INVESTIGATIONS ON MYCORRHIZA IN SOUTH AUSTRALIA

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CONTENTS

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The particular objects of the present investigation follow from a short note on the occurrence of mycorrhiza by Mr. Samuel in 1926; they were two. The first was to find whether there was any correlation between the conspicuous occurrence of endotrophic mycorrhiza in oats and barley growing at Mt. Gambier, Corny Point and Penola, and the fact that these soils have been found deficient in available manganese (Samuel and Piper, 1929). The second was to endeavour to determine the mycorrhizal fungus in association with Pinus radiata in South Australia.

I. ENDOTROPHIC MYCORRHIZA.

1. Introduction.

Endotrophic mycorrhiza has been recorded in the roots of a large number of herbaceous plants, although by comparatively few workers (Janse, F.R. Jones, Peyronel et al.). In 1924 F.R. Jones pointed out that it was particularly abundant in the roots of certain legumes, imparting a yellowish or yellowish-green tinge to the roots. The only record of its being at all abundant in cereals, however, is by Peyronel, who reported in 1922 that he found it in the roots of many wheat plants in Italy. In some cases it was abundant and in others scarce. Generally speaking, he found mycorrhiza more plentiful in undisturbed lands than in cultivated fields; this is not so on the South Australian manganese deficient soils (Peyronel, 1929, Le micorrize delle essenze forestali. "L'Alpe", 1929 and 1924, loc. cit.).

In the roots of oat plants from the volcanic soil around Mt. Gambier, and the black clay soil at Penola, and in the roots of barley plants from the light calcareous soil of Corny Point, the endotrophic fungus is invariably present and attains a luxuriant development. Although various workers have described this type of mycorrhiza as becoming most abundant in the fine lateral roots at the later stages of growth of the host plants, in the cereal plants from manganese deficient soils in South

Root system of oat plant seven weeks old (from lowest soil temperature tank), Mt. Gambier soil. 7.9.32

Mycorrhiza —— occupy nearly 70% of total length of root system.

nat. size

Fig. 1

nat. size

Oat roots eight weeks old from Corny Pt. soil.

26·9·32

Mycorrhiza

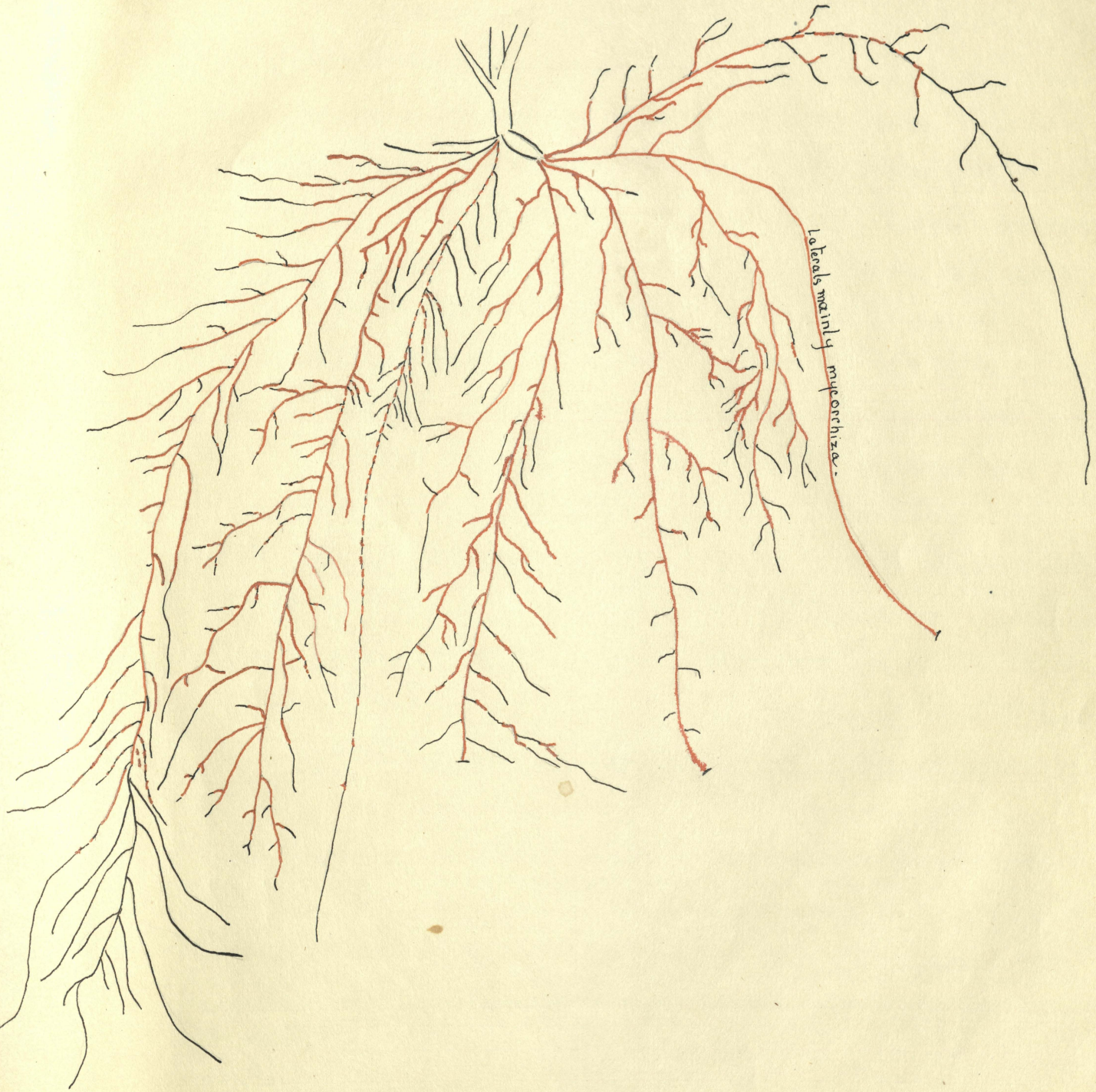


Fig. 2

Australia the fungus penetrates rapidly into the seedling roots and may have occupied 70% of the root system within seven weeks from germination (Figs. 1 and 2). It develops so luxuriantly, moreover, that the infected roots often acquire a pronounced yellowish-green colour, apparently much more marked, even, than was recorded for the legumes by Jones.

In contrast to this, the roots of cereal plants from other soils in South Australia are usually comparatively free fro the mycorrhizal endophyte until a rather late stage, when it gradually becomes established on fine lateral roots in the manner described for other countries. The striking difference between the occurrence of the fungus in the three manganese deficient soils as compared with normal soils showing no manganese deficiency, suggested that it was possibly the lack of manganese which in some way allowed the luxuriant development of the mycorrhiza on seedlings in the deficient soils. At the same time it was recognised that there were other factors which might be responsible. A lessened resistance on the part of the host cells could result from causes other than the deficiency of manganese, such, for example, as the disturbed ionic balance (K-Ca) claimed by Lůndegardh to be concerned in the production of disease symptoms in Dörrfleckenkrankheit (manganese deficiency disease) of oats. Or it might result from the fact that the mycorrhizal fungus is very much more abundant in the manganese deficient soils than in others, so that roots are exposed to infection on all sides from the time of their emergence. It was hoped that experiments on the addition of manganese to deficient soils, with careful comparison between the development of mycorrhiza in the roots of cereals growing in untreated, treated and non-liable soils, might provide some information on these questions.

2. <u>Description of endotrophic mycorrhiza of cereals</u>.

