 a	166 b	0	166 a	166 b
		Number of sporophores per hectare								
		Liesi, *Oxalis-Myrtillus* type, 6 replicates. F for N 15.8***[2] P 0.2 K 2.3 Significant interactions: none								
	0	0	0	0	0	0	0	0	0	0
	243 a	0	0	29	383	0	0	0	88	15
	243 b	781	309	88	766	339	177	722	236	236
N	243 c	236	147	221	575	324	251	589	15	516
		Vatinsuo, *Myrtillus* type, 6 replicates. F for N 4.6** P 0.4 K 1.9 Significant interactions: none								
	0	0	0	0	0	0	0	0	0	0
	243 a	0	29	0	15	29	0	15	0	0
	243 b	280	0	251	339	15	265	59	0	44
	243 c	265	0	0	44	501	0	634	0	0

[1] A mixture of finely ground rock phosphate and superphosphate, 10 per cent P, half of it water-soluble

[2] * Significant with a 5 per cent margin of error
** » 1 »
*** » 0.1 »

stating that the frequency $\frac{10}{21}$ is higher than the average, and a 4.9 % risk in saying that $\frac{2}{21}$ is lower.

Another test applied was to calculate the average number of *P. involutus* sporophores per plot, omitting those in which the species did not fruit. In Pikku Patasalo, given in increasing order of nitrogen applications, they were: 2, 1, 5, 15, the average for all of them being 9.6. Assuming that the number of these sporophores per plot follows the Poisson distribution (cf. FISHER 1954), the probability of obtaining one plot with two sporophores or less (unfertilized plots) is $e^{-9.6} + 9.6\, e^{-9.6} + \frac{9.6^2}{2!}\, e^{-9.6} \sim 0.0038$. The probability of obtain-

Table 6. The occurrence of *Paxillus involutus* sporophores in three dense mature pine stands in September 1969. Randomized circular plots, radius 6 m, except in Pikku Patasalo 20 m.

Kerimäki, Patasalo, *Myrtillus* type, 18 replicates, fertilization in 1967

Fertilizer	N, kg/ha		
	0	70	140
	Number of sporophores per hectare		
Calcium ammonium nitrate		54	133
NPK fertilizer[1]	10	49	10
Urea	0	15	29

Kerimäki, Pikku Patasalo, *Vaccinium* type, 7 replicates, fertilization in 1966

Fertilizer	N, kg/ha 0	80	160	240
	Number of sporophores per hectare			
Ammonium sulfate	2	2	11	33
NPK fertilizer [1]		0	3	39
Urea		0	17	99

Ruotsinkylä, *Calluna* type, 55 replicates, fertilization in 1965

Fertilizer	N, kg/ha 0	60	120	180
	Number of sporophores per hectare			
NPK fertilizer [1]	0	0	0	0

ing two plots with not more than one sporophore in each (N_{80} plots) is $(e^{-9.6} + 9.6\,e^{-9.6})^2 \sim 5\cdot10^{-7}$.

These results show unequivocally that nitrogen application to tree stands leads to increased fruiting of *Paxillus involutus.* It seems that small non-fruiting occurrences (p. 54) are stimulated to form a few sporophores, and that in places where fruiting already takes place sporophores will appear in great numbers. In extreme cases the increase may be explosive. For instance, in a dense mixed stand, 1/4 ha in area, (*Vaccinium* type, 96 kg/ha of nitrogen in 1962 as NPK fertilizer and 104 kg/ha of nitrogen in May 1969 as calcium ammonium nitrate) only 1—5 sporophores of *P. involutus* grew annually in 1966—1968, whereas one year later there were 990 in the same experimental plot.

3332. Cutting, scarification, draining

The data presented in Table 4 for the category »other stands» primarily represent the fruiting of *Paxillus involutus* in areas where regeneration cuttings had been done. Compared with the closed stands, the number of sporophores was here consistently greater on all sites, the ratio averaging 5:1. The difference becomes even clearer when we establish in which components of the ground mosaic they occurred in the closed stands. One third grew on windfall scars, ant hills etc. (Fig. 3, Table 7). Such parts formed a very small proportion of the ground mosaic in question, however, and thus the concentration of the sporophores in them is very striking. It is fully warranted to conclude that in its fruiting *P. involutus* avoids undisturbed natural forest and, especially, an unbroken ground surface. Many other investigators, too, have observed that the species frequently fruits in the exceptional sites mentioned (e.g. KARSTEN 1879, KAUFFMAN 1918, MICHAEL and HENNIG 1958).

On the basis of all transect surveys carried out in the present connection, the density of *P. involutus* sporophores in cutover areas is six-fold that in closed stands (Table 3). As in the fertilized plots, these sporophores may abound in cutover areas, too. There is also a difference in the time when the fructification takes place in these two groups of stands ($\chi^2 = 18.2^{**}$). In cutover areas fruiting was frequent even in the beginning of August, whereas in closed stands this stage was reached almost a month later (Fig. 4). The size of the sporophores showed no difference, however; in both the cases in question the fresh weight averaged 32 g, the largest specimens weighing about 200 g.

In scarified cutover areas the number of *Paxillus involutus* sporophores was greater than in corresponding areas with an unbroken ground surface. The following numbers show

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[1] 18 % N, 12 % P_2O_5, 6 % K_2O

Fig. 3. *Paxillus involutus* sporophore growing at the base of a birch stump, $^{1}/_{3}$ of natural size.

their mode of distribution; they are based on random observations made in five areas treated with scarification.

In patches	252
On the turf removed	56
On unbroken ground	92

The area of the patches and of the turf removed from them each covered about five per cent of the total area in question, and thus it can be seen that these sporophores quite clearly tended to occur in the patches. The mechanical disturbance of the ground surface either stimulated sporophore formation or promoted underground growth of the fungus as well. The frequent occurrence of these sporophores in fertilized forests with an unbroken ground surface points to the latter alternative. Further consideration will be given to this point in connection with the studies carried out on plots isolated from tree roots.

Table 7. Details of the location of *Paxillus involutus* sporophores in the closed stands of Table 4, site types CT — *lehto*.

Location	Total number	Per cent
Windfall scars	25	12.6
Stumps	10	5.1
Base of tree	17	8.6
Ant hill	13	6.6
Rock and boulders	9	4.5
Normal ground	124	62.6
Total	198	100.0

In draining, just as in scarification, the ground surface is also broken, but more effectively. And correspondingly, very large numbers of sporophores of *P. involutus* were found along ditches. This was true for both peaty and mineral soils. For long stretches their average number frequently exceeded one per meter of ditch. The difference in comparison with closed stands is so great as to be visible at a glance. Even years after draining most of the sporophores along ditches grow on the spoil banks only, or in their immediate vicinity. Special attention is called to the fact that they are concentrated on the highest parts of the spoil banks (Fig. 5), which are probably relatively well aerated.

On some occasions it was observed that *Paxillus involutus* fruited abundantly on the spoil banks a mere couple of months after draining. Usually, however, in such a short time few if any of its sporophores appear, but propagation takes place in the first few years after draining, as indicated by observations made in successive years in certain places. This is also shown by the data as a whole (Fig. 6). From these data it can be concluded that the number of sporophores along ditches reaches a peak 3—4 years after draining. The peak is followed by a clear decrease, although in favourable years the sporophores in question may still be abundant even ten years after draining. Along older ditches they are not much more common than in closed stands. In cutover areas and fertilized forests the course of development is obviously similar to that along ditches.

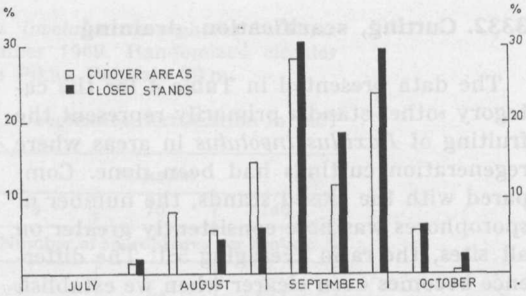

Fig. 4. Relative numbers of *Paxillus involutus* sporophores in 1968 on the permanent sample plots.

Fig. 5. Mass occurrence of *Paxillus involutus* sporophores on a heap of soil removed from the roadside ditch three years earlier. None in the surrounding closed stand (7 c, *Vaccinium* type).

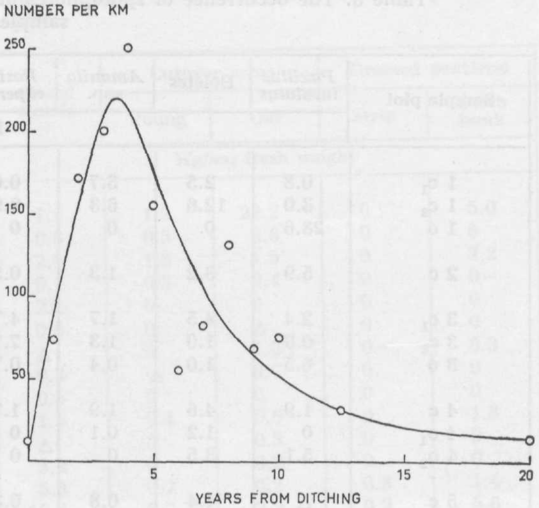

Fig. 6. The occurrence of *Paxillus involutus* sporophores along different ditch banks and roadsides (to a width of 5 m from ditch) carrying trees.

334. Occurrence of other macrofungi

The occurrence of other macrofungi was studied on the 22 permanent sample plots. On a few of these (those not selected at random, see p. 11) *Paxillus involutus* was very common but otherwise its density was about the same as in our forests in general. For other species, the selection of all the plots was random. Thus, the results, presented in Tables 8 and 9, must reveal some essential features of the occurrence of other macrofungi, too.

In the category of closed stands, species of *Russula, Lactarius, Cortinarius, Boletus* (in a broad sense) and *Amanita* were dominant. Their contribution to the total mushroom yield was 76 per cent. *Rozites caperata*, too, was abundant on many sample plots. *Polyporus ovinus* was extremely abundant in the spruce plot 3 c$_2$. The genera *Cantharellus, Hydnum, Hygrophorus* and *Tricholoma* were rather poorly represented in these plots, and the species *Clitopilus prunulus* and *Gomphidius glutinosus* were quite rare. A feature common to all the genera mentioned, and most of their species, is that they are either proven or suspected mycorrhiza formers (Trappe 1962).

In old cutover areas the mushroom flora was completely different. The species and genera mentioned above occurred in small numbers or not at all, and only near the edge of the forest. More frequently represented were such species as *Clitocybe gibba, Hygrophoropsis aurantiaca* and *Stropharia hornemannii*. They are obvious saprophytes. Among mycorrhizal fungi, in addition to *Paxillus involutus*, the species *Tylopilus felleus*, which in 1968 was exceptionally abundant, *Laccaria laccata* and *Lycoperdon gemmatum* were more frequent. *Thelephora terrestris* was not collected for weighing, but the observations carried out indicated that this species was frequent in these cutover areas.

In recently cut areas, the mushroom yield was low, and the species characteristic of old cutover areas were absent. It seems to take some years before they are able to invade the areas in question or their mycelia attain the necessary vigor to produce sporophores. The decrease in the fruiting of forest species, on the other hand, took place abruptly, in the growing season immediately following cutting, even if not all the trees were cut. This is the normal consequence of heavy cutting (Melin 1925, p. 115, Romell 1930). Even the mushrooms found in the vicinity of aspen, which is inclined to sucker, are absent in the

Table 8. The occurrence of sporophores of various fungi in 1968 on the permanent sample plots.

Sample plot	*Paxillus involutus*	Boletes	*Amanita* spp.	*Rozites caperata*	*Cortinarius* spp.	*Russula* spp.	*Lactarius* spp.	Others	Total
	Kg/ha, fresh weight								
1 c_1	0.8	2.5	3.7	0.6	6.1	10.2	16.3	7.4	47.6
1 c_2	3.0	12.6	6.3	9.9	27.5	19.3	28.4	5.2	112.2
1 o	28.6	0	0	0	2.3	17.0	12.7	2.3	62.9
2 c	5.9	3.2	1.3	0.5	2.7	12.8	22.6	2.1	51.1
3 c_1	2.4	4.5	1.7	4.7	7.1	11.4	4.1	3.0	38.8
3 c_2	0.3	1.0	1.3	7.7	16.3	21.8	5.9	36.2	90.5
3 o	6.5	1.0	0.4	0.7	0	3.7	1.1	2.1	15.6
4 c	1.9	4.6	1.9	1.7	12.3	17.1	4.5	9.5	53.5
4 o_1	0	1.2	0.1	0	0.6	3.5	0.5	0.2	6.1
4 o_2	5.1	3.5	0	0	0	0	0.8	4.2	13.7
5 c	△	1.4	0.8	0.3	0.7	4.2	0.7	0.5	8.7
5 o	39.3	2.7	△	0.3	0.1	1.8	0.8	8.0	53.0
6 c	0	0	5.9	0.5	2.9	97.3	1.9	4.9	113.4
6 o	11.9	9.0	1.6	0	0.5	2.5	1.6	6.6	33.8
7 c	0	1.9	0.4	5.7	2.5	22.4	0.5	1.6	35.0
7 o	24.5	4.8	0.5	0	0.3	1.0	0.3	6.7	38.0
8 c	△	2.8	1.0	6.4	2.9	14.5	0.5	1.7	29.8
8 o	1.8	2.0	0.5	0	△	3.7	1.0	1.2	10.3
9 c	0	0	0	0	0.5	0.9	13.7	0.6	15.8
9 d	4.4	5.0	3.1	0	1.2	6.9	50.9	3.7	75.2
10 c	0	0	0	0	0.2	0	8.3	0	8.5
10 d	5.4	0	0.9	0	1.6	4.6	84.1	0.3	96.9
Average	4.1	2.7	1.2	2.3	3.7	11.4	4.7	4.7	34.8
Average in 1969	11.2	1.0	0.3	△	0.7	0.4	3.0	4.4	21.0

growing season immediately following cutting (Thesleff 1919, p. 75).

Dwarf-shrub pine swamp, on which the peatland sample plots were located, is a site type where few mushrooms grow in Finland (Tuomikoski 1960). The areas of this study had been drained nine years earlier, employing 40 m ditch spacing. Such draining produces only slow changes in the water and oxygen relations and the ground vegetation in the middle parts of the strips (Heikurainen 1955, Sarasto 1957, Lähde 1969). Correspondingly, the mushroom yield was negligible, although along the ditches, on the other hand, it was considerable. A similar difference could be observed for the species composition. This agrees with the general experience that the spoil banks and sides of ditches usually give high mushroom yields (e.g. Rautavaara 1947).

The results emphasize the effect of cutting on the fruiting of macrofungi. Although there were seedlings, suckers and even large trees in the cutover areas, the mushrooms that were dominant in closed stands, most of them obviously obligate symbionts, occurred in small numbers only, or were altogether absent. Saprophytic species increased to some extent after cutting. The greatest increase was observed in the species *Paxillus involutus*, *Laccaria laccata* and *Thelephora terrestris*, which have all been considered facultative symbionts.

Table 9. The occurrence of sporophores of various fungi in 1968 in different stand types of the permanent sample plots.

Genus, species	Ecologic type [1]	Closed stands	Cutover areas		Drained peatland	
			Young	Old	Strip	Ditch bank
		Kg/ha, fresh weight				
Paxillus involutus	m	1.1	1.3	22.2	0	5.0
Tylopilus felleus	m	0.6	0.5	2.6	0	0
Other Boletes	m	2.1	1.3	1.5	0	2.2
Hygrophoropsis aurantiaca	s	0.1	0.3	2.4	0	0
Polyporus ovinus	(m)	2.1	0	0	0	0
Hygrophorus spp.	(m)	0.6	0	△	0	0
Laccaria laccata	m	△	0	1.2	0	0.3
Clitocybe spp.	(m)	0.3	△	0.7	0	0
Tricholoma spp.	m	0.5	0	0	0	0
Amanita spp.	m	1.5	0.4	0.5	0	1.8
Stropharia hornemannii	s	△	0	0.3	0	0
Rozites caperata	(m)	3.2	0	0.2	0	0
Cortinarius spp.	m	5.3	0.2	0.2	0.3	1.4
Russula spp.	m	15.4	3.7	2.2	0.2	5.6
Lactarius spp.	m	5.4	0.9	1.2	9.7	69.7
Cantharellus cibarius	m	0.5	△	0.1	0	0
Hydnum repandum	(m)	0.1	0	0	0	0
Lycoperdon gemmatum	(m)	△	0	0.2	0	0
Others		1.1	0.5	1.3	0.2	1.4
Total		39.9	9.1	36.8	10.4	87.4

[1] m Proven symbiont
(m) Suspected symbiont
s Humus saprophyte

34. THE EFFECT OF ISOLATION FROM ROOTS ON THE FORMATION OF SPOROPHORES

341. Method and material

In addition to transect surveys, another method by which the relations between *Paxillus involutus* and trees were studied was by cutting tree roots extending to places where its sporophores had occurred and observing whether the fungus continued to fruit or not. Similar isolated plots have been used for studying the influence of root competition and shading on tree seedlings (see Aaltonen 1948) and on at least one occasion in a sporophore study (Romell 1938).

Root studies are needed to determine the depth to which it is necessary to extend the barrier. In Finland, in contrast to more southern countries, the average root depth is not great, only 4—10 cm (Kalela 1949, Heikurainen 1955). Among the main tree species, birch has the deepest root systems and spruce the shallowest, pine being intermediate (Laitakari 1927, 1934). The average root depth is maximal in till soils and minimal in peat. In peatlands, even when drained, roots that penetrate deeper than 20 cm into the soil are exceptional (Heikurainen 1955, 1958). In mineral soils deep roots are found in all soil classes, frequently at depths exceeding one meter (Laitakari 1927, 1934). As even such deep roots may be infected with mycorrhizal fungi (Werlich and Lyr 1957, Mikola and Laiho 1962), isolation with a trench surrounding the plot to be studied is somewhat unreliable.

In most cases the isolation was done from the sides only. A trench 20—40 cm wide was dug around the plot with a spade and a mattock. In this trench a polythene sheet, 0.09 mm thick, was arranged so that it reached from the bottom to the ground level. The trench was then filled in (see Figs. 7 and 8). In connection with digging, the presence of

Fig. 7. Isolated plot 1 in stand 7 o, *Vaccinium* type, 8 m². Trenching April 28, 1968, to a depth of 0.5 m. In 1968 three *Paxillus involutus* sporophores appeared on the inner margin of the trench but none in the center, in 1969 10 and 15, respectively.

roots was observed in order to determine the depth necessary for isolation. Usually, it seemed sufficient to dig 30—50 cm into the mineral soil, but on some occasions trenches had to be dug almost one meter deep.

Seventeen of the trenched plots were located on peat free from snags or on mineral soil undergoing paludification. In such places it is possible to cut all the roots present. The rest of the plots were on stoneless sand or fine sand. Here, isolation was not always complete, for examination carried out later revealed a few intact roots at depths between 0.5 and 1 m. Generally speaking, however, the great majority of the tree roots were cut. Thus, for instance, the ground vegetation became exceptionally rank in the growing season following isolation. This is what occurs when root competition is eliminated (see Aaltonen 1948).

How long such a plot remains isolated depends on the growth rate of the roots. During one growing season it may be more than a meter (Laitakari 1934), the average growth, however, being much less than this and deep roots growing slower than superficial ones (Sirén 1950). In the present study the roots that had been cut branched profusely and grew 20—100 cm during the two study years. Usually, this growth took place along the polythene sheets; on some occasions, however, new tips reached the isolated plots by growing under the edge of the sheet.

Some of the plots were isolated from below as well. Most of these were in banks of soil made by excavators. The usual procedure was as follows: a dead stump with roots and soil attached, and on which *Paxillus involutus* had fruited the previous year, was pried out from its site, which was covered with a polythene sheet 0.20 mm thick. The stump was then returned to its original position and, when required, soil shoveled around it.

Most of the other isolation plots, too, were located exactly where the species had fruited the previous year. But a few were set up without exact knowledge of the location of the sporophores. For the sake of comparison a few plots were also isolated in which the species had not fruited during at least the two previous years.

The size of the plots isolated from the sides only varied from 3 to 60 m², and that of those isolated from below as well from 1 to 4 m². Abreast with the plots isolated from trees, others were established in which trees or tree seedlings grew inside the trench (Table 10). Most of the isolation experiments were carried out in the same stands in which the oc-

Fig. 8. *Paxillus involutus* fruiting on the inner (left) and outer (right) margins of the trench. Isolated plot 13, *Vaccinium* type. Trenching May 6 to a depth of 0.5 m, photo Oct. 10, 1968.

Table 10. Information on the plots isolated from tree roots. Trenching and six isolations from below were made April 28 – July 11, 1968, the rest June 25 – July 20, 1969.

Locality	Stand [1]	Trenched plots				Plots isolated from below as well	
		Treeless		Stocked			
		Number	m^2	Number	m^2	Number	m^3
Suomusjärvi	1 c_1	—	—	—	—	10	2.7
	2 c	6	97	4	40	—	—
	6 o	3	34	—	—	1	0.3
	7 c	5	118	2	66	4	1.9
	7 o	5	71	5	39	2	1.3
	9 d	5	43	4	30	2	0.6
	10 d	4	25	2	18	5	1.3
Juupajoki		6	87	4	51	—	—
Total		34	475	21	244	24	8.1

[1] See Table 2

currence of *Paxillus involutus* sporophores was being studied (Table 2).

The plots were mainly isolated in the early summer of 1968, and the fruiting of fungi was studied on them during the growing season of the same and the following year. Observations were made three times a month except in Juupajoki, where the plots were visited only twice during the growing season. At each visit all mature and nearly mature sporophores were collected from the plots; in addition, digging was carried out in order to check that isolation was effective. The exact position of the sporophores was also recorded. For this purpose the trenched plots, including their immediate surroundings, were divided into the following parts: center, inner margin of trench, outer margin of trench and control belt. Both margins of the trench (together making the trench belt) were 0.5 m wide and the control belt extended three meters from the trench.

342. Sporophores of *Paxillus involutus*

Paxillus involutus fruited in the immediate vicinity of most of the isolated plots (Table 11). Thus, the location of these plots must be considered suitable. Furthermore, the species also fruited inside isolated plots that were treeless. The reason for this must have been either failure to secure complete isolation, or that the sporophores are able to grow without any connection with trees.

Closer examination of the data revealed that 36 sporophores of *P. involutus* appeared in the centers of treeless trenched plots in the summer of isolation. None of these plots, how-

Table 11. The frequency of *Paxillus involutus* sporophores on plots isolated from tree roots.

Location	Year of isolation	Following year
Trenched plots		
Control belt	15	26
Outer margin of trench	18	24
Inner margin of trench	17	25
Center	6	23
Number of plots	34	34
Plots isolated from below as well		
Control belt	20	5
Center	1	2
Number of plots	24	6

ever, was located in a waterlogged area, where root systems are shallow.

The following year (1969) the frequency of these sporophores was as great in the centers of the plots as around them. The number, 226, is significantly higher than that recorded in the year of isolation, even if allowance is made for the fact that in the stands in question there was a 1.6-fold increase in the number of these sporophores that year as compared with the preceding year. Assuming that these annual numbers follow the Poisson distribution (cf. Snedecor and Cochran 1967), we obtain

$$t = \frac{226 - 2.6 \cdot 36}{\sqrt{226 + 2.6 \cdot 36}} \sim 7.4^{***}.$$

Such an increase supports the assumption that these sporophores were connected with living tree roots. If that was so, it would explain why the sporophores appeared earlier on the trench belt than in the center, that is, farther away from the tree roots.

Paxillus involutus also fruited in three plots isolated from below as well. The total number of these sporophores was 55, and they were of normal size. In one of these plots two pieces of polythene sheeting had been used, and on this occasion mycorrhizae formed by *P. involutus* were abundant in the birch roots growing near the seam; in addition, thick rhizomorphs were seen to lead in the direction of the sporophores. In the other two plots there was a small hole in the polythene sheet, around which mycelium and rhizomorphs of *P. involutus* were observed. When these sheets were replaced by undamaged ones, the sporophores died and no new ones appeared, although the change could be effected without much damage.

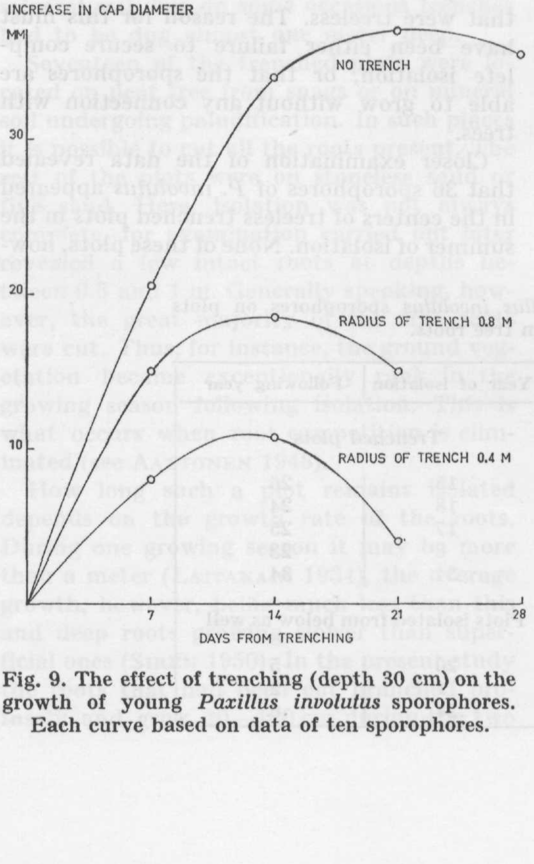

Fig. 9. The effect of trenching (depth 30 cm) on the growth of young *Paxillus involutus* sporophores. Each curve based on data of ten sporophores.

A great number of isolation trenches were also opened up again. Examination established that new roots had grown in abundance outside the polythene sheets. On many occasions only mycorrhizae formed by *P. involutus* were found, accompanied with abundant rhizomorphs (Fig. 25), which in many cases led into the isolated plots under the edges of the sheets. Thus, the trenches had not prevented *P. involutus* from forming rhizomorphal connections. This, in addition, took place astonishingly soon after isolation. For example, in a plot a sporophore was found ten weeks after it had been isolated to a depth of 80 cm. When the trench was opened, it could be seen that the yellow-brown rhizomorphs had grown along the surface of the sheet down to the very bottom of the trench. On this occasion the ground water table was rather far from the soil surface, it is true, and the sand used for filling the trench was more loosely packed than in natural soil. In waterlogged plots, growth beneath the barrier was less frequent.

