A Study of the Mycorrhiza of Abies firma, S. et Z., with special Reference to its mycorrhizal Fungus, Cantharellus floccosus, SCHW.

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With Plates II–V and 36 Text-figures.

Contents.

Introduction.

In the late spring of 1923, when we made an excursion to Kurama and its vicinity, about seven miles north from Kyoto, I found a mass of orange colored mushrooms, *Cantharellus floccosus* (nom. jap. *Usu-take*), which by careful digging was found to be actually attached to the mycorrhizal roots of a fir tree, *Abies firma*, standing nearby.

Though the mycorrhiza formation of *Abies firma* has been already described by Tubeuf ('96), Noelle ('10) and Mimura ('17), yet our knowledge of it is scanty, and especially the nature of the intimate relationship between fir tree and mushroom is quite unkown, so I resolved to carry out further investigations.

The study of the ectotrophic mycorrhiza has engaged the attention of numerous investigators since the appearance of Frank's first paper on the subject in 1885. Frank was really the father of mycorrhizal study. He first demonstrated the true morphological nature of the ectotrophic mycorrhiza and applied the name to it. He also advanced

the hypothesis that the fungus which is the cause of ectotrophic mycorrhiza is symbiotic with the root of the higher plant. Under the influence of this idea, numerous investigators have studied and discussed the nature of mycorrhiza. MÜLLER ('86), STAHL ('oo), TUBEUF ('03), PEKLO ('09), MELIN ('23–'24) and others have assumed it to be a symbiotic association, while WORONIN ('85), FUCHS ('11), WEYLAND ('12) and McDOUGALL ('14) considered it to be of parasitic nature. WORONIN, for instance, concluded after his research, that " das Umhüllen der Wurzeln mit einem Pilz-mantel habe ich für eine besondere Art von Parasitisum gehalten ". WEYLAND investigated it microchemically and stated his conviction in the following words : " dass, die ektotrophe Mykorrhiza tatsächlich ein echter Parasitisum des Pilzes auf der Pflanze ist, und mit einer Symbiose nichts gemein hat ". McDOUGALL discussed the question whether ectotrophic mycorrhiza represents symbiotic or parasitic association, and in conclusion he made the following statement : " There seems to be no good evidence that the root gets any food through, or from, the fungus, the evidence indicating, rather, that it is not. On the other hand, there is no question but fungus gets some food from the root."

As to the mycorrhizal roots of *Abies firma* I also could find no symbiotic relation with *Cantharellus floccosus*. On the contrary it must be considered in various respects as an instance of parasitism of the fungus on the roots.

In the histological investigation of the mycorrhiza, McDOUGALL has done some very instructive work. He divided the ectotrophic mycorrhiza into six forms. According to his idea, the mycorrhiza caused by *Cantharellus floccosus* clearly belongs to the sixth form, that is, heterotrophic mycorrhiza. He mentioned moreover a good illustration which clearly shows that *Russula* is a mycorrhizal fungus of *Tilia*.

Many investigators have studied the fungi which cause the ectotrophic mycorrhizas on the roots of various higher plants, and some species of *Russula, Cortinarius, Tricholoma, Boletus, Lactarius, Amanita, Agaricus, Geaster, Scleroderma*, etc., have been described as mycorrhizal

fungi. In recent years FUCHS (1911) and MELIN (1921–3) have made so-called " Synthesenversuch " in order to determine the true my-corrhizal fungi and the experiments of the latter have been successful.

As to the ecological conditions for the development of mycelium in the soil, SHANTZ and PIEMEISEL ('17) have reported some interesting cases. In the case of *Cantharellus floccosus* I also observed many in-teresting conditions for the mycelial development as well as for the production of fruiting bodies, which may be applicable to some other mushrooms.

Cantharellus floccosus is a wide-spread species, but, hitherto, it has not yet been described as a mycorrhizal fungus. In Japan, the mushroom occur not only in the vicinity of Kyoto, but also in Hok-kaido, Nikko and perhaps in many other places.

Presumably it has the same mycorrhizal relation with other species of *Abies*. I discovered specimen of the same produced from an infected root of *Abies Mayriana* MIYABE et KUDO.

NOAK ('89), PENNINGTON ('06), McDOUGALL ('14) and MELIN ('21–23) reported that one kind of tree may have a few or several kinds of fungi, each of which produces more or less different forms of my-corrhizas on the roots. In the case of *Abies firma*, I could find also four different types of mycorrhizas, which are described in the last chapter.

I. The relation between the root of Abies firma
S. et Z. and Cantharellus floccosus, SCHW.

1. General features of the occurrence of mushroom.

The fruiting body of *Cantharellus floccosus* is found, in my expe-rience, only in the area where the young roots of the *Abies* in ques-tion are distributed.

In order to make clear the origin of the mushroom I observed a great many buttons in detail, and I could distinguish the following four types differentiated according to their mycelial origination.

A. *Fruiting body originates directly from the infected root.*

The mycelium of *Cantharellus floccosus*, which advances along the surface of the young root, makes its luxuriant development especially on the young portion of the growing root, and numerous hyphae are given off radially from its surface into the surrounding soil, until the soil particles are cemented together into tolerable hardness. (Text fig. 1)

When such infected roots are found very near to the surface of the soil or in the interspaces of rocks, the fruiting bodies produced are always lacking in mycelial strands at their basal ends. (Fig. 7, Pl. III)

The fungous mantle, from which the fruiting body is produced, is very thick, in some cases measuring more than 3 mm in diameter. (Fig. 6, Pl. II)
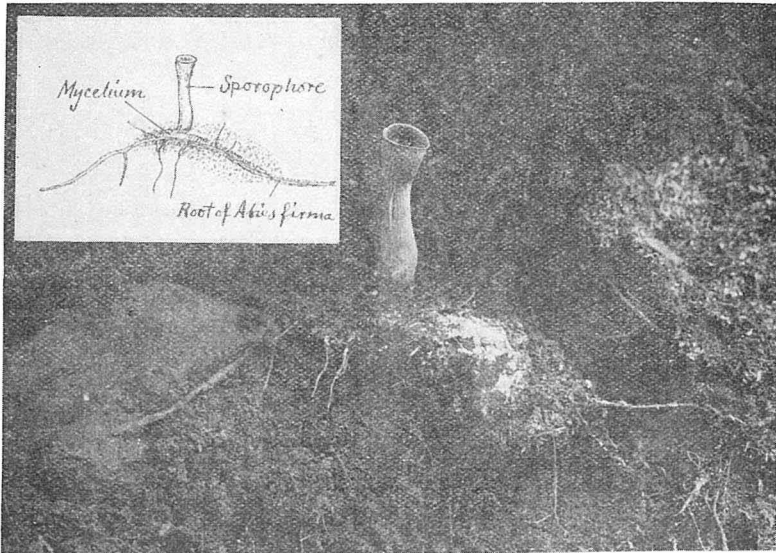
Fig. 1. A sporephore of *Cantharellus floccosus* has been produced directly from the mycelial mass formed surrounding a fir root. $\times \frac{1}{2}$.

In rare cases, a sporophore is produced from an infected portion of so large a root as that shown in Text figure 2, A[1].

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1) For the mycorrhizal relation of such a thick root, see p. 26.
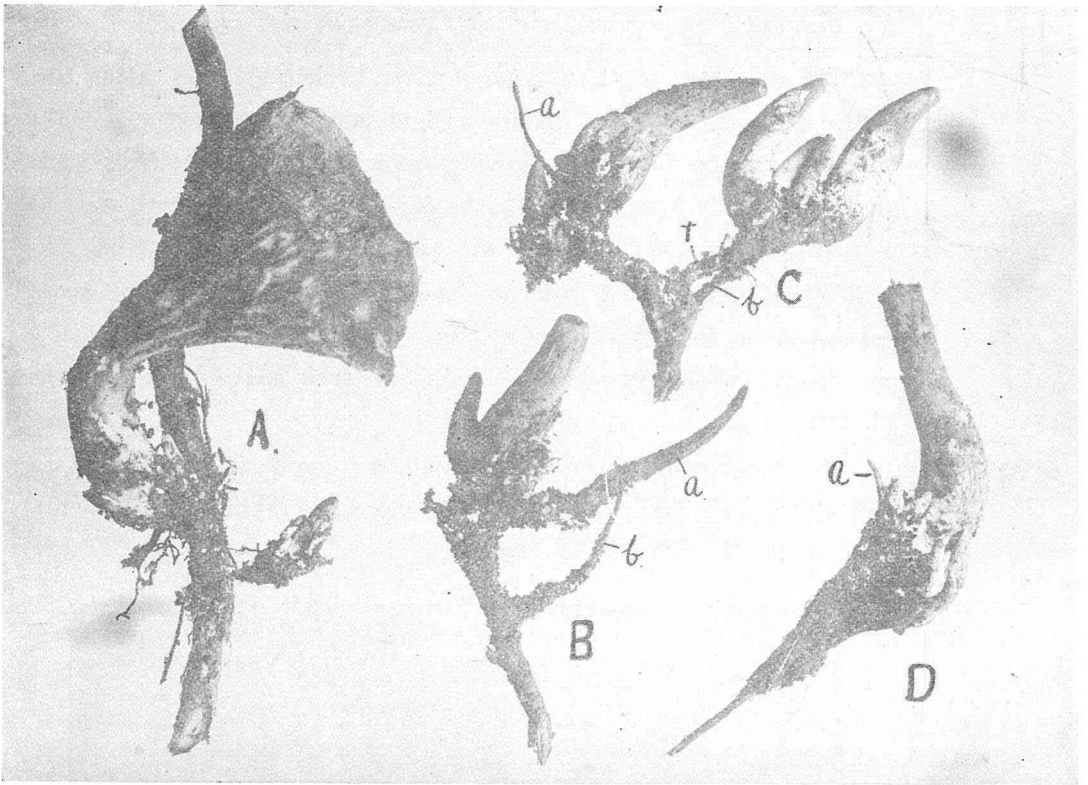
Fig. 2.   Sporophores of *Cantharellus floccosus* attach immediately
to the mycorrhizal roots.   × 1.2

In the Text fig. 2 B, two or more young fruiting bodies are seen
on the side of an infected root.   The middle portion of the root being
seriously infected, the apical portions (B, a & b) have withered, though
they were saved from mycelial infection.   Perhaps the death has
resulted from deprivation of food substances, caused by the fungous
infection.

Other examples of the same are shown in Fig. 7, a & b, Pl. III
and Text fig. 2, c.   In the last mentioned case several fruiting bodies
in different stages of development had sprung from a marginal portion
of a thick mantle which covered more than half the length of the
root.   The remaining portion of the root (C, a), though devoid of the
fungous mantle, had withered and turned dark brown in color.

A large rhizomorpha (r), which had sprung from the thick mantle, had spread along a side-branch of the root (C, b), and at the portion where the rhizomorpha and the mantle of the branch united into one, four young fruiting bodies were produced.

Text fig. 2, D, shows a large mycelial mass which has been formed arround a young root. Several fruiting bodies were produced at the upper margin of the mass. The tip of the root (D, a) was already of brown color, indicating the approach of withering.

All these roots were almost exposed upon the surface of the soil. Each of them bore a few or several small fruiting bodies upon the surface of the thick mantles.

B. *Fruiting body originates at the termination of the mycelial strand.*

When the infected roots are situated deeper in loam or raw-humus, there are found mycelial strands of various length and thickness. (Fig. 1 and 5c, Pl. II) They are first discernible as minute white knots.

In the case of *Cyathus fascicularis*, WALKER ('20) states that the primordium of the fruiting bodies originates slightly below the tip of the mycelial strands, but in *Cantharellus floccosus*, I could not meet with such a case.

C. *Fruiting body originates on a mycelial network which had been interwoven by the hyphae projected from the surfaces of numerous small mycorrhizas.*

The fruiting bodies are formed, frequently in clusters, upon the mycelial mass, which has originated from numerous small mycorrhizas. Fig. 6, Pl. III shows a good example of this type.

D. *Fruiting body originates as a side branch of an old fruiting body.*

Sometimes the upper portion of the fruiting body begins to perish for some reason. In such a case, one or several young fruiting bodies are formed as projections from the surface of its lower portion. Fig. 2, Pl. III shows such a case, where four young ones have been produced,

2. The development of the root.

In the fungous-infected roots or mycorrhizas of *Abies*, the infection
is superficial or commonly limited to the cortical layer of the roots.
This layer developes well at the young stage of the root, but after-
wards it shrivels and breaks down gradually from the surface of the
stelar cylinder, as is usually the case in woody plants. So the fungous
mycelium, which has infected the cortical layer, is also thrown away
from the host root. The fate of the mycelium depends, therefore,
very much on the presence of that layer on the root. It is therefore
necessary first to see, how long the cortical layer exists normally on
the young root and which stage of the root development is the most
favourable for the mycelial growth. Hence, I chose several uninfected
young roots of different thickness, and made cross handsections at
intervals of 10-30 mm from the apex backwards, and traced their
structural development successively.

Text fig. 3; 7 & 8, shows the cortical layer still in evidence at
a place where the secondary wood has already developed considerably.
Every stage of the root development, from the youngest stage onwards,
is fully indicated by the rate of development of the wood in the central
cylinder. (Text fig. 3)

As to fungous infection, I found that the mycelial development
was limited almost entirely to a relatively short range of the root,
namely from the stage where two primary vascular strands appeared,
(Text fig. 3; 2), to the stage, where several cell-layers of secondary
wood were formed in the central cylinder. (Text fig. 3; 8) The length
of the root from the apex to the portion just mentioned, is not the
same in each root, because the mode of their growth is variable,
depending chiefly on their environmental conditions. For instance,
some of them have a length of more than 25 centimeters from that
portion, while the others, grown in dry soil, always show a length
of less than 10 centimeters. For convenience I chose a normal root,
with the secondary wood fully developed at a point 24 centimeters

back from the apex, and obtained following results conserning the root characters.

(1)

	Distance from the tip.	Diam. of the root.	Color of the root.	Width of the cortical layer.	Quantity of starch in pericycle cells.
I.	mm. 3	mm. 1.67	white	mm. 0.50	0 — I
2.	10	,,	light brown	,,	I
3.	20	,,	,,	,,	2
4.	30	,,	brown	,,	3
5.	50	,,	,,	,,	4
	60	1.70	dark brown	,,	,,
6.	70	1.73	,,	,,	,,
	80	1.69	,,	0.46	,,
7.	90	1.37	,,	0.30	,,
	110	1.30	,,	0.26	,,
8.	130	1.15	,,	0.18	,,
9.	150	1.03	,,	0.13	,,
	180	1.20	,,	0.10	,,
10.	240	1.30	dark brown or reddish brown	0.10 — 0	,,

(2)

Woody structures in above mentioned materials :[1]

1. The protoxylem strands have appeared in two places in a central cylinder. (Text fig. 3; 1)

2. A few tracheids of primary wood have been added to both protoxylem strands.

3. Several tracheids of primary wood have been added to both protoxylem strands. (Text fig. 3; 2)

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1) During this investigation I owed much to JEFFREY's book and NOELLE's paper ('10), though their descriptions did not include, in detail, the root of *Abies firma*.

4. 10 or 12 tracheids of primary wood having been added, two groups of primary wood approaching the central resin canal. (Text fig. 3; 3)

5. The primary wood has attained its highest development.

6. A clear indication of the presence of the cambial layer has come out, which begins to give rise to secondary elements of wood and bast. (Text fig. 3; 4)

7. Interrupted lines of the secondary wood have appeared along both sides of the primary wood. (Text fig. 3; 5)

8. Two-cell-layered lines of the secondary wood have come in sight. (Text fig. 3; 6)

9. The secondary wood has taken a complete oval shape in the section. (Text fig. 3; 7)

10. Several-cell-layered secondary wood has been formed. (Text fig. 3; 8)

The apex of the growing root of *Abies firma* is of white color, but towards the older portion it becomes darker little by little, and at last it changes into reddish brown when the cortical layer is broken down.