The fungus found in oat roots bears the distinguishing organs - arbuscules, sporangioles and vesicles - which mark it as

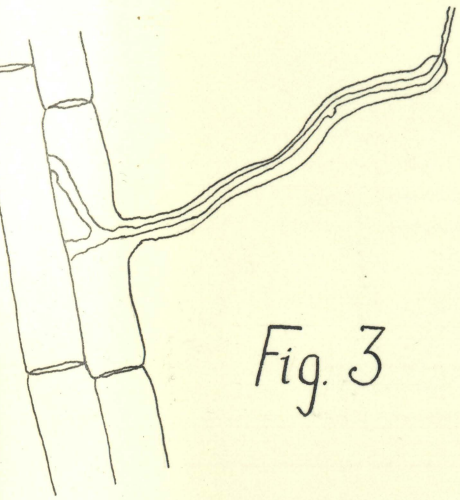


Fig. 3

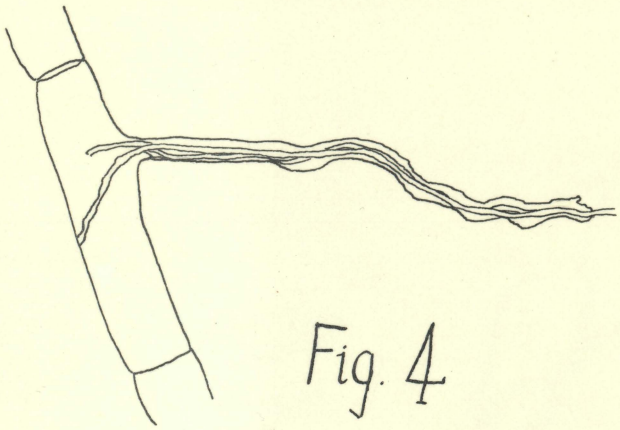
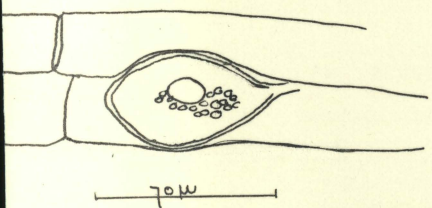
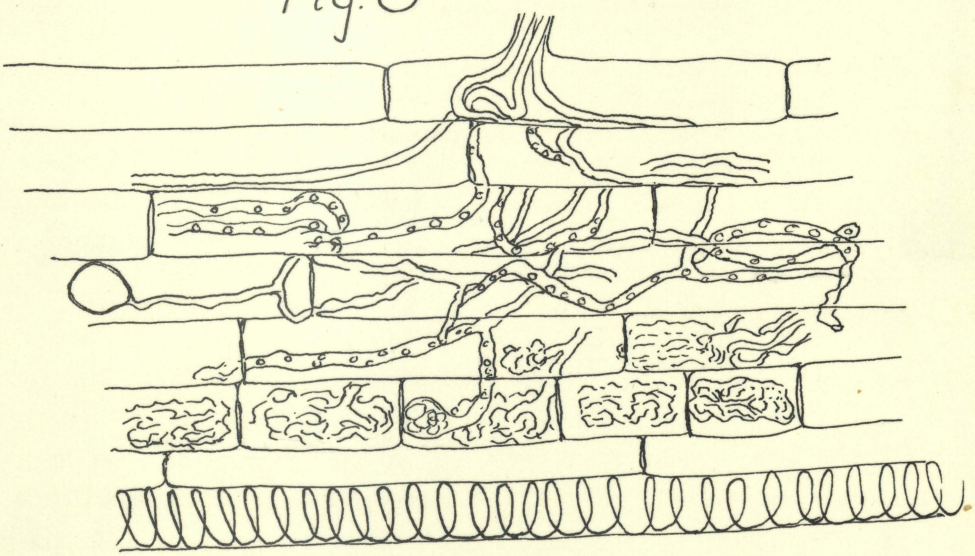


Fig. 4

Fig. 6

Fig. 5





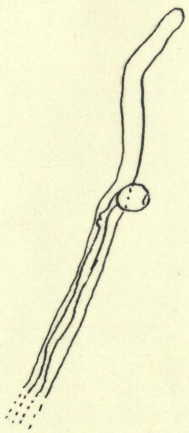


Fig. 7

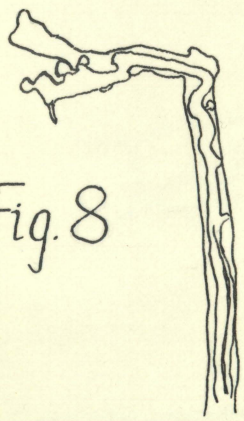


Fig. 8

Fig. 9

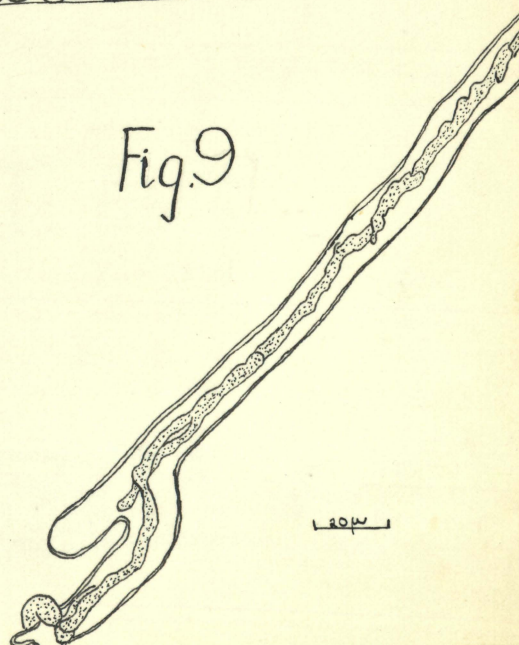
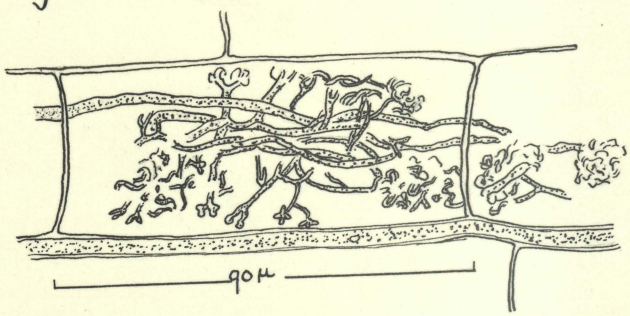


Fig. 10



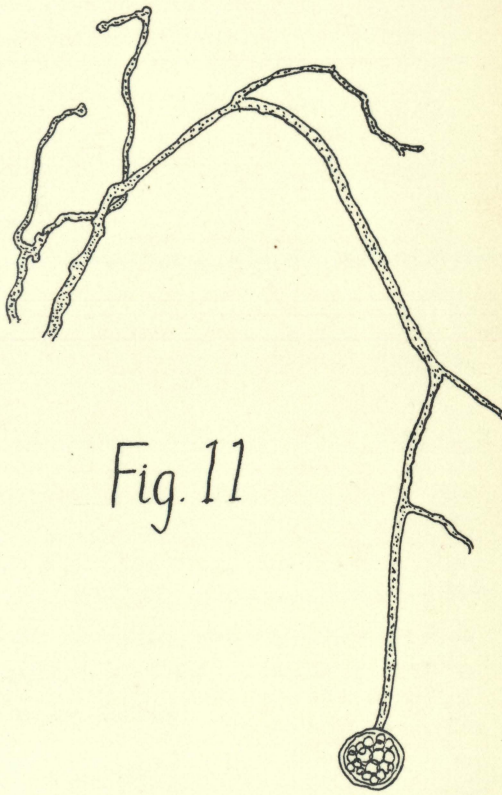
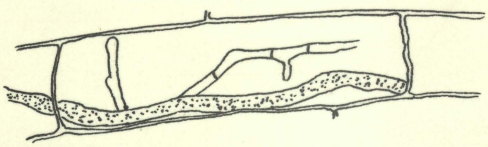


Fig. 11

Fig. 12



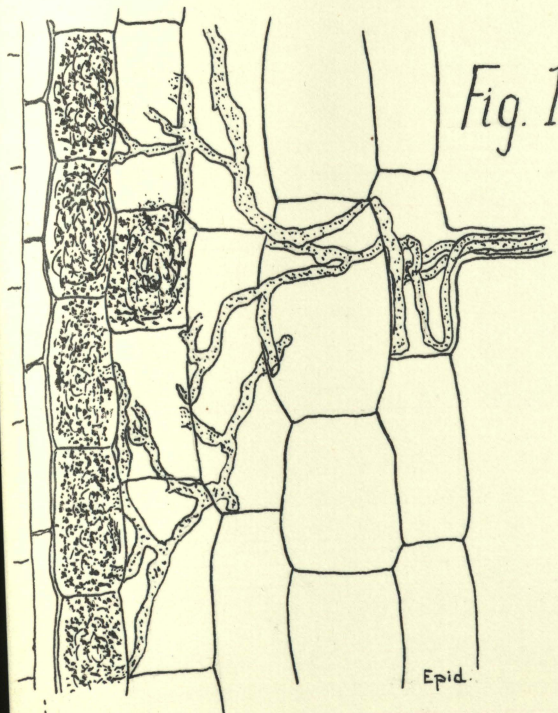


Fig. 13

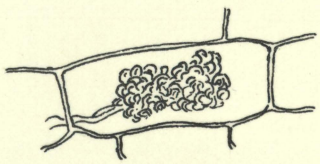


Fig. 14

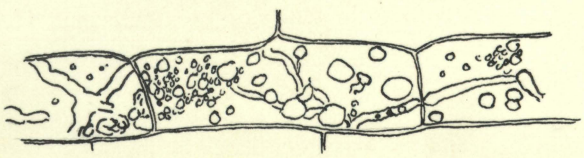


Fig. 15

15·9·32 L.s. root of three months old oat plant, grown in river sand.