Digging was also carried out in the center of isolation plots. Here, no living tree roots were found. Thus, the distance from *Paxillus involutus* sporophores to living tree roots was on many occasions at least 1—2 m. This probably need not be considered far for basidiomycetes forming rhizomorphs (cf. Harley 1968, p. 167). To check this, the connection between young sporophores and their surroundings was cut with a spade to a depth of about 30 cm. This measure was followed by arrest of the growth of the sporophores, which was the more distinct, the closer to them it was carried out (Fig. 9). At a distance of nearly one meter, sporophore growth remained almost undisturbed during the first few days; later, however, it decreased rapidly and evidently to the extent that energy supplies were exhausted. The results of this experiment demonstrate that in all probability *P. involutus* sporophores are regularly connected with mycorrhizae or mycelium located relatively far away.

Paxillus involutus fruited earlier on trenched plots than in the surrounding cutover areas ($\chi^2 = 16.9^{**}$). Growth into the latter areas, on the other hand, was earlier than into the corresponding closed stands (Fig. 4). The reason for this is probably to be sought in differences in the moisture conditions. For it was established with a few measurements that the water content of the soil was highest in the trenched plots and lowest in the closed stands; according to investigations made by others (see Aaltonen 1948, p. 372—375), this is generally the situation.

Comparison carried out on the occurrence of these sporophores in the control belts and in the trench belts (outer + inner margins) of the isolated plots revealed a certain rule. When they were lacking from the control belt, they were also sparse or absent in the trench belt, and, on the other hand, when they were abundant in the control belt, they were even more so in the trench belt (Fig. 10). Since, as stated on p. 23, the width of the trench belt was taken as one meter, the regression equation presented in Fig. 10 shows, as it stands, the correlation on an areal basis, too. It differs highly significantly ($t = 8.9^{***}$) from the equation that would have been obtained if the densities had been the same in the control belt and in the trench belt.

The average density of *Paxillus involutus* sporophores in the trench belt was about 7-fold that in the control belt (Table 12). This difference is still further emphasized by the fact that only one third of the width of the trench belt had been opened, and it was there that most of the sporophores were growing. As the result of isolation, the species thus began fruiting in the trench at a surprising rate. The question arises whether the trench, because of mechanical irritation, close contact to air or otherwise, gave the mycelia already present in the trench belt and its neighborhood an opportunity to start to fruit in the trench, or whether it also stimulated the mycelial growth of *Paxillus involutus*.

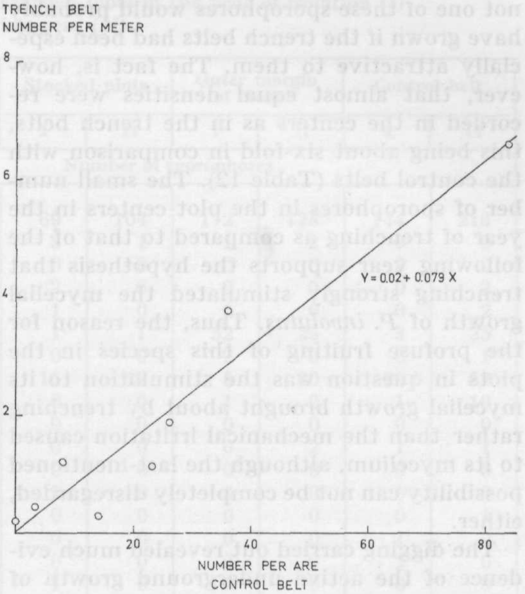

Fig. 10. The correlation between the abundance of *Paxillus involutus* sporophores in the trench belts and in the control belts in the year of isolation.

In the former case one would have expected many fewer sporophores in the control belts than outside them. This, however, was not the case. The ten trenched plots on sample plot 2 c had been selected at random and the average density of *P. involutus* sporophores in the control belts and in the plot 2 c was almost the same.

In the intact centers of the trenched plots

Table 12. The abundance of *Paxillus involutus* sporophores on plots isolated from tree roots.

Location	Area, m²	Year of trenching	Following year
		Number of sporophores per hectare	
Control belt	1 379	900	1 300
Outer margin of trench	200	6 800	7 900
Inner margin of trench	201	7 200	10 100
Center	274	1 300	8 200

not one of these sporophores would probably have grown if the trench belts had been especially attractive to them. The fact is, however, that almost equal densities were recorded in the centers as in the trench belts, this being about six-fold in comparison with the control belts (Table 12). The small number of sporophores in the plot centers in the year of trenching as compared to that of the following year supports the hypothesis that trenching strongly stimulated the mycelial growth of *P. involutus*. Thus, the reason for the profuse fruiting of this species in the plots in question was the stimulation to its mycelial growth brought about by trenching rather than the mechanical irritation caused to its mycelium, although the last-mentioned possibility can not be completely disregarded, either.

The digging carried out revealed much evidence of the active underground growth of *P. involutus*. Tree roots that had grown into the trenches bore abundant mycorrhizae formed by this species, sometimes even 50—100 per cent of the number of short roots. Mycelium and rhizomorphs, too, were abundant. Further, sporophores appeared on peat that had been moved from a trench onto unbroken ground about ten weeks earlier, and examination revealed that these pieces of peat were almost full of yellow-brown mycelium with strong connections down into the soil.

The results of these experiments are surprising. Although, generally speaking, isolation from living tree roots was successful, the density of *Paxillus involutus* sporophores was much greater in the isolated plots than in their surroundings. It appeared that the changes in the edaphic factors caused by the isolation process greatly promoted the growth of this species. On the other hand, isolation did not persist, the fungus being able to cross the barriers by growing under them. Thus, there is the possibility that all the sporophores in the isolated plots were in connection with living tree roots. This was impossible to confirm, but some indications in support of it were observed.

343. Sporophores of other macrofungi

In addition to *Paxillus involutus*, 217 sporophores representing 18 other species of mycorrhizal fungi were encountered in the immediate vicinity of the trenched plots in the year of isolation (Table 13). In the stocked plots the corresponding numbers were 59 and 7 and in the treeless ones 6 and 3. *Lactarius rufus* was the most frequent of these species. It was found in many plots, even those where only small pine seedlings were growing, but never in treeless ones. Likewise, *Laccaria laccata* was abundant in all except the treeless plots. Representatives of the genera *Boletus*, *Cortinarius* and *Russula*, too, were absent from the last-mentioned plots.

One year later 84 sporophores, representing eight species, were found on the treeless isolated plots. Thus there was a clear increase in comparison with the year of isolation. *Lactarius rufus* occurred in five of these plots, most of the sporophores growing just inside of the trench. Keeping in mind the slow growth rate of *L. rufus* (MODESS 1941) it can well be understood that its response to isolation was slower than that of *P. involutus*. It should be noted that, according to MODESS (op. cit., p. 87), *L. rufus* is a mycorrhiza-former of the second order, which means that it may be of minor importance as a symbiont in nature (cf. MELIN 1936, p. 1030).

In addition to *Paxillus involutus*, two further species that have been considered facultative were found growing on the isolated plots that were treeless, namely *Laccaria laccata* and *Xerocomus subtomentosus*. Near the sporophores of the latter, mycorrhizae were encountered to which the bright yellow rhizomorphs characteristic of the species were attached. Further, an experiment was made with *Thelephora terrestris*. Its sporophores were isolated from living tree roots with trenches 80 cm in depth, the diameter of the plots being 2 m. This resulted in the death of the sporophores. According to HACSKAYLO (1965), this also happens when the seedlings to which they are attached are shaded. In the light of these results, it seems that, in the ectomycorrhizal fungi that are considered facultative, sporophore formation takes place in more or less the same way as in obligate symbionts.

Only a few saprophytes were recorded. Since many of them have small sporophores, however, they tended to be overlooked. Such species were found not only in the trenched plots, but also in those that had been iso-

Table 13. The number of sporophores on trenched plots in the year of isolation (I) and the following year (II).

Genus, species	Ecologic type[1]	Treeless plots		Stocked plots		Outer margin of trench		Control belt	
		I	II	I	II	I	II	I	II
		Number of sporophores							
Paxillus involutus	m	181	430	69	104	172	178	144	218
Xerocomus subtomentosus	m	0	1	3	1	1	2	0	0
Tylopilus felleus	m	0	0	0	0	0	0	2	0
Leccinum scabrum	m	0	0	2	0	2	0	0	2
L. testaceo-scabrum	m	0	0	1	0	0	0	0	2
Hygrophoropsis aurantiaca	s	83	16	1	1	32	25	3	25
Hygrophorus sp.	(m)	0	0	0	0	2	0	0	1
Laccaria laccata	m	0	10	15	12	5	30	29	52
Clitocybe spp.	(m)	1	11	0	0	1	0	1	10
Armillariella mellea	w	0	6	0	0	0	0	0	0
Collybia butyracea	s	0	1	0	0	0	2	1	1
C. dryophila	s	2	0	0	0	0	0	0	0
Oudemansiella platyphylla	w	0	0	1	0	0	0	0	0
Amanita muscaria	m	0	0	0	0	0	0	0	1
A. porphyria	(m)	0	0	0	0	0	0	1	0
A. vaginata	(m)	0	0	0	0	0	0	2	0
Cystoderma amianthinum	s	0	0	1	0	0	0	0	0
Stropharia hornemannii	s	0	3	0	2	0	0	0	10
Hypholoma capnoides	w	0	46	0	8	2	35	1	57
Kuehneromyces mutabilis	w	0	0	0	0	0	0	50	0
Rozites caperata	(m)	0	0	0	0	0	0	2	0
Cortinarius spp.	m	0	12	2	0	5	2	2	2
Galerina spp.	s	0	5	0	1	4	2	0	0
Russula spp.	m	0	0	3	2	6	10	18	4
Lactarius rufus	m	0	39	33	44	90	77	33	122
L. spp.	m	4	0	0	3	6	0	9	1
Lycoperdon gemmatum	(m)	1	11	0	0	0	0	0	0
Area, m²		475	475	244	244	317	317	2 122	2 122

[1] m Proven symbiont
(m) Suspected symbiont
s Humus saprophyte
w Wood decomposer

lated from below as well. They were chiefly representatives of the genera *Armillariella, Mycena, Kuehneromyces* and *Galera.*

The results obtained from the isolation experiments help to explain the fruiting of *Paxillus involutus* in Finnish forests. Cutting, scarification and draining affect the soil in the same way as the isolations made and, consequently, they also stimulate the subterranean growth of the species, which leads to increased fruiting.

As compared to the trenching experiments carried out by Romell (1938), great similarities are seen in the results. In both studies sporophores appeared in great numbers in the trenches. With few exceptions mycorrhizal fungi did not fruit in the plots isolated from tree roots. Romell conducted his study in a closed stand on a poor site and, probably for this reason, *Paxillus involutus* was almost totally lacking from his material. In the fourth year after isolation, however, it formed a few sporophores in his plots, also. Romell (1939, p. 353) concluded that they were formed by saprophytic mycelia, but there remains the possibility that in his plots, too, rhizomorphal connections had formed by growth beneath the barrier. In the light of the present results, this even seems probable.

35. DISCUSSION

In closed forest stands ectomycorrhizal fungi fruit in abundance. In cutover areas, even if stocked with seedlings, suckers and mature trees, fewer sporophores are found and many of them belong to species which have been considered facultative. The question arises whether some symbionts require a more vigorous host than others.

Romell (1939, p. 354) stated that mycorrhizal fungi must be in connection with the roots of vigorous, mature trees in order to fruit. For *Paxillus involutus*, however, this is not the case. Sporophore formation is possible in this species even when the only hosts with which it can possibly be connected are small, stunted tree seedlings (p. 12). Similarly, *Lactarius rufus* fruited in trenched plots where only seedlings were growing. *Boletus communis* forms sporophores even in aseptic synthesis (Bryan and Zak 1961). Sporophore formation by mycorrhizal fungi is also common in forest nurseries and young plantations (e.g. Trappe and Strand 1969, Mikola 1970). Thus, although differences between species may exist, quite a number of ectomycorrhizal fungi can also fruit in association with relatively weak hosts. This emphasizes the importance of climatic and edaphic factors in their fruiting.

In *Paxillus involutus* the dependence of sporophore formation on climatic factors seems to be of minor importance. Fruiting is very common in cutover areas, but, on the other hand, it is not hindered by the shading of even dense tree stands. Thus, apart from host factors, it is primarily edaphic factors that regulate the occurrence of this mushroom.

Before further discussion, let us first recall that in closed stands in Finland the sporophores of *P. involutus* are most frequent on rich sites; on poor soil they are hardly found at all. After cutting, however, provided a few trees are left in the cutting area, the species fruits even on soils of the latter category. Sporophore formation is also stimulated when the site is scarified, drained or fertilized with nitrogen. An attempt was made to discover whether the sites of abundant fruiting have anything in common which is lacking from sites where fruiting of the species is sparse.

Poor sites with a closed tree stand are characterized by a very acid raw humus dominated by fungi, in which decomposition of organic matter is slow. Rich sites, on the other hand, are covered by less acid mull in which bacteria dominate; here decomposition of litter is active and nitrogen mobilization rapid (e.g. Hesselman 1926, Romell 1932, Aaltonen 1948).

Cutting brings great quantities of logging waste to the ground, roots die and those of the original microorganisms which can not adapt themselves to the changed conditions are destroyed. Thus, recently cut areas offer plenty of readily decomposable matter (Wittich 1952, Mikola 1954 b) and, in addition, better conditions for decomposition. As a result, nitrogen mobilization increases, the vegetation grows more luxuriant than before, the pH value of the soil rises and even raw humus takes on features of mull (e.g. Romell 1934, 1935, Aaltonen 1948; cf. Table 2).

As the result of scarification and draining the so-called true humus substances (Wittich 1952) are exposed. Decomposition of these substances is slow, but their high content of nitrogen (Viro 1962) makes rapid mobilization possible. In draining, in addition, the uppermost soil layer is covered with mineral soil, and this speeds up the decomposition of the organic matter involved (Wittich 1939).

On plots isolated from tree roots the ground vegetation becomes luxuriant, in the same way as in areas to which nitrogen has been applied (Romell 1938). Thus, nitrogen mobilization plays an important role in root isolation plots. Both measures are followed by an increase in the number of bacteria and other microorganisms which decompose organic matter, and this has an effect on the quantity and quality of the humus.

Dry summers are good *Paxillus* years (e.g. Tuomikoski 1959, Horak and Moser 1966). Despite this fact, *P. involutus* does not actually show any predilection for dry conditions. This is illustrated on p. 25. Moreover, in the dry summer of 1969 a few of its sporophores grew in the end of July, evidently supported by the spring moisture; the next did not appear until one month later, that is to say, two weeks after the rains had begun. Even now they occurred only on relatively moist habitats, particularly on the bottoms of ditches. In the end of September these sporophores were very abundant, although the crop of other mushrooms remained small. Probably during the dry spell large amounts of mycorrhizae, mycelia and soil organisms died. In this way material devoid of life but rich in nitrogen was formed. Because of its ability to grow out rapidly from mycorrhizae (p. 30), *Paxillus involutus* is probably superior to many other species in taking advantage of such a situation when conditions improve. The survival of the species is also promoted by the sclerotia (p. 31), which endure low humidity.

Thus, the conclusion can be drawn that *Paxillus involutus* fruits abundantly in places where decomposition of organic matter and nitrogen mobilization are relatively rapid. Such a situation may be caused by an increase in the amount of dead matter or improved conditions for decomposition. In such conditions bacterial populations increase, and in consequence of their activity the pH value of the soil usually rises (e.g. Romell 1934, Mikola 1954 b). Changes take place in the mobilization of other nutrients, as well as nitrogen, but no attention has been paid to them here, because they are not minimum factors in Finnish forest soils (Viro 1951) and because their application to the soil did not have any detectable influence on the fruiting of *P. involutus* (p. 15).

The response of *P. involutus* to the changes mentioned will be dealt with later. Only after the description of the pure culture experiments will it be possible to estimate their importance. In the present connection, it is enough to state that even favorable conditions for humus decomposition are not sufficient for the fruiting of the species unless trees are present. On the isolated plots its fruiting was abundant, it is true, but on each occasion where it could be established with certainty that mycorrhiza formation was precluded, no sporophores were found, even though conditions were favorable in other respects.

4. *PAXILLUS INVOLUTUS* IN PURE CULTURE

41. ISOLATION AND DESCRIPTION OF ISOLATES

411. Isolation

Quite a number of research workers, using the ordinary tissue culture method, have isolated *Paxillus involutus* from sporophóres (e.g. How 1942, Mikola 1955, Moser 1958 b, Pachlewski 1967 a, Shemakhanova 1967). This was easy to repeat in the present connection. If the sporophores are young and fresh, the species starts to grow from almost every seeding piece. Growth is visible as little as 1—2 days after plating on Hagem agar:

KH_2PO_4	0.5 g
NH_4Cl	0.5 »
$MgSO_4 \cdot 7\ H_2O$	0.5 »
$FeCl_3$ (1 % solution)	0.5 ml
Glucose	5.0 g
Malt extract	5.0 »
Agar	15.0 »
H_2O	1000 ml

Isolation of *P. filamentosus* is attended with some difficulty. In the present study it was less often successful, growth initiation required 1—2 weeks and the growth rate was slow (see Table 16).

From basidiospores *Paxillus involutus* has been isolated twice. On pure nutrient medium, however, their germination has not been successful, whether this be malt agar (Fries 1943) or the so-called »maximum nutrient medium» (Melin 1962). The former investigator used the yeast *Torulopsis sanguinea* as an activator, and the latter living pine roots. In this study germination was not attempted on agar, but basidiospores were placed in contact with the roots of nonmycorrhizal pine seedlings growing in autoclaved soil. From the fact that mycorrhizae were not formed, it was concluded that germination did not take place, for it has been established that monokaryotic mycelia, too, are able to form mycorrhizae (Fries 1942).

There are also certain difficulties in isolating symbionts from mycorrhizae. These include the problem of surface sterilization without destruction of the symbiont proper, as well as identification of the mycelia obtained. The latter is possible only in exceptional cases (Modess 1941, p. 10, Zak and Bryan 1963). In the present work, the characteristics of mycorrhizae formed by *Paxillus involutus* were studied by means of synthesis experiments (see p. 46). Mycorrhizae of similar appearance were then searched for near its sporophores. The identification of the mycelia isolated from the mycorrhizae was carried out by comparing them with strains isolated from sporophores. In addition, synthesis experiments were made.

Isolation of *Paxillus involutus* from mycorrhizae of different tree species met with success, and, indeed, was not even difficult. When clean, young, fresh mycorrhizae were plated without delay on Hagem agar, mycelium of *P. involutus* sometimes grew out within as little as 12 hours, and at latest in 24 hours (Table 14). Its growth was so rapid that other fungi were not able to hinder it in the beginning. Sometimes the species was able to get ahead so well that even pure cultures could be isolated from the edge of the colony. Other microorganisms were always present, however, and within 5—10 days after plating *P. involutus* was without exception overgrown by them.

If the mycorrhizae were rinsed with water, growth initiation of *P. involutus* was delayed to some extent. This was probably because they were left in a droplet of water which arrested oxygen uptake. The delay meant a decrease in the percentage of successful platings, because other microorganisms had more time to interfere.

When the same method of surface sterilization was used as that employed in isolating the symbiont from the ectendotrophic mycorrhiza of pine (Mikola 1965), *Paxillus involutus* was killed. Actually it did not even survive the separate ethanol and sublimate

Table 14. Isolation of *Paxillus involutus* from natural pine and birch mycorrhizae. Hagem agar, plating within 3 – 7 hrs after sampling.

Treatment	Number of mycorrhizae	Time to outgrowth, days	*Paxillus* colonies, per cent	
			Pure	Contaminated
None	1 000	1	0	66
Rinsing in water	1 000	1 – 2	0	57
70 % ethanol 5 sec.	200	5 – 10	3	6
60 sec.	200	—	0	0
0.1 % $HgCl_2$ 1 sec.	200	5 – 10	6	0
5 sec.	200	—	0	0
0.01 » 5 sec.	200	2 – 5	8	4
20 sec.	200	2 – 5	3	1
60 sec.	200	—	0	0
35 % H_2O_2 4 sec.	200	5	2	4
20 sec.	200	—	0	0

treatments included in the procedure which, in addition, must be regarded as relatively mild (cf. Pachlewski 1967 a). Only when the solutions were diluted to one tenth did the symbiont start to grow, and now in most cases as a pure culture. Growth initiation required 2—5 days, that is, longer than without sterilization. Even in this case, however, the species must be considered a rapidly emerging one. Other species of the same type include the formers of the C mycorrhiza (Melin 1936, p. 1021) and of the ectendotrophic mycorrhiza of pine (Mikola 1965). In some species growth initiation may require months (Melin, op. cit., p. 1025); some investigators have even rejected from their material all mycelia that have started growth in less than a week, considering them to be contaminants (Zak and Bryan 1963).

How (1942) also isolated *Paxillus involutus* from mycorrhizae. He used 0.1 % sublimate for surface sterilization. In the present study this treatment was found to be almost fatal, and so were those recommended by Pachlewski (1967 a) for similar mycorrhizae with a thin mantle. Thus, *P. involutus* must be regarded as sensitive to sterilizing agents.

Mycorrhizae formed by *P. involutus* are often connected with mycelial strands and rhizomorphs. Isolation of the species from these was not successful. However, it was not really tried either. It ought to be mentioned that certain other ectotrophic symbionts have been isolated from rhizomorphs, using both chemical sterilants (Levisohn 1955) and ultraviolet light (Shemakhanova 1967, p. 24).

In the vicinity of *Paxillus involutus* mycorrhizae sclerotia, too, were found, often in much the same numbers as mycorrhizae. More detailed examinations revealed that some of them were attached to mycelial strands connected with the mycorrhizae. These sclerotia were brown in color, round in shape and small in size (Fig. 24). Their mean diameter was 340 μ, but the size ranged from 100 to 800 μ. Their rind, unlike that of the sclerotia of *Cenococcum*, was thin and flexible, the inner parts being filled with pseudoparenchyma (Fig. 12). It was possible to isolate *P. involutus* from these sclerotia. Like the mycorrhizae, they, too, were only able to withstand weak surface sterilization. In addition, if they were submerged, however little, in the agar, the mycelium did not start to grow. This was also true of the mycorrhizae.

412. Description of isolates

A total of 46 isolates of *Paxillus involutus* were retained. Most of these were from sporophores, some, however, being from mycorrhizae and sclerotia (Table 15).

The sporophores came from tree stands of different kinds and from plots isolated from tree roots. The identity of the isolates obtained from them is indisputable. As stated earlier, they were used for comparison in the identification of other isolates. These comparisons revealed neither macroscopic nor microscopic differences. Only some uncertain anastomoses were detected but the same was the case

Table 15. *Paxillus* strains used in this study, isolated in the fall of 1968 if not otherwise mentioned in the list on the next page.

Species	Source of isolate	Number of strains
P. involutus	Sporophore	40
	Sclerotium	2
	Pine mycorrhiza	1
	Spruce »	1
	Birch »	1
	Aspen »	1
P. filamentosus	Sporophore	6
	Alder mycorrhiza	5

when sporophore strains were grown side by side. Thus, the inclination of the species to anastomose must be regarded as very small. The identification was ascertained by means of synthesis experiments. On the basis of these studies, the isolates retained from mycorrhizae and sclerotia, too, must be considered to belong to the species *Paxillus involutus*.

For the sake of comparison some isolates were also retained from sporophores of *P. filamentosus* as well as from mycorrhizae of gray alder (*Alnus incana* (L.) Moench) in their immediate surroundings. These were all similar between each other, and they also seemed, on the basis of rough microscopic examination, to be similar to those of *P. involutus*. As regards their symbiotic ability and growth rate, on the other hand, they were different.

The thickness of the aerial hyphae of *Paxillus involutus* isolates on Hagem agar ranged from 2 to 10 μ, being on an average 5 μ. The hyphae have septa and numerous clamp connections (Fig. 11). The amount of aerial mycelium was profuse. In most isolates it was erect and formed thin strands, while the rest of them formed a low velvety mat without strands. The color of the mycelium was yellow-brown, although variation occurred in this respect, too (Fig. 23). Similar descriptions have been given by How (1942) and Mikola (1955).

The amount of submerged mycelium was very small on agar, but in liquid culture it was more abundant. The hyphae were twisted and relatively thin (2—7 μ), but clamp connections were abundant in this case, too.

The isolates did not form chlamydospores or any other mycelial spores, but only sclerotia. In the velvety type of mycelium, however, sclerotia were not formed. The formation of sclerotia commenced with an accumulation of hyphae. This took place either on the surface of the agar or above it. First, profuse branching took place and the hyphae grew thicker, and before long the inner parts of this formation had changed into a pseudoparenchyma (Fig. 12). The rind remained thin and flexible. The earliest fully developed

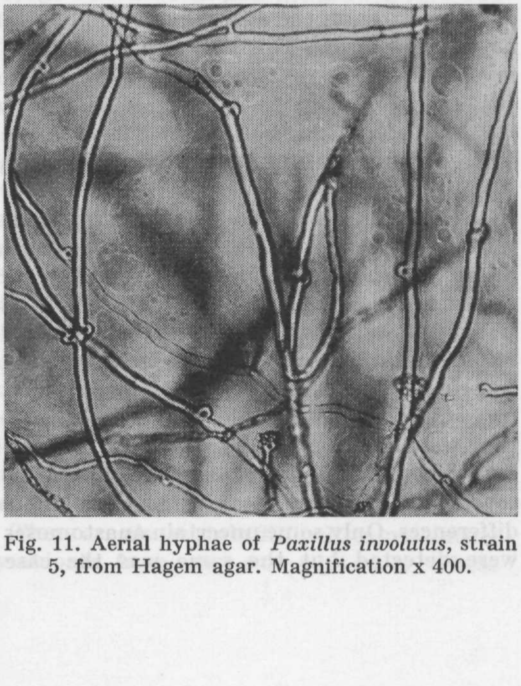

Fig. 11. Aerial hyphae of *Paxillus involutus*, strain 5, from Hagem agar. Magnification x 400.