A section, taken near the apex of the young root, shows that the central cylinder, which takes up about half of the diameter of the root, is sharply separated from the cortex by the presence of the well marked endodermis; and within that layer lies a broad encircling pericycle which abuts inside upon the primary phloem. The pericycle is composed of small, nucleated cells, about 0.03 mm in diameter, and these cells contain a great quantity of starch grains when they are old.

In the pericycle tissue there appears much mucilagenous fluid[1]

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1) The mucilage seems chiefly of pectic nature, as some microchemical tests indicate : Ruthenium red colors it deep red ; anilin blue and congo red color it ; corallin-soda reaction shows negative result ; iodine-potassium iodide gives it light yellow color ; chlorine zinc iodide gives a yellowish brown color ; xanthoprotein reaction, MILLON's reaction and biuret reaction show negative results.
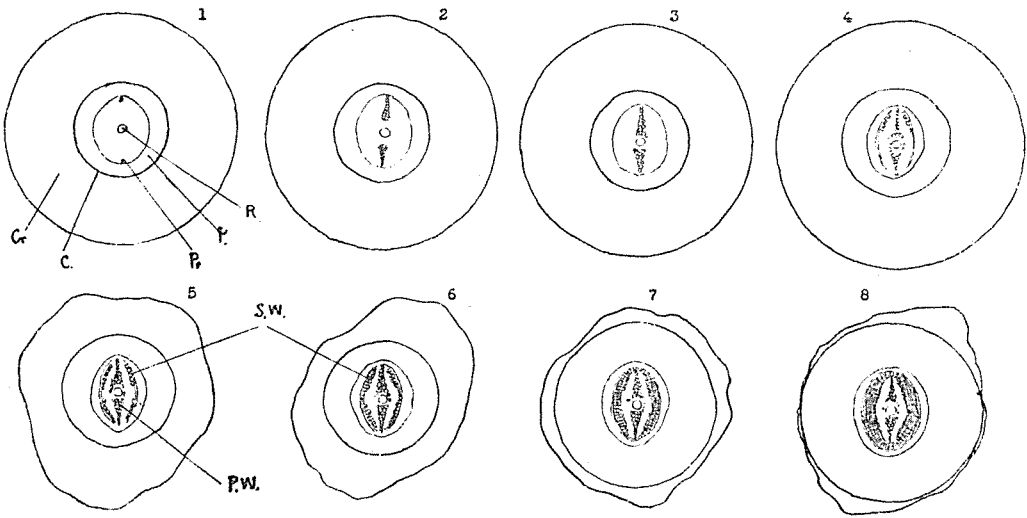
Fig. 3. Diagramatic representations of the development of wood in a root of *Abies firma*.
C, Central cylinder; Cr, cortex; P, pericycle; Pr, protoxylem strands; P. W, primary wood; R, central resin canal; S. W, secondary wood.

which made it easy for me in field observations to distinguish the fir roots from those of other plants.

The epidermal or the so-called piliferous layer, from which a few root-hairs are given off, dies at an early stage of the development, and its cell wall turns a brownish color. The cells of this layer are far smaller than those of the cortex. The middle and inner parts of the latter are composed of larger cells, 0.046-0.11 mm in diameter, with large intercellular spaces. Their cells are provided with small quantities of cytoplasmic substances and their walls are hyaline when they are young, but turn brown with age.

As the cortical layer stands in intimate relation with the mycor-rhizal development, I measured its width in relation to the diameter of the root in many young roots at the same stage of development, and obtained the following results :— See table in next page !

In a word, the larger root has a very much broader cortex. Calculation shows that a root, which has a diameter of 2.3 mm has more than 26 times the cortical volume of a root which

measures 0.46 mm in diameter.

Root diameter.	Width of the cortical layer.	Total area of the cortex.
mm 2.3	mm 0.66	mm^2 3.40
2.1	0.60	2.82
1.77	0.54	2.10
1.54	0.41	1.45
1.15	0.31	0.82
0.54	0.15	0.18
0.46	0.13	0.13

3. Histological features of mycorrhiza.

A superficial examination of the infected root, with low-powered microscope, reveals a network of mycelium over the surface and numerous hyphae or bundles of hyphae projecting from it. In external appearance it looks like an ectotrophic mycorrhiza. But the mycelial filaments not only attain intercellularly to the innermost cells of the cortical tissue, but also enter into the cell cavity of the cortical cells dissolving their walls. (Text fig. 4 and 5)

In rare cases the mycelium invades the outer layer of the pericycle tissue of old roots. In such cases, sometimes, fruiting bodies are produced from this portion. (Text fig. 2, A)

The novel thing about this mycorrhiza is that, in rare cases, the mycelium invades the whole central cylinder, and the tissue may be demolished at last.

Not only does the mycelium invade the young roots as above mentioned, but also it infects any rootlets which may come out from the infected roots, and transforms them into smaller mycorrhizas. The latter are frequently congregated together into a considerable mass cemented by the mycelial filaments produced from each. (Fig. 3, Pl II and Fig. 6, Pl. III) As to the other kind of mycorrhizas of *Abies* in general, see p. 64–77.
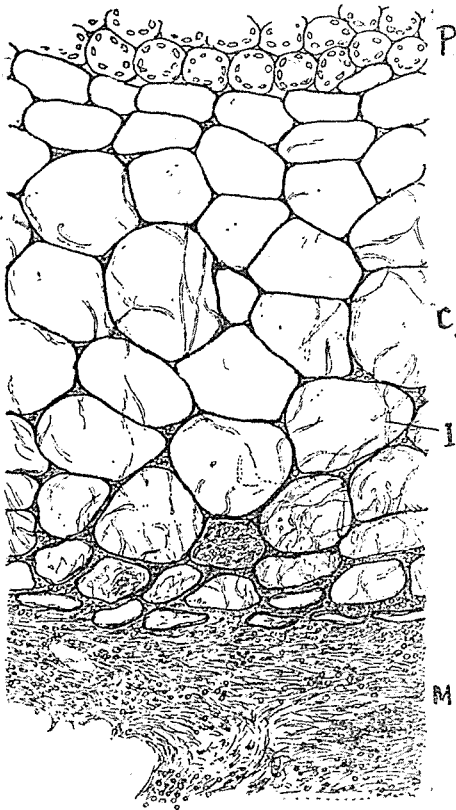
Fig. 4. A cross section of a mycorrhiza
caused by *Cantharellus floccosus*. C, cortical
tissue; I, intracellular hyphae; M, fungous
mantle; P, pericycle. ╳ 270.
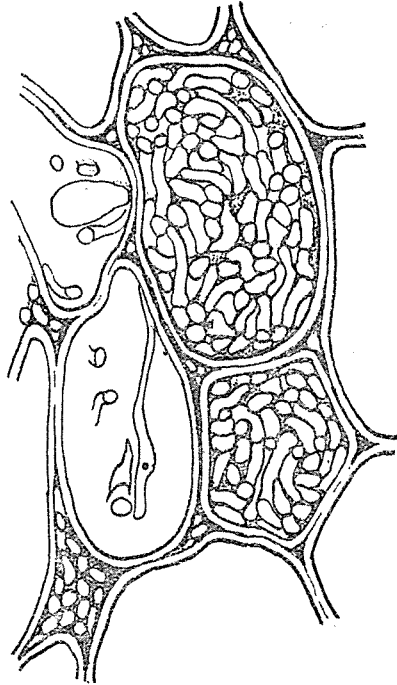
Fig. 5. Two cortical cells which
have been filled up with mycelium.
╳ 900.

The hyphae projected from the surface of the fungous mantle are
hyaline, well branched filaments with clump-connections, and are 3.3–
14μ in diameter. In most cases, the mycelium branches abundantly,
turning at once in the direction of the growth of the main branch,
and then coming to lie near and parallel to it.

Text fig. 6 shows the characteristics of the mycelium. They are
binucleated and provided with abundant clump-connections. The clumps
appear quite constantly in connection with the branching of the main
filaments as well as between the branches. There are several forms of

clumps as shown in this figure.   Hyphal anastomoses also occur occasionally. (Text fig. 6 ; 12–13)
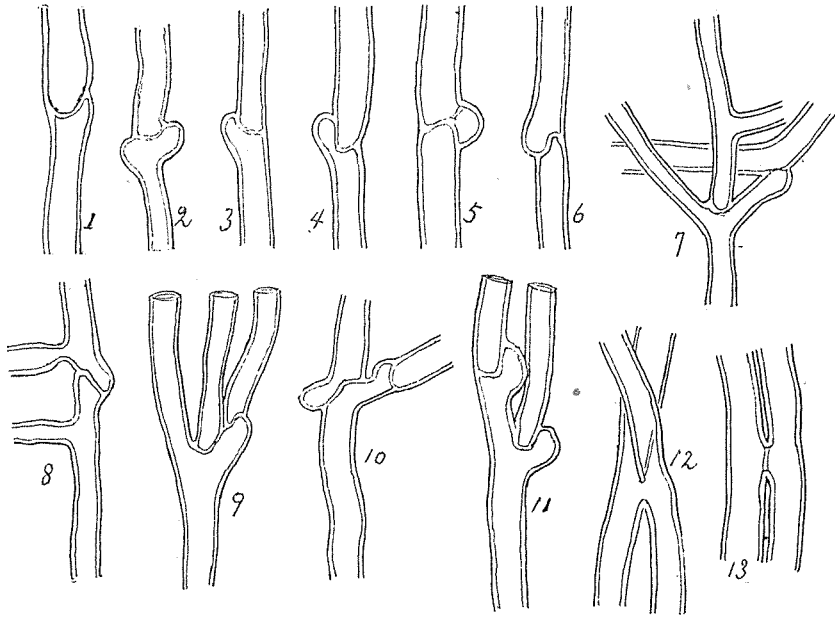
Fig. 6.   1–11, Clump connections; 12–13, anastomosing hyphae.

The filaments set free from the mantle begin to show a tendency to combine to form mycelial strands or rhizomorphas, even if they are spread upon rotten leaves or stones, and much more conspicuously in loam.   They are produced in most cases from the surfaces of those infected roots or mycorrhizas which are lying slightly deeper below the surface of the soil.   Occasionally the network of the mycelial strands is found immediately in contact with numerous infected roots.   Fig. 4, Pl. III shows one part of such a network.

As the filaments develope further these strands enlarge, until they measure 0.2–1 mm. or in some cases 3 mm. in diameter.   They are made up of loosely associated filaments in considerable numbers lying almost parallel to each other, and surrounded by more or less densely associated filaments which form a cortical layer.

4. Microchemical investigation.

While the infected roots of *Abies* are so much injured by the mycelium, the uninfected ones continue their growth without any impediment. This fact made me conceive that the mycelium of *Cantharellus*

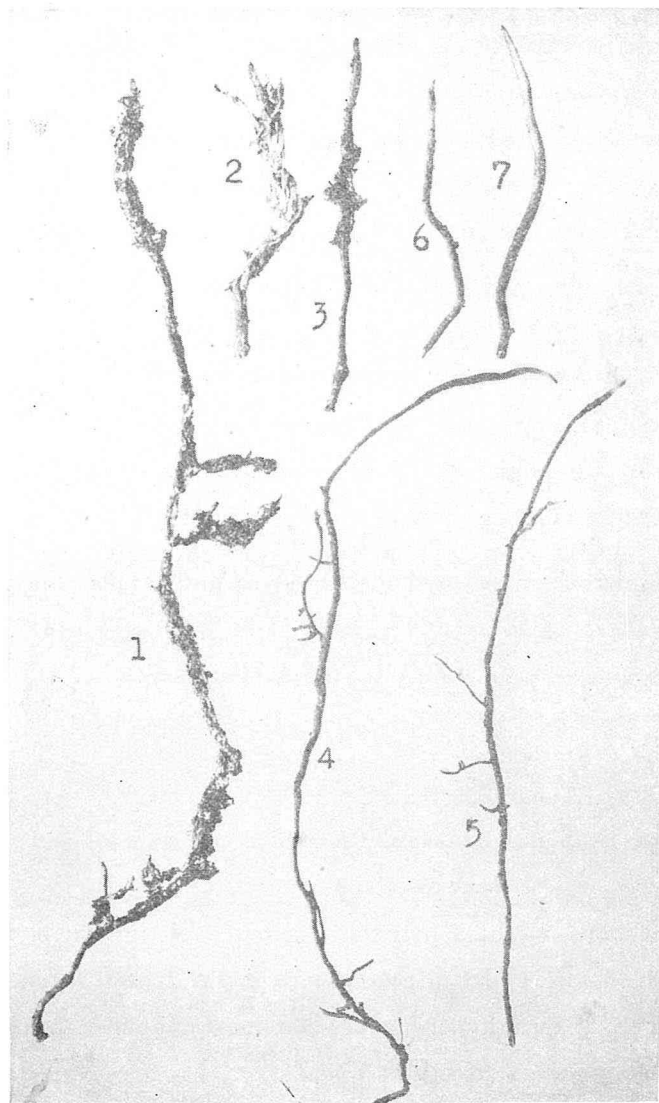Fig. 7. 1–3, Young roots of *Abies firma* which have been infected and, 4–7, uninfected.

floccosus is not symbiotic, but parasitic on the young roots of the *Abies* in question.

In order to make clear the nutritive relation between the root and the mycelium, I made microchemical tests upon both infected and uninfected roots as has been already done by WEYLAND ('12) and REX-HAUSEN ('20). WEYLAND concluded after his researches that the mycorrhizal fungi are parasite upon higher plants, while REXHAUSEN supported the view that, at any rate, they exchange their food substances.

For my investigation, I collected a considerable number of roots, both infected and uninfected, from one stock of fir tree. A part of them are shown in Text fig. 7. Numerous free-hand sections were made in fresh condition and used for the test.

As it is absolutely necessary for comparison to select roots in the same stage of development, I paid much attention to the woody structure of both sections as the basis of comparison.

The microchemical methods employed and results obtained.

I. Starch:

For the detection of starch I used the following exclusively :—
Iodine-potassium iodide solution.

Iodine	1 g.
5 % potassium iodide solution	100 cc.

Infected roots. Starch grains are found in the cells of pericycle, pith and rays, and not in cortical cells.

    a. Sections cut at a portion, where ten or twelve primary tracheids are found in two groups in the central cylinder, show a small quantity of minute starch grains in each cell of the pericycle. Text fig. 8; 1 and a.

    b. Sections cut at a portion where many primary tracheids are found in two groups, show a small quantity of rather small

starch grains, $3.2 \times 5 - 4 \times 6.6\mu$, in each pericycle cell. Text fig. 8; 2 and b.

c. Sections cut at a portion, where one-cell-layered secondary wood has developed, show rather large starch grains, $3.2 \times 6.5 - 6.5 \times 12.5\mu$, dispersed in the pericycle cells. Text fig. 8; 3 and c.

d. Sections cut at a portion where two-cell-layered secondary wood has developed, show a tolerable number of large grains, $8 \times 10 - 9.5 \times 13\mu$, in each pericycle cell. Text fig. 8; 4 and d.

e. Sections cut far back from the apex of the root where no serious infection has taken place, show large starch grains, $8 - 22\mu$ in length, filling the pericycle cells.

Uninfected roots. They always show a vigorous growth compared with the infected roots.

a′. Sections cut at the same stage of development as a, show rather large starch grains, $6 \times 6.4 - 6 5 \times 8.2\mu$, occupying almost half the volume of each pericycle cells. Text fig. 8; 1 and a′.

b′. Sections cut at the same satge as b, show large starch grains, $6.5 \times 7.8 - 12 \times 13.3\mu$, filling up each pericycle cell. Text fig. 8; 2 and b′.

c′. Sections cut at the same stage as c, d and e, show very large grains, $8 - 22\mu$ in length, filling up each pericycle cell.