Fig. 16

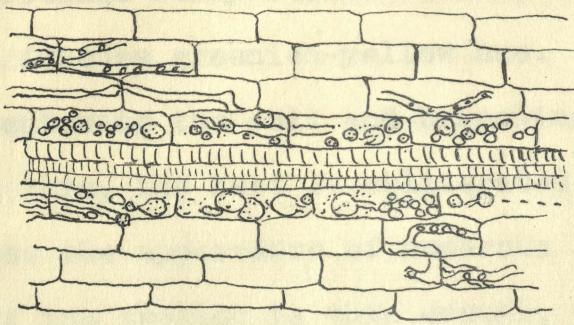
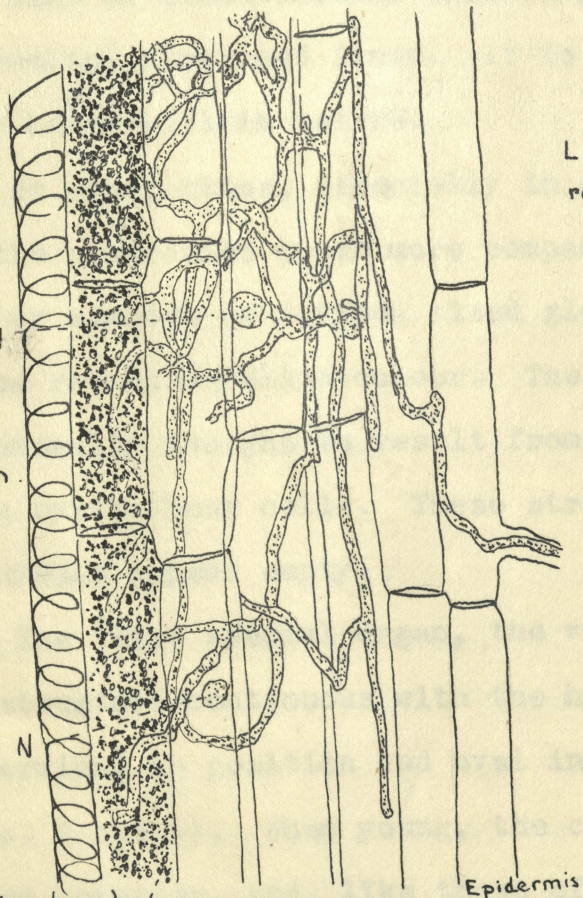


Fig. 17

8·11·32.



L.s. cortex of oat mycorrhiza; corona root of plant seven weeks old in Mt. Gambier soil. From a fresh hand section.

an endotrophic mycorrhiza fungus. The hyphae are coarse, usually about 7 microns in diameter, thin-walled, non-septate, at first granular and more or less colourless or almost yellow.

The usual mode of entry is through a root hair (Figs. 3, 4, 7, 8 and 9) of which the whole length is often traversed; when the hypha reaches the epidermal cell, it generally branches, the various branches spread out into other cortical cells and in the one or two layers outside the endodermis, the characteristic organs called arbuscules by Gallaud are formed (Fig. 6). These are indistinct bushy masses filling the cells, sometimes giving them an intense greenish-yellow hue. It is possible to see some hyphae entering the cell and branching, but unless the section is very thin, the rest is obliterated by the dense mass that often has the appearance of numerous small granules (Figs. 13 and 17). If the section is thin enough, however, the arbuscule is seen as a mass of dichotomously branching hyphae (Fig. 10), the branches getting finer and finer. It is probable that arbuscules are partly haustorial in nature.

At other times, especially in older mycorrhiza, in place of the arbuscules occur more compact, irregular bodies (Fig. 14) or numbers of various sized globules (Figs. 15 and 16) of the same greenish-yellow colour. These are the sporangioles and are generally thought to result from the digestion of the arbuscules by the host cells. These structures are still found when the hyphae appear empty.

The third special organ, the vesicle (Janse) is an enlarged structure continuous with the hypha, thicker walled, usually terminal in position and oval in shape, with a well marked apex (Figs. 5 and 6). When young, the contents are densely protoplasmic and granular, and, like those of the hyphae, stain densely with cotton blue; at this stage hyphae and vesicles stain light brown with iodine. Later on, the vesicles are filled with oil globules; sometimes one of these may be almost as large as the

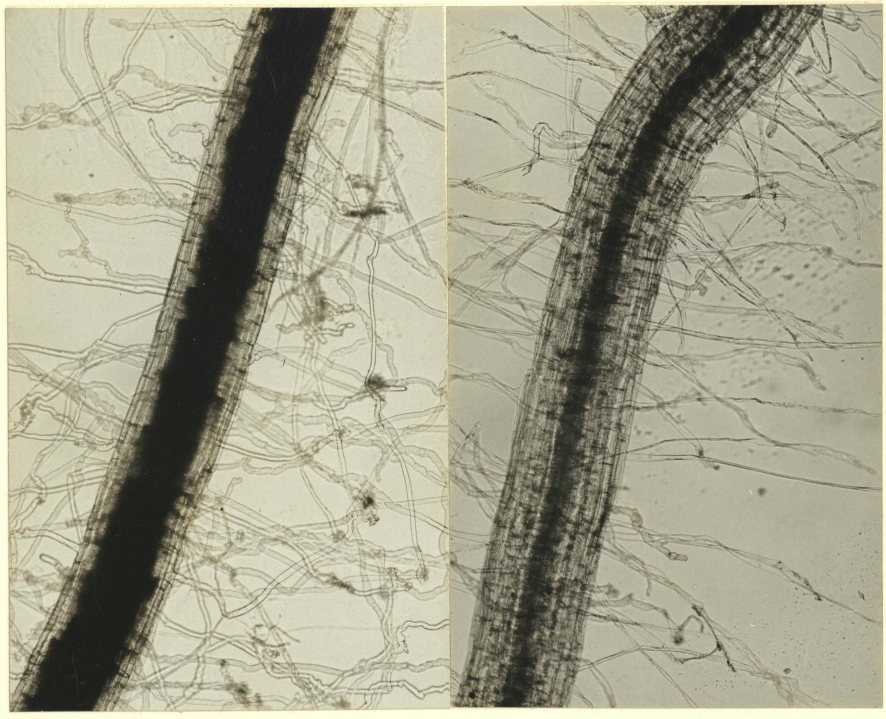
vesicle; the hyphae, also, now contain small oil globules, as recorded for Lolium temulentum L. (McLennan, 1926), this is shown by their staining with Sudan III or the osmic acid in Flemming's fixing solution; very often there are oil globules free in the cortical cells, which undoubtedly come from the fungus. Peyronel (1923) has found vesicles full of spores which are freed by the dehiscence of the wall.

For most purposes, fresh sections of the root were mounted in water or in glycerine jelly. The mycelium stains very well in the tissues by heating sections on a slide in lactophenol to clear them, then heating in a lactophenol solution of cotton blue and afterwards differentiating by heating in lactophenol again. All parts of the fungus stained deeply with Heidenhain's Iron Alum Haematoxylin; beyond this, the cytology of the fungus has not been studied in killed and fixed material.

Peyronel's experience (1924) of finding two distinct mycorrhizal endophytes in the same tissues was remembered, but only occasionally was a Rhizoctonia-like fungus observed (Fig. 12) . Once or twice (cp. p.13) a much finer mycelium was found in the roots of oats with plentiful arbuscules, both with and without the usual coarse hyphae. It was uncertain whether the arbuscules were attached to the fine mycelium, but the "pelotons" or coils of hyphae characteristic of the Rhizoctonia type were not seen.