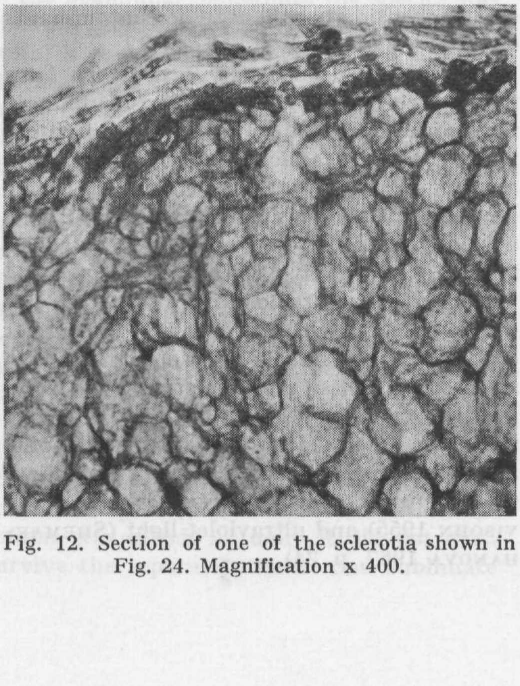

Fig. 12. Section of one of the sclerotia shown in Fig. 24. Magnification x 400.

sclerotia, which were similar to those encountered in nature, were found as little as one month after seeding. In agar dishes incubated for three months at a temperature of 15° C, sclerotia were formed in exceptionally large numbers, up to 200 per cm^2.

42. EXPERIMENTAL METHODS

The physiology of mycorrhizal fungi has been studied to quite a large extent, in particular by Melin (e.g. 1925, 1946, 1955, 1963) and his school (e.g. Modess 1941, Björkman 1942, Fries 1943, Norkrans 1950). In all their studies a similar methodology has been employed. The same methodology was also used in the present investigation, although with the modifications introduced by Mikola (1948) in his study of *Cenococcum*.

The basal solution[1], a medium also used by Mikola, was employed in most of the experiments, as such or modified. The chemicals were mainly products of Fluka, Merck or British Drug Houses and the quality usually Pro Analysi. The media were autoclaved (20 minutes at 120° C) after preparation. On most occasions 100-ml erlenmeyer flasks were used with 25 ml of nutrient solution, on which the inoculum material, which had been grown on Hagem agar, was placed floating. The cultures were incubated in darkness at room temperature (24—26° C). The duration of the incubation was usually 26—28 days. Exceptions and other details are stated in connection with each experiment.

Nine strains of *Paxillus involutus*, presented in the list below and in Fig. 23, were chosen for the pure culture studies. An attempt was made to take strains differing in age and mycorrhiza formation. In some of the experiments additional strains were included, together with representatives of other mycorrhizal fungi, humus saprophytes and even pathogens. The species of these three latter groups had all been isolated a few years earlier by Dr Mikola for the collection of the Department of Silviculture, University of Helsinki.

These fungi are cultured on Hagem agar and transferred once a month. The growth of the *Paxillus* strains was followed during the period in which most of the pure culture experiments were made. These observations mostly revealed no great changes (Table 16). Some strains, however, showed great changes in growth rate and were uncertain in growth initiation. This has also been observed earlier (Mikola 1954 a, Moser 1958 a), and is particularly apt to occur in old colonies. Therefore, young colonies are recommended as inoculum when mycorrhizal fungi are cultured (Moser 1962).

In the present work the age of the inoculum was 10—20 days, and only the edge of the colonies was used. It is nevertheless obvious that differences in the quality of the inoculum

List of the main strains of *Paxillus involutus* studied in pure culture. Isolation by the author from Suomusjärvi in fall 1968 if not otherwise stated.

Strain 1. Isolated by Dr Mikola from sporophore growing on ditch bank, spruce swamp, Juupajoki, fall 1960. Originally active symbiont, but in 1967 lost the ability to form mycorrhizae in semiaseptic synthesis.

Strain 3. Isolated from sporophore growing in cutting area, *Vaccinium* type, fall 1966. Originally nonmycorrhizal.

Strain 4. Isolated from sporophore growing on ditch bank, *Myrtillus* type, fall 1966. Originally nonmycorrhizal.

Strain 5. Isolated by Dr Mikola from sporophore growing in mixed stand, Vihti, fall 1966. Active symbiont.

Strain 7. Isolated from sporophore growing on roadside, *Calluna* type, fall 1966. Originally active symbiont, but in 1968 lost the ability to form mycorrhizae in semiaseptic synthesis.

Strain 8. Isolated from sclerotium, dwarf-shrub pine swamp. Active symbiont.

Strain 12. Isolated from sporophore growing in plot isolated from tree roots. Active symbiont.

Strain 18. Isolated from sporophore growing in plot isolated from tree roots. Active symbiont.

Strain 24. Isolated from sporophore growing in cutting area, *Oxalis-Myrtillus* type. Originally nonmycorrhizal.

[1]

Glucose	20	g
NH_4 tartrate	5	»
KH_2PO_4	1	»
$MgSO_4 \cdot 7\ H_2O$	0.5	»
$FeCl_3$ (1 % solution)	0.5	ml
$ZnSO_4 \cdot 7\ H_2O$	0.5	»
$MnSO_4 \cdot 4\ H_2O$	0.5	»
$CaCl_2$ (0.1 M solution)	5	»
Thiamine	50	γ
H_2O	ad 1000	ml

Table 16. Radial growth of *Paxillus* strains used in this study. Hagem slants, winter of 1968—69.

Species	Strain	Month						
		XI	XII	I	II	III	IV	V
		Growth, mm in 12 days						
P. involutus	Strains in Fig. 23	12	14	12	17	17	13	13
	Other strains	10	13	12	13	17	14	10
P. filamentosus	All strains	1	2	3	3	3	1	2

caused considerable differences in growth between both various strains and various experiments.

The homogeneity of the pieces used as inoculum can be improved by growing them for a short time on agar and selecting those which show the most even start (NORKRANS 1950, p. 12) or by using larger pieces. In the present study rather large discs, 5 mm in diameter, were used. They were removed with a sterile cork borer. These discs worked well, but their weight (0.8 mg dry matter) caused inaccuracy in the results when growth was small. On these occasions particular emphasis was placed on ocular inspection of the cultures.

In similar studies six replicates have been usual (LINDEBERG 1944, MIKOLA 1948, NORKRANS 1950). Considering that differences between strains are often great (e.g. MODESS 1941, MIKOLA 1948, 1957, MELIN 1959 b, MOSER 1959)), as many strains as possible were included in the present study, even at the expense of the replicates (5 in number). The results are given only as means. Presentation of standard errors was not considered worth while, because of the great differences between strains and because repeated experiments of these kinds, even with the same strain, are apt to give results which differ significantly from each other (cf. MODESS 1941, p. 31).

43. RELATION TO ENVIROMENTAL FACTORS

431. Hydrogen ion concentration

The relation of *Paxillus involutus* to the pH of the substrate was studied, using the method presented by MODESS (1941, p. 96—97). Fig. 13 shows the results obtained. The fungus grew well, and, consequently, as the quantity of nutrient solution in each flask was only 25 ml, the pH changed considerably in certain cases.

Optimum initial pH values from 3.1 to 6.4 were recorded for the strains included. The species was capable of growth over the whole range studied, that is, from pH 2.2 to 7.8. Correspondingly, the range of good growth was also wide, from pH 2.7 to 6.4. The differences between the various strains in their relation to pH were very great. However, this is not uncommon. The same phenomenon is known in many other species of fungi (e.g. MIKOLA 1957).

Pigmentation varied to a considerable extent in this experiment. It was clearly dependent on the pH, reaching its maximum around pH 6.0—6.5. The heaviest pigmentation was recorded for strain 18 (Fig. 14).

The optimum pH value of mycorrhizal fungi is usually rather low, and this is especially true of species growing in raw humus. For *Cenococcum graniforme*, for example, it is about pH 4.0 and for *Corticium bicolor* [1] even lower (MIKOLA 1948, 1962).

The pH optimum of most of the mycorrhizal fungi studied by SHEMAKHANOVA (1967) lay between pH 3.7 and 4.1, and this held for *Paxillus involutus*, too. For the species studied by MELIN (1925) the corresponding value was between pH 4 and 5, while, according to MIKOLA (1965), it was slightly more than pH 5 for the symbiont isolated from the ectendotrophic mycorrhiza of pine. MODESS's (1941) material included several species with optimum pH values exceeding 5.5.

Comparison of the results cited and those obtained for *Paxillus involutus* reveals that

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[1] *Corticium bicolor* is believed to be the species which forms the bright yellow mycorrhiza of raw humus (MELIN 1959 a, MIKOLA 1962)

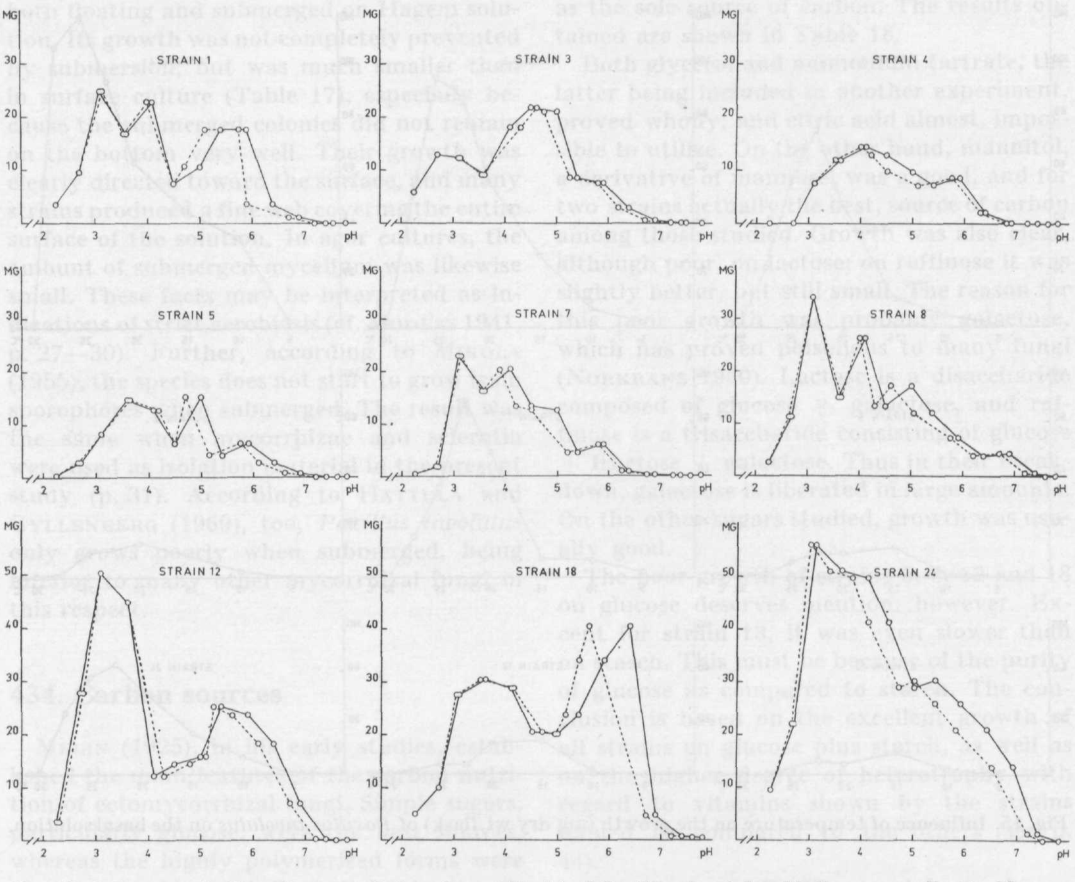

Fig. 13. Influence of pH on the growth (mg dry wt/flask) of *Paxillus involutus*. Medium according to MODESS (1941, p. 96–97). O——O Initial pH. O·········O Final pH.

strains of the latter between them cover the entire range of variation in all the species cited. Some strains grow well over a wide pH range; in this respect they resemble some litter-decomposing species (e.g. LINDEBERG 1944, HINTIKKA 1960 b) and even *Mycelium radicis atrovirens*, which is not at all susceptible to variation in the pH of its substrate (MELIN 1925, MIKOLA 1965).

432. Temperature

The lowest temperature at which culture of *Paxillus involutus* was attempted was 5° C.

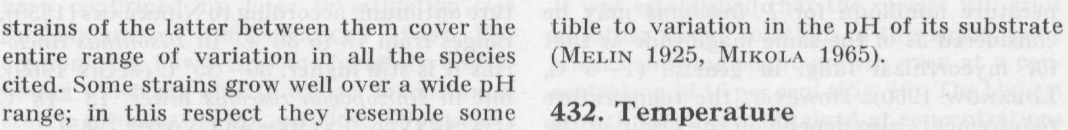

Fig. 14. Representative flasks of *Paxillus involutus*, strain 18, at the termination of the pH experiment. Initial pH from left 2.2, 3.1, 4.1, 5.0, 5.9, 6.4, and 6.9. Note heavy pigmentation and high yield in pH 6.4.

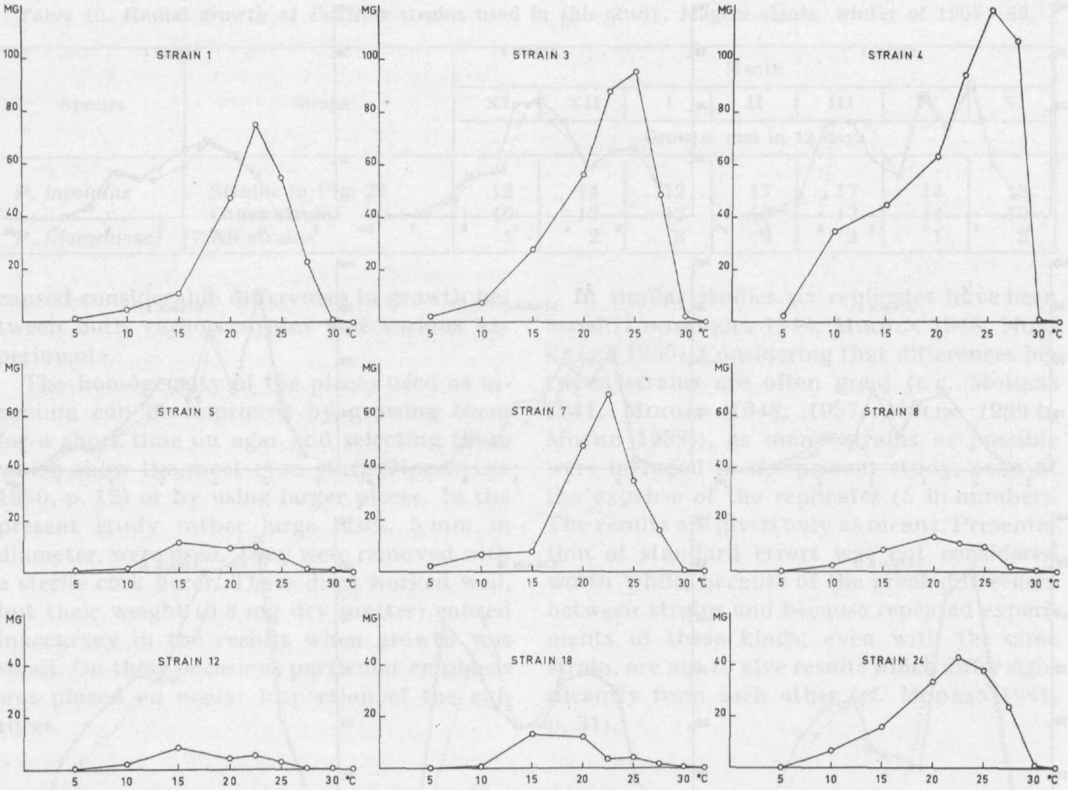

Fig. 15. Influence of temperature on the growth (mg dry wt/flask) of *Paxillus involutus* on the basal solution.

All nine strains did grow at this temperature, although slowly (Fig. 15). Thus, the temperature minimum for *P. involutus* may be considered as of the same magnitude as that for mycorrhizal fungi in general (1—5° C, Lobanow 1960). However, the temperature requirements also depend on the origin of the strains in question. According to Moser (1958 a), the temperature minimum for a *Paxillus involutus* strain isolated from a valley was 2—8° C, whereas that of a strain isolated from a mountain range was —2 to 4° C.

The temperature maximum was about 30° C. All but one of the strains grew at least slightly at this temperature, and even that one was not killed. At 32° C, however, all strains died.

The temperature optimum ranged from 15 to 25° C. For other mycorrhizal fungi similar optimum values have been obtained: i.e., 25° C for *Boletus* species (Melin 1925) and *Cenococcum graniforme* and slightly less for *Amanita* and *Lactarius* (Mikola 1948). In the mycorrhizal *Tricholoma* species the temperature optimum, according to Norkrans (1950), ranges from 18 to 30° C. In *Pisolithus tinctorius* it is still higher, 30—35° C (Marx 1969), but in *Rhizopogon roseolus* lower, 13—18° C (Hacskaylo, Palmer and Vozzo 1965).

The optimum temperature of *Paxillus involutus* is much higher than the temperatures normally prevailing in the soil during the growing season. This holds for soil fungi in general, and even for psycrophilic species (Hintikka 1964). Consequently, the changes taking place in the soil temperature after cutting, for example, probably do not essentially influence the relations of mycorrhizal fungi.

433. Oxygen

In order to illustrate the relation of *Paxillus involutus* to oxygen, the species was grown both floating and submerged on Hagem solution. Its growth was not completely prevented by submersion, but was much smaller than in surface culture (Table 17), especially because the submerged colonies did not remain on the bottom very well. Their growth was clearly directed toward the surface, and many strains produced a fine web covering the entire surface of the solution. In agar cultures, the amount of submerged mycelium was likewise small. These facts may be interpreted as indications of strict aerobiosis (cf. Modess 1941, p. 27—30). Further, according to Mikola (1955), the species does not start to grow from sporophores when submerged. The result was the same when mycorrhizae and sclerotia were used as isolation material in the present study (p. 31). According to Hattula and Gyllenberg (1969), too, *Paxillus involutus* only grows poorly when submerged, being inferior to many other mycorrhizal fungi in this respect.

434. Carbon sources

Melin (1925), in his early studies, established the main features of the carbon nutrition of ectomycorrhizal fungi. Simple sugars, particularly glucose, were the best sources, whereas the highly polymerized forms were almost (starch) or totally (cellulose) unavailable. Generally speaking, these results have been confirmed by later investigation (see Shemakhanova 1967, Harley 1969). Exceptions are, however, some facultative symbionts which are able to utilize, for instance, starch (Mikola 1948), cellulose (Norkrans 1950) or lignin (Lindeberg 1948).

The carbon nutrition of *Paxillus involutus* does not seem to have been directly studied previously. In the present connection quite a few experiments were made on the subject. The fungus was grown, for instance, on the basal solution with various carbon compounds as the sole source of carbon. The results obtained are shown in Table 18.

Both glycerol and ammonium tartrate, the latter being included in another experiment, proved wholly, and citric acid almost, impossible to utilize. On the other hand, mannitol, a derivative of mannose, was a good, and for two strains actually the best, source of carbon among those studied. Growth was also clear, although poor, on lactose; on raffinose it was slightly better, but still small. The reason for this poor growth was probably galactose, which has proved poisonous to many fungi (Norkrans 1950). Lactose is a disaccharide composed of glucose + galactose, and raffinose is a trisaccharide consisting of glucose + fructose + galactose. Thus in their breakdown, galactose is liberated in large amounts. On the other sugars studied, growth was usually good.

The poor growth of strains 5, 8, 12 and 18 on glucose deserves mention, however. Except for strain 18, it was even slower than on starch. This must be because of the purity of glucose as compared to starch. The conclusion is based on the excellent growth of all strains on glucose plus starch, as well as on the higher degree of heterotrophy with regard to vitamins shown by the strains named as compared to the others (see p. 44).

The relation of *Paxillus involutus* to glucose was studied in two additional experiments. It was established that the species will grow in rather strong solutions of this sugar, some strains showing slight growth even at a concentration of 16 per cent (Fig. 16). The highest growth rates were obtained at concentrations of 1—2 per cent, and without differences between the strains tested.

At a glucose concentration of 0.5 % *Paxillus involutus* and a few other mycorrhizal fungi were allowed to consume all the sugar. From these experiments the following economic coefficients were established:

Table 17. Superficial and submerged growth of *Paxillus involutus*. Hagem solution, 50 ml/flask.

Inoculum	pH		Strain									Mean
	Initial	Final	1	3	4	5	7	8	12	18	24	
			Growth, mg dry wt per flask									
Floating	4.8	2.3—2.6	79	54	67	38	46	41	47	60	79	56.7
Submerged	4.8	2.8—3.8	19	14	5	14	11	14	15	19	12	13.7

Table 18. The growth of *Paxillus involutus* on certain carbon compounds. Basal solution with 2 g $(NH_4)_2SO_4$ instead of NH_4 tartrate. Carbon compounds were in each case given in amounts corresponding to 2 % glucose solution.

Carbon compound	pH		Strain									
	Initial	Final	1	3	4	5	7	8	12	18	24	Mean
			Growth, mg dry wt per flask									
None	4.3	4.2—4.3	△	△	△	△	△	△	△	△	△	0.1
Glycerol	4.5	4.1—4.3	1	△	△	△	△	△	△	△	△	0.3
Mannitol	4.4	2.1—4.0	5	1	45	46	66	29	52	36	86	40.6
Citric acid + Na citrate	4.8	4.5—4.6	1	1	6	1	4	2	1	2	6	2.6
Glucose	4.0	2.2—3.2	51	31	88	5	39	4	11	15	50	32.8
Sucrose	4.1	2.1—2.5	43	34	55	48	58	30	38	64	72	49.1
Lactose	4.4	3.9—4.2	1	1	1	1	1	5	2	1	1	1.6
Maltose	4.2	2.1—3.1	19	16	51	38	27	32	36	60	81	39.9
Raffinose	4.2	3.0—4.1	3	4	3	1	8	11	2	4	4	4.2
Dextrin	4.4	2.3—3.2	10	9	12	23	10	24	27	41	26	20.1
Starch	4.2	2.7—3.2	22	17	18	10	27	11	18	13	19	17.1
½ Glucose + ½ starch	4.1	2.1—2.6	91	35	67	31	52	44	48	74	99	60.2
Cellulose	4.2	4.3—4.4	0	0	0	0	0	0	0	0	0	0

Suillus bovinus	0.35
S. luteus	0.22
S. variegatus	0.26
Paxillus filamentosus	0.31
P. involutus 1	0.29
P. involutus 3	0.28
P. involutus 4	0.27
P. involutus 5	0.29
P. involutus 7	0.26
P. involutus 8	0.29
P. involutus 12	0.30
P. involutus 18	0.26
P. involutus 24	0.30
Laccaria laccata	0.42
Amanita muscaria	0.32
Tricholoma flavobrunneum	0.34
Corticium bicolor[1]	0.24
Genococcum graniforme	0.45
Ectendotrophic symbiont[2]	0.28

The economic coefficient of *Paxillus involutus* is of the same magnitude as that of many other mycorrhizal fungi as well as of litter-decomposing species (e.g. MIKOLA 1956); i.e., slightly more than one fourth of the glucose consumed is converted into mycelium.

The growth of *Paxillus involutus* on starch proved to be good. On dextrin, a hydrolysis product of starch, it was still better. In another experiment, in which the species was allowed to utilize all the starch, the growth obtained was 23 mg/100 mg. The starch was not natural, however, but so-called soluble, partially depolymerized starch prepared by acid treatment (cf. COCHRANE 1958).

In a further experiment the carbon source was casein hydrolyzate. All strains grew on it, although slowly and only producing about 5 mg/100 mg.

In the experiment presented in Table 18, cellulose was given as a piece of filter paper. According to NORKRANS (1950, p. 66), however, this is not the best way of studying the ability of mycorrhizal fungi to decompose cellulose. For this reason another experiment was carried, out, employing her method. The filter paper (Munktell No. 3) was macerated in a Bühler homogenizer into a fiber suspension, which was then rinsed in distilled water. After drying at 60° C, 125 mg of the matter was weighed out per flask, either with starter glucose (0.5 g/l) or alone. Otherwise, the medium was that mentioned in Table 18.

When starter glucose had not been provided, only a few hyphae grew out of the inoculum discs. When starter glucose had been given, on the other hand, growth began at a good rate, and judging by eye, just as well as on the control medium without any cellulose. But when the sugar had been used up, the growth of *Paxillus involutus* stopped even in the flasks containing cellulose. On some occasions continued growth was suspected, but dry weight determinations did not confirm this; the amount of dry matter present corresponded exactly to the quantity of cellulose originally supplied plus the growth made possible by the starter glucose.

In some cases growth was measured by determining the total nitrogen content of the

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[1] Isolated from the bright yellow mycorrhiza of raw humus (see MIKOLA 1962)

[2] Isolated from the ectendotrophic mycorrhiza typical of nurseries (see MIKOLA 1965, LAIHO 1965)

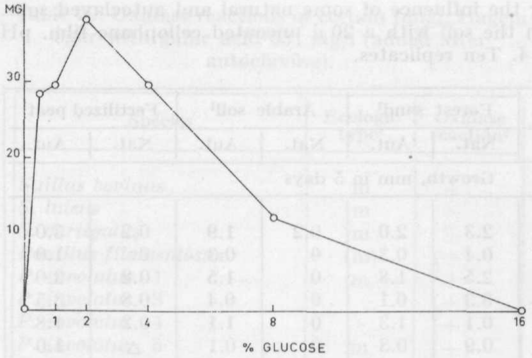

Fig. 16. Influence of glucose concentration on the growth (mg dry wt/flask) of *Paxillus involutus* (the usual 9 strains). Basal solution with varying glucose concentrations and with 0.5 ml of 1 % Fe citrate instead of $FeCl_3$.

dry matter (cf. Norkrans 1950, p. 61), but this method, too, gave no indication of any growth of *P. involutus* on cellulose during the four months that the experiment lasted. The symbionts mentioned on p. 38 were also included in this experiment, and with similar results.

When cellobiose was used as a source of carbon, the growth of *Paxillus involutus* (25 mg/100 mg) was almost as good as on glucose. This shows that the structural unit of cellulose is available to the species, but its polymers are not. Thus, for instance, tylose (a hydrolysis product of cellulose, soluble in water and containing about 100 units of cellobiose) could not be utilized either alone or when given with starter glucose. This was also the case with Na carboxymethylcellulose.