In a word, uninfected roots surpass exceedingly the infected ones in the number as well as in the size of their starch grains when sections at the same stage of development are compared.

II. Sugar.

1. Alpha-naphtol.

Twenty per cent alcoholic solution of alphanaphtol was poured on the sections and after the excess of solution had been sucked off with blotting paper, 2 or 3 drops of concentrated sulphuric acid were added. Sugar colored violet to purple.

Infected root.   Mycelium colored deep violet.

Cortex colored light violet to light purple.

Central cylinder colored violet to purple.
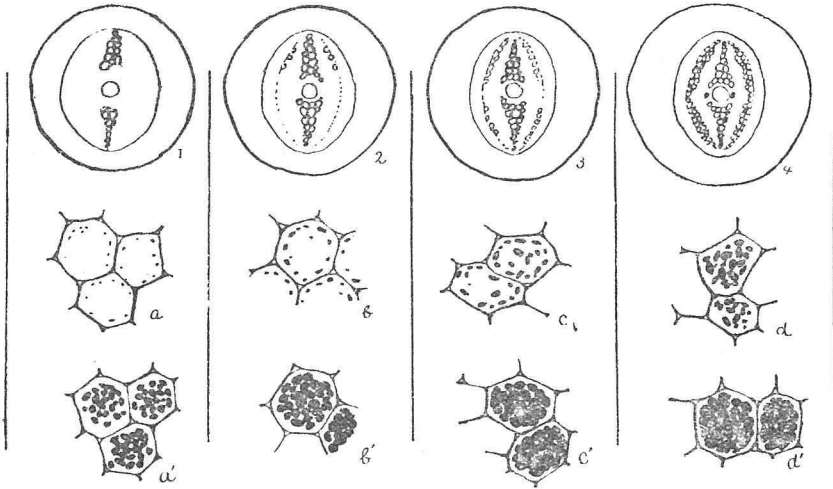
Fig. 8.   The quantitative difference of starch in the infected and uninfected roots, compared in the same stage of development, is diagramatically represented. a–d, Pericycle cells of seriously infected root in four stages, which are shown by the development of the central cylinder, 1–4; a'–d', those of uninfected root in the same stages as a–d.

Uninfected root.   Cortex colored light violet to light purple.

Central cylinder colored violet to purple.

2.   A. MAYER's method.

Saturated solution of copper sulphate was poured on sections which, after a while, were quickly washed with water and the following mixture were added : 10 g. of crystallized Rochelle salt and 10 g. of caustic soda in 10 g. water. When the sections were examined, 2 drops of glycerin was added and they were heated moderately.

Infected root.   Mycelium, light reddish brown.

Cortex, light reddish brown.

Central cylinder, reddish drown.

Uninfected root.   Cortex, light reddish brown.

Central cylinder, reddish brown.

A saturated solution of copper sulphate and caustic potash, and FEHLING's solution were also employed for the test. But the method of A. MAYER was by far the best in the present investigation.

The quantity of sugar in the tissue of infected root was proved to be almost the same as that in uninfected root.

The reason why the mycelium turns deep violet when it is treated with alphanaphtol and concentrated sulphuric acid, may be that by the treatment with a concentrated mineral acid, the glycogen is broken up into a great quantity of hexose.

III. Glycogen.

For the detection of glycogen, I used the following iodine-potassium iodide solution:

1 g. iodine and 0.3 g. potassium iodide in 45 cc. water.

Infected root. When the sections were treated with this reagent, the fungous mantle and hyphae which were comprised in the cortical tissue, gave a reddish brown color. The color faded on heating.

A. FISCHER's tannin-safranin-staining method proved to be abortive for this investigation.

IV. Tannic substances.

1. Potassium bichromate.

Small pieces of fresh materials were dipped in concentrated aqueous solution of potassium bichromate for 2 days, rinsed with water, and free hand sections made. The sections were examined in glycerin. Through this treatment the tannic substances were clearly revealable in a brown to dark brown color.

2. Copper acetate.

Small pieces of fresh material were steeped in 7 % aqueous solution of copper acetate for 8 days, and freehand sections made. They were then placed upon the slide, covered with 0.5 % solution of ferric acetate, rinsed with water, and one or two drops of glycerin were added to them. Through this treatment tannic substances gave a blue to dark blue color.

Infected root. Cells which were filled with tannic substances were found dispersed in the tissue of the central cylinder, mostly at its

margin and in the central portion. The peripheral tissue of the peri-
cycle was also rich in this substance.

Uninfected root. The tannic cells were found dispersed in the
central cylinder as in the infected root, but the peripheral tissue con-
tained fewer of them than the infected root.

In a word, the infected root contains much more
tannic substance in the central cylinder than the
uninfected root.

V. Albuminous substances.

In order to detect albuminous substances I used the following methods :— xanthopro-
tein reaction, picric acid reaction, eosin reaction, and iodine-potassium iodide reac-
tion. By these reactions I got the following results :—

Infected root. Fungous mantle contains much albuminous substance
in the mycelium. In the central cylinder it is contained exclusively
in the sieve portion and in the pericycle tissue. The cortical tissue is
very poor in protein contents.

Uninfected root. The quantity of albuminous substance in the
cortex and the central cylinder is almost the same as in infected root.

In a word, though the mantle contains plenty of albuminous sub-
stance, there is no noticeable difference in this respect in the root
itself, whether infected or uninfected.

VI. Ammonium.

NESSLER'S reaction. The formation of yellowish brown color upon the addition of
NESSLER'S reagent indicates the presence of ammonium.

Infected root.   Mycelial mantle, light brown.

Cortical tissue, no color.

Central cylinder, brown, peripheral tissue deeper color.

Uninfected root. Cortical tissue, no color.

Central cylinder, dark brown, peripheral tissue much
darker color.

In a word, the uninfected root shows much more am-
monium in the central cylinder than the infected root.

VII. Nitrates and Nitrites.

The presence of nitrates and nitrites in the material was detected with diphenylamin-sulphuric acid : o.1 g. diphenylamin in 10 cc. of concentrated sulphuric acid.

Infected root.   Mycelial mantle, light blue.

Cortical layer, light blue.

Central cylinder, light blue.

Uninfected root.   Cortical layer, deep blue.

Central cylinder, light blue.

The quantity of nitrates or nitrites is certainly greater in uninfected than in infected roots, when sections at the same stage of development are compared.

VIII. Potassium.

In order to detect potassium in the materials, I employed MOLISCH's cobalt nitrite method. The procedure was as follows :— 2 gm. cobalt nitrate (I used it instead of cobalt nitrite), 2.5 gm. sodium nitrite and 1 cc. glacial acetic acid were dissolved in 6.5 cc. of water. This solution was then diluted to $\frac{1}{2}$ its concentration with water. Free-hand sections were dipped in the reagent for 3 or 4 minutes, and then rinsed with 10 % solution of acetic acid. Then the materials were put into a mixture of ammonium sulphide (sp. gr. 0.96) and glycerin.

For this investigation very thin sections were used exclusively, but the fully formed dark precipitate obtained in each case made me hesitate to form any conclusion as to the difference between the infected and uninfected roots as regards their potassium content.

The mycelium, cortical layer and central cylinder of the infected roots were colored as dark as the cortical layer and the central cylinder of uninfected roots.

IX. Phosphate.

I used fresh FRESENIUS's solution for the investigation.

Infected root.   Mycelium, light yellow.

Cortical tissue, no color.

Central cylinder, yellow.

Uninfected root.   Cortical tissue, no color.

Central cylinder, yellow.

After the materials were rinsed with water they were treated with 2 % solution of
phenylhydrazine chloride as a reducing reagent. The color of the materials changed
to blue or dark blue. As a reducing reagent, I used also 20 % solution of pyro-
gallol with tolerably good results.

From this investigation, I concluded that there is no notable
quantitative difference in the phosphor in the two kinds of materials.

X. Calcium.

For the detection of calcium in both of the kinds of root in question, I used the
following reagents :
    1.   2 % solution of sulphuric acid.
    2.   5 % aqueous solution of ammonium oxalate.
    3.   5 % solution of oxalic acid.
    4.   2 % oxalic acid solution and alcohol.
    5.   3 % solution of ammonium carbonate.

Through these reagents I could find no quantitative difference in
the calcium in these two kinds of materials.

XI. Magnesium.

I used 0.1 % aqueous solution of sodium-ammonium-phosphate, as
a reagent, but I could not get any noticeable effect.

XII. Sulphur.

I used nitroprussic sodium and barium chloride but without result.

XIII. Iron.

I used 2 % solution of potassium ferrocyanide and 5 % solution
of hydrochloric acid, but, I could not find any quantitative difference
in the two kinds of materials.

The question as to whether mycorrhiza represents symbiotic associ-
ation or parasitic association has been hitherto much discussed, and
some particular evidence has been presented on both sides.

It is however worth while in the first place to consider just what
the difference is between symbiosis and parasitism.

By parasitism is usually understood a condition in which one
organism obtains nourishment at the expence of another living

organism. Symbiosis has been defined as a condition in which two organisms live in intimate relationship with each other in such a way that both are mutually benefited by the association. In the case of plants, it is usually taken for granted that this benefit has to do with obtaining food.

The microchemical investigation of infected and uninfected roots proves that the former contain obviously less starch in the pericycle tissue than the latter. Moreover, the quantity of starch accumulated in the pericycle varies in proportion to the degree of fungous infection. A root which has been invaded violently by the fungus, contains only a small amount of it, while a slightly infected one contains a great quantity of it. Even in one and the same root, which has been heavily infected in some distinct area we can observe the fact that the amount of starch diminishes locally according to the degree of infection.

In a word, a quantity of the starch accumulated in the pericycle tissue is consumed by the infecting fungus.

On the other hand, a large amount of glycogen is found accumulated in the cytoplasm of the hyphae which constitute the fungous mantle, intercellular mycelium and intracellular mycelial network.

It is quite conceivable therefore that the source of this glycogen is, not only the already formed starch, but also the carbohydrates accumulated by the root and absorbed, in the form of sugars, by the hyphae of the mycorrhiza.

The amount of ammonium salts contained in the cortical tissue of the uninfected root always exceeds that in the infected root.

In the same way, the uninfected root contains more nitrates or nitrites in the cortical tissue than the infected root. It seems therefore conclusive that the mycelium absorbs even nitrogenous substances from the root, contrary to the general view, that the mycorrhizal mycelium assimilates the humous nitrogen and supplies it to the root.

As to potassium, calcium and phosphates contents, I could not any notable difference between infected and uninfected roots.

In fact, to sum up, the root of *Abies* seems not to be benefited by the association with the mycelium of *Cantharellus floccosus*. On the contrary, it is only the fungus that is benefited by the root by obtaining sugar as a source of carbohydrates, and ammonium salts, nitrates or nitrites as sources of nitrogen. Not only from the morphological features, but also from the microchemical behaviors, I do not hesitate therefore to conclude that the mycorrhiza on *Abies firma* caused by the mycelium of *Cantharellus floccosus* is, contrary to FRANK's idea, not a symbiotic association, but an instance of parasitism, just as WORONIN, FUCHS, WEYLAND and McDOUGALL have stated in many other cases, though at the same time it is not inconceivable that a symbiotic association might result, according to the combination of species of fungi as well as of roots.

5. The mode of infection and the formation of mycorrhiza.

The problem of the fungous infection of young roots has been discussed by a few authors.

FRANK ('85) stated that fungi, which cause mycorrhiza on young root, are found spreading in all layers of soil.

MÜLLER ('86) declared that mycorrhizal fungi and their rhizomorpha-like hyphal masses are found in layers of rotten leaves. They build a network in rawhumus and lives as saprophytes upon them. The roots of *Fagus*, which spread in such a network, are infected by the mycelium and transformed into mycorrhizas.

Whether the mycelium of *Cantharellus floccosus* can live also as a saprophyte on rotten leaves for a long time, without reacting on the living root of the host plant, is not yet experimentally proved.

The mycelial filaments, produced from numerous mycorrhizas, frequently interweave into a compact network as mentioned in the previous chapter. Young fir-roots which have grown through it, may be destined to be infected and transformed into mycorrhizas. The filaments which interweave into a network are all combined with the living fir-roots, and no filament growing free from them may occur. This fact is proved by the following experiments :— If a mother root, which bears numerous mycorrhizas and the network, is cut, both the mycorrhizas and network perish. In the same way, when the whole tree is cut down, no mushroom is produced afterwards at that place.[1]

I hesitate, now, to believe that this mycorrhizal fungus lives as a saprophyte on rotten leaves for a long time without relation to living roots and serves for further infection.

FRANK reported that a young branchlet of *Fagus*, which has been brought forth from an infected mother root, is destined to be covered by the fungous mantle which has come up from that of the latter. In the case of *Abies firma*, I observed frequently the same fact in larger roots. Text fig. 9 shows a seriously infected mother root which bears three infected lateral branches. (B_1-B_3). Among them a branch B_3 has been covered by a fungous mantle only at its basal portion, and its cortical cells have been invaded more heavily towards the mother root.

This fact shows clearly that it has been infected by the mycelium which had extended from the fungous mantle of the mother root.

---

1) I observed an instructive case at Kurama. A large fir tree, about 73 years old, under which I counted 47 mushrooms in 1923 and 32 in 1924, was cut down by the people in the spring of 1925. All the mycelia then died, and I could find no mushrooms at that place in the fall.
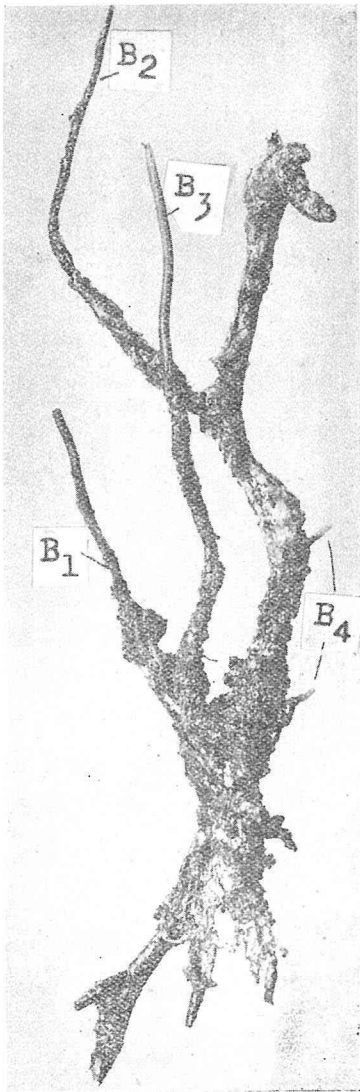
Fig. 9.

In order to study the mode of further infection of the fungus, I employed the glass plate method which was introduced by McDOUGALL ('14). The humus and leafmold were scraped away from a small area until young roots were uncovered. A glass plate of about 11 × 16 cm. size was then placed over these roots, pressed down firmly, and covered with soil and stone. Much care was taken not to leave air spaces beneath the glass, and then to cover it well with the soil; otherwise the roots would wither and die.

After one year, I obtained two instructive results which are shown by the photograph. (Fig. 1, 2, Pl. II) These two figures show a pure white mycelial network stretching irregularly over the surface of yellow clay. Now, the novel thing is that the mycelial network has stretched from the frame-work of fir roots like the webbed foot of a water-bird. These networks were found only in the area which has been occupied by the young roots of the *Abies* in question. The mycelium first advances covering the root from the basal portion toward the apex, and then expands to both sides, as is clearly seen in figure 2, Pl. II.

The figure shows moreover that a number of rhizomorphas have been proliferated upon the surface of the soil, and one of them is

provided with a young carpophore at its extremity. These rhizomorphas are those which have been given rise to by subterranean infected roots.

Besides the above-mentioned modes of infection, the rhizomorphalike hyphal bundle is clearly of use for further infection as MÜLLER described.

It is prolonged far away from the infected root, as if searching for host roots, and the infection may take place when it meets with them.