From some examinations of fresh sections of Vicia sativa roots (which at Glen Osmond nearly all contain strikingly large amounts of the mycorrhizal fungus) it was seen that the oat mycorrhizas did not contain many vesicles in comparison. Transverse septa were also seen in the hyphae in the Vicia root.

An oat root which has become invaded by the mycorrhizal fungus cannot, at first, be safely distinguished by the naked eye. Certainly, the brightyellow colour is perceptible, but many of the mycorrhizas do not possess it, especially in certain soils



Root containing mycorrhizal fungus. Normal root.

Fig. 18

like Glen Osmond soil. A microscopic examination of the roots whole, mounted only in water, usually reveals the presence of the fungus; the central tissues of the root become indistinct owing to the thick, irregular, yellow-green sheath of arbuscules formed around the vascular cylinder (Fig. 18). The vascular system is never penetrated by the fungus, nor are the young cells near the root tip entered; frequently, also, a fully invaded root bears a lateral which contains no fungus, or else none for some distance from the parent root. Evidently the endogenous origin of the root is the cause of this freedom.

Often, extra-radical hyphae are seen in the soil about mycorrhizal roots. These hyphae give off branches, sometimes only thin ones, and occasionally bear vesicles. They have the characters of the fungus in the roots, and there seems little doubt that they are the same. Such a fungus has never been isolated in pure culture and proved to form this type of endotrophic mycorrhiza (Fig. 11).

3. Methods.

For the experiments on the mycorrhiza of oats, the plants were, for the most part, grown in ordinary earthenware pots in the open. Wherever soil had to be heat-sterilized, it was always put into pots and watered for some days before planting, in order to remove any harmful substances formed by the heating of the humus.

In the experiments involving the rendering available of manganese to plants growing in manganese deficient soils, not only was direct addition of manganese sulphate used - dry or in solution - but also the methods of partial sterilization, acid treatment, and the waterlogging treatment suggested by Mr. Piper (1931).

Where it was necessary to reintroduce the mycorrhizal fungus into soil deprived of it by heat-sterilization, a small amount (50-70 grms.) of fresh Mt. Gambier soil was added. In

another section of the work, well washed roots of oat plants abundantly infected, were also successfully used as inoculum.

The greatest difficulty was experienced in devising some way of recording the amount of mycorrhizal infection in roots. No work of this nature appears to have been attempted previously, except on a small scale by Janse (1896), working with the roots of Coffea, in which case he was able to develop a colour reaction using potassium hydroxide to show up infected parts of the roots; and more recently, Magrou (1921) employed a method of embedding roots in paraffin wax and cutting a large number of transverse sections at short intervals, examining them microscopically for the fungus and treating the results statistically.

In the present work, no macroscopic method of recording was found practicable. The occurrence of the yellowish-green colour in infected roots was not sufficiently sharply defined to be of use. Fortunately, however, a fairly accurate picture of distribution was possible by microscopic examination of the roots mounted whole in water, thus saving the labour of cutting sections. The difference in appearance between infected and non-infected roots is illustrated in Fig. 18. In thick roots, however, and to find early stages of infection, resort had frequently to be made to sections; in such cases, the presence of hyphae in the hairs is a helpful guide, Figs. 3, 4 and 9.

An attempt was made to develop a method of comparison by sampling. The roots were washed and floated out in clean water, and then lifted up and from the wet tassel a sample removed by cutting across with scissors at the widest part so as to obtain 1 cm. lengths of root at the level where the greatest number of laterals occurred (about halfway up). A definite number of these laterals were then examined under the microscope, and an assessment made of the degree of infection observed

according to the following arbitrary scale:-

Infection rating	Interpretation
0	no fungus present
1	0-25% of the length of the root invaded
2	25-50% " " " " " " "
3	50-75% " " " " " " "
4	75-100% " " " " " " "

Results obtained by this method are presented on pages 8,9,10 & 11 ; they were found to be too crude to show up well all but the most obvious differences, however, and in order to gain a clearer picture of the progress of infection the method of completely "mapping" the root system was adopted. This was only possible, of course, since most of the plants examined were seedlings, or were grown in 4" or 6" pots. It would be an exceedingly laborious method for larger root systems. The roots, after being washed free from earth, were spread out on a tile, the complete root system sketched on paper, and then the roots taken in sections about 2"-2½" long.so as to fit comfortably on a slide, and passed in review under the microscope and the position of mycorrhizal infection recorded on the root map in red ink. Maps obtained by this method are presented in Figs. 19-32; they provide a basis for fairly accurate quantitative work by measurement if desired.

4. Possible relation between mycorrhiza and deficiency of manganese in the soil.

a. Pot experiments on soil treatment.

In the first experiment, oats were grown in Mt. Gambier soil treated in the following ways:- (1) watering on 50 c.c. of a 2% solution of $MnSO_4$, $4H_2O$ per 6" pot after germination, (2) partially sterilizing in an autoclave at one atmosphere for one and a half hours, some of these pots being kept as controls and the others mixed with a soil inoculum of the fungus; (3) there were also controls of untreated fresh and stored soils.

After six weeks, when the untreated controls were showing manganese deficiency symptoms, the roots were examined. The plants grown in untreated soil, of course, had formed

mycorrhiza, even where the soil had been in storage for a season or so, but in the two soils treated to provide available manganese, mycorrhiza was also found; there was none in the uninoculated sterile soil. Plants of the same experiment examined by the sample method nearly three months later showed a corresponding distribution of mycorrhiza; the figures are given in the following table.

TABLE I - showing the effect of various treatments for rendering manganese available on the amount of endotrophic mycorrhiza in the roots of Algerian oats grown 4½ months in Mt. Gambier soil.

Treatment	No. of roots examined	Average "infection rating"	Corresponding approx. to following percentage infection
Fresh soil	20	2.5	50%
Fresh soil stored 1 year	40	3.2	67%
$MnSO_4$ added in solution	10	3.4	72%
Soil sterilized and reinoculated	20	0.25	<20%
Soil sterilized	20	0	0

Another experiment of the same nature also included soils acidified with HCl and soil water-logged for a week before planting. Both these treatments favour reduction of highly oxidised manganese in the soil (Piper, 1931). It was not known how the fungus might be influenced by these two treatments, so some of the pots received an inoculum of fresh soil before being planted, but results showed that some of the fungus, at all events, survived both treatments. Soils with manganese added, both dry and in solution, and sterilized soil with an inoculum of fresh soil were also in the series.

Twelve weeks later, when the control plants were showing characteristic signs of manganese deficiency, some from each set were examined by the sample method and the figures were as set out in Table II. Mycorrhiza occurred in all, whether supplied

with manganese or not, and persisted for another two months when the experiment was stopped.

TABLE II - showing the effect of further soil treatment for rendering manganese available, on the amount of endotrophic mycorrhiza in the roots of Algerian oats grown twelve weeks in Mt. Gambier soil.

Treatment	No. of roots examined	Average "infection rating"	Corresponding approx. to following percentage infection
Fresh soil	20	2.5	50%
$MnSO_4$ added dry with seed	20	2.5	50%
Soil water-logged without reinoculation	20	2.9	60%
HCl added, soil not reinoculated	20	1.3	20%
Soil sterilized and reinoculated	20	0.85	<20%

It will be noted that in neither experiment has the addition of manganese sulphate to the soil resulted in any diminution of the amount of endotrophic mycorrhiza present. Nor has rendering manganese available by water-logging the soil had any depressing effect on the amount of mycorrhiza. On the other hand, there has been some reduction as a result of treating the soil with HCl, and a considerable reduction when the soil was sterilized and reinoculated. There is reason to believe from later experiments, however, that this effect is due to a reduction in the amount of mycorrhizal fungus in the soil rather than to any effect of the manganese rendered available in treatment.

b. Examination of barley from treated field plots.

Arrangements were made for periodical samples of barley plants from the untreated and manganese-treated plots at Corny Point, established under the direction of the Department of Agriculture, to be sent by post for examination. Samples were received fortnightly during August and September. Mycorrhiza was constantly found in both sets and no marked differences in amount could be detected, in the later samples, at any rate.