Correspondingly, the ability of *Paxillus involutus* to grow on lignin was also studied. The lignin experimented with was Li lignosulfonate, which had been prepared from birch wood at the Keskuslaboratorio Oy and had a molecular weight of about 40 000. One gram per liter of this lignin was added to the medium mentioned in Table 18, either alone or with starter glucose. Lignin did not interfere with the utilization of starter glucose, but when the latter had been consumed, growth did not continue during the four months covered by the experiment. The same result was also obtained for the other symbionts mentioned on the preceding page.

The ability of a symbiont to grow saprophytically in forest humus can also be studied directly. The humus used has to be sterilized, and this is usually done in an autoclave. Heating, however, changes the natural properties of humus, and autoclaved forest humus has often proved extremely poisonous to mycorrhizal fungi (e.g. Melin 1925, 1946, Shemakhanova 1967).

These changes can be evaluated by the method presented by Molin (1957), for instance. The humus sample to be studied is placed in the lid of a petri dish. On the sample an uncoated cellophane film is placed, which in turn is covered with a layer of agar onto which a test fungus is transferred. Last, the culture is covered with the inverted bottom part of the dish. The humus is now in diffusive contact with the agar until microorganisms grow through the cellophane film, which takes 3—5 days.

An example of the results obtained in such experiments is given in Table 19. All the species studied grew on natural forest soil, but not on arable soil or fertilized peat. On the last-mentioned substrates, without exception, growth was better after autoclaving, whereas on forest humus the result was different for different species. Thus *Tricholoma flavobrunneum* regularly throve better on autoclaved soil, but *Paxillus involutus* did not show any growth on forest humus that had been autoclaved, although it grew very well on it if it was untreated.

The above results emphasize the need to ascertain whether the humus which is studied as the carbon source of mycorrhizal fungi contains inhibitory substances or not. This can be done either by the cellophane method or by growing the fungi on autoclaved humus reinforced with glucose and mineral nutrients. For this purpose the usual nine *Paxillus involutus* strains were transferred to autoclaved birch leaves moistened with the nutrient solution mentioned in Table 18 (0.5 g/l of glucose). No growth occurred. Consequently, the leaves must have contained some inhibitory substances, which had to be removed. This can be done, for instance, either by growing certain saprophytes and then killing them at a relatively low temperature or by extracting (Melin 1934, 1946). After extraction, one of the strains started to grow on these leaves even without nutrient solution, and with the solution seven did so. Little growth occurred, however, and the leaves did not lose weight during the four

Table 19. Radial growth of certain symbionts under the influence of some natural and autoclaved soils. Inoculation on a 2×2 cm^2 agar plate isolated from the soil with a 20 μ uncoated cellophane film. pH adjusted to level 4. Ten replicates.

Species	Agar	Forest humus[1]		Forest sand[1]		Arable soil[1]		Fertilized peat[1]	
		Nat.	Aut.	Nat.	Aut.	Nat.	Aut.	Nat.	Aut.
		Growth, mm in 5 days							
Suillus bovinus	3.0	0.9	1.0	2.3	2.0	0.2	1.9	0.2	3.0
S. luteus	0.5	0.1	△	0.4	0.3	0	0.3	0	1.0
S. variegatus	4.0	0.7	0.8	2.5	1.8	0	1.5	0.2	2.0
Paxillus involutus 1	6.0	1.4	0	3.3	0.1	0	0.4	0.8	3.5
Tricholoma flavobrunneum	2.2	△	1.1	0.1	1.3	0	1.1	0.2	1.8
Amanita muscaria	2.0	0.6	△	0.9	0.3	0	0.1	△	1.0
Corticium bicolor	0.3	△	△	0.3	0.1	0	0	0	0.3
Cenococcum graniforme	0.5	0.2	0.4	0.2	0	0	0.3	0	0.4
Ectendotrophic symbiont	2.1	0.3	0.1	0.8	0.3	0	1.6	0.2	1.8

[1] See Table 27

months of the experiment. In extraction, soluble carbon compounds are lost, and this, of course, renders the material less able to support growth. If, on the other hand, *P. involutus* starts to grow on unextracted material, it can grow quite well. Thus, in an experiment carried out by Mikola (1954 a), it caused a 6 % decrease in the weight of birch leaves within two months.

In the present work, too, the species grew on unextracted birch leaves when these were added as the sole source of carbon (5 g/l) to the nutrient solution mentioned in Table 18. Apart from submerged mycelium, all the strains grew at a rate of 1—2 mg/flask (25 ml of solution) during four months. On decayed spruce wood (the same from which the mycorrhizae seen in Fig. 26 were taken), 74 % of which was lignin, the corresponding growth was 0.5—1 mg. This limited growth and the unchanged color of the substrate, the latter fact also having been observed by Mikola (1954 a), indicate that *Paxillus involutus* did not decompose cellulose or lignin in these tests either.

In culturing inoculation material, it was observed that *Paxillus involutus* grows well on peat moistened with glucose solution. This had already been noticed by Moser (1958 c). According to Shemakhanova (1967, p. 102), *P. involutus* decomposes peat in the presence of glucose, but neither the rate of decomposition nor the compounds attacked are specified. She reports that *Suillus bovinus*, *S. luteus* and *Xerocomus subtomentosus* have the same ability.

On gallic acid agar, the last-mentioned species as well as some other symbionts have been demonstrated to give a positive reaction (Lindeberg 1948), i.e., a dark oxidation zone is formed around the colonies because of the phenoloxidases released. When Lindeberg's method was used in the present connection, all the *Paxillus involutus* strains studied showed a slight positive reaction (Table 20). According to Sundman (1965, p. 29), however, it is probable that the energy-liberating metabolism of lignin is dependent on enzymes other than phenoloxidases. Thus, it remains uncertain whether the positive reaction obtained in this so-called Bavendamm experiment is a sufficient indication of the ability of the species to decompose lignin.

The ability of ectomycorrhizal fungi to decompose complex carbon compounds other than lignin has also been studied by means of enzymatic methods. Norkrans (1950) found indications of high cellulase activity in *Tricholoma fumosum* and of weaker activity in other mycorrhizal *Tricholoma* species. She presented the hypothesis that the difference in cellulase formation between mycorrhizal and litter-decomposing fungi is quantitative rather than qualitative.

Experiments by Lyr (1963 b) point to the same situation in regard to some other enzymes. He observed that many ectomycorrhizal fungi secrete cellulase, xylase, amylase and proteinase. In wood-decomposers the activity of these enzymes is high and in mycorrhizal fungi low, but still real, on certain occasions even reaching the level found in litter-decom-

Table 20. Oxidase reactions of certain fungi. Hagem agar with gallic acid 851 mg/l (added after autoclaving).

Species	Ecologic type[1]	Oxidase reaction[2]
Suillus bovinus	m	+
S. luteus	m	–
S. variegatus	m	+
Paxillus filamentosus	(m)	(+)
P. involutus 1	m	+
P. involutus 3		(+)
P. involutus 4		(+)
P. involutus 5	m	(+)
P. involutus 7	m	+
P. involutus 8	m	+
P. involutus 12	m	+
P. involutus 18	m	(+)
P. involutus 24		+
Laccaria laccata	m	–
Tricholoma flavobrunneum	m	–
Amanita muscaria	m	–
Corticium bicolor	m	(+)
Cenococcum graniforme	m	+
Ectendotrophic symbiont	m	–
Hygrophoropsis aurantiaca	s	+
Collybia butyracea	s	+++
C. dryophila	s	++
Marasmius androsaceus	s	+++
M. perforans	s	++
Stropharia hornemannii	s	++
Pholiota carbonaria	s	++
Clavaria ligula	s	+++
Mycelium radicis atrovirens	r	++
Paxillus atrotomentosus	w	+
Armillariella mellea	w	+++
Hypholoma capnoides	w	+++
H. sublateritium	w	+++
Fomes annosus	w	+++
Stereum sanguinolentum	w	+

[1] m Proven symbiont
(m) Suspected symbiont
s Humus saprophyte
r Root colonizer
w Wood decomposer

[2] – No reaction
(+) Very slight reaction
\+ Slight reaction
++ Moderate reaction
+++ Strong reaction

posers. Further, as suggested by Lyr, it may be that the mycelia growing in soil have a higher enzymatic activity than those growing on artificial media; on the other hand, the species from which pure cultures have been obtained may primarily represent facultative symbionts.

Quite recently, Lundeberg (1970) studied the enzymatic properties of a number of mycorrhizal fungi, including *Paxillus involutus*. The latter did not form any significant amounts of cellulase, pectinase, laccase or proteinase. Thus, it is safe to say that the species is a typical ectomycorrhizal fungus in respect of its carbon nutrition. Nevertheless, like *Cenococcum graniforme* (Mikola 1948), it may be relatively good at utilizing such carbohydrates as cellobiose and starch. In comparison with *Mycelium radicis atrovirens* (Schelling 1950) its abilities are limited and the difference from saprophytic humus fungi is extremely clear. Many species of this category decompose both cellulose and lignin (e.g. Lindeberg 1944, Mikola 1956, Hintikka 1960 a, b), while *Paxillus involutus* can not break down either. Owing to the versatile and heterogeneous organic composition of litter and forest humus, however, the species may nevertheless be able to live saprophytically in forest soil.

435. Nitrogen sources

The ability of the various ectomycorrhizal fungi to utilize different nitrogen sources is relatively good. For the species studied by Melin (1925), inorganic ammonium salts were a good source of nitrogen, nitrates being inferior to them to varying degrees. Nucleic acids, too, could be broken down, as well as peptones and asparagine, whereas most amino acids were more or less unsuitable. Proteolytic enzymes were also shown to be formed. Generally speaking, the results of later research follow similar lines (see Harley 1969). It may be mentioned, however, that many mycorrhizal *Tricholoma* species completely lack the ability to utilize nitrates (Norkrans 1950).

The ability of *Paxillus involutus* to utilize various sources of nitrogen was tested by growing the species on basal solution with different nitrogen sources. The experiments showed that both ammonium and nitrate ions can serve as the sole source of nitrogen (Table 21). Ammonium tartrate was slightly superior to inorganic ammonium salts; this is probably mainly due to the fact that the pH of the latter fell rapidly to a level unfavorable for growth. On urea, growth evidently suffered from too high a pH. In addition, the colonies were dense, with folded surfaces, i.e. different from those normally found at the same pH. The same phenomenon has

Table 21. The growth of *Paxillus involutus* on certain nitrogen compounds. Basal solution with 0.35 g KH_2PO_4 and 0.15 g K_2HPO_4. One third of the amount of water was replaced by a buffer mixture containing 299 ml M/15 KH_2PO_4 and 1 ml 0.1 N HCl. In each case nitrogen compounds were given to correspond to 0.3 g nitrogen per liter.

Nitrogen compound	pH		Strain									Mean
	Initial	Final	1	3	4	5	7	8	12	18	24	
			Growth, mg dry wt per flask									
None	4.9	4.8–4.9	△	1	△	△	△	1	1	1	△	0.6
KNO_3	4.9	4.7–5.7	13	25	33	6	23	25	27	14	42	23.0
NH_4NO_3	4.8	2.3–3.4	44	26	84	13	26	15	18	8	17	27.7
$(NH_4)_2SO_4$	4.8	2.2–3.4	46	35	108	12	10	29	29	62	61	43.5
NH_4 tartrate	5.2	2.8–4.3	54	47	145	25	18	23	77	46	51	54.0
Aspartic acid	4.9	4.4–4.9	△	△	8	3	2	6	3	1	4	2.9
Glycine	4.8	4.5–4.8	1	4	10	1	1	3	1	1	2	2.7
Nucleic acid	4.7	3.9–4.5	7	10	9	14	5	7	10	6	12	8.9
Urea	6.3	5.9–6.3	23	15	5	10	29	11	5	10	1	12.1
Casein hydrolyzate	5.1	3.2–4.9	73	22	108	76	28	80	78	52	56	63.7
Peptone	5.0	3.3–4.1	47	45	95	48	73	69	51	45	78	61.3

been observed in the case of *Cenococcum graniforme* (Mikola 1948). For the mycorrhizal *Tricholoma* species, on the other hand, urea is a good source of nitrogen, for some species even the best (Norkrans 1950). On nucleic acid *Paxillus involutus* showed distinct, but slow, growth. On casein hydrolyzate and peptone its growth was very good, but not much better than on the inorganic nitrogen sources studied. On amines, Lundeberg (1970) has shown that the species grows poorly, and on nitrite not at all.

According to Hattula (1968), the amino acid composition of *Paxillus involutus* sporophores is quite rich; at least 22 amino acids are included. According to the same author, these amino acids are also found in mycelium grown with ammonium ion as the sole source of nitrogen. Thus, the species is able to synthesize a considerable number of amino acids. On the other hand, its ability to split them is perhaps not very good, because its growth on amino acids was slow (Table 22). The only exceptions were arginine, on which growth was good, and glutamic acid, on which it was excellent. In the latter case this is easy to understand, because it is believed that assimilation of ammonium begins with the formation of glutamic acid (Cochrane 1958, p. 246).

Table 22. The growth of *Paxillus involutus* on certain nitrogen compounds. Basal solution with nitrogen compounds in each case to correspond to 0.15 g nitrogen per liter.

Nitrogen compound	pH		Strain									Mean
	Initial	Final	1	3	4	5	7	8	12	18	24	
			Growth, mg dry wt per flask									
None	3.9	3.4–3.9	△	1	2	2	2	△	1	2	2	1.4
Leucine	4.2	3.5–3.9	2	4	6	3	3	4	2	2	4	3.4
Tyrosine	4.1	3.7–3.9	△	0	3	2	2	2	1	1	2	1.5
Glutamic acid	4.3	3.9–5.5	59	72	61	31	61	27	53	40	112	57.4
Proline	4.0	4.0–4.1	1	2	1	2	1	△	1	2	2	1.2
Valine	4.1	3.4–4.0	6	12	5	3	3	2	2	2	6	4.4
Arginine	4.0	2.6–3.5	44	68	79	16	63	9	16	31	79	44.8
Histidine	4.1	3.6–3.9	2	2	4	2	2	3	2	2	2	2.5
Lysine	4.0	3.8–4.0	3	4	2	2	1	△	2	2	1	1.9
All the above amino acids [1]	4.2	3.2–4.5	41	11	97	47	58	33	35	25	87	48.2
Casein hydrolyzate	4.0	2.9–3.4	43	39	63	47	45	55	46	37	54	47.6
$(NH_4)_2SO_4$	4.0	2.2–2.6	50	20	72	24	34	25	23	48	57	39.0

[1] In approximately the same proportion as found in casein, see Mikola 1948, p. 31

On the whole, the ability of *Paxillus involutus* to use various nitrogen sources is similar to that of other ectomycorrhizal fungi (cf. Lundeberg 1970). Minor differences, of course, do exist. Thus, for instance, its growth on amino acids is more limited than that of *Cenococcum graniforme* (Mikola 1948) and, further, relatively weak on casein hydrolyzate as compared to ammonium ion.

In their relation to nitrogen, in contrast to their carbon nutrition, ectomycorrhizal fungi are much like other soil fungi. Thus, for instance, many litter-decomposing species grow only weakly, or not at all, on nitrate (e.g. Lindeberg 1944, Norkrans 1950). The ability of these fungi to utilize organically bound soil nitrogen was quite recently studied by Lundeberg (1970). This nitrogen, which is very tightly bound (cf. Wittich 1952, Russell 1961, p. 289), was unavailable to half of the litter-decomposers studied. On the other hand, none of the mycorrhizal fungi tested succeeded in releasing it from humus agar. In joint culture with pine seedlings on raw humus, however, a few of the *Suillus* isolates produced a net mineralization of the bound humus nitrogen. The two *Paxillus involutus* strains included in the study failed to do so in both experiments. Thus the species does not release nitrogen from raw humus, but otherwise its ability to utilize various nitrogen sources is good. In this light the distinct concentration of its sporophores in places where nitrogen mobilization is active is readily understandable.

436. Vitamins

The relation of *Paxillus involutus* to vitamins was studied on the basal solution (p. 33) from which thiamine was excluded. The species grew slightly even in this control solution (Table 23). This was probably due to vitamins transferred with the inoculum discs, although they had been raised on agar without added vitamins. In addition, they were smaller (0.5 mg) than those normally used. Growth began without delay after inoculation, but soon stopped.

Of the ten vitamins studied, thiamine caused a clear response. In all its combinations, the growth of *Paxillus involutus* was about 10-fold that obtained in the controls. The remaining nine vitamins only occasionally enhanced the growth. This experiment shows that the ability of *P. involutus* to synthesize the vitamins it requires is usually good; only for thiamine is it clearly heterotrophic.

Table 23. The influence of certain vitamins on the growth of *Paxillus involutus*. Basal solution without thiamine. Inoculum raised on Hagem agar lacking thiamine and malt extract.

Added per flask	pH		Strain									Mean
	Initial	Final	1	3	4	5	7	8	12	18	24	
			Growth, mg dry wt per flask									
None	4.3	4.2—4.3	3	4	4	3	2	3	△	△	2	2.3
Thiamine 1 γ	4.3	3.0—4.3	38	25	117	5	38	13	1	10	16	29.2
Lactoflavine 1 γ	4.3	4.1—4.3	4	4	4	2	2	3	△	△	3	2.5
Choline 15 γ	4.3	4.1—4.3	3	4	3	1	2	5	1	5	2	2.9
Niacin 1 γ	4.3	4.1—4.3	3	4	4	1	1	3	1	2	1	2.3
Adermin 1γ	4.3	4.1—4.3	4	4	4	1	1	3	2	3	2	2.7
Biotin 10 mγ	4.3	4.1—4.3	3	4	4	1	2	3	1	1	1	2.3
β-alanine 1 γ	4.3	4.0—4.3	5	7	7	3	2	10	2	2	4	4.6
Inositol 1 mg	4.3	4.2—4.3	4	4	3	2	2	2	1	2	2	2.4
Ca pantothenate 1 γ	4.3	4.0—4.3	4	6	21	3	3	3	2	1	4	5.0
Folic acid 1 γ	4.3	4.1—4.4	5	3	3	1	9	2	1	1	3	3.2
Thiamine + biotin	4.3	3.2—4.3	40	50	102	2	30	4	7	13	7	28.2
Thiamine + choline	4.3	3.2—4.2	41	46	112	6	19	12	8	25	12	31.2
Thiamine + niacin	4.3	3.2—4.3	34	51	110	8	47	8	3	2	8	30.1
Biotin + choline + niacin	4.3	4.1—4.3	4	3	4	1	6	3	2	2	3	3.0
Thiamine + biotin + choline + niacin	4.3	3.5—4.3	44	31	77	5	33	7	5	6	11	24.5
All the above vitamins	4.3	3.2—4.2	51	31	101	10	22	45	12	6	16	32.4

Closer examination, however, reveals slight heterotrophy with regard to other vitamins, too. The growth of strains 5, 8 and 18, and especially of strain 12, when only thiamine had been applied, was much inferior to that obtained when the other nine vitamins had also been added. The relatively great heterotrophy of these strains with regard to vitamins is also seen in other experiments. When glucose was used as carbon source and the medium included no other organic substances, the growth of the four strains mentioned was poor (Fig. 15, Table 18). On the other hand, on Hagem agar, which includes malt extract, they grew as well as the other strains (Fig. 23, Table 17). It deserves note that the strains in question were very active mycorrhiza-formers.

According to MOSER (1958 c), *Paxillus involutus* requires thiamine and probably also biotin from the substrate. According to SHEMAKHANOVA (1967, p. 115), the species does not synthesize thiamine, but does form biotin, niacin, pantothenic acid and pyridoxine. These results are in good agreement with those obtained in the present work.

Of the other mycorrhizal fungi, *Cenococcum graniforme* seems to be heterotrophic to a higher degree than *Paxillus involutus*; many of the vitamins listed in Table 23 promote its growth even alone, especially in the case of slow-growing strains (MIKOLA 1948). With respect to thiamine, on the other hand, *Cenococcum* is to some extent autotrophic, although its ability to synthesize this vitamin depends, for instance, on the availability of oxygen.

Correspondingly, all the other mycorrhizal fungi studied have shown either partial or complete heterotrophy with respect to thiamine. In various connections heterotrophy has been indicated, in addition, with regard to pantothenic acid, niacin, biotin and inositol (see MELIN 1953, 1962).

Heterotrophy with respect to thiamine is characteristic of the whole order Agaricales (COCHRANE 1958). Thus, for example, the *Marasmius* species that decompose litter are heterotrophic with regard to thiamine and *M. androsaceus* to biotin as well (LINDEBERG 1944). Many wood-decaying fungi, too, for instance *Fomes annosus*, are completely heterotrophic in respect to thiamine (FRIES 1938). On the other hand, the imperfect fungus *Mycelium radicis atrovirens*, which frequently lives on tree roots, is autotrophic in its relation to thiamine (MELIN and NORKRANS 1942).

437. Other fungi

The vigor of *Paxillus involutus* in relation to other species of fungi was studied by growing it with them in pairs on Hagem agar in the same petri dish. The couple to be studied was transferred simultaneously, and the inoculum discs were placed at a distance of 20 mm from each other. The nine strains of *P. involutus* (p. 33) were tested against the fungi mentioned in Table 20.

In comparison with other mycorrhizal fungi, *Paxillus involutus* was usually the more vigorous. *Suillus* species restricted its growth efficiently, however. *Cenococcum graniforme* and *Tricholoma flavobrunneum* actually stimulated the growth of *Paxillus involutus*, and it continued unimpeded over their colonies, whereupon their own growth came to an end. *Corticium bicolor*, on the other hand, continued growth even when overgrown by *P. involutus*.

When grown on extract of *Paxillus involutus* mycelium, *Cenococcum graniforme* has previously been shown to suffer to some extent (MIKOLA 1948). This latter species, like many other mycorrhizal fungi, forms antibiotics (SANTORO and CASIDA 1962, KRYWOLAP, GRAND and CASIDA 1964), but it seems that *P. involutus* is not susceptible to them.

In the presence of litter-decomposing basidiomycetes the growth of *Paxillus involutus* was weak. Only the slow-growing *Stropharia hornemannii* presented no obstacle to its growth. It was most severely limited by *Hygrophoropsis aurantiaca*, *Collybia dryophila* and *Marasmius perforans*. In addition, the two species of *Collybia* studied changed the yellow-brown color of the *P. involutus* mycelium to white.

When grown with *M. r. atrovirens*, *P. involutus* usually spread rapidly over the entire surface of the agar. This, however, did not prevent the *atrovirens* mycelium from doing the same.

Among the species growing on wood, *Fomes annosus* and *Stereum sanguinolentum* seemed strongly to limit the growth of *Paxillus* *involutus*. On some occasions, however, a zone of inhibition remained between the colonies and, therefore, *P. involutus* may be able to protect tree roots from *Fomes annosus* in the same way as *Suillus variegatus* (Rypáček 1963) and *S. bovinus* (Hyppel 1968). In any case, the mantle protects the roots mechanically (Zak 1964). The rest of the species proved equal in vigor to *P. involutus*, except *Armillariella mellea*, over colonies of which it was able to grow.

Of the strains of *Paxillus involutus*, those that grew rapidly were better able to overpower competitors than those that grew slowly. Probably none of them secretes any notable amounts of toxic substances or antibiotics; this conclusion is based on the fact that inhibitory zones between the colonies were very infrequent. On the other hand, the species does not seem to be susceptible to substances secreted by other species of fungi, either.

5. ON THE BEHAVIOR OF *PAXILLUS INVOLUTUS* AS A MYCORRHIZAL SYMBIONT

51. METHODS AND MATERIAL

511. Synthesis experiments

For many years, aseptic synthesis continued to be employed in exactly the form in which it was developed by Melin (1936). Recently, however, it has been considerably modified. Various substrates and nutrient solutions have been used (e.g. Hacskaylo 1953, Lundeberg 1963, Shemakhanova 1967), and the tops of the seedlings kept in the open air (e.g. Trappe 1967 b). Moreover, synthesis is successful even in large test tubes and on plain water agar without added nutrients (Pachlewski 1967 b).

After preliminary trials, aseptic syntheses were made in 200 × 32 mm test tubes. The tubes were filled with 50 g of 0.5—2 mm quartz sand and 33 ml of the nutrient solution described below[1], i.e., the solution used by Mikola (1948). Half of it was placed in a small glass cylinder and thus there was no need to open the tubes during the experiments. This arrangement left plenty of room for vertical growth of the roots and the fungus. The seed was sterilized in 35 % H_2O_2 for ten minutes (cf. Trappe 1961) and germinated on water agar. Inoculation was performed from colonies grown on Hagem agar. To ensure that the inoculum would take, the tubes were kept in darkness for the first ten days, and when brought back into the light, were shaded with aluminum foil. The tubes were kept in a glass greenhouse without artificial light, and were cooled with water, 16—22° C, when necessary.

The mycorrhizal nature of a fungus can be ascertained only with aseptic synthesis (e. g. Modess 1941, Melin 1953). All the 46 strains of *Paxillus involutus* isolated in the present study were tested with regard to their relations to pine, and some of them with respect to other tree species, too. Aseptic synthesis was also used to study the influence of light conditions as well as of the concentration of sugar and of nitrogen on the formation of these mycorrhizae. In this experiment the procedure followed was that of Björkman (1942).

Aseptic synthesis has also been used to measure the symbiotic efficiency of fungi (e.g. Marx and Zak 1965). In the present study the aim was to discover whether, in symbiosis with pine seedlings, *P. involutus* exhibits any parasitic features.

Semiaseptic synthesis requires elimination of contamination through other mycorrhizal fungi. Otherwise this synthesis is easy to carry out, thus making extensive experiments possible; certain modifications of the method can even be used in nurseries (see Mikola 1970).

The results of this synthesis, however, are unpredictable. Certain species take, others do not. In the present work only three of nine mycorrhizal fungi were successfully inoculated (Table 24). The ectendotrophic symbiont of pine has already been shown to be reliable as an inoculum (Mikola 1965, Laiho 1965), and now *Corticium bicolor* and *Paxillus involutus* were found to be similar to it. The other six species did not take at all, although the soils used contained relatively small amounts of growth inhibitors (Table 19). Neither did they take from inoculum raised on humus. Aseptic synthesis, on the other hand, is normally successful on these species, and in nature they very likely are active mycorrhiza formers.