Recently MELIN ('24) has stated that the spores of the mycorrhizal fungi germinate only when they meet with phosphatide which has been excreted by the living roots of host plants. In some cases, hyphae which have germinated directly from the spores, according to his idea, may be of use for infection.

The fir-roots, which have been thus infected by the mycelium of *Cantharellus floccosus* may be sooner or later transformed into mycorrhizas.

A large young root, which has been given off from an infected mother root, is frequently found to be an endotrophic mycorrhiza, lacking a mantle over its surface, though it is transformed, sooner or later, into an ecto-endotrophic mycorrhiza by the gradual development of the fungous mantle. In this case the inward infection precedes the formation of the mantle as reported by MÖLLER ('03).

But sometimes the mycelium advances in a mass constructing a pure white mantle, toward the apex of the root as shown in fig. 3, Pl. III. Microtomic section of such specimens shows that tips of the roots, which are not yet covered with the thick mantle, are not only bare, but also lacking inwardly infected mycelia. In such cases, the formation of the mantle precedes the inward infection, as described by FRANK ('85) and McDOUGALL ('14).

The speed of advancement of the fungous mantle is measurable in the case of the latter, by the use of the glass-plate method.

McDOUGALL ('14) supposed that the development of the fungous mantle of an ectotrophic mycorrhiza may take place very rapidly; probably only a day or two is needed for the formation of a complete mantle.

Most ectotrophic mycorrhizas have ordinarily too small mantle to be observed for this purpose. But the mycorrhiza caused by this fungus frequently occurs in a large size and moreover it is pure white in color as above mentioned, so that it is very favourable for the investigation.

On July 29, I noticed, under the glass plate, an infected root over which a pure white mantle had advanced to a portion 33 mm. from the tip. The root proved to be capable of further growth as its color had not yet turned yellow or brown. The mycelium also showed that it had been advancing over the surface of the root, in tolerable thickness, toward its apex. I made then a mark upon the glass plate with a glass-cutter, in order to recognize the point to which the mantle had advanced.

On August 27, it was shown by measurement that the mycelium had advanced 29 mm in 30 days, while the root had elongated 12 mm. The mantle must have advanced therefore almost one mm, on an average, in a day.

The speed of advancement naturally varies chiefly with the environmental conditions, and it should be noted that the elongation of the mycelinm in this case took during a very dry period. Had there been plenty of moisture the mycelium would have grown more rapidly perhaps.

6. The fate of the infected roots.

The growth of the young roots of *Abies firma* is gradually retarded as the infection proceeds, while the uninfected ones grow unin-

terruptedly. Roots, of which the growth has entirely ceased, are found to be those which have been fatally infected.

Moreover, the roots, the growth of which suddenly diminishes in summer, are, in almost all cases, those which have been much invaded by the mycelium. From these facts, it seems very probable that t h e inhibition of growth may be chiefly owing to direct injury caused by the mycelial infection. The more the infection advances, the more the growth is retarded.

In order to study the rate of growth of both infected and normal roots of *Abies* more precisely, I employed the glass plate method already mentioned.

Almost every month the rate of growth was measured and marked with a glass-cutter on the glass plate as the root tip advanced. Sometimes it was necessary to remove the glass plate for observation in detail.

The experiment was made chiefly in two places at Kurama. One place was situated in a rather dry area on a mountain side sloping to

	Place of experiment.	Number of roots used in the experiments.	Roots which had lost the capacity for growth.	Roots which had still maintained the capacity.
Exper. I (July 1)				
After 30 days.	Mount. side	24	15	9
Exper. II (July 30)				
After 30 days.	Mount. side	22	20	2
	Valley side	6	2	4
Exper. III. (Aug. 29)				
After 32 days.	Mount. side	4	3	1
	Valley side	4	1	3
Exper. IV (Oct. 1)				
After 42 days.	Mount. side	8	2	6
	Valley side	5	0	5

	Place of experiment.	Number of roots used in the experiments.	Roots which had lost the capacity for growth.	Roots which had still maintained the capacity.
Exper. V. (Nov. 11)				
After 44 days.	Mount. side	6	1	5
	Valley side	4	1	3
Exper. VI (Dec. 25)				
After 50 days.	Mount. side	4	1	3
	Valley side	6	5	1
Exper. VII (Febr. 12)				
After 70 days.	Mount. side	4	0	4
	Valley side	5	0	5
Exper. VIII (April 22)				
After 41 days.	Mount. side	4	1	3
	Valley side	4	0	4

the south and covered with low deciduous trees, and the other was in humid soil on a valley side covered with large coniferous trees. The temperature in the former was always higher than in the latter.

The difference in the temperature and the soil moisture in these two places may have had different effects upon the growth of the roots as shown in the table. Ordinarily the mycelial infection does not take place violently in roots which are lying in humid soil, as the mycelium grows very feebly in such conditions. On the other hand it makes luxuriant development in rather dry soil and thereby it attacks them violently.

Roots, which are free from mycelial infection as in the case of those on the valley side, would show almost continuous growth with some seasonal fluctuations (PFEFFER II p. 262–3 and McDOUGALL 1917). On the other hand the growth of those which have been attacked by the mycelium gradually diminishes, until they are absolutely enfeebled and die.

The mycelial development began to be rapid on the mountain side from May or June. A considerable number of roots in the surface layer of the soil were fatally infected in this season, and about 60–90 % of them are found dying off during July and August. (Fig. 4, Pl. II)

Young roots, brought from the infected mother root are always destined to die by the immediately following infection. Text fig. 9 (p. 40) shows this clearly. Among five lateral roots, the oldest one (B_1) has perished already by the fatal infection of the mycelium The second one (B_2) has also been nearly killed. The third one (B_3) has been so much invaded, that no further elongation can take place. About two thirds of the whole length of it has been covered by the fungous mantle, and the fatal inward infection has already gone little by little into its basal portion. Two young rootlets (B_4) have peeped out almost simultaneously from the surface of the fungous mantle of the latter. In the same way several new lateral roots may be given rise to successively from the surface of an infected mother root, while the latter may in turn be killed by the fatal infection. Such a replacement of new roots may continue during the summer. In the following seasons as the injury done by the fungous parasitism decreases, the roots show a rapid growth and it is just these roots which may be heavily infected by the mycelium and produce the fruiting bodies of *Cantharellus floccosus* in the following summer.

7. Relation between the diameter of the infected root and the weight of the fruiting body which is produced from it.

In the previous chapter I stated that the fruiting bodies are produced directly or indirectly from the infected roots of the *Abies* in question.

When a considerable number of the infected roots from which the mushrooms have been produced are examined in detail, one may

point out a relation between the diameter of the root and the size of the mushroom which has been produced from it, as the mushroom seems to develope at the expense of the root. However, as the environmental conditions have much to do with the development of the mushroom, sometimes such a relation is not realised.

In order to understand the relation, I measured on the one hand, the diameter of the infected root of an *Abies* with the aid of a micrometer, and on the other hand I measured the weight and length of the mushroom which had been produced from it, and obtained the following result :—

Table 1.

Thickness of cortical layer. (mm).	Weight of mushroom. (g)						Total.
	1−3	3−5	5−7	7−9	9−11	11−13	
0.5 − 1.0	6	.	.	.	.	.	6
1.0 − 1.5	6	4	.	.	.	.	10
1.5 − 2.0	.	1	3	3	2	1	10
2.0 − 2.5	.	.	.	.	.	2	2
Total.	12	5	3	3	2	3	28

Table 2.

Thickness of cortical layer. (mm).	Length of the mushroom. (cm)						Total.
	4−5	5−6	6−7	7−8	8−9	9−10	
0.5 − 1.0	.	5	1	.	.	.	6
1.0 − 1.5	5	2	1	2	.	.	10
1.5 − 2.0	.	.	.	4	3	3	10
2.0 − 2.5	.	.	.	.	.	2	2
Total.	5	7	2	6	3	5	28

These tables show, that (1) larger mushrooms seem to be produced generally from larger roots with thick cortical layer, and vice versa, while (2) the length of

the mushrooms is not so intimately related with the thickness of the cortex, as the length itself is concerned chiefly with the environmental conditions during the growth.

II. Ecological conditions of mushroom production.

1. Cantharellus floccosus does not occur in too wet soil.

As already mentioned, the formation of the mycelial masses and the occurrence of fruiting bodies is due naturally to the existence of living fir roots in the superficial layer of soil. But the topography and the soil conditions play also an important role. I was able to find an instructive case in this respect.

On a valley side at Kurama, there is a large fir tree, about 45 years old, in a mixed wood of *Chamaecyparis* and *Cryptomeria*. To the south from that tree there is a wide area of damp soil, down the slope towards the west, a small stream, and up the slope a large out-crop of chert. (Text fig. 10) The area north of the tree is covered by a rough under-growth of shrubs. The soil there is rather thin over the native rock. It is yellow clay in nature, mixed with pebbles and stones of various size. and covered with a thin layer of rawhumus or moss. Though the fir roots, as I could find by digging, were widely distributed in the superficial layer around that tree, the formation of the mycelial bed and the occurrence of *Cantharellus floccosus*, to my astonishment, were quite restricted to the northern area. I could find neither mushrooms nor even a trace of a mycelial mass in the wet soil.

The superficial layer of this damp soil contains only 45–55 % of water in summer, but the mycelium of *Cantharellus floccosus* seems not to be able to spread well under such conditions, though some other kinds of mycorrhizas could be found on the fir-roots there.

Obliquely downwards from the tree, there was a mycelial bed (M_2), an optical section of which along line c–d is shown in Text figure 11.
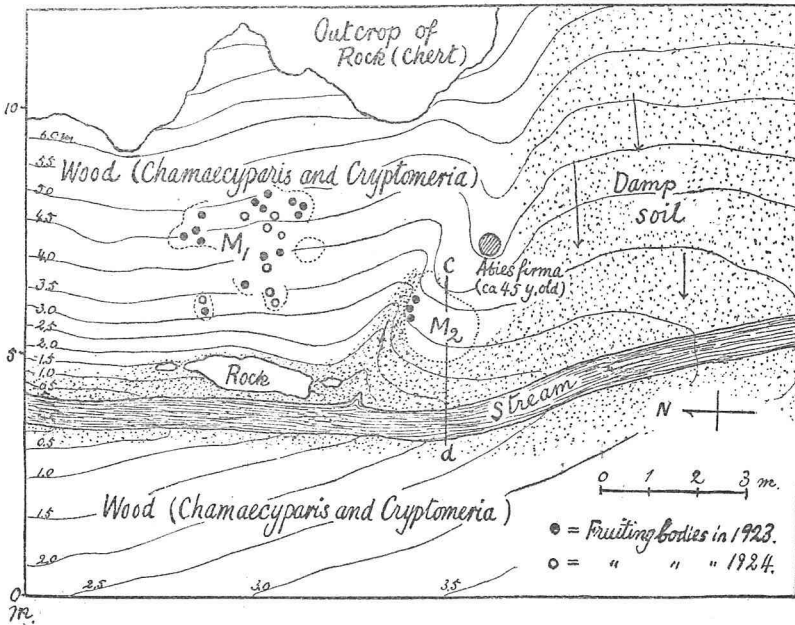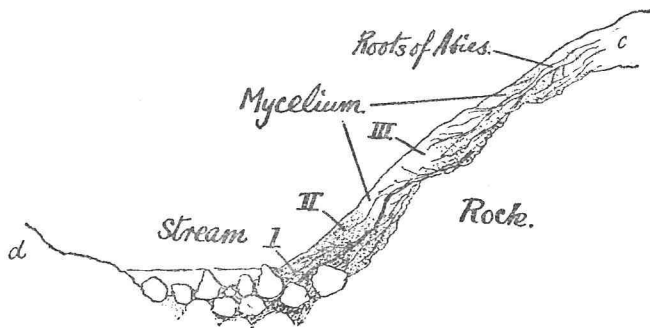
Fig. 10.

Fig. 11.

A considerable number of fir roots were found in the soil over the rock. As the slope approaches the stream, the soil moisture increases by degrees. On the other hand, the development of the mycelium decreased towards the lower portion of the slope, as the following table shows :— (See next page.)

Besides these examples, I noticed instances of the same in several other places.

	Amount of water in the soil.	Development of the mycelium.
I. (Lower part)	56 %	—
II. (Middle part)	44 %	— ?
III. (Upper part)	35 %	+

2. The existence of young fir-roots near the surface of the soil is necessary for the production of mushrooms.

Several times I have stated that the existence of the fir-root near the surface of the soil is a most important factor for the formation of mycelial beds as well as for the production of mushrooms.

Roots which are lying deep in the soil are not infected by the mycelium and for this reason such roots are not concerned with the production of mushrooms. But when roots which have been imbedded deep in the soil and free from the mycelium of *Cantharellus floccosus* are once brought near the surface of the soil artificially or by any other cause, those roots can be infected by the mycelium and produce mushrooms. Such cases have been observed several times at Kurama and in its vicinity.

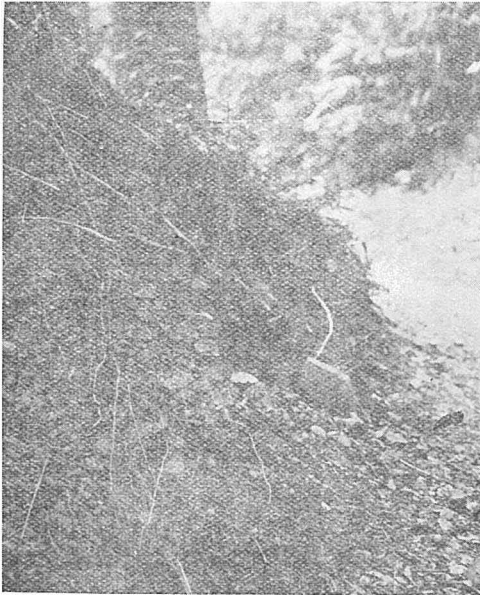Text fig. 12 shows, in the center of the figure, a mushroom which I saw on

Fig 12. *Cantharellus floccosus* occurring on a spot, which was exposed to the air in the previous year, as a large amount of soil was removed from there.

a path. Near the path there is a large fir tree. On the 15th of Oc-

tober, 1923, a lot of soil was dug and removed from the path, so that, a considerable number of the fir-roots were exposed to the air. After a year, a mushroom was produced from that very place. On digging up the soil below the mushroom I found numerous mycorrhizas which had been formed during a year. Text fig. 13 illustrates the condition diagramatically.
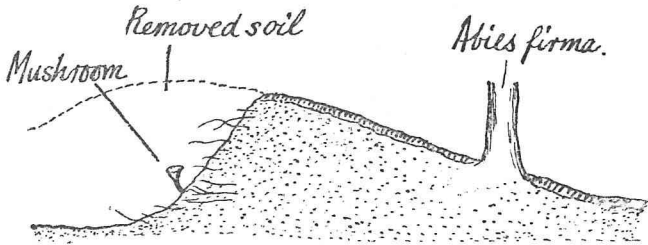

Fig. 13.

I may introduce here still another example. Text fig. 14 shows a mushroom produced just beside a large *Chamaecyparis*. On digging up the soil below the mushroom I found not a root of that tree, but a heavily infected fir-root which had wandered along and been lifted up to the surface of the soil. The root had been infected by mycelium and produced a mushroom just near the trunk. Text fig. 15 illustrates this diagramatically.