The following are typical records obtained by the "sampling" method. The samples were taken from six plants of each plot; the infection ratings are for two sets of twenty roots in both cases.

7/9/32.

No manganese plot.

4, 4, 3, 4, 4, 4, 4, 0, 4, 1, 4, 4, 2, 3, 4, 4,
0, 3, 0, 4 = 60 average 3.0

4, 3, 4, 3, 4, 1, 4, 4, 3, 4, 4, 0, 4, 4, 4, 3,
3, 4, 0, 4 = 64 3.2

Manganese manured plot.

3, 3, 4, 2, 4, 4, 4, 4, 3, 4, 4, 3, 0, 4, 4, 0,
2, 2, 3, 4 = 61 average 3.0

4, 3, 4, 4, 4, 4, 2, 4, 1, 3, 4, 0, 4, 4, 0, 4,
3, 1, 4, 2 = 59 3.0

From the experiments in the preceding two sections it is evident that deficiency of manganese alone is not the factor leading to the unusually abundant endotrophic mycorrhiza formation in the roots of cereals growing on manganese deficient soils. The addition of soluble manganese salts and treatments to render available the insoluble manganese actually present did not in any way reduce the amount of mycorrhizal infection, except in those cases where the treatment used might have been expected to reduce the amount of mycorrhizal fungus in the soil itself. Added interest therefore attaches to a comparison of the progress of mycorrhizal infections in manganese-deficient and normal soils.

5. Comparison of mycorrhiza in Mt. Gambier and Glen Osmond soils.

a. Soil mixtures.

It was desired to throw some light on the behaviour of the fungus in mixtures of two soils, the one especially favouring mycorrhiza development in oats and the other believed not to do so. Two series of mixtures were therefore prepared, each with pure Mt. Gambier soil at one extreme, and either pure Gawler River sand or Glen Osmond soil from the Waite Institute fallows

at the other extreme, with three intergrades in each case. After three months, the roots were examined by the method of sampling; the figures showed a decrease from a large amount of mycorrhiza in the Mt. Gambier soil to almost zero in pure sand. In the Mt. Gambier-Glen Osmond series, there was a slight progressive decrease from the quantity in the Mt. Gambier soil, but quite half this amount in pure Glen Osmond soil. The figures obtained on sampling are given in Table III. It is interesting to note the trace of mycorrhiza formation in pure river sand.

TABLE III - showing the amount of mycorrhiza formed on roots of Algerian oats grown in two separate series of soil mixtures.

Soil mixtures		No. of roots examined	Average "infection rating"	Corresponding approx. to following percentage infection of laterals
Mt. Gambier	Glen Osmond			
100% (bulk)	0	20	2.5	50
50	50	"	2.8	57
25	75	"	2.8	57
5	95	"	2.4	48
0	100	"	1.6	27
Mt. Gambier	Sand			
100	0	20	2.5	50
50	50	"	2.0	37
25	75	"	1.3	20
5	95	"	0.3	<20
0	100	"	0.2	<20

The decreasing infection in the sand series suggests that infection is fairly localized, or in other words, that the fungus does not grow far along the root once it has entered, and also that sand is probably not a favourable medium for the growth of the hyphae outside the roots. It would seem likely, too, that the comparative freedom from mycorrhiza of oat roots in Glen Osmond field soils is due less to some special resistance of the root cells when grown in this medium than to mere scarcity of the mycorrhizal fungus in it.

b. The fungus in the two soils after sterilizing.

It was thought that by removing the mycorrhizal fungus

naturally occurring in the soil by sterilization, and then reintroducing a known proportion of soil inoculum (a quarter by volume of fresh Mt. Gambier soil was used), it would be possible to obtain a comparison between the amount of mycorrhiza formed in Mt. Gambier and in Glen Osmond soils, which would not be affected by initial differences in the quantity of active mycelium. It seemed likely that Mt. Gambier soil might be more favourable to the growth of this particular fungus than Glen Osmond soil. But after fifteen weeks, when the roots were examined by the sample method, there was no excess of mycorrhiza on the oats from the Mt. Gambier soil over that in oats from Glen Osmond soil; in fact no important difference could be detected and the uninoculated control pots had remained free of mycorrhiza.

The conditions were not truly comparable to field conditions, of course, for the process of heat sterilization, besides affecting the chemical nature of the soil, has a profound effect on the biological soil population, and this biotic factor may be the important one determining the amount of the mycorrhizal fungus to survive in the soils under consideration.

It is to be regretted that samples from this series were not washed out periodically to follow the comparative development of the mycorrhiza in the two soils in the early stages. The results, as they stand, do little more than support the conclusions drawn in the previous section.

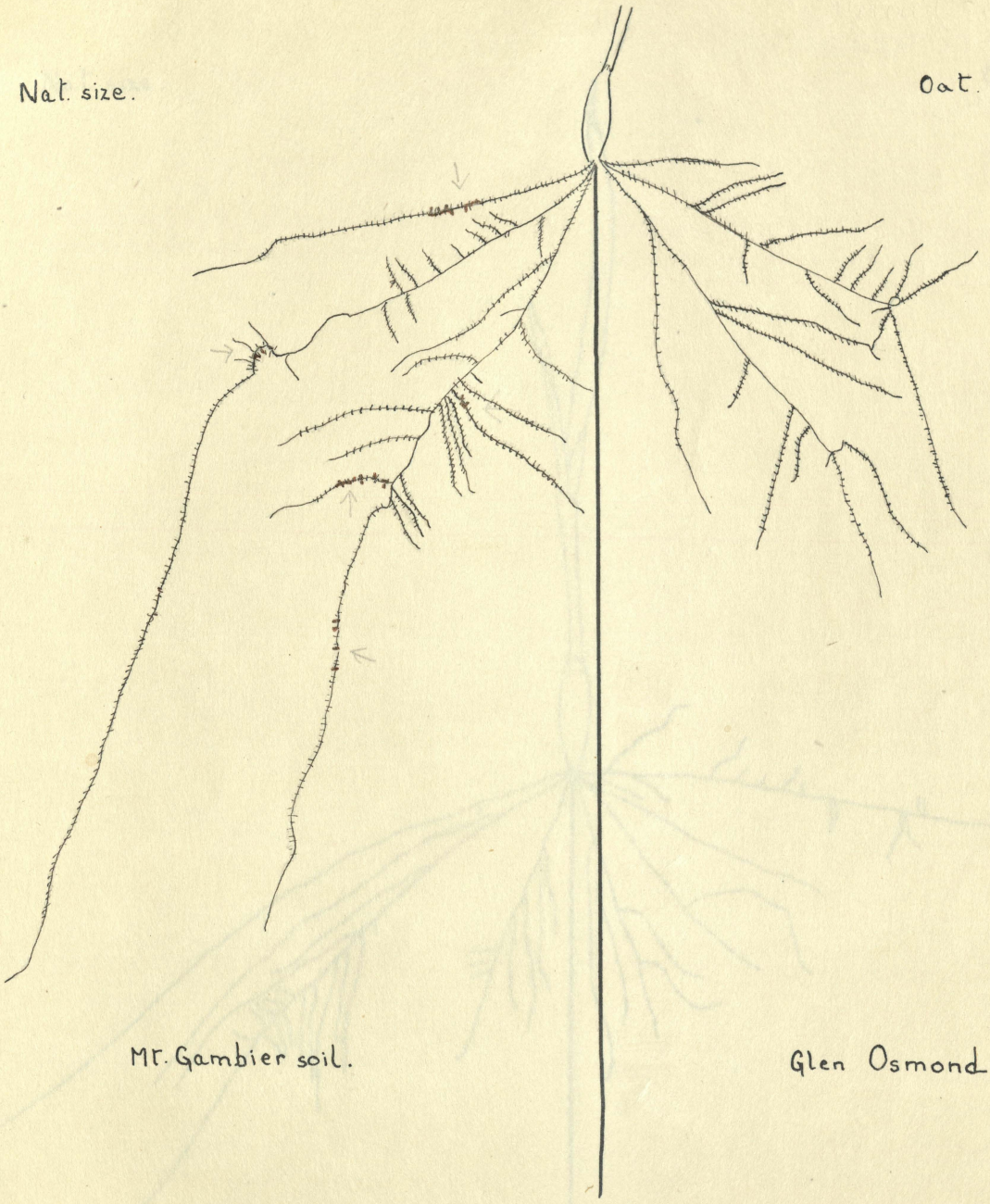
c. <u>Progress of mycorrhiza formation in the two soils</u>.