As *Paxillus involutus* proved suitable for

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[1]

Glucose	2.5	g
$(NH_4)_2SO_4$	0.25	»
KH_2PO_4	0.5	»
$CaCl_2$	0.05	»
$MgSO_4 \cdot 7\ H_2O$	0.15	»
NaCl	0.025	»
$(NH_4)_2HPO_4$	0.025	»
Fe^{III} citrate (1 % solution)	1.2	ml
Thiamine	25	γ
H_2O	1000	ml

Table 24. Taking of inoculations from agar in semiaseptic synthesis. Pine seedlings, 3″ clay pots, soils forest sand and fertilized peat (see Table 27).

Species	Number of inoculations attempted	Takes, per cent
Suillus bovinus	303	0
S. luteus	310	0
S. variegatus	315	0
Paxillus involutus 1	620	39
Tricholoma flavobrunneum	193	0
Amanita muscaria	100	0
Corticium bicolor	331	24
Cenococcum graniforme	225	0
Ectendotrophic symbiont	128	57

use in semiaseptic syntheses, the aims striven for in such experiments were the same as in aseptic syntheses. Initially, the seedlings were grown in autoclaved soil. They were not inoculated until at least two months old in order that they should be susceptible to mycorrhizal infection (Laiho and Mikola 1964). For inoculum, colonies grown on Hagem agar were used. On a few occasions, living seedlings with mycorrhizae formed by *P. involutus* were also used.

Inoculation could be successfully done into the pots in which the seedlings had been raised (Fig. 17). On such occasions the soil contained abundant microorganisms. Usually the seedlings were raised in boxes and then potted into recently autoclaved soil. The roots (rinsed and cut to a length of 8 cm) were placed against the wall of the pot and the inoculum against the roots. This is illustrated in Fig. 17. In order to discover whether contamination occurred through mycorrhizal fungi, some pots were not inoculated. All the pots were kept in the greenhouse and watered daily. In some cases mantles formed by *Paxillus involutus* were observed as little as four days after inoculation. Later, on many occasions, infection reached practically all the short roots as well as a considerable proportion of the long roots.

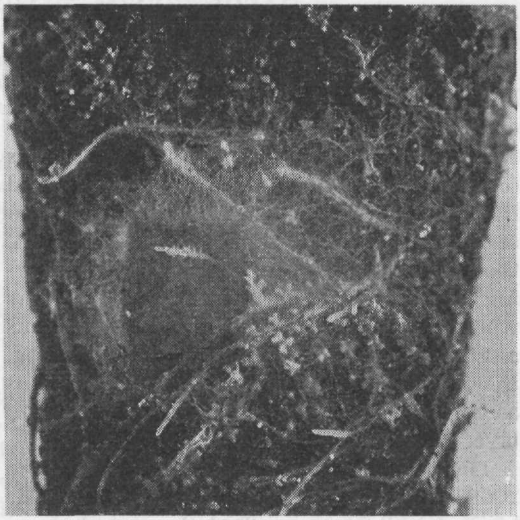

Fig. 17. Typical view (pot removed) of semiaseptic synthesis between *Paxillus involutus* and pine. Seeding Feb. 29 on autoclaved soil, inoculation from Hagem agar with strain 5 April 29, photo May 10, 1968. Note inoculum with outgrowing hyphae and a number of mature mycorrhizae. Magnification x 2.

512. Planting experiments

The planting experiments were carried out in order to find out to what extent *Paxillus involutus* survives on roots of tree seedlings planted out on different sites and how far the seedlings themselves develop. For this purpose pine seed was sown at the end of February on autoclaved fertilized peat in the greenhouse. Inoculation was done at the end of April, when the seedlings were transferred from boxes into 3″ clay pots, 10 seedlings to each. The intention had been to inoculate with a few other symbionts, in addition to *P. involutus*, but owing to their poor success (Table 24) and because of the slow development of *Corticium* mycorrhizae, they were not included in this study. As similar experiments with seedlings inoculated with the ectendotrophic symbiont of pine have previously been carried out (Laiho 1967, Mikola 1967), only two kinds of control seedlings were included here, viz. nonmycorrhizal ones and those inoculated with humus from *Myrtillus* type.

The seedlings were planted out 4—6 weeks after inoculation. The planting sites comprised closed stands, cutover areas and areas treated with prescribed burning, all of which were of *Vaccinium* or *Myrtillus* type, as well as arable land of medium quality. In all of these soils mycorrhizal fungi occurred naturally. Thus, the influence of *Paxillus* mycorrhizae on the development of the seedlings could not be studied here without interference from other symbionts.

These experiments were carried out in 11 locations. The seedlings were planted in blocks, each comprising 15 squares. The squares were randomized between the three treatments (classes of seedlings), which were replicated five times. Each replicate consisted of 2—5 seedlings planted with a spacing of 10 cm, the distance to the seedlings of the neighboring squares being 50 cm. In this way the whole block was planted in as homogeneous a soil as possible. In connection with planting, the ground vegetation and part of the humus as well were removed and the topmost soil layer was scarified.

The planting was carried out in 1968 and 1969. In the former year two blocks of the kind described were planted on each location and in the latter year one. Their total number was thus 33, and each treatment was represented by 450 seedlings. In addition, a total of 200 pine seedlings inoculated with *Paxillus involutus* and a similar number of nonmycorrhizal seedlings were planted in sites differing from those mentioned, especially on dry pine heaths.

Two thirds of the seedlings were removed from the soil after one growing season, and the rest of them after two growing seasons. The seedlings of a block were lifted at one time. In this connection the dry weight of the seedlings was determined and observations made on their root systems. The latter will be described in the next section.

513. Root studies

In mycorrhizal studies it is customary to count the number of short roots on root samples (e.g. MELIN 1927, HEIKURAINEN 1955, MIKOLA and LAIHO 1962). Each individual tip of a branched short root is counted (HATCH 1937, BJÖRKMAN 1942, SHEMAKHANOVA 1967). Even this does not give accurate quantitative results, however, because mycorrhizae are of different sizes (e.g. A and B mycorrhizae, MELIN 1927). In addition, long roots are also usually infected (BJÖRKMAN 1942, ROBERTSON 1954, MIKOLA 1965, WILCOX 1967), and this infection is quite impossible to count accurately.

Accurate or not, counting always is a time-consuming procedure. On the other hand, *Paxillus* mycorrhizae do not keep their color well and the chances of recognizing them rapidly diminish. Therefore, they must be examined promptly. Methods requiring lengthy rinsing (e.g. HEIKURAINEN 1955) or sifting (e.g. MARKS, DITCHBURN and FOSTER 1967) make identification difficult. Actually, identification is most reliable if the roots are cleaned on the spot by gentle shaking. With this procedure, only the relative numbers of different short roots can be determined. The checkings carried out indicated that even a rapid estimate gives fairly accurate results when made by the same experienced observer on all occasions.

In brief, the root studies reported here were done in the following ways:

Aseptic experiments: Seedlings were examined under a binocular microscope at magnifications of 4—20. The numbers of short roots and of *Paxillus* mycorrhizae were counted.

Semiaseptic experiments: Seedlings were examined under the microscope as before, except that in some cases a hand lens was used on the spot in the greenhouse. Usually the short roots were not counted but the frequency of *Paxillus* mycorrhizae was estimated as a percentage of the number of short roots.

Test plantings: The proportion of *Paxillus* mycorrhizae at the time of planting was estimated as above as an average for each block. When these experiments were discontinued, a similar estimate was made for each transplant. Usually, this was made on the spot, although the seedlings were afterwards brought to the laboratory for other determinations.

Stands: From each permanent sample plot 20—50 seedlings of about the same size were lifted at random, using a planting mattock. On other occasions only a few samples were taken. When seedlings were not present, thin long roots of mature trees were sampled. The samples were cleaned by shaking them gently, and the relative number of *Paxillus* mycorrhizae, if any, was estimated. The procedure used for identification of these mycorrhizae is described on p. 51.

Some samples were studied in the laboratory in greater detail. Observations were made, for example, on the structure of the mantle as well as on the occurrence of rhizomorphs and sclerotia. Representative parts of the root systems were fixed in Karpetchenko-Navashin's solution (JOHANSEN 1940), embedded in paraffin, sectioned at 7 μ and stained with safranin-fast green double staining (MIKOLA and PERSIDSKY 1951). In all, about 500 short roots, 50 sclerotia and 20 rhizomorphs were sectioned.

52. DESCRIPTION OF *PAXILLUS* MYCORRHIZAE

It was established from the syntheses made that *Paxillus involutus* forms ectotrophic mycorrhizae with quite a normal structure. Mycorrhiza formation clearly begins with a mantle, but the Hartig net may remain incomplete. The species seems to be especially inclined to infect long roots as well. *P. involutus* exerts, like ectomycorrhizal fungi perhaps in general (cf. MARX and BRYAN 1970), a controlling influence on the color and appearance of the mycorrhizae, but in size, branching and internal structure *Paxillus* mycorrhizae show features varying with the tree species. In pine, for example, they dichotomize profusely (Figs. 24 and 25). A more detailed description of the mycorrhizae formed by *Paxillus involutus* is as follows:

Mantle: Normally well developed and yellow-brown in color, average thickness, found by measurement, 40 μ (10 – 100 μ). Structure prosenchymatic. The surface is composed of loosely woven hyphae running in all directions and having abundant clamp connections.

Hartig net: Normal in structure, the thickness of the hyphae 2 – 6 μ. The Hartig net becomes weaker in the deeper layers of the cortex and does not usually reach the endoderm.

Rhizomorphs: Though not abundant, rhizomorphs and mycelial strands are attached to these mycorrhizae. The hyphae at the center have a wide lumen (Ø up to 50 μ), and the total number of hyphae ranges from tens to thousands.

Intracellular infection: This was not found in living cells, nor were there other signs of one-sided parasitism.

Mycorrhizae formed by *P. involutus* have also been described by How (1942) and by SHEMAKHANOVA (1963, 1967). Their descriptions agree with the present one. These mycorrhizae are usually of type A of MELIN's (1927) classification, type B being only found on rare occasions. In DOMINIK'S (1966) classification, they are nearest to the genus Bd. The above description is based on mycorrhizae formed in synthesis experiments, but it also applies to those formed by the species in nature. Illustrations are given in Figs. 17—20 and 24—26.

53. IDENTIFICATION OF MYCORRHIZAE

531. General

The occurrence of its sporophores provides unequivocal information on the distribution of a given species. In the case of mycorrhizal fungi, such information has been used in estimating the relative importance of the various species as symbionts (e.g. BRYAN and ZAK 1961). But sources of error are numerous. For instance, even when its mycorrhizae are abundant a species does not fruit every year, and perhaps the majority of species never do so. Thus there is a great risk of overestimating the importance of those forming sporophores (BOWEN 1965), and investigations into the numerical ratio between mycorrhizae and sporophores formed by one and the same species are certainly needed. For this purpose, among others, mycorrhizae must also be identified.

Until recently, the classification of mycorrhizae introduced by MELIN (1927) has been in wide use. In this classification only Dn mycorrhizae are formed by a certain symbiont (*Cenococcum graniforme*). But many scientists working on mycorrhizae are of the opinion that the possibilities to identify many more are good (e.g. PEYRONEL 1963). MELIN's classification has been modified by dividing ectotrophic mycorrhizae into 12 subtypes with a total of 50 genera (DOMINIK 1966), but this classification, too, is purely morphologic. Morphologic similarity does not necessarily mean physiologic similarity, however. On the other hand, in this classification one and the same mycorrhiza may come to be placed in different genera, depending on its age, for instance (TRAPPE 1967 a).

There is only one theoretically correct way to identify mycorrhizae, viz. the use of aseptic synthesis. This method is also feasible in practice, because in many of their characteristics synthesized mycorrhizae are similar to those formed in nature (p. 51; cf. TRAPPE 1967 a). On the other hand, synthesis experiments have certain limitations and, therefore, MELIN (1936) recommended observations on the occurrence of sporophores in order to focus the

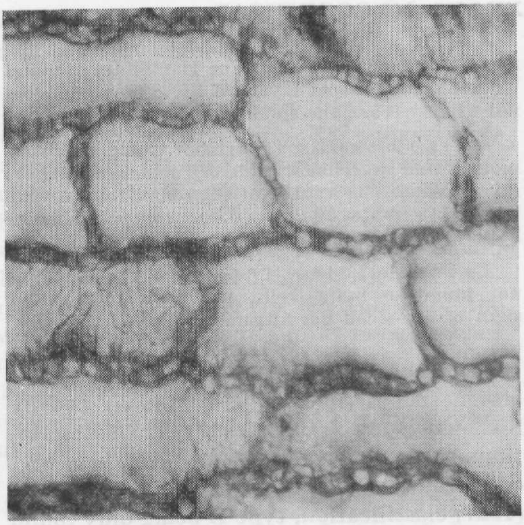

Fig. 18. Tangential section of one of the mycorrhizae in Fig. 24. Magnification x 400.

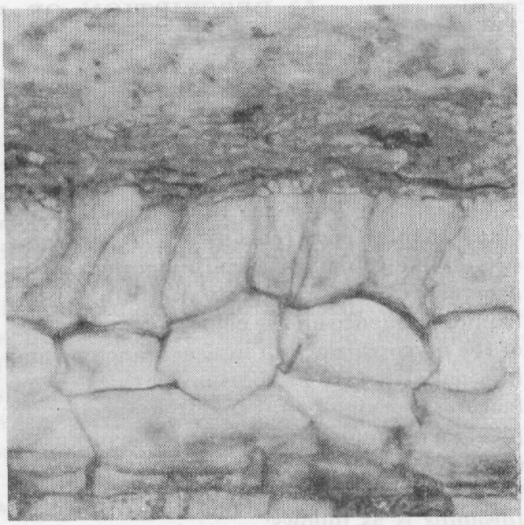

Fig. 19. Longitudinal section of a semiaseptically synthesized (*Paxillus involutus*, strain 5) birch mycorrhiza. Magnification x 400.

more comprehensive investigations on the most probable mycorrhiza formers.

These observations can be made, for example, by recording the species of sporophores that grow in the vicinity of certain characteristic mycorrhizae (LOBANOW 1960, p. 127) or — as is usually done — vice versa. If certain characteristic mycorrhizae are repeatedly found in the vicinity of one and the same mushroom, this strongly suggests their identity. For an even more positive identification mycelial connections between them can be sought. The possibility of detecting such connections has been doubted (see PEYRONEL 1963), and not without reason (MELIN 1936, p. 1031), but in recent years there seem to have been a number of reliable observations (e.g. ROTHMAYR 1962, MEYER 1963, ZAK and BRYAN 1963, DOMINIK 1966, FASSI and FONTANA 1966, 1967, SCHRAMM 1966, BAKSHI 1967, FONTANA and CENTRELLA 1967). Success depends on whether the fungus has any special characteristics (color of the mycelium, formation of rhizomorphs, etc.), as well as on soil conditions (e.g. MEYER 1963, PEYRONEL 1963). The procedure usually recommended is to take samples of 3—4 dm³ to the laboratory without removing the sporophores; here the chances of detecting connections with the aid of lenses are relatively good (LOBANOW 1960, DOMINIK 1966, SHEMAKHANOVA 1967).

If no connections are found, the mycelium of the sporophore and that of the mantle can be compared with each other. According to PEYRONEL (1963), they are so similar that, for instance, broken mantles of mycorrhizae formed by *Lactarius* species leak »milk» like the sporophores. In addition, some my-

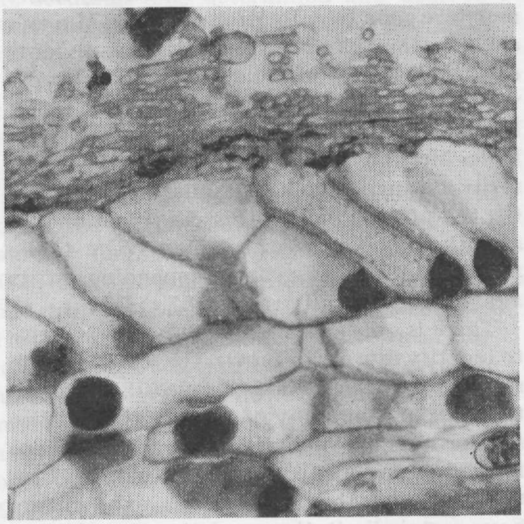

Fig. 20. Longitudinal section of an aspen mycorrhiza formed in nature by *Paxillus involutus*. Magnification x 400.

corrhizal fungi have a characteristic odor (SINGER 1962, p. 16). The existence of clamp connections and other special characteristics is always easy to check. Fluorescence and chemical indicators, the latter of which are much used in fungus taxonomy, certainly deserve mention here, too, although their use in mycorrhizal identification has hardly yet begun.

532. *Paxillus* mycorrhizae

As mentioned in the preface, the author's attention was attracted by certain yellow-brown mycorrhizae which were found in the vicinity of the sporophores of *Paxillus involutus*. Later, it turned out that these mycorrhizae can always be found near the mushrooms of this species. In a cursory examination of soil samples 3 × 3 × 3 dm³ in size taken from the base of 50 sporadically occurring sporophores selected at random, these mycorrhizae were found on 28 occasions. In samples taken at a distance of 20 m from the same sporophores they were not found at all. Thus, their concentration near these sporophores is very clear. If they had been homogeneously distributed throughout these areas (probability of occurrence $\frac{28}{100}\Big)$, the probability of getting 50 empty samples is almost nil, or $\left(1 - \frac{28}{100}\right)^{50} \sim 10^{-7}$.

Direct mycelial connections were not detected. There were both mycelium and rhizomorphs near the sporophores, it is true, but farther away they disappeared almost completely; on the other hand, mycelial connections between the mycorrhizae and their surroundings were not profuse. Therefore, *Paxillus involutus* is not a very promising species for such a search, but in favorable places such as rocks and fens, for instance, these connections might be discovered.

The mycelium at the base of the sporophores of *Paxillus involutus* and on the surface of the yellow-brown mycorrhizae mentioned appeared similar. This, however, can not be accepted as completely convincing identification, because other species with similar mycelia may exist. Additional evidence was obtained, however, when sclerotia similar to those formed by *P. involutus* were found around these mycorrhizae.

For the identification of *Paxillus* mycorrhizae another stringent test could be applied. If clean, young representatives of these particular mycorrhizae were transferred — rather immediately on the spot — to Hagem agar, the yellow-brown mycelium of the symbiont emerged from most of them within 12—24 hours (Table 14). Because no corresponding growth was observed from any of the other mycorrhizae tested, this check must be considered quite reliable.

The color of ectomycorrhizae is usually not a good characteristic (TRAPPE 1967 a), since it is inclined to change for various reasons. But there are exceptions to this rule, e.g. *Cenococcum graniforme*, with its jet black mycorrhizae. The mycorrhizae formed by *Paxillus involutus* also seem to have a typical color, although, as shown in Figs. 23—26, they are not of quite the same color. Immediately after they have been removed from the soil their color is characteristic, however, but in the course of a few hours it fades, even if the sample does not dry up (cf. SINGER 1962, p. 110).

The possibility that *Paxillus involutus* may also form completely different mycorrhizae must, of course, be taken into consideration. According to the syntheses made, however, changes in the experimental conditions, even if great (p. 56), do not provoke clear changes in the characteristics of these mycorrhizae. Observations made in nature confirm the results of these experiments. Thus, for instance, the appearance of these mycorrhizae was the same all the year round. It is recommended, however, to carry out these identification studies in the fall, when sporophores are visible and roots growing.

The mycorrhizae formed by *Paxillus involutus* were first identified by How (1942). In the present study they were identified from root material of various types. This was done on the basis of color and general appearance. They were compared to the corresponding characteristics of mycorrhizae formed in synthesis experiments. These studies were mainly carried out in the field, either with the naked eye or with a hand lens. In order to check the validity of the identification, on each occasion a few clean, vigorous individuals were transferred to Hagem slants and examined after 1—2 days for the yellow-brown mycelium of this particular symbiont. Some samples were also brought to the laboratory, as mentioned on p. 48. There, certain representative cases were examined with regard to mycelial characteristics and inner structure. These controls revealed no erroneous identifications.

54. MYCORRHIZAL HOSTS OF *PAXILLUS INVOLUTUS*

Paxillus involutus has been conclusively established to be a symbiont of *Pinus silvestris* (Shemakhanova 1963, 1967) and *P. cembra* (Moser 1967 a). Whether other tree species, too, can serve as its hosts was a question studied with synthesis experiments and observations in the field. Aseptic syntheses were performed with eight tree species. *Paxillus involutus* formed ectotrophic mycorrhizae with all these species (Table 25). With semi-

Table 25. Host species of *Paxillus involutus.*

Host	Aseptic synthesis, strain		Semi-aseptic synthesis, strain		Observations in nature	
	4	5	4	5	Own[1]	Others
Pinaceae						
Larix decidua Mill.						Peyronel 1922, How 1942, Kalmár 1950
Picea abies (L.) Karst.	—	+	—	+	+	Chastukhin 1967, Moser 1967 a
P. engelmannii Parry	—	+	—	+		
P. sitchensis (Bong.) Carr.			—	+		
Pinus cembra L.						Moser 1958 b
P. contorta Dougl.						Trappe 1969
P. ponderosa Laws.			—	+		
P. silvestris L.	—	+	—	+	+	Kreisel 1954, Pachlewski and Pachlewska 1960, Moser 1967 a
P. strobus L.			—	+		
Pseudotsuga menziesii (Mirb.) Franco	—	+	—	+		Gilmour 1958, Trappe 1969
Tsuga heterophylla (Raf.) Sarg.			—	+		Trappe 1969
Betulaceae						
Alnus glutinosa (L.) Gaertn.	—	+	—	+	—	
A. incana (L). Moench	—	+	—	+	—	
A. rubra Bong.						Trappe 1969
A. viridis Dc.						Horak 1963
Betula glandulosa Michx.						Lange 1957
B. nana L.					+	
B. pubescens Ehrh.	—	+	—	+	+	Lange 1948, Trappe 1969
B. tortuosa Led.						Lange 1946
B. verrucosa Ehrh.	—	+	—	+	+	
B. spp.						Schwöbel 1956
Fagaceae						
Fagus silvatica L.						Kalmár 1950
Pyrus communis L.						Dominik 1950
Quercus spp.						Kalmár 1950, Lobanow 1960
Rhamnaceae						
Rhamnus purschiana Dc.						Trappe 1969
Salicaeae						
Populus tremula L.					+	Becker 1956
P. trichocarpa Torr. and Gray						Trappe 1969
P. spp.						Fontana 1961
Salix acutifolia Willd.						Komirnaya and Fursayev 1953
S. cinerea L.					+	
S. phylicifolia L.					+	

[1] Based on sporophores and mycorrhizae

aseptic synthesis 12 tree species were tested. These syntheses, too, were all successful.

The results of these syntheses could be verified and the host list of *Paxillus involutus* enlarged through field observations. In the vicinity of its sporophores a search was made for mycorrhizae similar to those formed by *P. involutus*, and the lower partner was identified. Except for *Alnus*, all the species studied were found to be infected with this fungus.

Although the associations assumed by other scientists are mainly based on the fruiting of *Paxillus involutus* in the vicinity of certain tree species, there is little reason in this case to doubt their validity. On the contrary, it is very likely that the host list of *P. involutus* is considerably larger than that shown in Table 25. Consequently, as assumed earlier by Horak (1963) and Moser (1967 a), the species must be considered an unspecialized symbiont. The host list presented is as extensive as, for example, that for *Amanita muscaria*, some *Boletus* species, *Scleroderma aurantium* (Trappe 1962), *Thelephora terrestris* (Marx and Bryan 1970) and the ectendotrophic symbiont of pine (Laiho 1965). Only *Cenococcum graniforme* has a much longer list, but this species also has herbs as hosts (Trappe 1964).

As already mentioned, *Paxillus involutus* was not found to form mycorrhizae on alder in nature; in addition, its sporophores were never found in pure alder stands. In mixed stands they were encountered, even in the immediate surroundings of alder roots twisted around the roots of birch. On many occasions the latter were wholly invested with *P. involutus*, but even then the alder roots lacked this infection. On the other hand, the synthesis experiments carried out were not convincing, either. In semiaseptic synthesis a strong inoculum, a living pine seedling with *Paxillus* mycorrhizae, was required before a few of these mycorrhizae were finally formed. In aseptic synthesis, too, mycorrhiza formation by *P. involutus* was very weak and infrequent on alder.

On the basis of these studies *Paxillus involutus* can not be considered an active symbiont of *Alnus incana* or *A. glutinosa*. In Finnish conditions *Paxillus filamentosus* is associated with these tree species in nature. Near its sporophores, it was easy to find alder mycorrhizae which had the characteristics of those formed by *Paxillus involutus* but a much thicker mantle (30—120 μ, average 70 μ). From these mycorrhizae strains with all the characters of *P. filamentosus* were isolated (p. 32).

Evidently *Paxillus filamentosus* also grows in North America (Singer 1964 b), and it is possible that the observation on *Alnus rubra* (Table 25) concerns this species. On the other hand, Horak (1963) also states that *Paxillus involutus* is associated with alder; root studies were not made, however, and thus it can not be said whether the species formed mycorrhizae with *Alnus viridis*, with some other hosts possibly present (admixed tree species, bushes, dwarf-shrubs) or not at all.

The ability of *Paxillus involutus* to form mycorrhizae probably varies from host to host. Willow is another species that is only infected with difficulty; this conclusion is based on the fact that only very few *Paxillus* mycorrhizae were found in its roots, which grew twisted around infected birch roots. On the other hand, *Salix* is not a highly mycotrophic genus either (cf. Lobanow 1960, p. 103). Inoculation of ponderosa pine and western hemlock was successful only with mycorrhizal seedlings. On the other hand, inoculation of Scots pine, Norway spruce and white and common birch is very easy. In nature, it seems to be easiest to find *Paxillus* mycorrhizae on birch roots.

Despite the general conformity of the results of aseptic and semiaseptic synthesis (Table 25), differences were also observed. In semiaseptic syntheses either fully developed mycorrhizae were formed or the inoculum died. In aseptic syntheses *Paxillus involutus* always grew well, although there were instances on which mycorrhizae were not formed. In all these cases, too, the fungus had a tendency to grow along the roots, however, forming loose wefts here and there around them. The interpretation of such a phenomenon is problematic and partially subjective (cf. Modess 1941, p. 56, Marx and Bryan 1970).