The mushrooms occur generally around the fir tree a little away from the center, and the mode of their occurrence seems to depend very much on
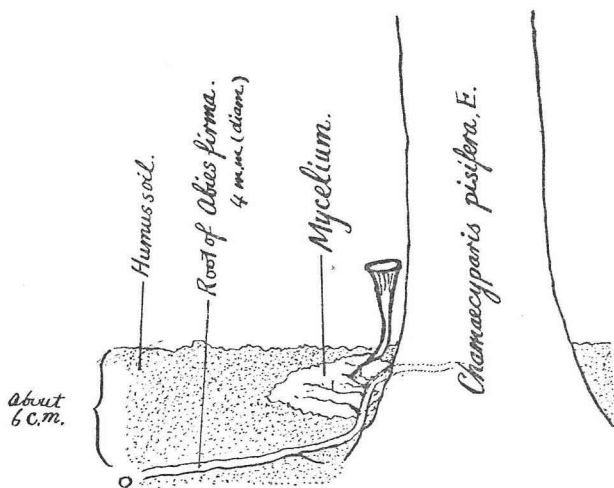

Fig. 14.

Fig. 15.

the topography of the soil. When *Abies firma* is standing on a slope, more mushrooms always occur on the lower portion of the slope than on the portion above the trunk. This may be due to the fact that in the lower portion of the slope the young roots of *Abies* in question are liable to come up to the surface layer of the soil more easily than in the upper portion.

I once found a very instructive case, in which a considerable number of mushrooms were produced during two years (17 in 1923, 26 in 1924) only on the lower part of the slope, ca 4–7 meters from the mother fir-tree, about 36 years of age. (Text fig. 16)

Fig. 16

● = Fruiting bodies in 1923.
○ = " " " 1924.
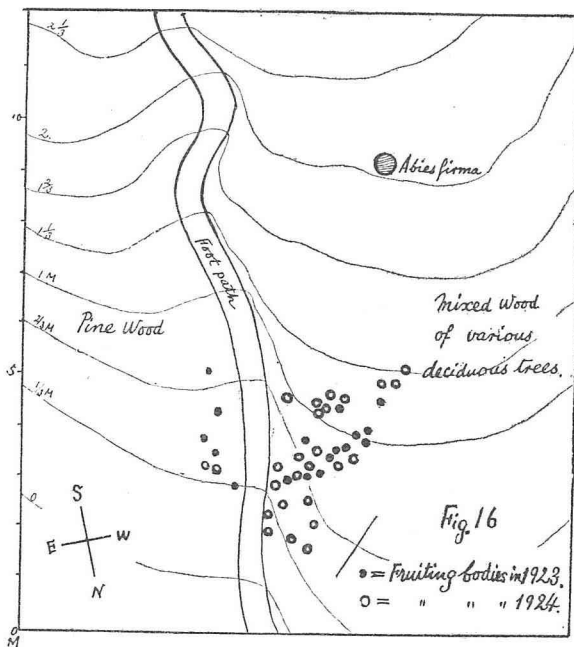
The following table shows of the examples which have been obtained in several places.

Number of mushrooms occurring around a fir standing on a slope.

Fir-tree observed.	Upper portion of slope.	Lower portion of slope.
No. 1	0	1
„ 2	0	3
„ 3	0	7
„ 4	0	7
„ 5	0	9
„ 6	0	43
„ 7	8	17
„ 8	12	34

On a declivity, the roots of *Abies* possibly come up to the superficial layer of the soil more than when they are on a plane. When there is a steep slope just near a fir-tree, then a considerable number of roots appears on the surface at that point, and a well developed mycelial bed is frequently met with clusters of fruiting bodies. Text fig. 17 shows an example of this.
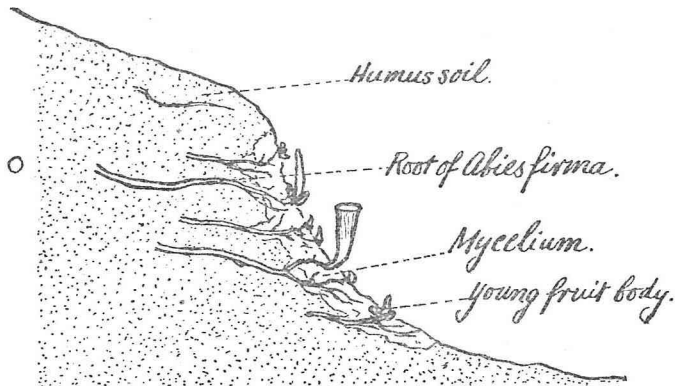

Fig. 17.

When the soil is of compact yellow clay, the fir roots must lie more superficially than usual for the production of fruiting bodies. It is less than 20 cm. in most cases. But when the roots in question
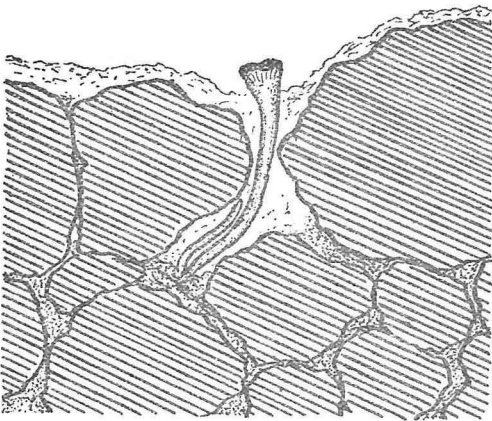
Fig. 18.

are lying in a pile of stones the mushrooms may be produced even from a depth of 26 cm. below the surface. Text fig. 18 illustrates this diagramatically.

Perhaps aeration is of considerable importance in determining the formation of a fruiting body.

3. The occurrence of mushrooms shows, to a certain extent, the localisation of the young root in the superficial layer of the soil.
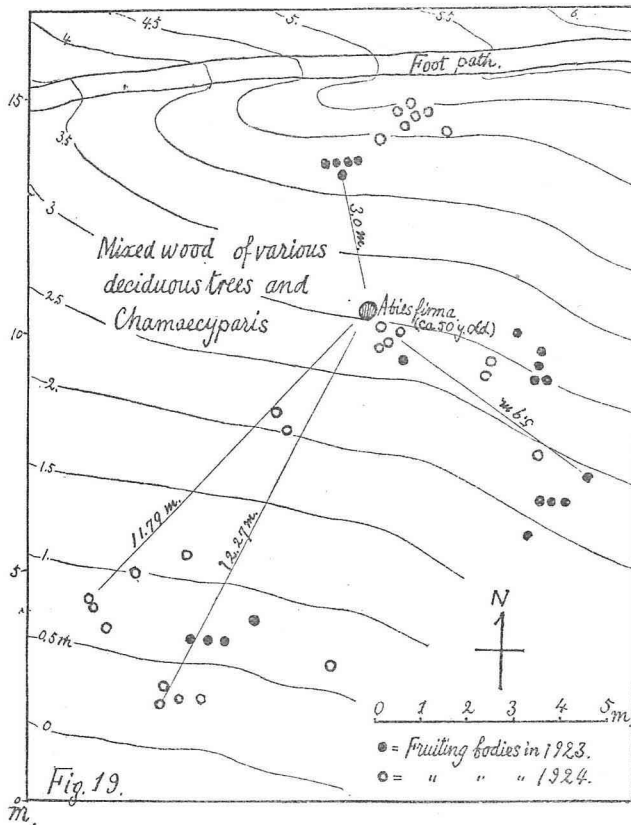

Fig. 19.

Not all firs produce mushrooms from their roots. For the pro-
duction of them environmental conditions must be favourable. If the
conditions are right, almost every growing root lying in the superficial
layer of the soil is infected by the mycelium first, and then produces
mushrooms. Therefore one may follow, to a certain extent, the
localisation of the young roots by the occurrence of mushrooms. Text
fig. 19 shows the localisation of the growing roots of a fir, about 50
years of age. standing on a slope.

In older trees the mushrooms always occur further
away from the trunk than in relatively young ones.
This fact shows that in older trees the roots have extended further
than in the younger ones.

4. Cantharellus floccosus does not form a fairy ring.

The mushroom cannot occur apart from the infected roots of
Abies. The distribution of the roots in the superficial layer of soil is
however too dispersed to make a compact circular mycelial bed as in
the fungi which form the fairy ring. It is only occasionally that the
fir roots come up to the surface of the soil in clusters, so as to allow
the mushrooms to occur in line or arc form as shown in Text fig. 20.

Fig. 20.   Mushrooms occurring in line.

5. Relation between the age of fir tree and the occurrence of mushrooms.

In my field observations, I noticed that *Cantharellus floccosus* does not occur around young fir-trees.

In order to get some knowledge of the relation between the age of trees and mushroom production, I counted, on the one hand, the number of mushrooms near each tree, and on the other hand, the number of their annual rings by the aid of an incrementborer. The results obtained are as follows :—

Ages of fir trees.	Fruiting bodies occurring in 1923.	Fruiting bodies occurring in 1924.
ca 73	47	32
„ 50	20	26
„ 36	17	26
„ 45	13	4
„ 40	9	
„ 42	6	0
„ 50		13
„ 67		27
„ 43		22
„ 72		32
„ 55		16
„ 42		18
„ 35		7

As the existence of fir roots in the superficial layer of the soil is a necessary factor for mushroom production, it is quite reasonable that the older trees bear more mushrooms, since they have more roots lying superficially than the younger ones. At Kurama the production of mushrooms is restricted to fir trees of at least more than thirty years of age.

III. Cantharellus floccosus.

1. Description of the fruiting body.

Cantharellus floccosus has been hitherto described by Saccardo (Syll. V, 491), Marshall and other systematists. The mushroom grows in clusters or solitary on the infected roots of *Abies firma* and perhaps of the other species of this genus. It is abundant in fir woods in Japan and has long been noticed by collectors and others. It is regarded as one of the edible mushrooms in America (Marshall 1919), but in Japan I have heard little of its being actually eaten by common people.

The plants are usually 6–8 cm. in height, 5–35 gr. in weight, the cap 2–7 cm. in breadth, and the stem rather short and smooth. Sometimes it developes so enormously as to attain 14 cm. high, and more than 100 gr. in weight.

The pileus is fleshy, funnel- or trumpet-shaped and usually pervious to the base of the stem. The surface of the depression is scaly and when wet it becomes more or less mucilaginous. The margin of the pileus is rather thick, blunt and usually more or less inrolled. It is often wavy or repand in the case of a fully expanded pileus. The color of the depression is rust red, thick rust red, cinnamon or brownish amber yellow.

All these characteristics of the pileus are observable in normally developed mushrooms, but in open soil it frequently takes on a tubular shape in maturity.

The gills are blunt, narrow, forked or anastomosing irregularly. The color of the gills is reddish salmon, buff, gamboge yellow, light cinnamon or reddish salmon.

The spores are faintly brownish in mass, elliptical, $6.2 — 7.1 \times 12.3 — 15.7\mu$ and granulated.

The stem is rounded or more or less compressed, $8 — 18$ mm. $\times$ $10 — 20$ mm. in diameters, and the size of its basal portion is variable according to the mode of origin.

The mushrooms produced directly from an infected root are usually provided with a base large in comparison to the upper portion of the stem but those originating at the termination of the mycelial strand do not show such a contrast.

In the development of the pileus, light is of real importance at the very initial stage.

According to BULLER ('22), if the primordium of the fruiting body of *Polyporus squamosus* has formed the pileus already, even in a most rudimentary condition, it continues to develope, attains a considerable size, produces a hymenial tube and liberates millions of spores, even if it is entirely shut off from the light. If however, the primordium, yet devoid of the trace of the pileus, developes further in the dark, it remains absolutely sterile.

In the case of *Cantharellus floccosus* also we can see not seldom the mushrooms which exist in the sterile condition in the dark interspaces of rocks. They are pure white in color and deviod of even a trace of the hymenium, though they have already attained several centimeters in length. It is interesting to note, that sometimes the sterile fruiting body can branch in its termination, as if it represents only a thickened rhizomorpha. (Fig. 1, Pl. III)

The young fruiting bodies are prolonged usually until their upper end reaches the light, and then their pilei begin to develope.

2. The mode of development of the fruiting body.

Since the appearance of BLIZZARD's paper in 1917, the exogenous origin of the lamellae in some species of Agaricaceae has evoked considerable attention. He described it in *Omphalia, Clitocybe* and *Clitopilus*. Since then the same fact has been reported in *Mycena, Hygrophorus* and *Entoloma* by DOUGLAS ('18), and in *Pluteus* by WALKER ('19).

In 1923, I collected fruiting bodies of *Cantharellus floccosus* in all

stages of development at Kurama during June and July. The young
white button and the upper portion of the rather elongated fruiting
bodies were immediately fixed at the place where they were collected.

As fixing solutions, FLEMMING's weaker solution and the chromo-
acetic solution were employed, and both of them proved to be almost
the same. DELAFIELD's haematoxylin and DOUGLAS's basic fuchsin were
employed for staining, but the latter was found to be far the better
for the study in question. The hyphal wall and its contents were
stained quite distinctly by the latter and it proved also good for
photography.

Primordium of the Basidiocarp. The undifferentiated basidiocarps
are slender, tapering toward the apex. They are usually curved or
bent in various directions. Those studied measured 0.6 mm. in dia-
meter and 1–2.4 mm. in length. (Fig. 1. Pl. IV). At this stage of
development they consist of a homogenous weft of slender threads,
measuring 5–6μ in diameter. Their general direction is almost parallel
with the axis of the young fruiting body, and no differentiation can
be found at this stage of development. The young fruiting body in-
creases in size by the continued growth and branching of hyphae
which compose the undifferentiated basidiocarp. The young fruiting
body becomes then somewhat spindle-shaped in longitudinal section, as
shown in fig. 2, Pl. IV. The interwoven hyphae assume generally a
longitudinal direction throughout the central portion of the fruiting
body, while on the surface the peripheral hyphae exhibit already a
strong inclination to turn outward.

The hyphae, which have diverged from the peripheral tissue of
the basidiocarp at an oblique angle of about 45° outward, branch a
little more densely toward the apex of the fruiting body.

Structure of the stipe. The interwoven central hyphae continue
their growth upward rapidly. At the same time the hyphae, which
have turned outward to the periphery of the fruiting body, become
more compact by branching and stain much denser. (Fig. 3, Pl. IV)
The cortical layer of the stipe, which is white or red in appearance,

is thus formed. The layer is composed of thin filaments, 2.5–3.5μ in diameter, and the filaments are neither parallel with each other nor do they reach the same level. The subcortical layer of the stipe is very rough in texture and is composed of thin filaments, 1.5μ in diameter.

When the fruiting body attains to a certain size, the central hyphae elongate less rapidly as compared with those of the periphery, and thus the upper end of the fruiting body becomes as shown in Fig. 4, Pl. IV. At this stage of development, only the cortical layer is found, in the section at both sides of the fruiting body, and the hymenophore primordium has not yet come into existence.

Hymenophore primordium, palisade layer, and development of the lamellae. By continued growth of the peripheral hyphae upward, the upper end of the fruiting body becomes concave. (Fig. 5, Pl. IV) At this stage of development the hymenophore primordium originates in just the same way as the cortical layer formed at the peripheral growing point.

At the peripheral portion of the upper end of the fruiting body, the zone of primordial elements organizes a definite layer of rather parallel threads which becomes more or less even on the surface since the ends of the hyphae reach the same level. A little later, it thus results in forming a compact layer of parallel threads almost perpendicular to the surface. The hyphal elements of this layer are slender, cylindrical and septate threads, 2.5–3.3μ in diameter. The terminal cells are longer than the rest of the same thread and slightly larger, so that they give a more or less clavate appearance to the threads. The cells are rich in protoplasmic contents, and present an appearance of active growth.

As the peripheral portion of the upper end of the fruiting body continues its further growth upward, and forms a new zone of hymenophore primordium at the outer side of the growing point, the central concave portion begins to be perforated, as shown in figure 7, Pl. IV. At this stage of development, the differentiation of the palisade layer

appears next to the newly formed hymenophore primordium.   The
hyphal elements of the palisade layer are, like those of the hymeno-
phore primordium, slender, cylindrical and septated, but the terminal
cell of the thread becomes much larger, 4.2–5.7μ in diameter, and
takes a clavate form.   Fig. 6, Pl. IV shows a cross section of the layer.