On account of the differences observed between mycorrhiza of oat seedlings in Glen Osmond and Mt. Gambier soils, it was decided to make careful comparative records of the stages of invasion in these two soils.

Accordingly, pots with a partition of paraffined cardboard down the centre were set up; Algerian oats were ger-

Nat. size.

Oat. 21.9.32.



Mt. Gambier soil.

Glen Osmond soil.

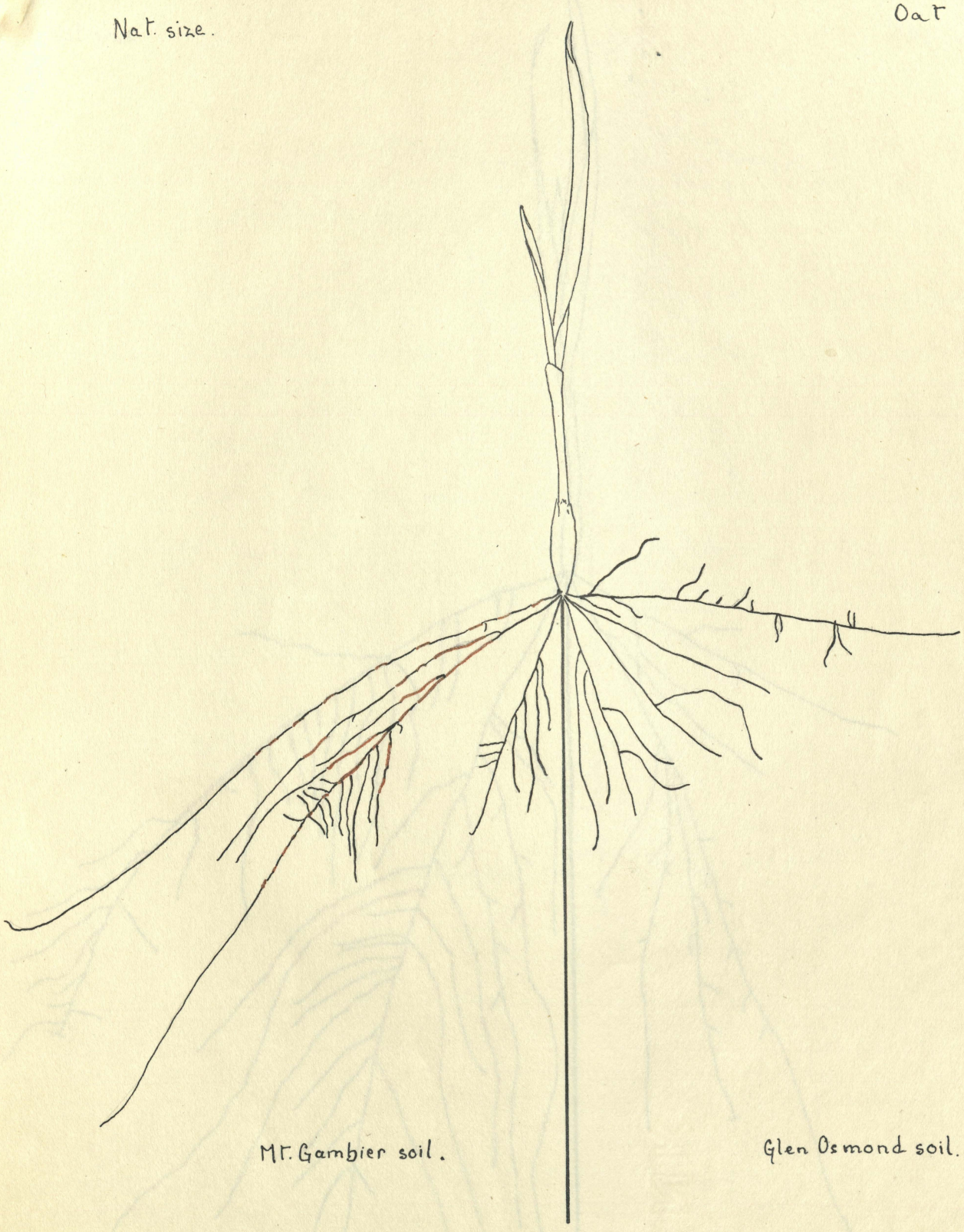
Mycorrhiza

First week.

Fig. 19

Nat. size.

Oat 28.9.32



Mt. Gambier soil.

Glen Osmond soil.

Second week

Mycorrhiza

Fig20

Nat. size

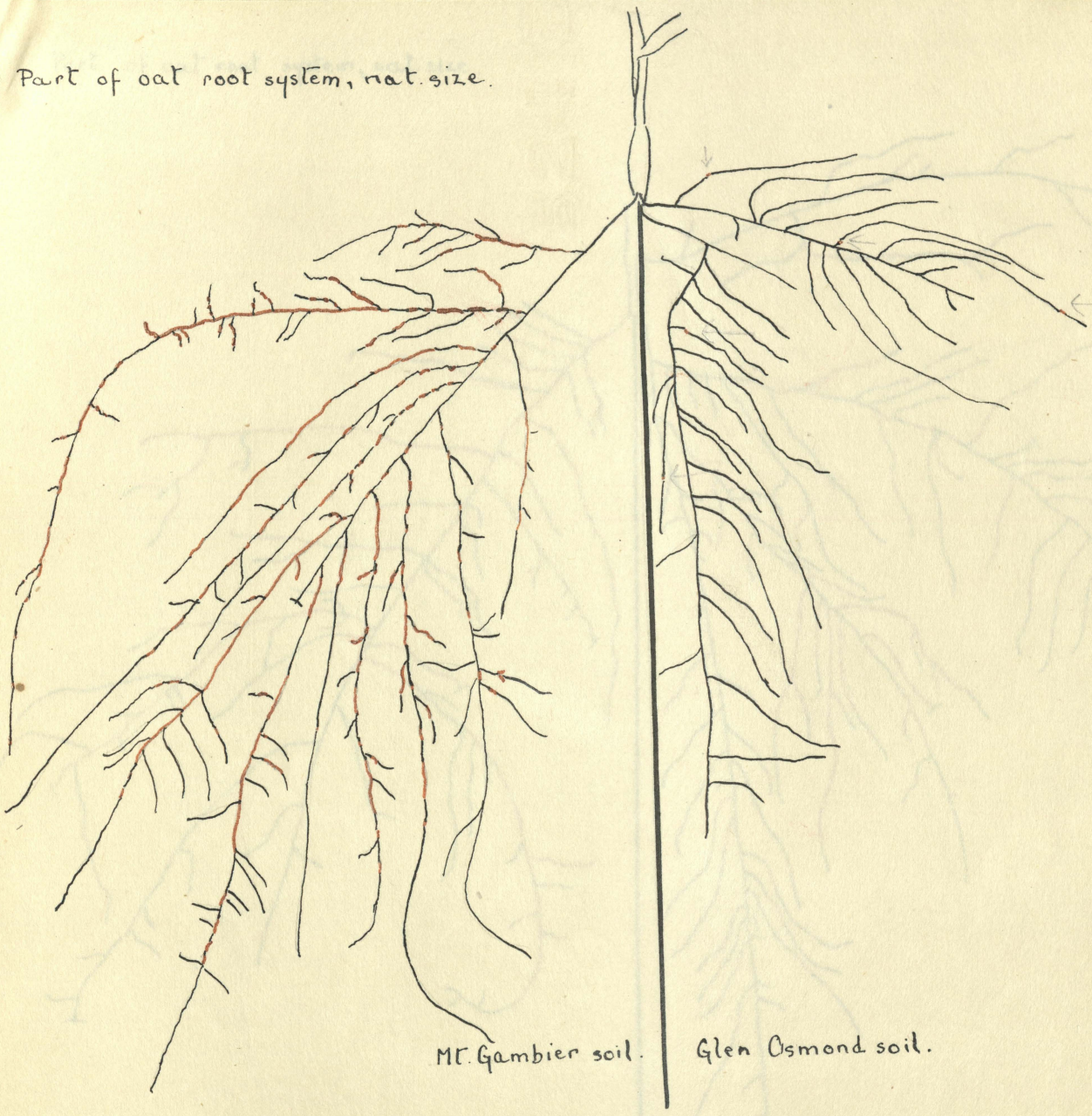
Oat. 5·10·32

Third week. Mycorrhiza —

Fig. 21

Part of oat root system, nat. size.