The mycorrhizal activity of *Paxillus involutus* also varies from strain to strain. Of the 46 strains studied, two (numbers 1 and 7) lost their ability to form mycorrhizae in semiaseptic synthesis in the course of the experiments, aseptic synthesis, however, being successful even after this, though slowly and irregularly. Three other strains (3, 4 and 24) did not form mycorrhizae at all, although tested several times with both methods. These inactive strains had a velvety mycelium, and showed no tendency to form rhizomorphs or sclerotia. As, however, they were not physiologically different from the other strains, there is no basis for concluding that saprophytic strains of this species exist, as in the case of *Xerocomus subtomentosus*, for instance (Lundeberg 1970).

55. OCCURRENCE OF *PAXILLUS* MYCORRHIZAE IN NATURE

In various connections in the foregoing, scattered data have been presented on the occurrence of mycorrhizae formed by *Paxillus involutus*. Their distribution was also mapped systematically in order to obtain a general idea of the species as a mycorrhiza-former. Thus, 800 root samples were collected in connection with the transect surveys. *Paxillus* mycorrhizae were most frequently encountered along ditches and in cutover areas, i.e., in places where the sporophore density was relatively high (Table 3). A closer look (p. 51) revealed that these mycorrhizae are heavily concentrated in the immediate vicinity of *Paxillus involutus* sporophores.

Mycorrhizal investigations were also carried out on the permanent sample plots. The relative number of *Paxillus* mycorrhizae was closely correlated with the density of the corresponding sporophores (Fig. 21). Even when closed stands are excluded, the correlation is almost the same ($y = -31 + 74\,x$; $r = 0.86^{***}$). The root systems of the remaining sample plots were of roughly the same magnitude, and the dependence in question can also be expressed as follows: the greater the density of *Paxillus* mycorrhizae, the more numerous are its sporophores, or, the formation of a sporophore requires the interaction of a certain number of mycorrhizae.

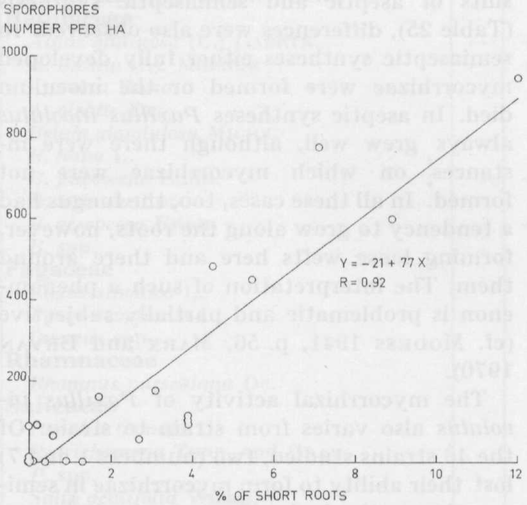

Fig. 21. The occurrence of *Paxillus involutus* mycorrhizae and sporophores on the permanent sample plots in 1968.

Assessment of this number on each occasion would involve detailed quantitative root studies. The studies made in the present connection were not quantitative and, therefore, only a few comments can be made. Thus, fruiting does not bring to light all the sites where *P. involutus* exists, for *Paxillus* mycorrhizae were encountered in sample plots 4 o_1, 6 c and 7 c, although no sporophores appeared in 1968. These mycorrhizae were also found in sample plots 5 c and 8 c in places where the species did not fruit. In sample plots 4 o_1 and 5 c the frequency of *Paxillus* mycorrhizae was relatively large, 1.3 and 0.9 %, respectively, and in 1969 the species also fruited fairly abundantly on these two plots (111 and 19 sporophores per hectare). The other three plots mentioned also remained empty that year. On these plots only a few *Paxillus* mycorrhizae were found in seedlings growing near decayed stumps.

On the other hand, *Paxillus involutus* fruited on three of the sample plots, the root samples of which did not include the corresponding mycorrhizae. This is liable to happen when a few sporophores are in question. For instance, on many of the sample plots studied by Björkman (1942), sporophores of boletes occurred when no C mycorrhizae were encountered, and vice versa.

Although *Paxillus* mycorrhizae do occur sporadically, they typically appear in very large numbers. It is easy, for instance, to find seedlings with roots infected by this species alone. In tree roots growing in decayed wood it is often altogether dominant (Fig. 26). In the area used for peat winning (p. 12) most mycorrhizae over an area of several ares were formed by *P. involutus*. Their dominance around plots isolated from tree roots (Fig. 25) should also be noted.

In light mineral soils *Paxillus* mycorrhizae are encountered down to a depth of at least 20 cm. In the cavities remaining after roots have decayed, the species follows new roots to much deeper soil layers and in the trenches of the isolated plots it formed mycorrhizae at a depth of 50 cm. Thus the mycorrhizae formed by *P. involutus* are at least not more superficial than ectotrophic mycorrhizae in general, although the behavior of the species in pure culture (p. 37) might lead one to expect them to be so. The mycorrhizae formed by *P. filamentosus* tend to occur at depths of 20—30 cm, even in heavy soils. These observations support Lyr's (1963 a) conclusion that the vertical distribution of mycorrhizae depends on factors other than the oxygen content of the soil.

Comparison between *Paxillus involutus* and some other relatively well-known mycorrhizal fungi reveals certain differences. Thus, *Cenococcum graniforme* forms a few scattered mycorrhizae in almost any root sample, but is dominant only under conditions completely unfavorable for other species (e.g. Dominik 1966, p. 52). In the same way C mycorrhizae, too, are found here and there in most root samples (e.g. Melin 1927, Björkman 1942, Heikurainen 1955). The bright yellow K mycorrhiza is frequent in the raw humus of closed coniferous forests with a thick moss carpet, but disappears after cutting (Mikola 1962), i.e. its behavior is just the reverse of that of *Paxillus* mycorrhizae. The ectendotrophic mycorrhiza of pine, on the other hand, thrives especially in nurseries (Mikola 1965), but also in burned ground (Mikola, Laiho, Erikäinen and Kuvaja 1964).

56. THE INFLUENCE OF CERTAIN ECOLOGIC FACTORS ON THE FORMATION OF *PAXILLUS* MYCORRHIZAE

561. Light, nitrogen, glucose

In order to study the factors mentioned, aseptic synthesis tubes were shaded from daylight to the 40 and 15 % levels. The nitrogen and glucose concentrations were exactly the same as those used by Mikola (1948).

The seedlings did not grow much, but the influence of each factor studied was nevertheless clear (Table 26). Increases in both illumination and glucose improved root growth, whereas addition of nitrogen weakened it.

Table 26. Root development of pine seedlings in aseptic synthesis with *Paxillus involutus* 5. Explanations in Table 29.

Glucose, g/l	Light 15 %			Light 40 %		
	$(NH_4)_2SO_4$, g/l					
	0	0.25	1.0	0	0.25	1.0
	Number of mycorrhizae					
0.5	7	4	2	46	8	4
2.5	51	19	2	31	46	4
	Number of short roots					
0.5	9	6	4	185	22	7
2.5	69	25	7	263	124	19
	Root dry weight, mg					
0.5	2	2	1	15	6	3
2.5	5	3	3	16	19	5

At the highest level of nitrogen combined with the lower levels of light and glucose, only a few branches were formed.

As it is a rapidly growing species, *Paxillus involutus* soon came into contact with the roots. This was the situation in all the synthesis tubes. The first mycorrhizae were formed within 1—2 months of inoculation. Table 26 gives their number at the end of the experiment; the numbers may be slightly overestimated because of the difficulty of distinguishing mycorrhizae from loose mantle-like wefts. In the present connection all short roots that seemed to have a mantle were considered to be mycorrhizae. Representative specimens were sectioned, and it was established that normal ectotrophic mycorrhizae had really formed in all treatments. In addition to a mantle, they had a Hartig net. A balanced symbiosis was maintained even when the seedling concerned was relatively weak (light on the lower level) and the fungus relatively virulent (nitrogen at the highest level, cf. Melin 1925, p. 100). *Paxillus involutus* is thus an active fungus, with medium virulence in its relation to pine roots. This experiment did not reveal the lowest level of light at which formation of its mycorrhiza is possible.

562. Substrate

Mycorrhiza formation in various substrates was studied with semiaseptic experiments. It was most common in garden peat (Table 27), which had been strongly fertilized before the experiment was started. On the other hand, mycorrhizae were also formed in very poor substrates, whether organic or inorganic. The »activity» (»inherent ability of a fungus to form a mycorrhiza», Trappe 1962) of *Paxillus involutus* towards pine roots was extremely high in these experiments; this conclusion is drawn from the fact that the first mycorrhizae were formed within one week of inoculation. The virulence, however, was medium in these cases, too.

The experiments mentioned also involved considerable variation with regard to the pH of the substrate. The variation was still further increased by lowering the pH with weak sulfuric acid. The lowest value at which the seedlings remained alive was 2.7, and at this pH mycorrhizae were still formed. On the other hand, mycorrhizae were also formed when the pH of the substrate had been raised to pH 8.3 by application of lime (even 200 g/l of soil). As in the former experiments, *P. involutus* formed ectotrophic mycorrhizae with only a slight variation in color. According to Dominik (1966, p. 76), the pH of the substrate does not influence the morphologic characteristics of mycorrhizae in general either, nor are the pH limits determined for fungal symbionts in pure culture experiments valid for mycorrhiza formation. An extreme example of this is the ectendotrophic symbiont of pine (Mikola 1965).

Table 27. Mycorrhizal development of pine seedlings in semiaseptic synthesis with *Paxillus involutus* 5. Seeding on fertilized peat Feb. 29, transfer to the substrate studied April 23 (shoot weight about 30 mg), inoculation May 3, termination and soil analyses Oct. 2, 1968. Ten replicates.

Substrate	Properties of the substrate						*Paxillus* mycorrhizae, per cent		Shoot dry weight, mg
	pH	Loss on ignition, %	Total nitrogen, %	Exchangeable			Average	Variation	
				K	P	Ca			
				mg/l					
Vermiculate	7.9	3	△	59	5	240	15	0—30	54
Quartz sand, 0.5—2 mm	6.7	△	△	5	3	90	8	0—20	35
Arable soil	5.8	6	0.2	90	4	340	34	0—90	132
Forest sand [1]	4.8	5	0.1	61	8	390	38	0—70	157
Forest humus [2]	4.4	91	1.1	68	10	220	27	0—60	138
Milled pine bark	4.8	99	0.1	35	3	200	20	0—90	48
Composted spruce bark	5.8	86	0.8	91	16	2 250	18	0—70	98
Sphagnum peat [3]	4.2	94	0.7	20	4	125	23	0—100	104
Fertilized peat [4]	5.9	89	1.4	240	45	1 450	41	0—100	144

[1] From sample plot 7 o, see Table 2

[2] From sample plots 3 c

[3] From sample plot 9 d, degree of humification 2

[4] »Kultaturve», *Sphagnum* peat, degree of humification 4, commercial product of Finnhumus OY

57. SURVIVAL OF *PAXILLUS* MYCORRHIZAE IN TRANSPLANTS

Mycorrhizal fungi can be inoculated in the field in many ways. The methods used and the results obtained have been discussed lately by Mikola (1970). In the present study, living pine seedlings with mycorrhizae formed by *Paxillus involutus* were used. The rearing, inoculation and planting out of these seedlings are described on p. 47. Table 28 gives information on the survival of this particular symbiont.

In the fall following planting, *P. involutus* was often more strongly associated with the roots than at the time of planting. The original mycorrhizae had survived well and numerous new ones had been formed.

In the course of the following growing season the proportion of *Paxillus* mycorrhizae decreased on all occasions. In general, *P. involutus* was not replaced by other symbionts in old mycorrhizae, but it did not form new ones to the same extent as other species. Being the sole symbiont of the seedlings at the time of planting, it had an advantage over other species, especially because it usually grows along long roots and it is from these that the new short roots often acquire the primary infection (cf. Melin 1927, Robertson 1954, Wilcox 1967). In the second growing season, on the contrary, many other symbionts also had a living connection with the long roots.

Examination of the data by sites shows that in closed stands *Paxillus involutus* succeeded well in keeping its position on these seedlings. This contrasts with the occurrence of its sporophores and mycorrhizae in such stands (see p. 54). The reason for this discrepancy may be the fact that the surface soil was scarified in connection with planting.

The good survival of the species in cutover areas is in harmony with its natural distribution. The best survival, however, was noted in burned-over areas, on arable land and in nurseries. In addition, root growth was best in these sites. In spite of this, the fine roots of many seedlings were completely invested with *Paxillus involutus*. This supports the conclusion drawn on p. 12 that the absence of its sporophores on arable land and burned-over sites is not due to unsuitability of the soil.

These seedlings were also planted in fertilized garden peat, which in Finland is used in forest nurseries, for instance; the peat is spread on top of the mineral soil. The survival of *P. involutus* was good. It only disappeared from seedlings transferred to polythene greenhouses. This was probably because of the temperature, which in air reached as much as 50° C and in the topmost soil layer 30° C. In addition, the relative humidity was near saturation.

The fact that *Paxillus involutus* survived so well on arable land, in burned-over areas and in nurseries is no surprise. They are all sites in which the population of mycorrhizal fungi is restricted (e.g. Björkman 1962, Zak and Marx 1964, Göbl 1967). Being common in cutover areas and also able to live in forest nurseries, the species can be characterized as a symbiont specifically adapted to the regeneration stage of forests. From the nursery it can be transferred to various planting sites, where its survival is fairly good. Although the seedlings were not followed for more than two growing seasons after planting, it seems very likely that *P. involutus* is not replaced by other ectomycorrhizal fungi more often than symbionts under conditions where competition between species is hard.

Table 28. Relative number of *Paxillus* mycorrhizae in 450 pine transplants after one (I) and two (II) seasons in the field. Seeding in the greenhouse Feb. 28, inoculation April 25, planting May – June, (*Paxillus* mycorrhizae 5 – 100 %, average 40 %), termination in October.

Planting site	*Paxillus* mycorrhizae, per cent	
	I	II
Closed stands	40	15
Cutover areas	28	18
Burned-over cut areas	67	14
Arable land	62	23

58. THE EFFECT OF *PAXILLUS* MYCORRHIZAE ON THE GROWTH OF PINE SEEDLINGS

One of the most important tasks of present-day mycorrhizal study is to measure the symbiotic efficiency of various mycorrhizal fungi (e.g. MIKOLA 1970). As *Paxillus involutus* is a common species around the world, and in addition is very suitable for use in forestation, there is a relatively great need for information on its symbiotic properties.

Paxillus involutus is one of the many species that has been tested for host response in the Soviet Union (see SHEMAKHANOVA 1967, p. 204). In these experiments inoculation has generally had a favorable influence on the development of oak seedlings, but the effect of *P. involutus* is not specified. MOSER (1963) employed a mixed inoculum of *Suillus*, *Paxillus* and *Amanita* and observed that it promoted the initial development of *Pinus cembra* near the alpine forest line. This was also the case when *P. involutus* was used alone, in particular in soils with low microbial activity or where local symbionts were unsuitable or completely lacking.

In his first aseptic syntheses with raw humus as the source of nitrogen, LUNDEBERG (1967) found that *Paxillus involutus* had a deleterious effect on pine seedlings and even killed some of them. In his later (1970) syntheses, however, this was not the case, but the seedlings inoculated with *P. involutus* developed like the uninoculated control seedlings. In these experiments the species did not form mycorrhizae.

In the aseptic syntheses of the present work *Paxillus involutus* grew very well in the tubes. A few of the seedlings died, but not more than among the controls. In contrast, *Mycelium radicis atrovirens*, a common colonizer of tree roots in nature, rapidly killed all the seedlings.

It was easy in these syntheses to demonstrate the effect of light, nitrogen and glucose on the development of pine seedlings. Inoculation, on the contrary, did not necessarily have any visible effect, but shoot weights (Table 29), for instance, were of the same magnitude irrespective of whether the seedlings had been inoculated or not, or whether mycorrhizae had formed or not (strain 4 did not form mycorrhizae, strain 5 did).

In another experiment (Table 30) the response of pine seedlings to a larger number of strains and to various species was studied. Significant differences were established ex-

Table 29. Shoot development (mg dry wt) of pine seedlings in aseptic synthesis. Nutrient solution on p. 46 with the modifications indicated. Seeding April 10, inoculation May 9, termination Oct. 7, 1968. Four replicates.

F for inoculation	1.8
F for nitrogen	24.4***
F for glucose	7.5**
F for light	119.5***
F for inoculation x nitrogen	2.7*
F for nitrogen x light	15.3***
F for glucose x light	8.2**

Inoculum	Light 15 %			Light 40 %		
	$(NH_4)_2SO_4$, g/l					
	0	0.25	1.0	0	0.25	1.0
	Glucose 0.5 g/l					
None	10	11	9	19	18	7
Paxillus involutus 4	9	12	8	17	29	15
Paxillus involutus 5	9	10	8	18	15	11
	Glucose 2.5 g/l					
None	13	8	7	24	27	11
Paxillus involutus 4	9	8	9	20	34	15
Paxillus involutus 5	12	9	9	24	27	11

pressly for root development. Mycorrhizal fungi promote branching of roots (e.g. Hatch 1937) and consequently the number of short roots in mycorrhizal seedlings was relatively large. The corresponding difference can be seen even more clearly from the root weights, in which the mantles with the attached mycelium are included. The differences in shoot weights were of a similar nature, but much smaller.

The experimental conditions (short duration, small vessel size, nutrients limited in amount and in a form readily available to roots) did not give the fungus much chance to promote the growth of the seedlings but rather encouraged it, particularly in the first experiment (Table 29), to injure them. The growth responses recorded must therefore be regarded as underestimates and the true mycorrhizal nature of *Paxillus involutus* firmly established.

Of the 450 nonmycorrhizal pine seedlings planted in the field (p. 48) 9 % died, whereas of those inoculated with *Paxillus involutus* or with forest humus only 4 % died. If the numbers of dead seedlings are paired by blocks, it appears that the mortality of the nonmycorrhizal seedlings was significantly higher than that of seedlings inoculated with *P. involutus* (t = 3.68***). Most of these seedlings died soon after planting, probably owing to lack of water. Correspondingly, the mortality was very high on dry pine heaths, namely 91 and 85 %, respectively.

Planting involves a certain shock to seedlings, disturbing their water economy especially. If, at the time of planting, the seedlings bear mycorrhizae which survive in the planting site, this critical stage becomes shorter. As, however, in Finnish conditions, one month is enough for mycorrhiza formation (Mikola 1967), seedlings planted without

Table 30. The development of pine seedlings in aseptic synthesis. Seeding April 29, inoculation May 30, termination Sept. 8, 1969. Nutrient solution on p. 46, five replicates.

Inoculation	Species, strain						Mean
	Number of mycorrhizae. F for rows 13.2***						
None	0	0	0	0	0	0	0
P. involutus, active strains	31	21	19	18	16	11	19.2
» less active strains	11	10	7	6	5	2	6.8
» nonmycorrhizal strains	0	0	0	0	0	0	0
Other symbionts [1]	0	6	15	6	1	4	5.3
	Number of short roots. F for rows 5.4***						
None	11	29	9	15	10	24	16.1
P. involutus, active strains	46	43	46	35	42	31	40.3
» less active strains	30	30	28	38	30	15	26.9
» nonmycorrhizal strains	9	8	29	6	12	15	13.2
Other symbionts [1]	38	27	24	17	34	43	30.4
	Root dry weight, mg. F for rows 15.2***						
None	4	4	4	4	4	4	3.8
P. involutus, active strains	6	11	11	7	10	7	8.5
» less active strains	7	9	10	6	8	7	7.8
» nonmycorrhizal strains	5	4	4	4	4	6	4.6
Other symbionts [1]	8	7	7	10	8	9	8.2
	Shoot dry weight, mg. F for rows 3.2*						
None	18	19	15	17	17	18	17.3
P. involutus, active strains	14	20	18	15	23	21	18.5
» less active strains	21	19	20	19	21	15	18.9
» nonmycorrhizal strains	15	18	19	14	12	18	16.1
Other symbionts [1]	22	21	24	18	18	24	21.1

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[1] *Suillus bovinus*, *S. variegatus*, *Laccaria laccata*, *Corticium bicolor*, *Cenococcum graniforme*, the ectendotrophic symbiont of pine

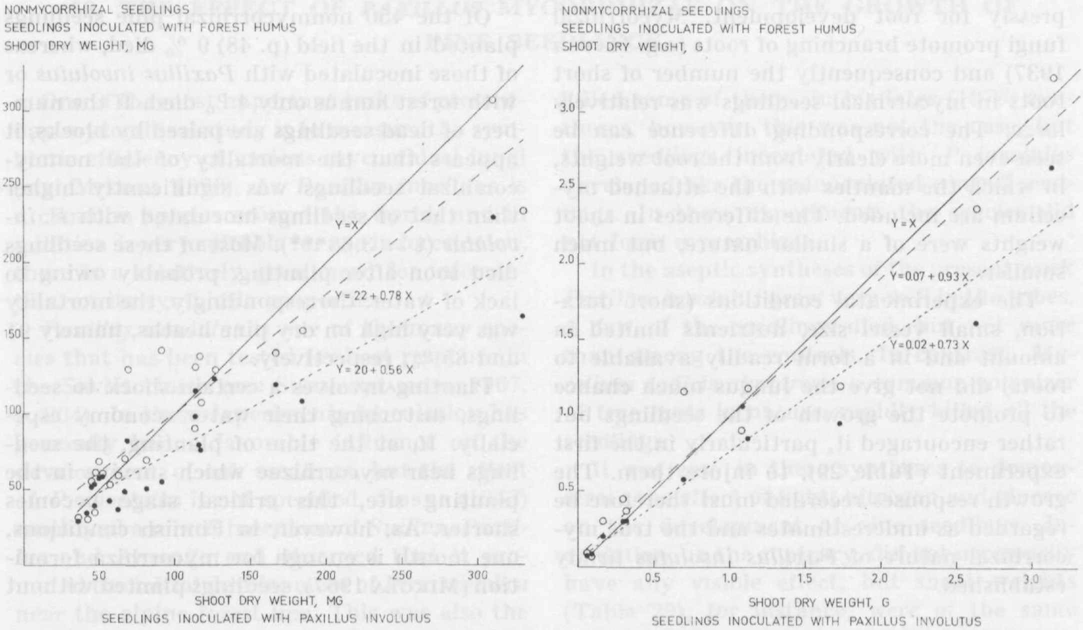

Fig. 22. Nonmycorrhizal pine transplants (●………●) and those inoculated with forest humus (○— —○) as compared to corresponding transplants inoculated with *Paxillus involutus* (——) after one (on the left) and two (on the right) years in different planting sites. For further information, see text.

mycorrhizae also have good chances to survive. Their initial development, however, will presumably be slowed down.

The development of these seedlings was studied by plotting their weights in a coordinate system so that the straight line $y = x$ describes the seedlings inoculated with *Paxillus involutus*. If, in a certain block, the seedlings that were nonmycorrhizal when planted out were smaller than those that were inoculated with *P. involutus* (the usual situation), the point representing them is located below this line (Fig. 22). Thus, in addition to differences in mortality, differences also existed in the development of the seedlings that survived. At the end of the first growing season after planting out, the weight of the initially nonmycorrhizal seedlings averaged 73 % and at the end of the second growing season 75 % of the weight of those inoculated with *P. involutus*. In both years the difference in weight between the two classes of seedlings was statistically significant. If the weights are replaced by their logarithms in order better to meet the requirements of regression analysis, and the regression coefficients compared to the value 1, the respective t-values are 2.69* and 2.65*.

Because of the great variation within and between replicate treatments, further analysis of the data is not justified. This variation must be largely due to differences in the size and vitality of the seedlings and, in particular, in the number of mycorrhizae at the time of planting.

In the literature there are numerous examples of astounding responses of tree seedlings to inoculation with ectomycorrhizal fungi (see Levisohn 1958). By comparison, *Paxillus involutus* is a less efficient symbiont. Two points, however, must not be forgotten. In the present case the nonmycorrhizal control seedlings, too, soon became infected, and, in addition, the beneficial effect of mycorrhizae shows up particularly in soils deficient in readily soluble phosphorus (McComb 1943, Ritter and Lyr 1965, Bowen and Theodorou 1967, Trappe and Strand 1969). In Finnish forest soils there is no such deficiency (Cajander 1949, Viro 1951).

The response of seedlings to the ectendotrophic symbiont of pine has been studied in part in the same sites (Laiho 1967) as *Paxillus involutus* in the present connection. In burned-over areas and on arable land the response was greater than that recorded for *P. involutus* but it should be noted that the survival of the ectendotrophic symbiont was also almost complete.

In order to compare *Paxillus involutus* directly with other species, seedlings inoculated with forest humus were included in these test plantings. Their weight averaged 99 % of those inoculated with *P. involutus*. As mixed populations usually give a better response than individual species (see Mikola 1970), this comparison indicates that *P. involutus* is a relatively beneficial symbiont. On the other hand, of course, the result depends on the fungal population. This population formed C, Dn and K mycorrhizae to a minor extent. *Thelephora terrestris* fruited in many of the pots, and it probably formed the bulk of these mycorrhizae (cf. Marx, Bryan and Grand 1970). At the time of planting they were present in similar numbers to *Paxillus* mycorrhizae.

Thus it can be concluded that *Paxillus involutus* is not harmful to pine seedlings, even when the seedling is relatively weak and the fungus relatively virulent. Its mycelium and rhizomorphs grow profusely in the soil and consequently its opportunities to absorb water and nutrients are relatively good (cf. Harley 1969). However, the many natural seedlings examined with *Paxillus involutus* as almost the only symbiont were neither stunted nor particularly vigorous. The experimental evidence also supports the view that the species is about average in its symbiotic efficiency.

6. DISCUSSION

61. ECOLOGIC ASPECTS

Previous investigations have established that *Paxillus involutus* is an ectomycorrhizal fungus. In the present study this could be confirmed. The species forms ordinary ectotrophic mycorrhizae with many tree species. On the other hand, pure cultures were isolated from its sporophores, which repeatedly failed to form mycorrhizae in synthesis experiments. As, in addition, the literature includes several statements on the fruiting of the species in treeless areas and on decaying material in forests, special attention was paid to the question of whether the species fruits in Finnish conditions independently of trees and other hosts. For this purpose numerous sites of every treeless type were searched, but no such sporophore was discovered. Wherever there were sporophores, there were at least seedlings of trees in the vicinity, and they regularly bore *Paxillus* mycorrhizae.