As the cellular element of the palisade layer increases more in
size, a great tension is produced within this structure.   This peripheral
tension is relieved to some extent by the palisade layer itself being
thrown into folds, but the inner layer becomes more sparse thereby.
(Fig. 7–11, Pl. IV)   The folds become very conspicuous a little later,
and thus the lamellae of the *Cantharellus floccosus* are formed.   The
cellular elements of the fold consist of basidia, 10μ in diameter, and
paraphisis, and lack cystidia.

Pileus.   As above mentioned, when the fruiting body attains to a
certain size, the central hyphae of its upper end stop their elongation,
while the peripheral hyphae continue their growth further upwards.
In this way the upper end of the fruiting body becomes concave.   The
thick border of this slight depression differentiates into pileus.   At the
same time the hymenophore primordium is organized at the peripheral
portion of the pileus. (Fig. 7, Pl. IV)   By continued growth and
branching of the hyphae, the pileus begins to elongate obliquely out-
ward, forming a new zone of the hymenophore primordinm always
laterally.   In this way the further elongation of the pileus is realised,
and on the other hand the depression becomes deeper and deeper.
The inner side of the depression is the scaled surface of the pileus.

By the gradual elongation of the pileus, a new zone of the
hymenophore primordium is organized ; and the older portion of the
primordial zone differentiates into palisade layer, and the older portion
of the latter differentiates in turn into a portion of the fold.

3. Food storage hyphae?

In 1918 DOUGLAS found in the interwoven hyphae of a young

fruiting body of *Hygrophorus miniatus* a kind of hypha, rather large in diameter and having a strong affinity for stain. He described it as a food storage hypha. I found also such a hypha in the young fruiting body of *Cantharellus floccosus*.

The hyphae are fully demonstrated when the longitudinal hand-section of the fresh button are mounted with glycerin-iodine or with glycerin after being colored with an aqueous solution of fuchsin or eosin.

The microchemical test with MILLON's reagent, conc. nitric acid, picric acid, biuret reaction and eosin, proved the content of the hyphae to be of protein nature.

They are $4.5-6.3\mu$ in diameter and more richly provided with protein substances than the elements of the interwoven hyphae. They are long cells provide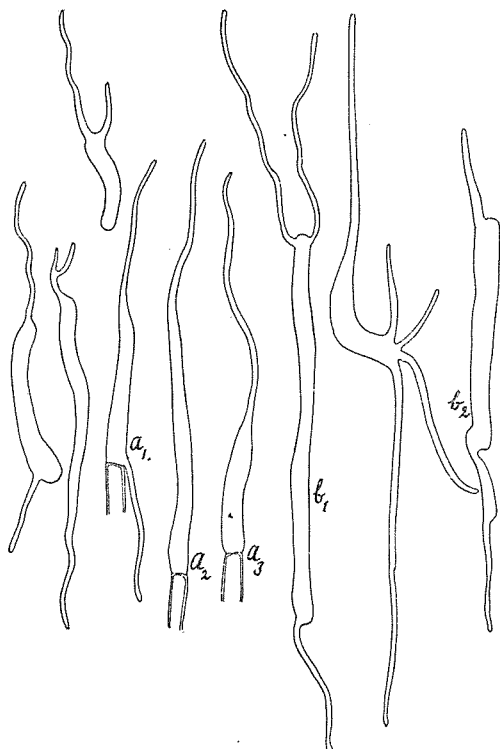d with two nuclei each at their thick portion and give rise to one or more branches at one end or at both. (Text fig. 21) The branches are also richly provided with protoplasmic substances. The novel thing is that they are entirely lacking in a septum though some of them are very long.

As for the origin of the hyphae, it may be assumed that they are produced occasionally from cells provided with an exceptionally large amount of nutrient substances.

About the function of these hyphae I cannot

Fig. 21.   ×200.

say much, but they probably function just as nutritive elements for the other rapidly growing hyphae, as DOUGLAS has stated.

4. Cantharellus floccosus is a mycorrhizal fungus also of Abies Mayriana MIYABE et KUDO.

On July 4, 1924, I found several fruiting bodies of this fungus around a large *Abies Mayriana* MIYABE et KUDO, in Nopporo, Hokkaido. (Fig. 5, Pl. III) They were yet young, and the hymeni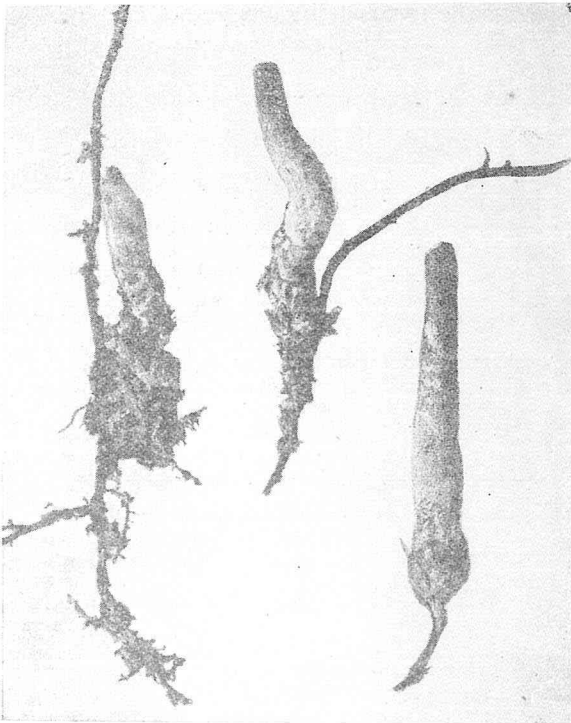al layer had not been developed. But the characteristic color, size and form were enough to determine them as young fruiting bodies of *Cantharellus floccosus*.[1]

On digging up these mushrooms, a fresh young mycorrhiza was found immediately below each one, and in every case the actual connection between the mycelium or mycelial strand of the mushroom and the mycorrhiza was easily demonstrated. (Text fig. 22) These mushrooms were found only in the area occupied by *A. Mayriana* in this district.

Fig. 22. Young fruiting bodies of *Cantharellus floccosus* attached to the mycorrhizal roots of *Abies Mayriana*. Natural size.

Besides these mycorrhizas from which the fruiting bodies had

1) This place was pointed out by Mr. S. IMAI, where he had already found the same mushroom in the previous year. I wish to express here my gratitude to Mr. IMAI for so kindly assisting me in this investigation during my stay in Hokkaido.

been produced, many other roots which had been heavily infected at their growing portions were found in the same place. (Text fig. 23)

Fig. 23.   Heavily infected roots of *Abies Mayriana*.   Natural size.
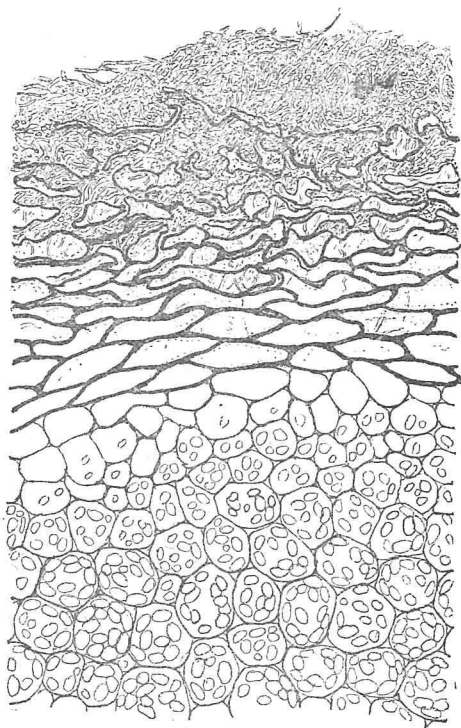

Fig. 24.   A cross section of the mycorrhiza of *Abies Mayriana* caused by *Cantharellus floccosus*.   × 100.

They were found, as in the case of *Abies firma*, only in a superficial layer of the soil.

In order to demonstrate the internal structure of the mycorrhiza, I made a cross section of it with a razor.   A thick fungous mantle, which was made up of closely interwoven hyphae, was found surrounding the cortical layer of the root.   On the inner side of the mantle, a considerable number of cortical cells, isolated like islands, were found scattered in it as the figure shows. (Text fig. 24)   The mycelium extended not only in between the cortical cells dissolving the middle lamella, but also entered into the cortical cells as in the case of *A. firma*.

In the form of mycorrhiza as well as in the mode of production of mushrooms, *A. Mayriana* resembles very much *A. firma*. Moreover the mushroom seems to occur in this district under the same environmental conditions as in the vicinity of Kyoto.

IV. Further investigation on mycorrhiza of Abies firma S. et Z.······Four types of mycorrhiza and their structure.

During my observation I noticed that most rootlets of fir tree, in the upper part of soil, are usually transformed into ectotrophic mycorrhizas. They are easily recognized by the characteristic clusters of numerous short stubby branches, the so-called coral-branching rootlets, or of numerous, irregularly bent, abnormally elongated rootlets. When alive, they have always a bright, fresh appearance, even when collected in mid-winter.

After detailed investigation, I could distinguish in the rootlets of *Abies firma* in this locality four types of small mycorrhizas, which I may denote as Forms A, B, C and D.

1. Form A.

These are infected rootlets or mycorrhizas caused by *Cantharellus floccosus*, which I have already described. They are found always 'in loam soil, where the fruiting bodies of the fungus occur.

This form is w h i t e i n c o l o r w h e n f r e s h. The particular character of this form is that it is a n a b n o r m a l l y s l e n d e r a n d i r r e g u l a r l y b e n t m y c o r r h i z a, i n t e r w o v e n as shown in Text fig. 25, A.

The hyphae, 3.3–14μ in thickness, constitute a network, covering the surface of the rootlet, and form a mantle which gives off filaments projecting out into the surrounding soil. Moreover, the mantle frequentry gives off what may be called a rhizomorpha-like hyphal bundle, which is prolonged far out from the mycorrhiza.
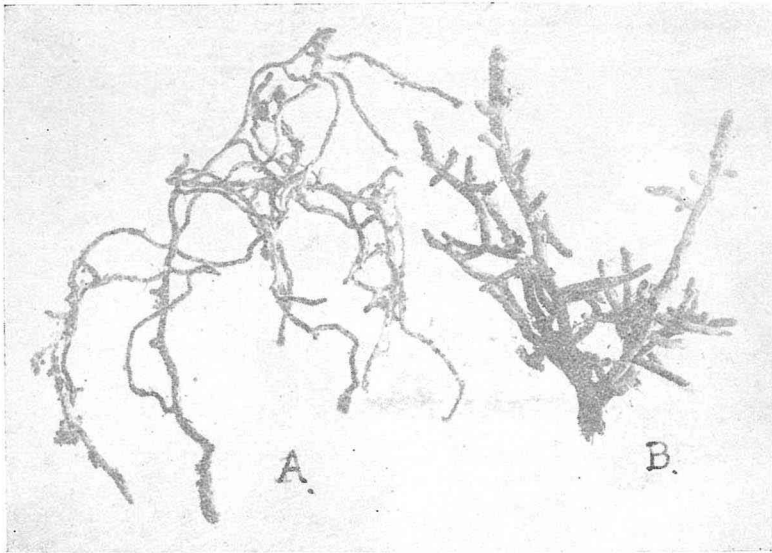
Fig. 25. A, mycorrhiza Form A; B, mycorrhiza Form B. ×2.5

The thickness of the mantle is very irregular : usually it is $6-15\mu$ and nearly even in thickness as shown in Text fig. 26; 5 & 6, but sometimes it reaches 50μ in some parts of the mantle. (Text fig. 26; 2) In cross sections of the mycorrhiza I frequently observed un-completed mantles which are shown diagramatically in Text fig. 26; 1, 2, 3 & 4.

The illustration shows that the mantles are not only uneven in thickness, but mantle-rings are more or less lacking in the bordering surface of the rootlets. Such a phenomenon may, perhaps, occur at any portion of a long mycorrhiza, according to circumstances, when the mantle advances over the surface of the rootlet.

The next character of this mycorrhiza is that, it contains only a small quantity of tannic substances compared with the other mycor-rhizas.

From the inner part of the mantle, hyphae penetrate inward bet-ween the cells of the root and extend into one or two layers of the cells, and are not minutely septated into short cells, so that almost
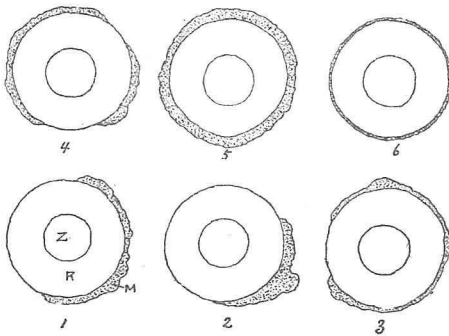
Fig. 26. Cross sections of the mycorrhiza Form A in *Abies firma*. Z; central cylinder; R, cortex; M, fungous mantle.

the same character of hyphae is observed here as in the mantle. (Fig. 1, Pl. V.)

2. Form B.

A second type of mycorrhiza is brown in color when fresh. It occurs always in rawhumus in the woods, in clusters of numerous, short, stubby, monopodially branched rootlets. (Text fig. 25, B) This mycorrhiza is, generally, 0.15–0.7 mm., sometimes 1 mm in diam., and is the largest of the four forms. When it is treated with one per cent solution of chromic acid or concentrated aqueous solution of potassium bichromate, it becomes much darker than the other mycorrhizas of *Abies firma*. Accordingly it contains more tannic substances than the other three forms. Superficial examination, with a low-powered microscope, reveals numerous short hyphae projecting out from the surface of it. They are only wanted at the growth point of the mycorrhiza when it is young.

Sometimes I found, besides the minute projections, long hair-like filaments, also projecting out from the mantle. The filaments, which are 3–3.5μ in thickness, transversely septated and hyaline, mingle into a network over the surface of the mycorrhiza, or join together into a thin bundle.

FRANK ('85) reported in the case of the mycorrhiza of *Fagus silvatica* that " Rhizomorpha-artige Stränge ", which are divided into smaller branches, are often produced from the mantle of ectotrophic mycorrhizas. MÜLLER ('86) described the same thing under the name of " Rhizomorpha-ähnlichen Hyphenmassen ". Also in the case of this mycorrhiza, I found these rhizomorpha-like strands. They are

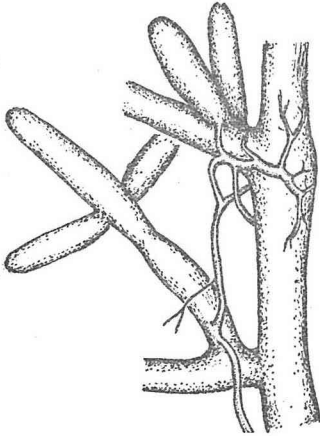50–150μ in diameter, and produced from the surfaces of the mantles. (Text fig. 27) The bundle is divided into smaller bundles or numerous filaments, and their terminations are connected firmly with other mycorrhizas or surrounding rotten leaves.

When thin hand-sections of the mycorrhizas are treated with an aqueous solution of ruthenium-red or iodine-potassium iodide solution, it is proved that the fungous mantle and the minute projections contain much glycogen. The minute projections are 12–19μ in length and 2.8–7μ in width, and are found to be unicellular, hyaline and flask-shaped bodies. Each of them is provided with minute sporelike bodies at its termination, and many of them contain plenty of cytoplasmic substances while others are poor in contents and mucilagenous substances are drifted off from the terminal pores which have been perforated by the discharge of the spore-like bodies. Over the surface of the mantle there is always suspended a mucilagenous substance which colors yellow with iodine-potassium iodide solution. The sporelike bodies are found in large numbers in this substance.