12-10-32



Fourth week. Mycorrhiza.

Fig. 22

Part of oat root system, nat. size.

4·31·32

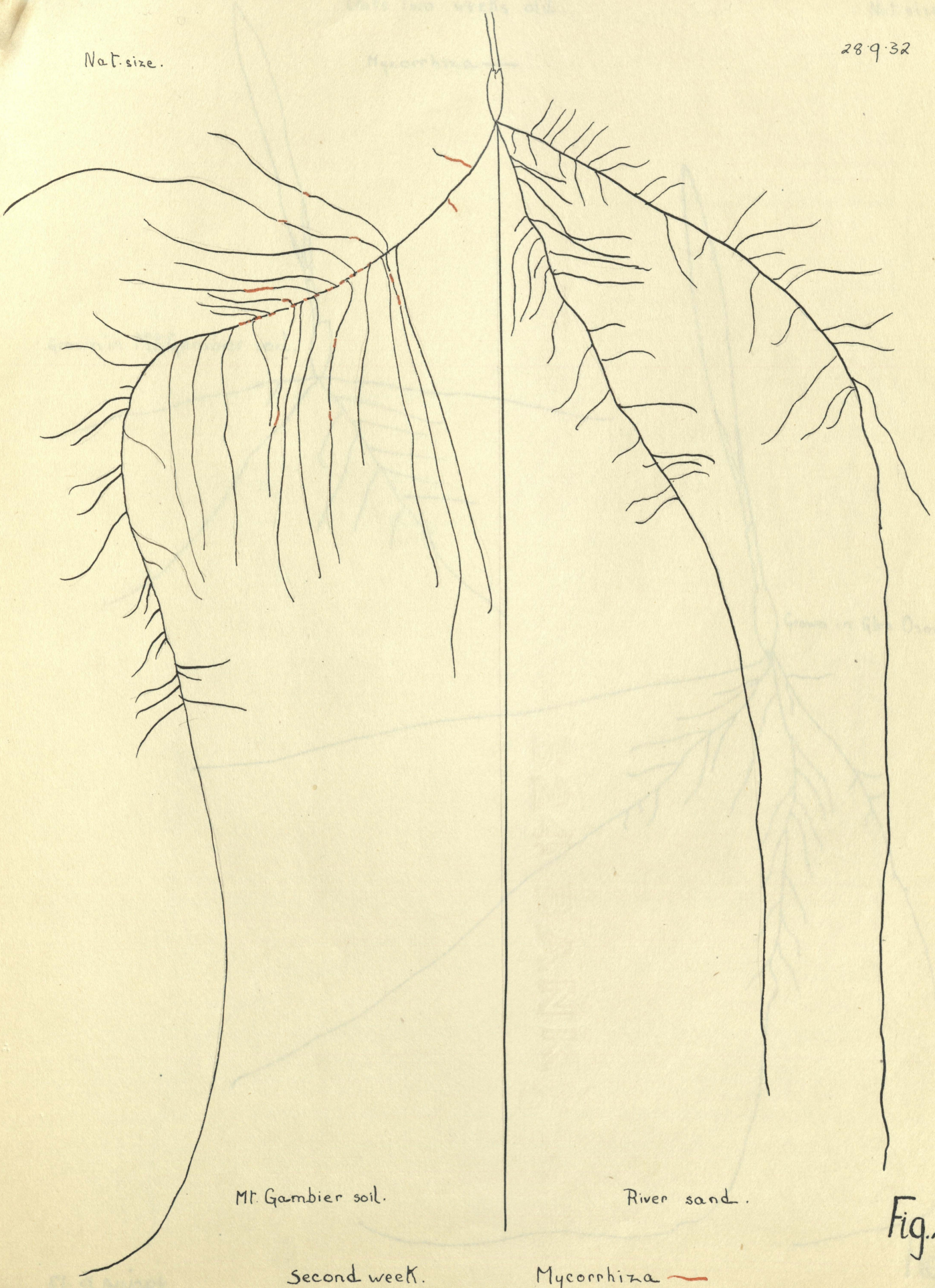
Mt. Gambier soil. | Glen Osmond soil.

Seventh week. Mycorrhiza —

Fig. 23

Nat. size.

28·9·32



Mt. Gambier soil.

River sand.

Fig. 24.

Second week.

Mycorrhiza —

23·9·32

Oats two weeks old.

Nat. size

Mycorrhiza

Grown in Mt. Gambier soil.

Grown in Glen Osmond soil.

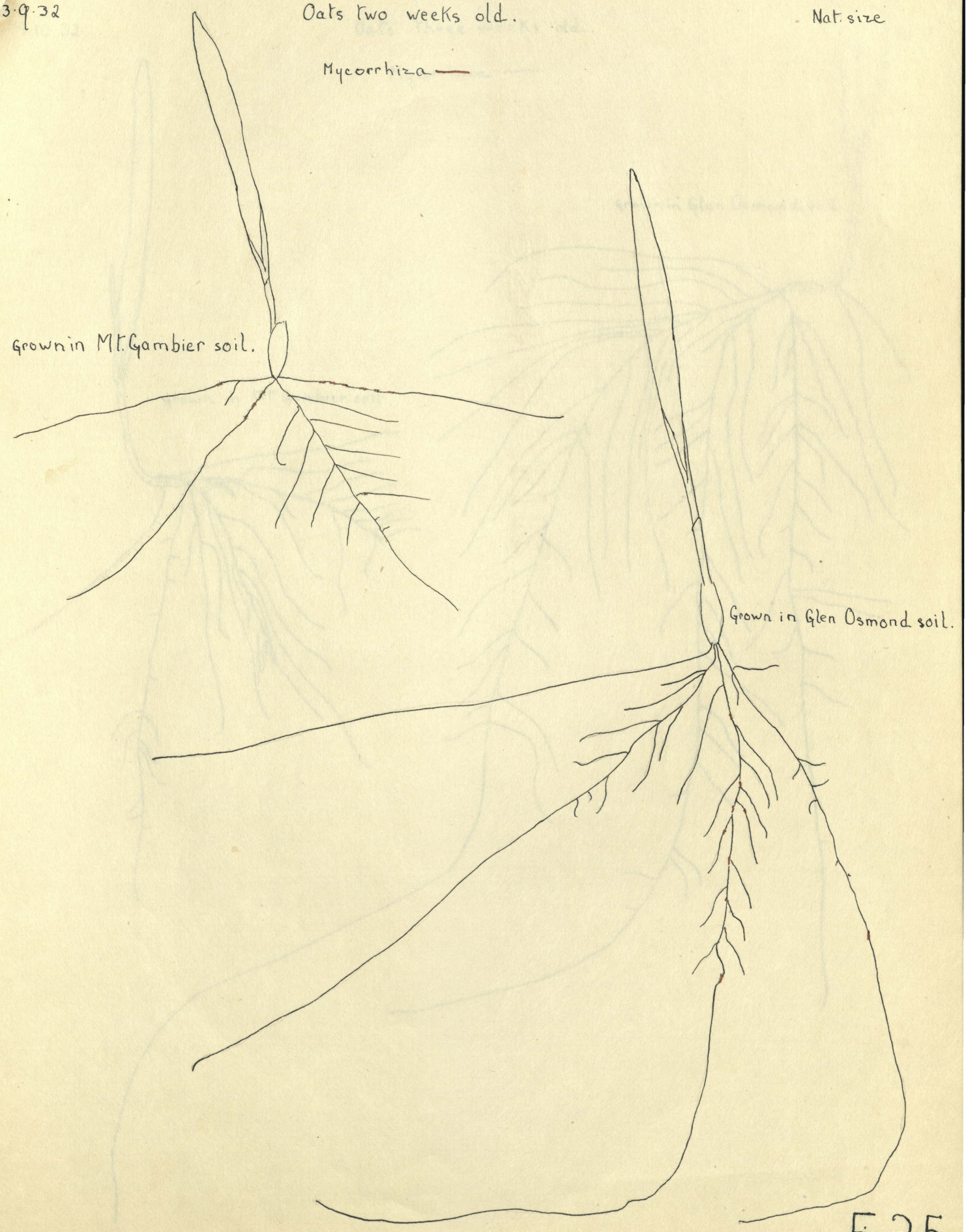


Fig. 25

1·10·32

Oats three weeks old.