In the presence of trees *P. involutus* fruits to varying extents. In closed stands on poor soil it hardly fruits at all and on good sites rather abundantly. After partial cutting, soil scarification and nitrogen fertilization, for instance, its sporophores appear in great numbers and tend to be increasingly numerous within the first 3—5 years. The factor responsible for this profuse fruiting can not be arrest of the underground growth of the species (cf. RAUTAVAARA 1947, p. 418). If this were the reason, the increased fruiting would be expected only to last one season. On the contrary, the subterranean growth was found to be very vigorous in places where fruiting was abundant. In addition, a positive correlation exists between the numbers of mycorrhizae and sporophores formed by *Paxillus involutus*.

On the occasions mentioned above, the species started to fruit in places where its sporophores were previously lacking. Consequently, it either spreads through spores or may be present in the absence of sporophores. Both alternatives are possible. Thus, for instance, in a large area used for peat winning and abandoned two years earlier, small birch seedlings were growing here and there, and about half of them were infected with *Paxillus involutus*. In this case air-borne spores were the most likely source of infection (cf. ROBERTSON 1954, MARX and ROSS 1970).

On the other hand, on many occasions *P. involutus* formed mycorrhizae without fruiting in the vicinity. These cases are difficult to detect, and small sporadic groups of these mycorrhizae certainly are not rare. As, in addition, the species forms sclerotia, it can effectively spread in soil even without the help of spores when conditions improve.

As discussed earlier (p. 28—29), conditions for the growth of *Paxillus involutus* in nature are improved by at least the following changes: increased bacterial activity, increased humus decomposition, increased nitrogen mobilization, and a rise in the pH value of the soil. If the significance of these changes is estimated from the behavior of the fungus in pure culture, the rise in pH must be considered of minor importance. The increased bacterial activity, on the other hand, may even have a direct effect, which may be of much greater importance, because *Paxillus involutus* has rather srong antagonists among the common saprophytic fungi. Its relations to bacteria, however, were not studied to any extent.

Paxillus involutus did not decompose cellulose or lignin in pure culture, but otherwise it exhibited a rather good ability to utilize different carbon sources. Thus it grows to some extent on birch leaves and decayed wood, for instance, and forest humus contains only relatively small amounts of substances inhibitory to its growth. After being attacked by white-rot fungi, such humus contains plenty of anthrone-positive substances (HINTIKKA 1970), which *P. involutus* probably can utilize. Thus, on certain occasions even humus may offer fairly good conditions for the growth of this species, although not good enough to enable it to fruit. Of course, this inability may depend on factors other than lack of energy (cf. LOBANOW 1960, p. 194).

Whether the stimulated growth of *Paxillus involutus* is a consequence of its taking part in the decomposition of humus in favorable conditions is a question that can not be definitely answered in the present study. But it is difficult to understand how, in the presence of vigorous hosts, the growth of an ectomycorrhizal fungus could be severely limited by a deficient carbon supply. Thus, the amount of available nitrogen must be regarded as the most important edaphic factor regulating the growth of *P. involutus*. When the amount of available nitrogen is more abundant than in raw humus, the fungus is stimulated to active growth, spreading extensively along roots and in the soil and forming numerous sporophores. According to MELIN (1925), a good supply of available nitrogen stimulates the growth of all mycorrhizal fungi, but this seems to be particularly true of *P. involutus*.

On the other hand, too great a supply of available nitrogen decreases mycorrhiza formation (BJÖRKMAN 1942) and above a certain limit even well-developed mycorrhizae start to elongate as nonmycorrhizal short roots (SLANKIS 1967). Where this limit lies for *Paxillus involutus* remains to be determined, but it certainly was not reached in any of the experimental stands.

The relatively abundant fruiting of *Paxillus involutus* on sites with a mull-like humus distinguishes it from the species typical of raw humus. The latter compete effectively for nutrients and form mycelia which are difficult to break down and, thus, many of them actually promote the formation of raw humus. A typical example is *Cenococcum graniforme* (MEYER 1964), the economic coefficient of which is exceptionally high, being as much as 0.56 (MIKOLA 1948, p. 23). In *P. involutus* this coefficient is 0.26—0.30 (p. 38) and the species does not appear to change the structure of humus (cf. ROMELL 1939, HINTIKKA 1970).

In synthesis experiments *Paxillus involutus* formed mycorrhizae with species of *Pinus*, *Picea*, *Pseudotsuga*, *Tsuga*, *Betula* and *Alnus*. Other genera were not tested. Observations made in nature also suggest that its host list is much longer, i.e., typical of what a facultative symbiont is supposed to have. On the other hand, its moderate symbiotic efficiency, great activity, medium virulence and good ability to compete with other symbionts are not typical of facultative species (see p. 7).

The most important characteristic of facultative symbionts is their ability to form sporophores when growing by a saprophytic mode of nutrition. At least in Finland this is not the situation in respect to *Paxillus involutus*. Probably some other species considered facultative also lack this ability. Thus, *Xerocomus subtomentosus*, *Laccaria laccata* and *Thelephora terrestris* seem to be dependent in their fruiting on mycorrhizal connections (p. 26). Mycorrhizal fungi which are able to form sporophores when growing by a saprophytic mode of nutrition probably exist, but in general the observations concerning them require verification. It may be of interest to note here that *X. subtomentosus*, for instance, seems to include both mycorrhizal and saprophytic strains, but not facultative ones (cf. LUNDEBERG 1970).

If, as is usually done, facultative symbionts are defined as species which can grow, or, this being difficult to detect, survive in soil without connection to a host, their number evidently is great. According to ROSENDAHL and WILDE (1942), mycorrhizal fungi in general survive saprophytically in soil, and according to KELLEY (1950), they do not actually differ from other groups of fungi. Anyway, it is generally known that at least several ectomycorrhizal species survive for decades after clearcutting, sometimes for as much as 150 years (see HARLEY 1969, p. 66). According to BJÖRKMAN (1949, p. 225), survival occurs in an »inactive» state, but LYR (1963 b) assumes that at least some of these fungi may take part in decomposition of litter.

According to several studies, many ectomycorrhizal fungi release small amounts of enzymes attacking complex carbon compounds (LINDEBERG 1948, NORKRANS 1950, LYR 1963 b). Thus, they are not wholly incapable of breaking down litter, especially as the mycelia in soil very likely have higher enzymatic activities than those grown on synthetic media (LYR 1963 b). According to LUNDEBERG (1970), the corresponding activity of *Paxillus involutus* is very limited and thus the species must be regarded as a typical ectomycorrhizal fungus. On the other hand, it is readily isolated and grows well on synthetic media, both facts which suggest a tendency toward saprophytic nutrition (Melin 1925, p. 116, Lyr 1963 b). Whether *Paxillus involutus* is able to grow in the soil without a host, and if so, to what extent and for how long, are questions that can not yet be answered, but the same is also true of the other ectomycorrhizal fungi.

62. METHODOLOGIC ASPECTS

Semiaseptic synthesis as compared to aseptic synthesis, gives results sooner and is easier to carry out. Further, all mycorrhizae are similar to those formed in nature. On the other hand, only a very limited number of symbionts can so far be successfully inoculated under semiaseptic conditions. This number will undoubtedly increase with improvement in methods (cf. Hacskaylo and Vozzo 1967). Contamination by other mycorrhizal fungi is another drawback (e. g. Henderson 1968, Marx and Bryan 1969), particularly in experiments of long duration.

In aseptic synthesis, in addition to mycorrhizae similar to those formed in nature, other types of infection may also occur. In the present study a few strains of *Paxillus involutus* formed only more or less loose mycelial wefts, the significance of which is difficult to interpret. In semiaseptic synthesis these strains did not take at all.

So far, some 80 species have been aseptically established to be ectomycorrhizal fungi (see Trappe 1962). Probably several times as many exist, however, for instance many of those in which synthesis has failed (cf. Modess 1941, Zak and Bryan 1963). On the other hand, the conditions in aseptic synthesis may be »too favorable»; that is, perhaps not all proven symbionts are actually capable of forming mycorrhizae in nature (Singer 1964 a). In the present study *Paxillus involutus* in aseptic culture was demonstrated to form mycorrhizae with alder, although these were weakly developed and few in number. Their existence could not be confirmed in nature, where *P. filamentosus* is specialized on alder. The synthesis was also successful semiaseptically when mycorrhizal pine seedlings were used as inoculum. Immediate and long-lasting contact with this inoculum and complete absence of other symbionts resulted in a slight infection even in alder. Thus, as suggested by Romell (1938), synthesis experiments seem to tell what fungus-host combinations are physiologically possible. According to Melin (1953), too, complementary observations in nature are needed in order to say whether a certain species is, for example, ecologically important or whether it forms mycorrhizae in nature at all.

As mentioned earlier, not all strains of *Paxillus involutus* formed mycorrhizae in synthesis experiments. Some of them lost the ability during the study, while others were »inactive» from the start. All the nine strains studied were incapable of utilizing cellulose and lignin, however, and, therefore, the inactive ones are better considered to have lost their ability to form mycorrhizae at isolation than to be saprophytes. Thus, the negative results in synthesis experiments should be confirmed with several other strains. Great differences between strains in other experiments, too, emphasize the need to use numerous strains in addition to adequate replication.

In the present study mycorrhizae formed by *Paxillus involutus* were identified in the field. The primary criteria used, general appearance and color, were learned through aseptic synthesis. As a check, a few representative specimens were placed on agar, on which this particular symbiont, even without surface sterilization, usually emerges within 24 hours. In connection with this work several other characteristic mycorrhizae were also observed. This supports Trappe's (1967 a) view that the possibility of identifying mycorrhizae is better than has hitherto been assumed.

As to whether ectomycorrhizal fungi form sporophores when growing by a saprophytic mode of nutrition, the need for thorough investigations must be emphasized. The occurrence of sporophores on meadows and pastureland as well as in cutover areas and gardens, etc., does not, even for species requiring woody host plants, necessarily mean that they have been formed by saprophytic mycelia. On sites of these kinds, seedlings of broad-leaved trees, for example, may be more or less hidden. If their absence is not carefully checked, an erroneous conclusion is drawn. For *Paxillus involutus* this has probably happened on several occasions (cf. SCHWÖBEL 1956, SINGER 1964 a). In the present study it was avoided by studying the roots of nearby trees and seedlings. They regularly bore mycorrhizae formed by *P. involutus*. Although sporophore formation by saprophytic mycelia is not impossible in the presence of mycorrhizae formed by the species in question, it certainly is not probable.

In this connection the value of trenching experiments is stressed. Finding entirely convincing examples in nature is chancy and laborious, and, therefore, such experiments are to be recommended. It must be kept in mind, however, that cutting the roots round a certain area and hindering their reentry into it with a barrier does not necessarily prevent new connections from being formed. Thus, in the present study *Paxillus involutus* tended to establish rhizomorphal connections underneath the barrier. For this reason isolation should preferably be done from below as well.

7. SUMMARY

Paxillus involutus is a mycorrhizal fungus with a wide host range. It includes species of at least the genera *Pinus*, *Picea*, *Pseudotsuga*, *Tsuga* and *Betula*, but the list evidently is much longer. The species forms ordinary ectotrophic mycorrhizae with a mantle and Hartig net. In semiaseptic synthesis they may even take as little as a week to form.

These mycorrhizae can be identified in the field. Identification is based on their general appearance and color, and it can be confirmed by plating vigorous and clean mycorrhizae without surface sterilization on agar, whereupon the yellow-brown mycelium of this particular symbiont grows out within 24 hours.

In all sites where *Paxillus involutus* fruited, trees, or at least tree seedlings, were present and they invariably bore mycorrhizae formed by it. In addition, a positive correlation was found between the numbers of mycorrhizae and sporophores formed by the species. It is concluded that *Paxillus involutus* does not form sporophores when growing by a saprophytic mode of nutrition.

In the presence of trees the species fruits to varying extents: in poor closed stands hardly at all and in fertile stands profusely. After partial cutting, soil scarification, draining and application of nitrogen, its fruiting increases markedly. The conclusion is drawn that the growth of *Paxillus involutus* in raw humus is arrested primarily as a result of deficiency of available nitrogen. When the amount of available nitrogen increases, the species becomes active, infecting roots and growing into the surrounding soil. In the opposite case *P. involutus* is replaced by species characteristic of raw humus.

In pure culture *Paxillus involutus* behaves in very much the same way as most of the other ectomycorrhizal fungi studied. The amount of submerged mycelium on agar is very limited, but the aerial mycelium profuse. In the latter, sclerotia are also formed. The pH and temperature requirements are on the whole normal, but great differences exist between individual strains. In addition to many simple carbon compounds, the species is also able to utilize starch. Cellulose and lignin are both useless, but the species grows to some extent on birch leaves and decayed wood. Nitrogen is utilized in the form of both ammonium and nitrate, and the species also grows on organic nitrogen sources, such as peptone and certain amino acids. With regard to vitamins it is heterotrophic almost exclusively in respect to thiamine. The species does not secrete any significant amounts of substances inhibiting the growth of other fungi.

If the term facultative is confined to symbionts that are able to form sporophores even from mycelia subsisting by a saprophytic mode of nutrition, it can not be applied to *Paxillus involutus*, but then neither is it merited by certain other species now considered facultative. If, on the other hand, mycorrhizal fungi that survive in soil without a host are considered facultative symbionts, *Paxillus involutus* may very well be included among them. It remains to be shown, however, whether it can grow in the soil without a host, and if so, for how long and to what extent.

Paxillus involutus forms a balanced symbiosis, even when the host is relatively weak and the fungus relatively virulent. The species survives rather well in pine seedlings planted in various sites; moreover, the initial development of these seedlings is better than that of seedlings transplanted when nonmycorrhizal. The symbiotic efficiency of the species in comparison with other mycorrhizal fungi requires additional studies, however.

This study was focused on the fruiting, behavior and attributes in pure culture, and mycorrhiza formation of *Paxillus involutus*. The results obtained by different approaches in general corresponded well. This study further serves to emphasize the need for thorough investigations concerning whether mycorrhizal fungi are capable of fruiting when subsisting by a saprophytic mode of nutrition, special attention being paid to the possible presence of mycorrhizae in the vicinity. In pure culture experiments several strains should be used. Semiaseptic synthesis is sometimes surprisingly rapid, its major handicap being the limited number of fungal symbionts that can be successfully inoculated. In both this and aseptic synthesis mycorrhizal associations can be formed whose existence in nature is questionable.

REFERENCES

AALTONEN, V. T. 1948. Boden und Wald. Paul Parey, Berlin and Hamburg.

ATKINSON, G. F. 1901. Studies of American fungi. Mushrooms. Edible, poisonous, etc. Andrus and Church, Ithaca, N.Y.

BAKSHI, B. K. 1967. Investigations on mycorrhiza forming fungi with special reference to conifers in India. U.S. Public Law 480, 83rd Congress Project, final technical report. Forest Research Institute and Colleges, Dehra Dun.

BECKER, G. 1956. Observations sur l'écologie des champignons supérieurs. Ann. Sci. Univ. Besançon (Ser. 2, Bot.) 7: 15—128.

BJÖRKMAN, E. 1942. Über die Bedingungen der Mykorrhizabildung bei Kiefer und Fichte. Symb. Bot. Upsal. 6.2.

—»— 1949. The ecological significance of the ectotrophic mycorrhizal association in forest trees. Sv. Bot. Tidskr. 43: 223—262.

—»— 1962. The influence of ectotrophic mycorrhiza on the development of forest tree plants after planting. Proc., IUFRO, 13th Congress, Vienna, Part 2, Vol. 1.

—»— 1968. Mycorrhiza research. IUFRO, 14th Congress, Munich, Vol. 10, p. 208.

BOWEN, G. D. 1965. Mycorrhiza inoculation in forestry practice. Austr. For. 29: 231—237.

—»— and THEODOROU, C. 1967. Studies on phosphate uptake by mycorrhizas. Pap., IUFRO, 14th Congress, Munich, Vol. 5, pp. 116—138.

BRESINSKY, A. and STANGL, J. 1965. Beiträge zur Revision M. Britzelmayrs »Hymenomyceten aus Südbayern» 2. Z. Pilzk. 31: 58—59.

BRYAN, W. C. and ZAK, B. 1961. Synthetic culture of mycorrhizae of southern pines. For. Sci. 7: 123—129.

BUCH, R. and KREISEL, H. 1957. Höhere Pilze der Leipziger Auenwäldern. Z. Pilzk. 23: 4—20.

CAJANDER, A. K. 1949. Forest types and their significance. Acta Forest. Fenn. 59.

CHASTUKHIN, V. YA. 1967. Biology and ecology of mycorrhiza-forming fungi. In: Imshenetskii, A. A. (ed.). Mycotrophy in plants (originally published in Russian in 1955), pp. 87—100. Israel Program for Scientific Translations, Jerusalem.

COCHRANE, V. W. 1958. Physiology of fungi. John Wiley, New York.

CORTIN, B. 1956. Svampar i färg. Almqvist and Wiksell, Stockholm.

DOMINIK, T. 1950. Badania mykotrofizmu dzikich grusz na terenie Polski z uwzględnieniem warunków bioekologicznych. Acta Soc. Bot. Polon. 20: 255—303.

—»— 1966. Studies on mycorrhizae (originally published in Polish in 1961). Sci. Publ. Foreign Coop. Center Cent. Inst. Sci., Tech. and Econ. Inform., Warsaw.

EISFELDER, I. 1954. Beiträge zur Kenntnis der Fauna in höheren Pilzen. Z. Pilzk. 16: 1—12.

FASSI, B. and FONTANA, A. 1966. Ricerche sulle micorrize ectotrofiche del pino strobo in vivaio. II. Micorrize di *Thelephora terrestris* EHRH. ex FRIES, di *Laccaria laccata* (SCOP.) BERK. et BR. e di *Hebeloma mesophaeum* PERS. ex FRIES. Summary: Researches on ectotrophic mycorrhizae of *Pinus strobus* in nurseries. II. Mycorrhizae of *Thelephora terrestris* EHRH. ex FRIES, of *Laccaria laccata* (SCOP.) BERK. et BR. and of *Hebeloma mesophaeum* PERS. ex FRIES. Allionia 12: 47—53.

—»— and —»— 1967. Sintesi micorrizica tra *Pinus strobus* e *Tuber maculatum*. Summary: Mycorrhizal synthesis between *Pinus strobus* (eastern white pine) and *Tuber maculatum*. Allionia 13: 177—186.

FISHER, R. A. 1954. Statistical methods for research workers. Oliver and Boyd, Edinburgh.

FONTANA, A. 1961. Primo contributo allo studio delle micorrize dei pioppi in Piemonte. Summary: First contribution to the study of poplar mycorrhizas in Piedmont. Allionia 7: 87—129.

—»— and CENTRELLA, E. 1967. Ectomicorrize prodotte da funghi ipogei. Summary: Ectomycorrhizae produced by hypogeous fungi. Allionia 13: 149—176.

FRIES, N. 1938. Über die Bedeutung von Wuchsstoffen für das Wachstum verschiedener Pilze. Sym. Bot. Upsal. 3.2.

—»— 1942. Einspormyzelien einiger Basidiomyceten als Mykorrhizabildner von Kiefer und Fichte. Sv. Bot. Tidskr. 36: 151—156.

—»— 1943. Untersuchungen über Sporenkeimung und Mycelentwicklung bodenbewohnender Hymenomyceten. Symb. Bot. Upsal. 6.4.

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

GERSCHLER, I. 1959. Vergleichend-ökologische Untersuchungen an Grosspilz-Standorten der Dresdener Heide. Z. Pilzk. 25: 77—103.

GILMOUR, J. W. 1958. Chlorosis of Douglas fir. New Zeal. J. For. 7: 94—106.

GÖBL, F. 1967. Mykorrhiza-Typen in Pflanzgärten. Pap., IUFRO, 14th Congress, Munich, Vol. 5, pp. 60—66.

GROVES, J. W. 1962. Edible and poisonous mushrooms of Canada. Duhamel, Ottawa.

HACSKAYLO, E. 1953. Pure culture syntheses of pine mycorrhizae in terra-lite. Mycologia 45: 971—975.

—»— 1965. *Thelephora terrestris* and mycorrhizae of Virginia pine. For. Sci. 11: 401—404.

—»— , PALMER, J. G., and VOZZO, J. A. 1965. Effect of temperature on growth and respiration of ectotrophic mycorrhizal fungi. Mycologia 57: 748—756.

Hacskaylo, E. and Vozzo, J. A. 1967. Inoculation of *Pinus caribaea* with pure cultures of mycorrhizal fungi in Puerto Rico. Pap., IUFRO, 14th Congress, Munich, Vol. 5, pp. 139—148.

Harley, J. L. 1968. Mycorrhiza. In: Ainsworth, G. C. and Sussman, A. S. (ed.). The fungi, Vol. 1, pp. 139—178. Academic Press, New York and London.

—»— 1969. The biology of mycorrhiza. 2nd edit. Leonard Hill, London.

Hatch, A. B. 1937. The physical basis of mycotrophy in *Pinus*. Black Rock Forest Bull. 6.

Hattula, M. L. 1968. Liuosmenetelmällä tuotetun sienimyseelin aminohappokoostumus. Unpublished licenciate thesis in the Dept of Microbiology, Univ. of Helsinki.

—»— and Gyllenberg, H. 1969. Adaptability to submerged culture and amino acid contents of certain fleshy fungi common in Finland. Karstenia 9: 39—45.

Heikurainen, L. 1955. Rämemännikön juuriston rakenne ja kuivatuksen vaikutus siihen. Referat: Der Wurzelaufbau der Kiefernbestände auf Reisermoorböden und seine Beeinflussung durch die Entwässerung. Acta Forest. Fenn. 65.3.

—»— 1958. Sekametsiköiden juuristoista ojitetulla suolla. Referat: Der Wurzelaufbau in Mischwäldern auf entwässerten Moorböden. Acta Forest. Fenn. 67.2.

—»— 1964. Improvement of forest growth on poorly drained peat soils. Intern. Rev. For. Res. 1: 39—113.

Helimäki, U. I. 1967. Tables and maps of precipitation in Finland, 1931—1960. The Meteorological Yearbook of Finland 66.2.

Henderson, G. S. 1968. The influence of mycorrhizae on nutrient uptake and growth of conifer seedlings. Ph. D. thesis, Cornell University.

Hesselman, H. 1926. Studier över barrskogens humustäcke, dess egenskaper och beroende av skogsvården. Referat: Studien über die Humusdecke des Nadelwaldes, ihre Eigenschaften und deren Abhängigkeit vom Waldbau. Medd. Stat. Skogsförsöksanst. 22.5.

Hintikka, V. 1960 a. Zur Ökologie einiger an Brandplätzen vorkommender Blätterpilzarten. Karstenia 5: 100—106.

—»— 1960 b. Das Verhalten einiger *Mycena*-Arten zum pH sowie deren Einfluss auf die Azidität der Humusschicht der Wälder. Karstenia 5: 107—121.

—»— 1964. Psychrophilic basidiomycetes decomposing forest litter under winter conditions. Comm. Inst. For. Fenn. 59.2.

—»— 1970. Studies on white-rot humus formed by higher fungi in forest soils. Comm. Inst. For. Fenn. 69.2.

Horak, E. 1963. Pilzökologische Untersuchungen in der subalpinen Stufe (Piceetum subalpinum und Rhodoreto vaccinietum) der rätischen Alpen. Schweiz. Anst. forstl. Versuchswesen 39.1.

—»— and Moser, M. 1966. Fungi Austroamericani VIII. Nova Hedvigia 10: 329—338.

How, J. E. 1942. The mycorrhizal relations of larch. III. Mycorrhiza formation in nature. Ann. Bot. N. S. 6: 103—129.

Hyppel, A. 1968. Effect of *Fomes annosus* on seedlings of *Picea abies* in the presence of *Boletus bovinus*. Studia Forestalia Suecica 66.

Imazeki, R. and Hongo, T. 1965. Colored illustrations of fungi of Japan. I. Higashi-Ku, Osaka.

Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill, New York and London.

Kalela, E. K. 1949. Männiköiden ja kuusikoiden juurisuhteista. Summary: On the horizontal roots in pine and spruce stand. I. Acta Forest. Fenn. 57.2.

Kalmár, Z. 1950. Kalapos gombáink (Hymenomycetes) mykorrhiza kapcsolatai. Magyar Agrár. Egyetem, Erdöm. Kar. Evkön. 1: 157—187.

Karsten, P. A. 1879. Rysslands, Finlands och den skandinaviska halföns hattsvampar. Bidrag till kännedom av Finl. natur. o. folk 32.

Kauffman, C. H. 1918. The Agaricaceae of Michigan. I. State Printers, Lansing.

Kelley, A. P. 1950. Mycotrophy in plants. Chronica Botanica, Waltham, Mass.

Kleijn, H. 1962. Grosses Fotobuch der Pilze. BLV Verlagsgesellschaft, München.

Kolkki, O. 1966. Tables and maps of temperature in Finland during 1931—1960. The Meteorological Yearbook of Finland 65.1 a.

Komirnaya, O. N. and Fursayev, A. D. 1953. Forest fungi of a tree plantation of semi-desert Zavolzh and questions of mycorrhiza formation. Bot. Zhur. 38: 426—428 (Russian).

Kreisel, H. 1954. Beobachtungen über die Pilzflora einiger Hoch- und Zwischenmoore Ost-Mecklenburgs. Wiss. Zeitschr. Univ. Greifswald 3: 291—300.

Krieger, L. C. C. 1967. The mushroom handbook. Dover, New York.

Krywolap, G. N., Grand, L. F., and Casida, L. E. 1964. The natural occurrence of an antibiotic in the mycorrhizal fungus *Cenococcum graniforme*. Canad. J. Microbiol. 10: 323—328.

Lähde, E. 1969. Biological activity in some natural and drained peat soils with special reference to oxidation-reduction conditions. Acta Forest. Fenn. 94.

Laiho, O. 1965. Further studies on the ectendotrophic mycorrhiza. Acta Forest. Fenn. 79.3.

—»— 1967. Field experiments with ectendotrophic Scotch pine seedlings. Pap., IUFRO, 14th Congress, Munich, Vol. 5. pp. 149—157.

—»— and Mikola, P. 1964. Studies on the effect of some eradicants on mycorrhizal development in forest nurseries. Acta Forest. Fenn. 77.2.