Microtomic sections of the mycorrhiza reveal them more clearly. (Text fig. 28 and Fig. 2, Pl. V)

The flask-shaped projections are the hyphae which have been differentiated from the filaments of the outermost layer of the mantle (Text fig. 29), and show themselves to be organs, each of which produces a single spore-like body. They contain plenty of protoplasmic substances and minute granules. (Fig. 5, a-j, Pl. V)

The sharp apex, which is more rich in contents, is rounded off first, and the neck begins to constrict and then differentiate into a
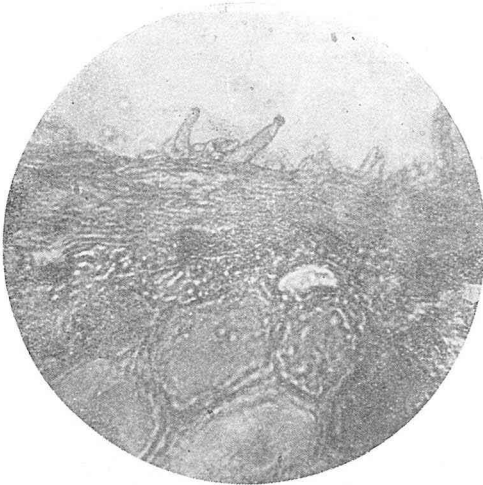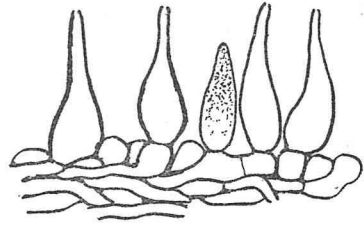
Fig. 27. Mycorrhiza, Form B which produced rhizomorpha-like hyphal bundles.   ×9.

Fig. 29. Outermost layer of fungous mantle of mycorrhiza, Form B, showing five projections.   × 1500.

Fig. 28. A cross section of mycorrhiza B.   × ca 800.

spore-like body. (a–e) This spore-like body discharges itself when it attains its full growth, leaving a minute pore at the ending of the organ. After the discharge is over the cytoplasmic substances seem to flow out of the pore. (Text fig. 29 and Fig. 5, l–m, Pl. V) The spore-like bodies are spheroidal or oval in shape, $1–1.5\mu$ in diameter. (Fig. 5, k, Pl. V) It is a question, whether these bodies are capable of germination and serve for further infection.

In MAGNUS's wall-map[1] appears a similar projection projected from the surface of the mycorrhiza of *Pinus silvestris*, and he names it "Absorptionshyphe". But in the case of mycorrhiza Form B, the projection is, though resembling it in the morphological sense, not an "Absorptionshyphe" but the organ which produces the spore-like body.

Hitherto, many of the fungi of ectotrophic mycorrhizas have been believed to be higher basidiomycetes, and little is known about this spore-like body which is produced directly from the mycorrhiza.

The fungous mantle is very thick, $13–49\mu$ in thickness, and some-

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1) In NEGER's Biologie der Pflanzen. 1913, P. 474, there is an illustration of MAGNUS's wall-map.

times it is divided into two layers. The outer layer is always thinner than the inner, and composed of larger filaments, $3-4\mu$ in thickness, while the inner one is made up of filaments of $2-3\mu$ in thickness.
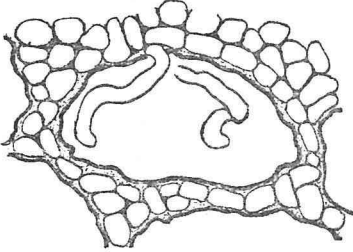

Fig. 30. Intracellular hyphae of a cortical cells. $\times 1500$.

The fungous filaments, which penetrate into the cortical layer of the root, are minutely septated and form the so-called HARTIG's network. The next characteristic of this mycorrhiza is that the filaments, which extend in between the root cells, enter occasionally into the cell cavities, and appear very much like endotrophic filaments. (Text fig. 30)

Development of the fungous mantle. In the case of mycorrhiza Form B, it is not so difficult to obtain specimens in its early stages, as in the case of the mycorrhiza of *Tilia americana*. (McDOUGALL 1914) I found a lot of good specimens on the first of October, 1923. Longitudinal sections from some of them are shown in Text fig. 31 and 32.
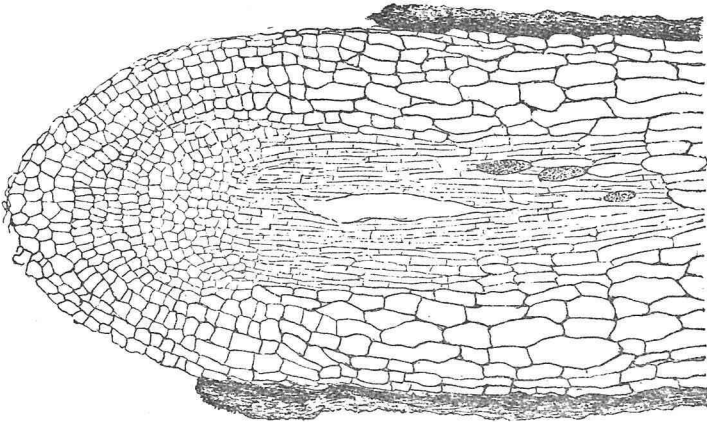

Fig. 31. Apex of a young mycorrhiza of which mantle has not yet been completed. Form B. $\times 100$.

It is clearly shown, that in the developing mantle the mycelial growth advances toward the tip of the root along its surface, as

McDOUGALL has stated. The same fact was also observed in mycor-
rhiza Form C which will be mentioned in the next section.

The marginal growth of the mantle along each side is not always
uniform, so that its final closing point can more or less deviate from
the tip of the rootlet. Toward the margin of the mantle, almost all
the hyphae are arranged nearly parallel to the advancing direction.
They are provided with a large amount of protoplasmic content,
showing the capacity for further growth. Those which constitute the
innermost layer of the fungous mantle have also rich contents. They
elongate inwards rapidly, penetrating into the cortical layer by dis-
solving out the middle-lamella. The cells of the outermost layer of
the cortex are thereby crowded apart first by the penetrating fila-
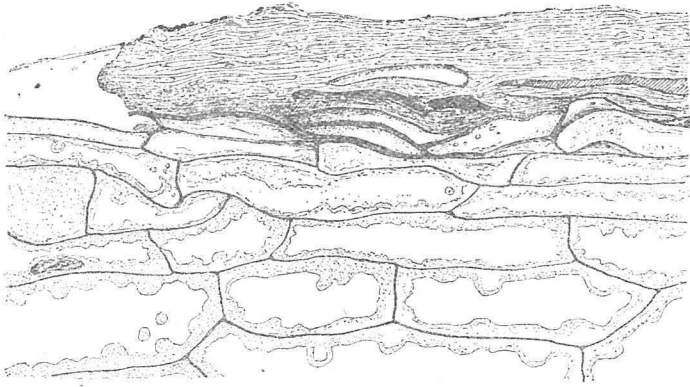ments, more or less simultaneously with the advance of the fungous
mantle. (Text fig. 32)

Fig. 32.   Marginal portion of the advancing
mantle,   Form B.   ×510.

The formation of HARTIG's network, between the cells of the cor-
tical tissue, clearly takes place at a point a little back of the margin.
The mycelial filaments which constitute it increase in number and the
cortical cells are thereby isolated from each other and crowded into
filamentous bodies or cut up into slender pieces as shown in the Text
fig. 33. On the other hand the hyphae penetrate deeper inwards
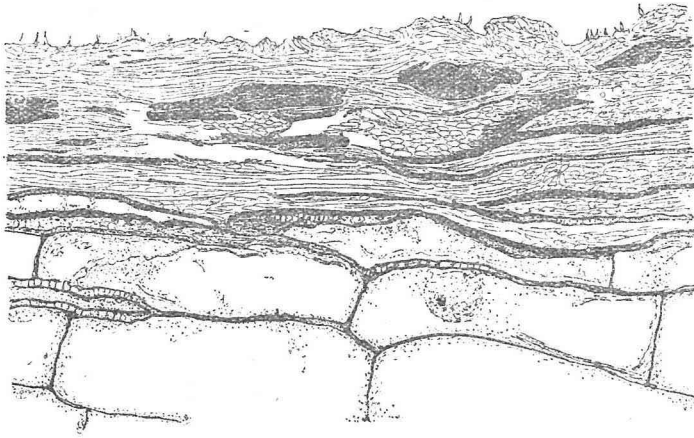until they reach the endodermis.

Fig. 33. Older portion of the fungous
mantle, Form B.　×510.

Thus the fungous mantle becomes thicker little by little by the inward addition of the element, though in the case of the larger mycorrhiza caused by *Cantharellus floccosus* the thickening of the mantle takes place by outward addition. The cells isolated first are seen as islands in the outer layer of such a thick mantle.

It has been, hitherto, a problem whether the mantle or the lichen structure is first formed on the rootlet by the infection. FRANK stated that an ectotrophic mycorrhiza is produced by a fungous filament applying itself to the side of a rootlet, and then branching and spreading until it covers the whole rootlet. He evidently believed that the mantle of fungous tissue is put on first, and that the formation of the lichen structure within the rootlet is a secondary process. MÖLLER, on the other hand, discussing the mycorrhiza of *Picea*, reports that infection starts in the root tip, and that the lichen structure within the root is formed first, and the mantle put on later. McDOUGALL agreed with FRANK and positively denied MÖLLER'S statement. In a word, there are two different ideas about the formation of the ectotrophic mycorrhiza.

In the case of the mycorrhiza Form B, a well developed

mantle begins to send off invading hyphae into the
cortical tissue and form a lichen structure after the
mantle has advanced rather far, though the cells of its
outermost layer are, sometimes, isolated like islands in the mantle
more or less simultaneously with its advance.   That is to say, the
formation of the fungous mantle here precedes that of
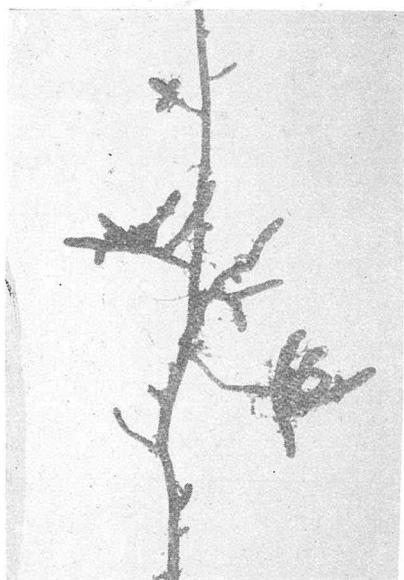the lichen structure.

3. Form C.

Fig. 34. Mycorrhiza
Form C.   × 2.5

This type was always found
on rather young trees abundantly
from spring to autumn.   The
mycorrhiza is r a t h e r l o n g,
i r r e g u l a r l y b e n t and 0.5–0.25
mm. in diameter. (Text fig. 34)

It is b r i g h t y e l l o w i n
c o l o r, w h e n y o u n g a n d
f r e s h, b u t c h a n g e s t o
b r o w n o r d a r k i n a g e.   A
superficial examination with the
microscope reveals, when young,
a smooth surface and short fila-
ments sparsely prejecting out from
it, although at a later stage, the
projecting filaments become numer-
ous and long and construct a network over the surface of it.   More-
over rhizomorpha-like hyphal bundles are always found connected
intimately with it.   The occurrence of such a bundle of hyphae may
be important in connection with the further infection by mycorrhizal
fungi of newly elongated rootlets of the host.   A n o l d m y c o r-
r h i z a a l w a y s p r o d u c e s a b u n d l e o f f i l a m e n t s w h i c h
e l o n g a t e a l o n g t h e m o t h e r r o o t o r i n a n y o t h e r d i r e c-
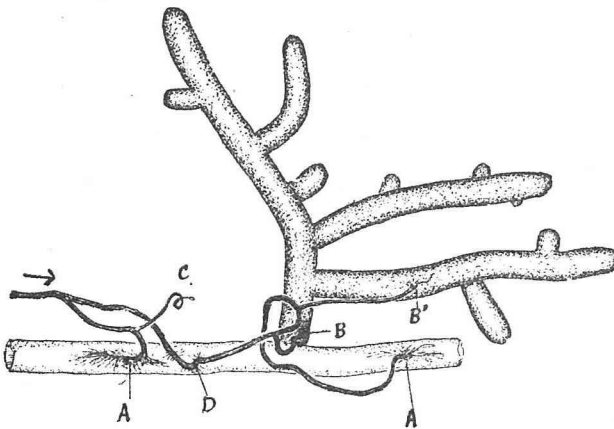t i o n, as if it searching for the host.   They are yellow in color, 0.15

Fig. 35.   Rhizomorpha-like hyphal bundle
attached to mycorrhizal root, Form C.   ×9.

−0.22 mm. in thickness, and send off branches as described by FRANK. (Text fig. 35)

When the branchlets of this bundle come in contact with the surface of a rather old root of *Abies firma*, only a small mass of mycelial out-growth is formed along that point. (A & D) If, however, they come in contact with young rootlets, or with completed young mycorrhiza mantles, the diffused mycelium of the bundle expands all over the surface until a new mantle is completed. (B & B′)   When the bundle does not find a root of *Abies firma*, it may dry up as shown in the figure. (C)

In order to see the internal structure of the mycorrhiza, microtomic sections were prepared.   Fresh materials were fixed with chromoacetic fixing solution, dehydrated and imbedded in paraffin. For staining, the tannic acid-fuchsin method introduced by DOUGLAS ('18) was employed.   By this staining the filaments which constitute the mantle and HARTIG's network and the cell-walls and the nuclei of the host cells, were colored a beautiful red.

The fungous mantle is found to be $14–29\mu$ in thickness and to consist of hyphae $1.4-1.7\mu$ in diameter.   Most of the mycorrhizas bear mantles divided into two layers, inner and outer, by the existence of an interposed thin layer which is colored deeper than the hyphal pseudoparenchymatous tissue. (Fig. 3, Pl. V)

Sometimes I observed, besides the two-layered mantle, a one-or three-layered one, as shown in Text fig. 36. In the three-layered mantle two interstitial layers can be distinguished which divide the thick mantle-complex into three almost equal parts, while in the one-layered one no such interstice is found and the mantle itself is only one third the thickness of the former. It is quite clear that each fo these layers is formed independently in different periods successively over the surface of the root or over the preexisting mantle, whereby the innermost is the older. Such an overlapping of mantles is, so far as I am aware, quite new to our k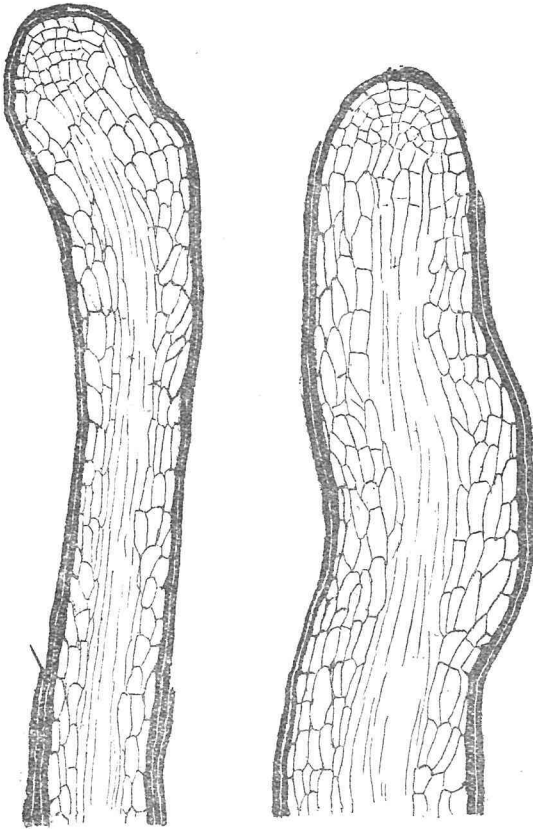nowledge, and it is very interesting to see that, on the part of new mantles, there is no great distinction made as to whether the surface to be covered is a root or an already formed mantle.