Laitakari, E. 1927. Männyn juuristo. Morfologinen tutkimus. Summary: The root system of pine (*Pinus silvestris*). A morphological investigation. Acta Forest. Fenn. 33.1.

—»— 1934. Koivun juuristo. Summary: The root system of birch (*Betula verrucosa* and *odorata*). Acta Forest. Fenn. 41.2.

Lange, J. E. 1939. Flora Agaricina Danica IV. Copenhagen.

Lange, M. 1944. Iakttagelser over svampefloraen paa brandpletter. Summary: Notes on fungi confined to burnt ground. Friesia 3: 58–61.

Lange, M. 1946. Mykologiske indtryck fra Lapland. Summary: Mycological impressions from Lapland. Friesia 3: 161–170.

—»— 1948. The agarics of Maglemose. Dansk Bot. Arkiv. 13.1.

—»— 1957. Macromycetes. III. Greenland Agaricales. Medd. om Grønland 148.2.

Levisohn, I. 1955. Isolation of ectotrophic mycorrhizal mycelia from rhizomorphs present in soil. Nature (Lond.) 176: 519.

—»— 1958. Effects of mycorrhiza on tree growth. Soils and Fertilizers 21: 73–82.

Lindeberg, G. 1944. Über die Physiologie ligninabbauender Bodenhymenomyzeten. Studien an schwedischen *Marasmius*-Arten. Symb. Bot. Upsal. 8.2.

—»— 1948. On the occurrence of polyphenol oxidases in soil-inhabiting basidiomycetes. Phys. Plant. 1: 196–205.

Lisiewska, M. 1965. Udział grzybów wyższych w grądach Wielkopolski. Summary: Higher fungi of the Querco-Carpinetum of the Wielkopolska province. Acta Mycologica 1: 169–268.

Lobanow, N. W. 1960. Mykotrophie der Holzpflanzen (originally published in Russian in 1953). Deutscher Verlag Wiss., Berlin.

Lundeberg, G. 1963. Die Beziehungen zwischen Kiefernsämlingen und Bodenpilzen-einige Untersuchungen mit einer neuen Wassersterilkulturmethode. In: Rawald, W. and Lyr, H. (ed.). Mykorrhiza, Intern. Mykorrhizasymp., Weimar 1960, pp. 185–189. Fischer, Jena.

—»— 1967. Raw humus as the nitrogen source for pine seedlings. Pap., IUFRO, 14th Congress, Munich, Vol. 5, pp. 112–115.

—»— 1970. Utilisation of various nitrogen sources, in particular bound soil nitrogen, by mycorrhizal fungi. Studia Forestalia Suecica 79.

Lyr, H. 1963 a. Über die Abnahme der Mykorrhiza- und Knöllchenfrequenz mit zunehmender Bodentiefe. In: Rawald, W. and Lyr, H. (ed.). Mykorrhiza, Intern. Mykorrhizasymp., Weimar 1960, pp. 303–313. Fischer, Jena.

—»— 1963 b. Zur Frage des Streuabbaues durch ektotrophe Mykorrhizapilze. In: Rawald, W. and Lyr, H. (ed.). Mykorrhiza, Intern. Mykorrhizasymp., Weimar 1960, pp. 123–145. Fischer, Jena.

McComb, A. L. 1943. Mycorrhizae and phosphorus nutrition of pine seedlings in a prairie soil nursery. Iowa Agr. Exp. Sta. Res. Bull. 314: 582–612.

Marks, G. C., Ditchburne, N., and Foster, R. C. 1967. A technique for making quantitative estimates of mycorrhiza populations in *Pinus radiata* forests and its application in Victoria. Pap., IUFRO, 14th Congress, Munich, Vol. 5, pp. 67–83.

Marx, D. H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59: 153–163.

—»— and Bryan, W. C. 1969. Studies on ectomycorrhizae of pine in an electronically air-filtered, air-conditioned, plant-growth room. Can. J. Bot. 47: 1903–1909.

Marx, D. H. and —»— 1970. Pure culture synthesis of ectomycorrhizae by *Thelephora terrestris* and *Pisolithus tinctorius* on different conifer hosts. Can. J. Bot. 48: 639–643.

—»—, —»—, and Grand, L. F. 1970. Colonization, isolation, and cultural descriptions of *Thelephora terrestris* and other ectomycorrhizal fungi of shortleaf pine seedlings grown in fumigated soil. Can. J. Bot. 48: 207–211.

—»— and Ross, E. W. 1970. Aseptic synthesis of ectomycorrhizae on *Pinus taeda* by basidiospores of *Thelephora terrestris*. Can. J. Bot. 48: 197–198.

—»— and Zak, B. 1965. Effect of pH on mycorrhizal formation of slash pine in aseptic culture. For. Sci. 11: 66–75.

Melin, E. 1925. Untersuchungen über die Bedeutung der Baummykorrhiza. Eine ökologisch-physiologische Studie. Fischer, Jena.

—»— 1927. Studier över barrträdsplantans utveckling i råhumus. II. Mykorrhizans utbildning hos tallplantan i olika råhumusformer. Referat: Studien über die Entwicklung der Nadelbaumpflanze in Rohhumus. II. Die Ausbildung der Mykorrhiza bei der Kiefernpflanze in verschiedenen Rohhumusformen. Medd. Stat. Skogsförsöksanst. 23: 433–494.

—»— 1934. En ny methodik för svampkulturer på steriliserad humus. Sv. Bot. Tidskr. 28: 441–442.

—»— 1936. Methoden der experimentellen Untersuchung mykotropher Pflanzen. In: Abderhalden, E. (ed.). Handbuch der biologischen Arbeitsmethoden, Vol. 11, pp. 1015–1108. Urban and Schwarzenberg, Berlin.

—»— 1946. Der Einfluss von Waldstreuextrakten auf das Wachstum von Bodenpilzen, mit besonderer Berücksichtigung der Wurzelpilze von Bäumen. Symb. Bot. Upsal. 8.3.

—»— 1953. Physiology of mycorrhizal relations in plants. Ann. Rev. Plant Phys. 4: 325–346.

—»— 1955. Nyare undersökningar över skogsträdens mykorrhizasvampar och det fysiologiska växelspelet mellan dem och trädens rötter. In: Inbjudningar till doktorspromotionerna, bifogad skrift. Almqvist and Wiksell, Uppsala.

—»— 1959 a. Mycorrhiza. In: Ruhland, W. (ed.) Handbuch der Pflanzenphysiologie, Vol. 11, pp. 605–638. Sprinter Verlag, Berlin, Göttingen and Heidelberg.

—»— 1959 b. Studies on the physiology of tree mycorrhizal basidiomycetes. I. Growth response to nucleic acid constituents. Sv. Bot. Tidskr. 53: 134–154.

—»— 1962. Physiological aspects of mycorrhizae of forest trees. In: Kozlowski, T. T. (ed.). Tree growth, pp. 247–263. Ronald Press, New York.

—»— 1963. Some effects of forest tree roots on mycorrhizal basidiomycetes. In: Nutman, P.S. and Mosse, B. (ed.). Symbiotic associations, pp. 125–145. Cambridge University Press.

—»— and Norkrans, B. 1942. Über den Einfluss der Pyrimidin- und der Thiazolkomponente des Aneurins auf das Wachstum von Wurzelpilzen. Sv. Bot. Tidskr. 36: 271—286.

Meyer, F. H. 1963. *Laccaria amethystina* (Bolt. ex. Fr.) Berk et Br., ein zur Mykorrhizabildung an der Buche befähigter Pilz. Ber. Deutsch. Bot. Ges. 76: 90—96.

—»— 1964. The role of the fungus *Cenococcum graniforme* (Sow.) Ferd. et Winge in the formation of mor. In: Jongerius, A. (ed.). Soil micromorphology, pp. 23—31. Elsevier, Amsterdam.

—»— 1966. Mycorrhiza and other plant symbiosis. In: Henry, S. M. (ed.). Symbiosis, Vol. 1, pp. 171—255. Academic Press, New York and London.

Michael, E. and Hennig, B. 1958. Handbuch für Pilzfreunde. I. Die wichtigsten und häufigsten Pilze. Fischer, Jena.

—»— and —»— 1967. Handbuch für Pilzfreunde. IV. Blätterpilze — Dunkelblättler. Fischer, Jena.

Mikola, P. 1948. On the physiology and ecology of *Cenococcum graniforme*, especially as a mycorrhizal fungus of birch. Comm. Inst. For. Fenn. 36.3.

—»— 1954 a. Metsämaan kantasienien kyvystä hajoittaa neulas- ja lehtikarikkeita. Summary: Experiments on the ability of forest soil basidiomycetes to decompose litter material. Comm. Inst. For. Fenn. 42.7.

—»— 1954 b. Kokeellisia tutkimuksia metsäkarikkeiden hajaantumisnopeudesta. Summary: Experiments on the rate of decomposition of forest litter. Comm. Inst. For. Fenn. 43.1.

—»— 1955. Metsämaan kantasienien puhdasviljely. Summary: Growing forest soil basidiomycetes in pure culture. Karstenia 3: 5—16.

—»— 1956. Studies on the decomposition of forest litter by basidiomycetes. Comm. Inst. For. Fenn. 48.2.

—»— 1957. Physiological variation in *Collybia butyracea* (Bull.) Fr. Karstenia 4: 10—13.

—»— 1962. The bright yellow mycorrhiza of raw humus. Proc., IUFRO, 13th Congress, Vienna, Part 2, Vol. 1.

—»— 1965. Studies on the ectendotrophic mycorrhiza of pine. Acta Forest. Fenn. 79.2.

—»— 1967. The effect of mycorrhizal inoculation on the growth and root respiration of Scotch pine seedlings. Pap., IUFRO, 14th Congress, Munich, Vol. 5. pp. 100—111.

—»— 1970. Mycorrhizal inoculation in afforestation. Int. Review of For. Res. 3: 123—196.

—»— and Laiho, O. 1962. Mycorrhizal relations in the raw humus layer of northern spruce forests. Comm. Inst. For. Fenn. 55.18.

—»— , —»—, Erikäinen, J., and Kuvaja, K. 1964. The effect of slash burning on the commencement of mycorrhizal association. Acta Forest. Fenn. 77.3.

—»— and Persidsky, D. J. 1951. Detection of mycorrhizal infection in early stages of seedling development. State Cons. and Soils Dept, Coll. Agr., Madison, Wis., Tech. Note 39.

Modess, O. 1941. Zur Kenntnis der Mykorrhizabildner von Kiefer und Fichte. Symb. Bot. Upsal. 5.1.

Molin, N. 1957. Om *Fomes annosus* spridningsbiologi. Summary: A study on the infection biology of *Fomes annosus*. Medd. Stat. Skogsförsöksinst. 47.3.

Moser, M. 1949. Untersuchungen über den Einfluss von Waldbränden auf die Pilzvegetation I. Sydovia 3: 336—383.

—»— 1956. Die Bedeutung der Mykorrhiza für Aufforstungen in Hochlagen. Forstwiss. Cbl. 75: 8—18.

—»— 1958 a. Der Einfluss tiefer Temperaturen auf das Wachstum und die Lebenstätigkeit höherer Pilze mit spezieller Berücksichtigung von Mykorrhizapilzen. Sydowia 12: 386—399.

—»— 1958 b. Die künstliche Mykorrhizaimpfung an Forstpflanzen. I. Erfahrungen bei der Reinkultur von Mykorrhizapilzen. Forstwiss. Cbl. 77: 32—40.

—»— 1958 c. Die künstliche Mykorrhizaimpfung von Forstpflanzen. II. Die Torfstreukultur von Mykorrhizapilzen. Forstwiss. Cbl. 77: 273—278.

—»— 1959. Beiträge zur Kenntnis der Wuchsstoffbeziehungen im Bereich ectotropher Mycorrhizen I. Archiv für Mikrobiologie 34: 251—269.

—»— 1962. Soziologische und ökologische Fragen der Mykorrhiza-Induzierung. Proc., IUFRO, 13th Congress, Vienna, Part 2, Vol. 1.

—»— 1963. Die Bedeutung der Mykorrhiza bei Aufforstungen unter besonderer Berücksichtigung von Hochlagen. In: Rawald, W. and Lyr, H. (ed.). Mykorrhiza, Intern. Mykorrhizasymp., Weimar 1960, pp. 407—424.

—»— 1967 a. Antwort. Z. Pilzk. 33: 41.

—»— 1967 b. Die Röhrlinge und Blätterpilze (Agaricales). In: Gams, H. (ed.). Kleine Kryptogamenflora, Vol. II b/2. Fischer, Stuttgart.

Norkrans, B. 1950. Studies in growth and cellulolytic enzymes of *Tricholoma* with special reference to mycorrhiza formation. Symb. Bot. Upsal. 11.1.

Nyyssönen, A., Kilkki, P., and Mikkola, E. 1967. On the precision of some methods of forest inventory. Acta Forest. Fenn. 81.4.

Orloś, H. 1967. Ecological studies on the mycoflora of some forest types in the Białowieża national park (originally published in Polish in 1961). Sci. Publ. Foreign Coop. Cent. Inst. Sci., Tech. and Econ. Inform., Warsaw.

Pachlewski, R. 1967 a. Investigations of pure culture of mycorrhizal fungi of pine. U.S. Public Law 480, 83rd Congress Project. Polish Forest Research Institute, Warsaw.

—»— 1967 b. Studies on mycorrhizal fungi of pine (*Pinus silvestris* L.) — *Lactarius rufus* (Scop. ex Fr.) Fr. and *Rhizopogon luteolus* Fr. and Nordh. under natural conditions and in pure cultures. Pap., IUFRO, 14th Congress, Munich, Vol. 5, pp. 12—28.

—»— and Pachlewska, J. 1960. Badania morfologiczno-anatomiczne mikoryz sozny (*Pinus silvestris* L.) w zespołach leśnych Białowieskiego parku narodowego. Referat: Morphologisch-anatomische Forschungen der Mykorrhizen der Kiefer (*Pinus silvestris* L.) in den Waldgesellschaften des bialowieser Nationalparks. Inst. Badawczy Leśn. 205: 3—48.

Peyronel, B. 1922. Altri nuovi casi di rapporti mikorizici tra fanerogame e basidiomyceti. Soc. Bot. Ital. Bull. 4: 50—52.

—»— 1963. Mykorrhizenstruktur und mykorrhizogene Pilze. In: Rewald, W. and Lyr. H. (ed.). Mykorrhiza, Intern. Mykorrhizasymp., Weimar 1960, pp. 15—25. Fischer, Jena.

Ramsbottom, J. 1951. A handbook of the larger British fungi. Jarrold, Norwich.

—»— 1953. Mushrooms and toadstools. A study of the activities of fungi. Collins, London.

Rautavaara, T. 1947. Suomen sienisato. Summary: Studies on the mushroom crop in Finland and its utilization. WSOY, Porvoo.

Ritter, G. and Lyr, H. 1965. The significance of mycorrhizal fungi for the utilization of different sources of phosphate by *Pinus silvestris* L. In: Macura, J. and Vancura, V. (ed.). Plant microbes relationships, pp. 277—282. Chech. Acad. Sci., Prague.

Robertson, N. F. 1954. Studies on the mycorrhiza of *Pinus silvestris*. I. The pattern of development of mycorrhizal roots and its significance for experimental studies. New Phytol. 53: 253—283.

Romell, L.-G. 1930. Blodriskan, en granens följesvamp. Referat: Der echte Reizker, ein Fichtenbegleiter. Sv. Bot. Tidskr. 24: 524—530.

—»— 1932. Mull and duff as biotic equilibria. Soil Sci. 34: 161—188.

—»— 1934. En biologisk teori för mårbildning och måraktivering. Stockholm.

—»— 1935. Ecological problems of the humus layer in the forest. Cornell Univ. Agr. Exp. Sta., Mem. 170.

—»— 1938. A trenching experiment in spruce forest and its bearing on problems of mycotrophy. Sv. Bot. Tidskr. 32: 89—99.

—»— 1939. Barrskogens marksvampar och deras roll i skogens liv. Summary: Soil fungi of softwood forests and their rôle in the forest's life. Sv. Skogsvårdsf. Tidskr. 37: 348—375.

Rosendahl, R. O. and Wilde, S. A. 1942. Occurrence of ectotrophic mycorrhizal fungi in soils of cut-over areas and sand dunes. Bull. Ecol. Soc. Amer. 23: 73—74.

Rothmayr, J. 1962. Kartoffelbovist und Mykorrhizenproblem. Allg. Forstzeitschr. 17: 444—445.

Runov, E. V. 1967. Experimental introduction of mycorrhizas into oak sowings in arid steppe. In: Imshenetskii, A. A. (ed.). Mycotrophy in plants (originally published in Russian in 1955), pp. 174—186. Israel Program for Scientific Translations, Jerusalem.

Russell, E. W. 1961. Soil conditions and plant growth. 9th edit. Longmans, London.

Rypáček, V. 1963. Die gegenseitigen Beziehungen zwischen Mykorrhizapilzen und holzzerstörenden Pilzen. In: Rawald, W. and Lyr, H. (ed.). Mykorrhiza, Intern. Mykorrhizasymp., Weimar 1960, pp. 233—240. Fischer, Jena.

Santoro, T. and Casida, L. E. 1962. Elaboration of antibiotics by *Boletus luteus* and certain other mycorrhizal fungi. Canad. J. Microbiol. 8: 43—48.

Sarasto, J. 1957. Metsän kasvattamiseksi ojitettujen soiden aluskasvullisuuden rakenteesta ja kehityksestä Suomen eteläpuoliskossa. Referat: Über Struktur und Entwicklung der Bodenvegetation auf für Walderziehung entwässerten Mooren in der südlichen Hälfte Finnlands. Acta Forest. Fenn. 65. 7.

Schelling, C. L. 1950. Die Verwertbarkeit verschiedener Kohlenstoffquellen durch *Mycelium radicis atrovirens* Melin. Schweiz. Z. Allg. Path. Bakteriol. 13: 570—574.

Schramm, J. R. 1966. Plant colonization studies on black wastes from antracite mining in Pennsylvania. Trans. Amer. Phil. Soc. 56.1.

Schulmann, O. 1960. Zur Kenntnis der Basidiomyceten Finnlands. Karstenia 5: 5—99.

Schwöbel, H. 1956. Beitrag zur Frage der Mykorrhizabildung. Z. Pilzk. 22: 20—23.

Shemakhanova, N. M. 1963. Bedingungen der Mykorrhizabildung bei *Pinus silvestris* mit *Boletus luteus* Fr. in Reinkultur. In: Rawald, W. and Lyr, H. (ed.). Mykorrhiza, Intern. Mykorrhizasymp., Weimar 1960, pp. 191—202.

—»— 1967. Mycotrophy of woody plants (originally published in Russian in 1962). Israel Program for Scientific Translations, Jerusalem.

Singer, R. 1946. The Boletineae of Florida with notes on extralimital species. IV. The lamellate families (Gomphidiaceae, Paxillaceae, Jugasporaceae). Farlowia 2: 527—564.

—»— 1954. Agaricales von Nahuel Huapi. Sydowia 8: 100—157.

—»— 1962. The Agaricales in modern taxonomy. Cramer, Weinheim.

—»— 1963. Der Ektotroph, seine Definition, geographische Verbreitung und Bedeutung in der Forstökologie. In: Rawald, W. and Lyr, H. (ed.). Mykorrhiza, Intern. Mykorrhizasymp., Weimar 1960, pp. 223—231. Fischer, Jena.

—»— 1964 a. Areal und Ökologie des Ektotrophs in Südamerika. Z. Pilzk. 30: 8—14.

—»— 1964 b. Boletes and related groups in South America. Nova Hedvigia 7: 93—132.

—»— and Moser, M. 1965. Forest mycology and forest communities in South America. Mycopathol. et Mycol. Appl. 26: 129—191.

Sirén, G. 1950. Alikasvoskuusten biologiaa. Summary: On the biology of undergrown spruce. Acta Forest. Fenn. 58.2.

Slankis, V. 1967. Renewed growth of ectotrophic mycorrhizae as an indication of an unstable symbiotic relationship. Pap., IUFRO, 14th Congress, Munich, Vol. 5, pp. 84—99.

Snedecor, G. W. and Cochran, W. G. 1967. Statistical methods. 6th edit. Iowa State University Press, Ames.

Sundman, V. 1965. A study of lignanolytic soil bacteria with special reference to α-conidendrin decomposition. Acta Polytechn. Scand. 40.

Thesleff, A. 1919. Studier öfver basidsvampfloran i sydöstra Finland. Bidr. till kännedom af Finl. natur o folk. 79.1.

Trappe, J. M. 1961. Strong hydrogen peroxide for sterilizing coats of tree seed and stimulating germination. J. For. 59: 828—829.

—»— 1962. Fungus associates of ectotrophic mycorrhizae. Bot. Rev. 28: 538—606.

Trappe, J. M. 1964. Mycorrhizal hosts and distribution of *Cenococcum graniforme*. Lloydia 27: 100—106.

—»— 1967 a. Principles of classifying ectotrophic mycorrhizae for identification of fungal symbionts. Pap., IUFRO, 14th Congress, Munich, Vol. 5, pp. 46—59.

—»— 1967 b. Pure culture synthesis of Douglas-fir mycorrhizae with species of *Hebeloma*, *Suillus*, *Rhizopogon*, and *Astraeus*. For. Sci. 13: 121—130.

—»— 1969. Personal communication.

—»— and Strand, R. F. 1969. Mycorrhizal deficiency in a Douglas-fir region nursery. For. Sci. 15: 381—389.

Tuomikoski, R. 1950. *Paxillus filamentosus* Fr. (*P. leptopus* Fr.) in Finnland gefunden. Karstenia 1: 76—77.

—»— 1959. Sienet värikuvina. WSOY, Porvoo.

—»— 1960. Soiden lakkisienistä. Suo 11: 90—91.

Uggla, E. 1958. Ecological effects of fire on North Swedish forests. Almqvist and Wiksell, Uppsala.

Viro, P. J. 1951. Nutrient status and fertility of forest soil. I. Pine stands. Comm. Inst. For. Fenn. 39.4.

—»— 1962. Forest site evaluation in Lapland. Comm. Inst. For. Fenn. 55.9.

—»— 1969. Prescribed burning in forestry. Comm. Inst. For. Fenn. 67.7.

Werlich, I. and Lyr, H. 1957. Über die Mykorrhizaausbildung von Kiefer (*Pinus silvestris* L.) und Buche (*Fagus silvatica* L.) auf verschiedenen Standorten. Arch. Forstw. 6: 1—23.

Wilcox, H. 1967. Seasonal patterns of root initiation and mycorrhizal development in *Pinus resinosa* Ait. Pap., IUFRO, 14th Congress, Munich, Vol. 5, pp. 29—39.

Witkamp, M. 1959. Seasonal fluctuations of the fungusflora in mull and mor of an oak forest. G. W. van der Wiel, Arnhem.

Wittich, W. 1939. Untersuchungen über den Verlauf der Streuzersetzung auf einem Boden mit Mullzustand. Forstarchiv. 15: 96—111.

—»— 1952. Der heutige Stand unseres Wissens vom Humus und neue Wege zur Lösung des Rohhumusproblems im Walde. Schriftenreihe d. Forstl. Fak. d. Univ. Göttingen 4.

Zak, B. 1964. Role of mycorrhizae in root disease. Ann. Rev. Phytopath. 2: 377—392.

—»— and Bryan, W. C. 1963. Isolation of fungal symbionts from pine mycorrhizae. For. Sci. 9: 270—278.

—»— and Marx, D. H. 1964. Isolation of mycorrhizal fungi from roots of individual slash pines. For. Sci. 10: 214—222.

Zerova, M. Ya. 1967. Mycorrhiza formation with forest trees in the Ukrainian SSR. In: Imshenetskii, A. A. (ed.). Mycotrophy in plants (originally published in Russian in 1955), pp. 39—60. Israel Program for Scientific Translations, Jerusalem.

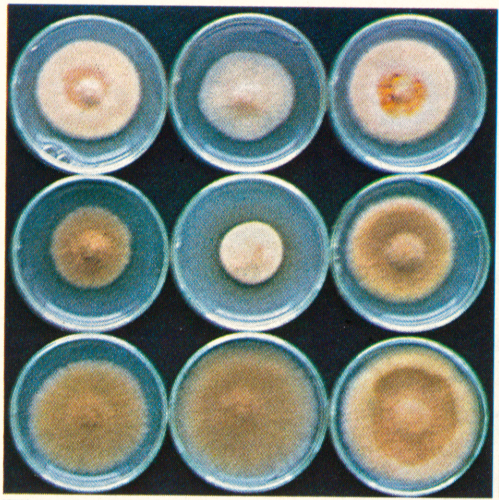

Fig. 23. *Paxillus involutus* strains 1, 3, 4 (upper row), 5, 7, 8 (middle row), 12, 18, and 24 (lower row) on Hagem agar. Age of colonies 16 days. Note differences in color.

Fig. 24. Pine mycorrhizae formed in aseptic synthesis with *Paxillus involutus*, strain 5. Seeding April 10, inoculation May 9, photo Oct. 8, 1968. Note rhizomorphs and sclerotia. Magnification x 6.

Fig. 25. A view of the outer side wall of the trench reexposed, just behind the polythene. Isolated plot 5 in stand 7 o, *Vaccinium* type. Trenching May 6, photo Oct. 10, 1968. New pine roots, grown against the polythene, with coralloid mycorrhizae formed by *Paxillus involutus* as well as thick rhizomorphs. $^2/_3$ of natural size.

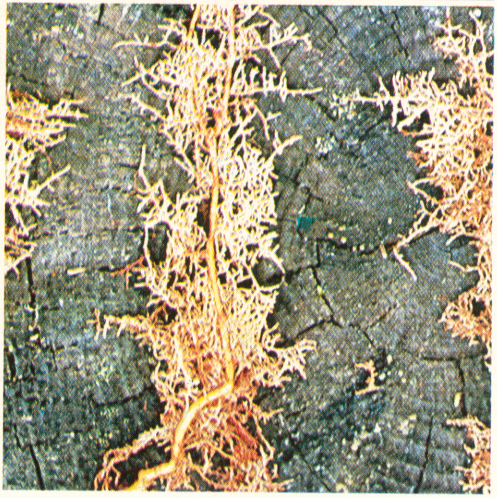

Fig. 26. Heavy *Paxillus involutus* infection on birch roots that had grown in decayed spruce wood. $^2/_3$ of natural size.

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