It seems very probable that some substances which cause the overlapping growth of mycelium are excreted from the surface directly from the root or indirectly through the mantles.

The fungous infection usually takes place not at the tip of the root, but a little back from it.

The fungous man-

Fig. 36.   A longitudinal section of mycorrhiza Form C.   ×75.

tle, certainly made first at that part of the young root where the infection has taken place, seems mostly to spread and advance along the surface of the root toward the apex of it.

In the same way, secondary or tertiary mantles, must advance also in the same direction over the older ones.

The structure, the thickness and the staining reaction of the hyphae of each layer are all the same. It may be concluded, therefore, that these layers are formed by the same species of mycorrhizal fungus in different periods. I could not hitherto meet with cases of the mycelia of different fungi lapping over each other, though it seems not impossible.

Fungous hyphae penetrate intercellularly and form the so-called HARTIG's network, so that the cells of the cortical tissue of the root are often separated from each other isolated as islands far out in the fungous mantle, as shown in Fig. 3, Pl. V. The hyphae in the HARTIG's network are almost the same in thickness as those of the mantle. Protoplasmic membranes of the cortical cells are separated from the walls and torn off into pieces of irregular forms.

Nuclei still existing in the cells are minutely granulated and seem to have been lost their vitality.

4. Form D.

A fourth type found in *Abies firma*, is brownish in color when fresh. In microtomic section some parts are much distinguished from the other forms described above. The structural difference is concerned principally with the fungous mantles.

The mantle, which consists of a pseudoparenchymatous tissue, is divided into two different layers. The outer layer is made up of easily distinguishable filaments, while the inner one is composed of almost undistinguishable filaments.

The filaments of the former are 2.8–7.0μ in diameter, and the walls of them are stained violet with DELAFIELD's haematoxylin.

They run almost longitudinally and some are given off from the

surface of the mantle. The filaments of the latter are 3–10μ in thickness and irregularly mingled with each other. Their cell walls are thinner than those of the former ·and are not colored with the same staining dye. The inner layer is always much thicker than the outer.

There are rapidly elongated root cells, but the outermost cells of the root are crowded apart by the fungus until some of them are isolated as islands far out in the fungous mantle. The fungus penetrates nearly to the central cylinder so that nearly all of the cortical cells are entirely separated from each other. The filaments, which consist of the so-called Hartig's network, are minutely septated and are 1.4–3μ in diameter. (Fig. 4, Pl. V)

So much for the mycorrhizas I have observed in *Abies firma*. It is often reported, that one kind of tree has many kinds of fungi as its mycorrhiza. Noak ('89), for example, reported that *Giaster fimbriatus* and *Agaricus terreus* cause mycorrhizas on *Pinus*, *Giaster fimbriatus* and *Cortinarius callisteus* on *Picea*, and *Agaricus terreus* and *Cortinarius caerulescence* Sch. on *Fagus silvatica*.

According to Pennington ('06) *Cortinarius* and *Russula emetica* cause mycorrhizas on red-oak and *Boletus speciosus* and *Trichoma transmutans* Pk. on the root of black-oak. McDougall ('14) reported that *Cortinarius* and *Boletus* cause different ectotrophic mycorrhizas, brown and white, on *Betula*. Moreover he described three kinds of mycorrhizas on *Carya ovata*, and four on *Quercus alba*. Melin ('21–'23), isolated three different fungi from ectotrophic mycorrhizas of *Pinus silvestris*, and asserted by what he called "Synthesenversuche" that eight different fungi can cause mycorrhizas on *Betula*.

In the case of *Abies firma* also the four forms of mycorrhizas described above may be caused by four different fungi, and among them, Form A is the mycelium of *Cantharellus floccosus*.

As I have been unable to find any fruiting bodies in these mycor-

rhizas other than *Cantharellus*. I can not identify them specifically by name.

Summary.

1. *Cantharellus floccosus* causes a mycorrhiza on the roots of *Abies firma* S. et Z.

2. Fruiting bodies of *Cantharellus floccosus* originate (1) directly on the infected root of *Abies firma*, (2) at the termination of the mycelial strands derived from the infected roots, (3) on a mycelial network interwoven by the hyphae projected from numerous small mycorrhizas, and rarely (4) as a side branch of an old fruiting body.

3. The mycorrhizal root caused by *Cantharellus floccosus* has not only a fungous mantle and HARTIG's network but always intracellular hyphae.

4. The fungous infection on young roots is, perhaps, caused not only by the mycelial filaments which have come from the spores, but also by the mycelial filaments and mycelial strands, both of which have been given off from the preexisting mycorrhiza. When the young roots branch off from the infected mother root, they may be infected directly by the mycelium of the mantle.

5. The rate of growth of the main lateral roots of *Abies firma*, which I measured by the glass-plate method of McDOUGALL, is diminished by the fungous infection. In summer a considerable number of them are killed by fatal infection of *Cantharellus floccosus*.

6. The microchemical investigation of both infected and uninfected roots shows the following results:

    1. The quantity of starch accumulated in the pericycle tissue is diminished by the infection of the fungus.

    2. The amount of ammonium salts contained in the cortical tissue of the uninfected root always surpasses that in the same tissue of the infected root.

3. The uninfected root contains more nitrates or nitrites in the
    cortical tissue than the infected root.

    It is obvious that these substances are used up by the infect-
    ing mycelium.   Only the fungus seems to be benefited by the
    root, obtaining sugar as a source of glycogen, and ammonium
    salts, nitrates or nitrites as sources of nitrogen.

    In a word, the mycorrhiza, caused by *Cantharellus floccosus*, is
    not a symbiotic association, but an instance of parasitism of the
    fungus on the root.

7.· Generally the larger fruiting bodies are produced from the larger
    infected roots, and on the contrary smaller ones are formed from
    the smaller roots.

8. The fruiting bodies do not occur under or near young fir trees,
    on account of the depth of the roots in the soil.

9. *Cantharellus floccosus* does not occur in damp soil.

10. The existence of young fir-roots in the superficial layer of the
    soil is an important factor for the production of *Cantharellus
    floccosus*.

11. The occurrence of *Cantharellus floccosus* indicates, to a certain
    extent, the localisation of the growing roots of *Abies firma* in
    the superficial layer of the soil.

12. Development of *Cantharellus floccosus*.

    (1) The primordium of the basidiocarp is a minute body which
         is made up of interwoven homogeneous hyphae.

    (2) As the first step of development, the cortical layer of the
         stipe differentiates.

    (3) When the fruiting body attains a certain size, the central
         hyphae begin to elongate less rapidly, while the peripheral
         hyphae continue their growth further upwards.

    (4) In the peripheral portion of the upper end of the fruiting
         body the primordia of both pileus and hymenophore appear
         almost simultaneously.

(5)   The hymenophore primordium originates exogeneously.

13.   The mycorrhiza of *Abies firma* is caused not only by *Cantharellus floccosus*, but also by some other kinds of fungi.   I have been able to distinguish 4 forms in all.   Of them, the Form B, an ectotrophic mycorrhiza that produces basidia-like projections from which spore-like bodies are discharged, is a particular one which is new to our knowledge.

14.   In most cases, the fungous mantle of the mycorrhiza in *Abies firma* advances, from the commencement in tolerable thickness toward the apex, along the .surface of the young root.

15.   In the case of the mycorrhiza Form B, the formation of the fungous mantle always precedes that of the lichen structure, but in the case of Form A (*Cantharellus floccosus*) sometimes the latter precedes the former.

16.   *Cantharellus floccosus* is a mycorrhizal fungus also of *Abies Mayriana* MIYABE et KUDO.

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Explanation of plates.

Plate   II.

Fig. 1.   An effect of the glass-plate method. Near the right end of the figure appears a young root which has been heavily infected by mycelium. In the central part of the figure there appears a considerable number of mycelial strands which have been given rise to by the underlying mycorrhizal roots. Near the lower corner of the left side is seen a bundle which is provided with a button at its termination. × 1.

Fig. 2.   An effect of the glass-plate method. Pure white mycelium has been formed along the young fir roots. × 1.

Fig. 3.   Young roots of *Abies firma* which have been heavily infected by mycelium of *Cantharellus floccosus*. × 1.

Fig. 4.   Young roots of *Abies firma* which have been killed by the infecting fungus. × 1.

Fig. 5, a—c. *Cantharellus floccosus* occurring at the end of long mycelial strand. × 1.

Fig. 6.   A cross section of a heavily infected root, showing a mycelial mantle in dark bordering ring, and also showing cells of cortex filled up with mycelium in dark spots.

Plate   III.

Fig. 1.   An abnormal fruiting body of *Cantharellus floccosus*, which beais several young buttons on its upper end. ×1.5

Fig. 2.   Four young fruiting bodies have been produced as side branches from the lower portion of an old fruiting body.

Fig. 3.   A young root which has been infected by mycelium of *Cantharellus floccosus*. Several fruiting bodies have been produced on the mycorrhizal root.   The mycelium is advancing, in a branched body, along the root toward its apex. ×1.5

Fig. 4.   Anastomosing mycelial strands. ×3.5

Fig. 5.   Four young fruiting bodies of *Cantharellus floccosus* produced from the mycorrhizal roots of *Abies Mayriana*.

Fig. 6.   Several fruiting bodies occurring on a mycelial mass composed of a considerable number of the small mycorrhizas. ×1.

Fig. 7a.   Several young fruiting bodies have been produced directly from the surface of the mycorrhizal root. ×1.

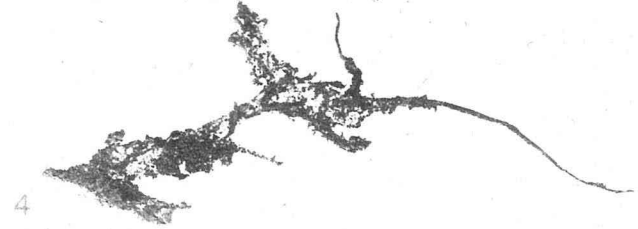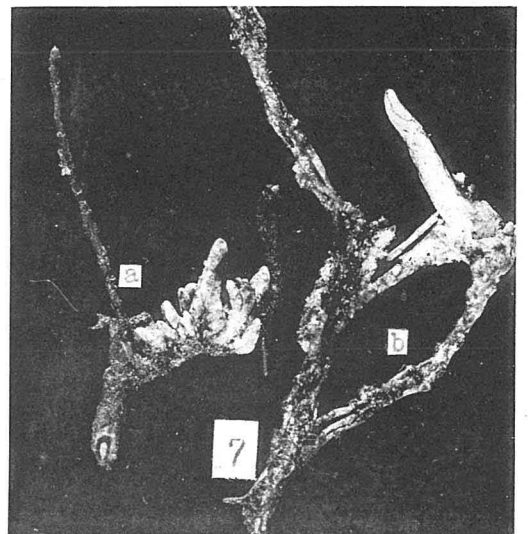Fig. 7b.   A young fruiting body produced at the junction of two rhizomorphas. ×1.

Plate   IV.
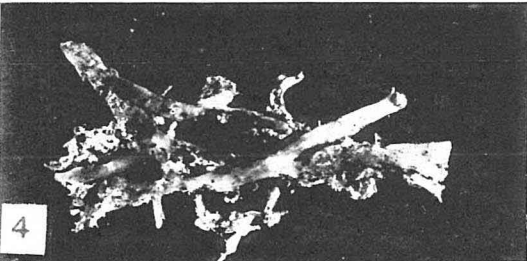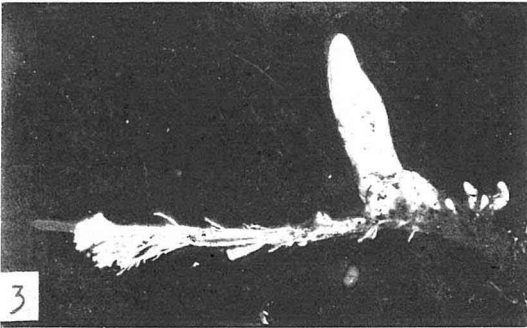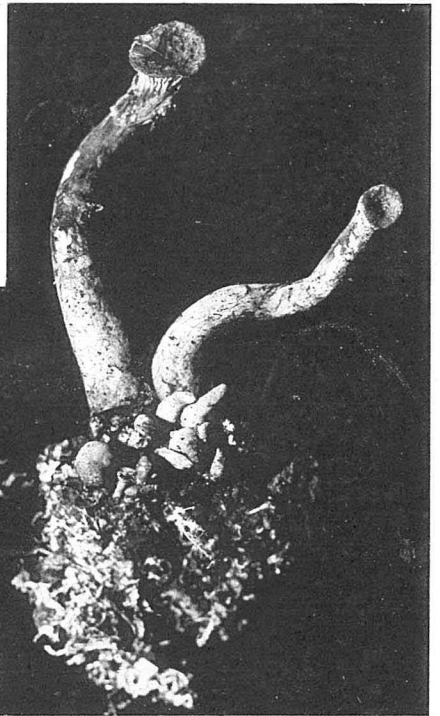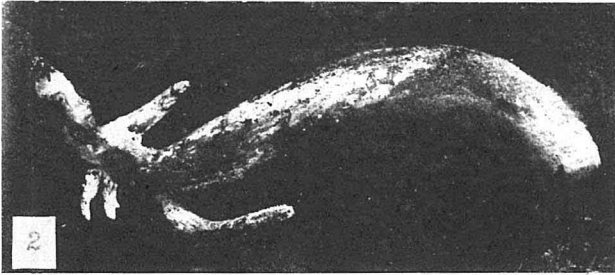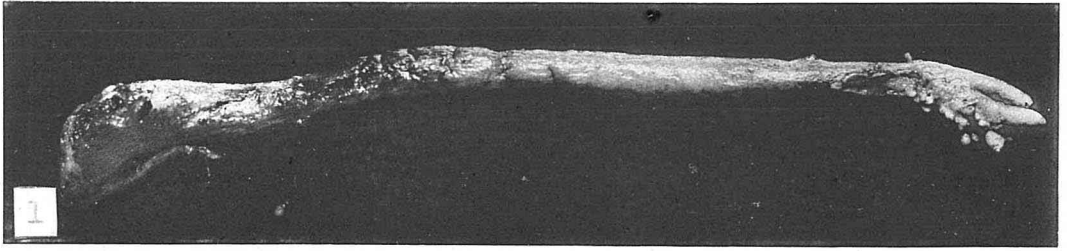
Fig. 1.   Median longitudinal section of very young fruiting body in which no differentiation has been taken place. ×15.

Fig. 2.   Median longitudinal section of the button, showing slight differentiation of the cortical layer. ×15.

Fig. 3.   Median longitudinal section of an elongated fruiting body, showing well developed cortical layer. ×15.

Fig. 4.   Median longitudinal section of very elongated fruiting body, which shows plain apex. ×15.

Fig. 5.   An older stage, which shows the differentiation of the hymenial primordium and the primordium of pileus. ×15.

Fig. 6.   Cross section of the fruiting body showing palisade layer in dark border. ×15.

Fig. 7.   Median longitudinal section of much advanced stage, showing a slight depression at the upper end. ×15.

Fig. 8.   Shows more advanced stage than Fig. 7. ×15.

Fig. 9.   Cross section of an old fruiting body, showing several folds of hymenial layer. ×15.

Fig. 10, 11.   Median section of pileus, showing well marked hymenial layer. ×15.

Plate   V.

Fig. 1.   A cross section of mycorrhiza, Form A. ×868.

Fig. 2.   A longitudinal section of mycorrhiza, Form B. ×867.

Fig. 3.   A longitudinal section of mycorrhiza, Form C. ×867.

Fig. 4.   A cross section of mycorrhiza, Form D. ×867.

Fig. 5.   Mycorrhiza Form B. K, spore-like bodies; a-j, several stages of development of the body; l-m, old projections which have discharged the bodies. ×1500.

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Pl. III.

Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B, Vol. II.

Pl. V.