Mycorrhiza —

Nat. siz

Grown in Glen Osmond soil.

Grown in Mt. Gambier soil.

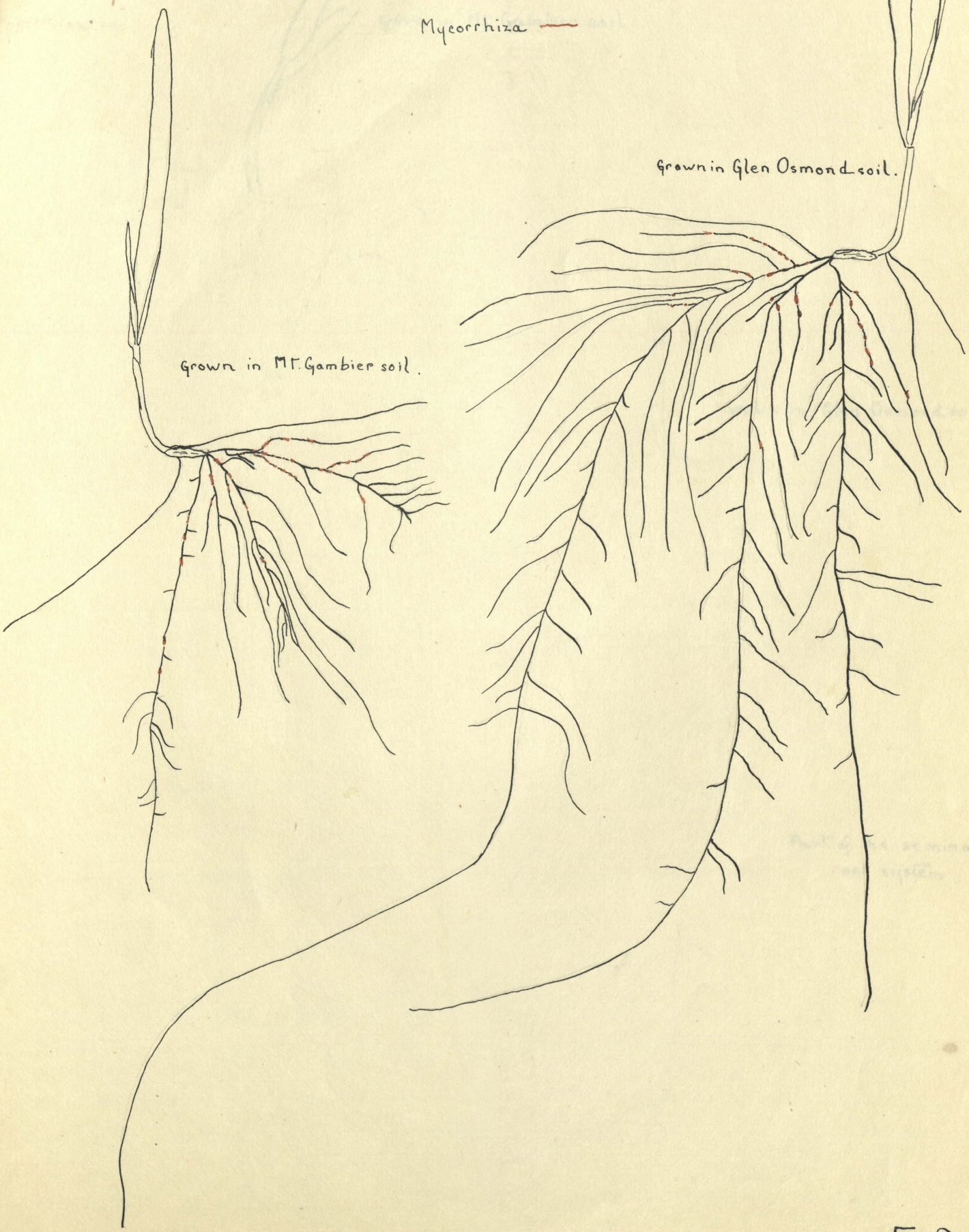


Fig. 26

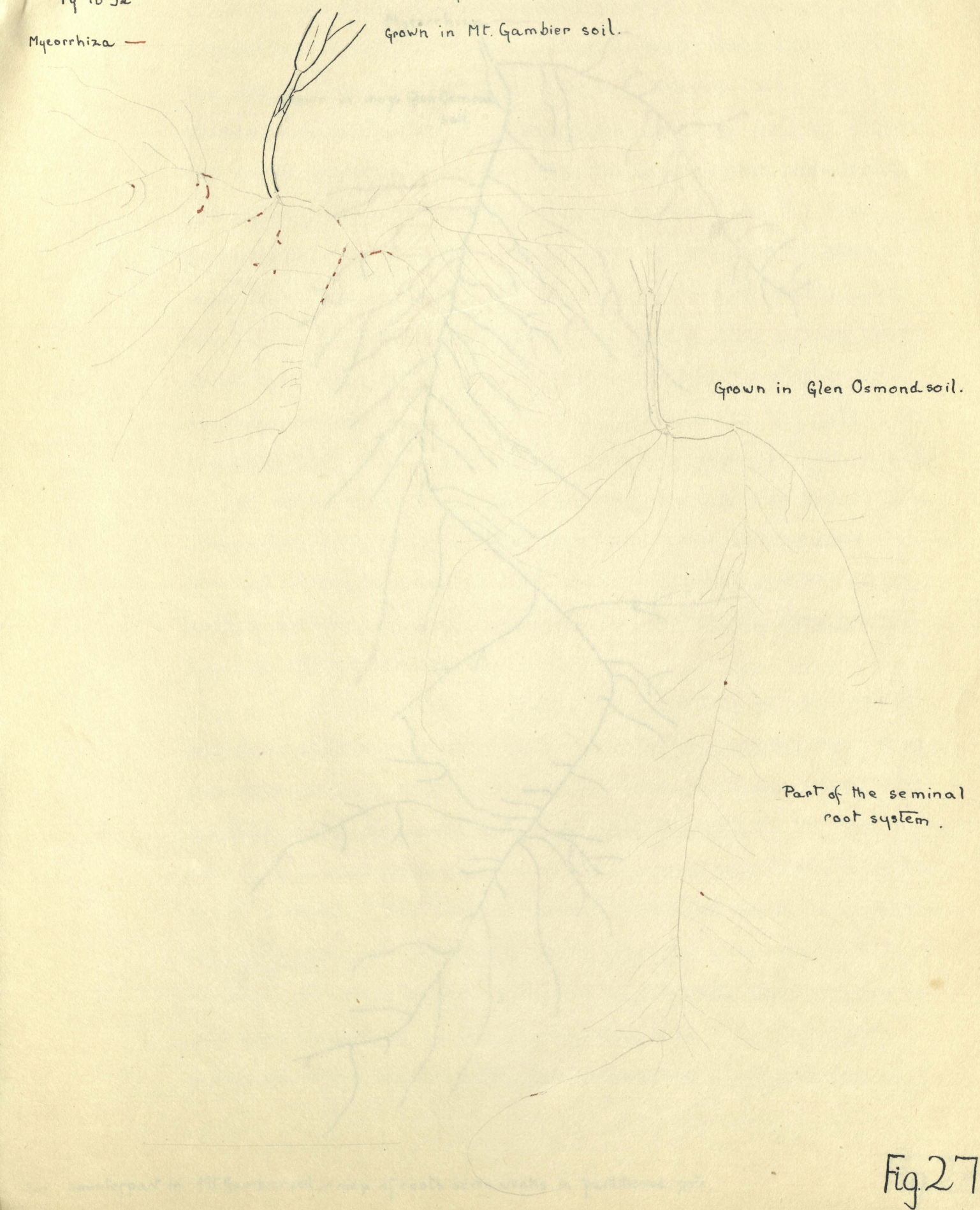
19·10·32

Oats five and a half weeks old.

Nat. size.

Mycorrhiza —

Grown in Mt. Gambier soil.



Grown in Glen Osmond soil.

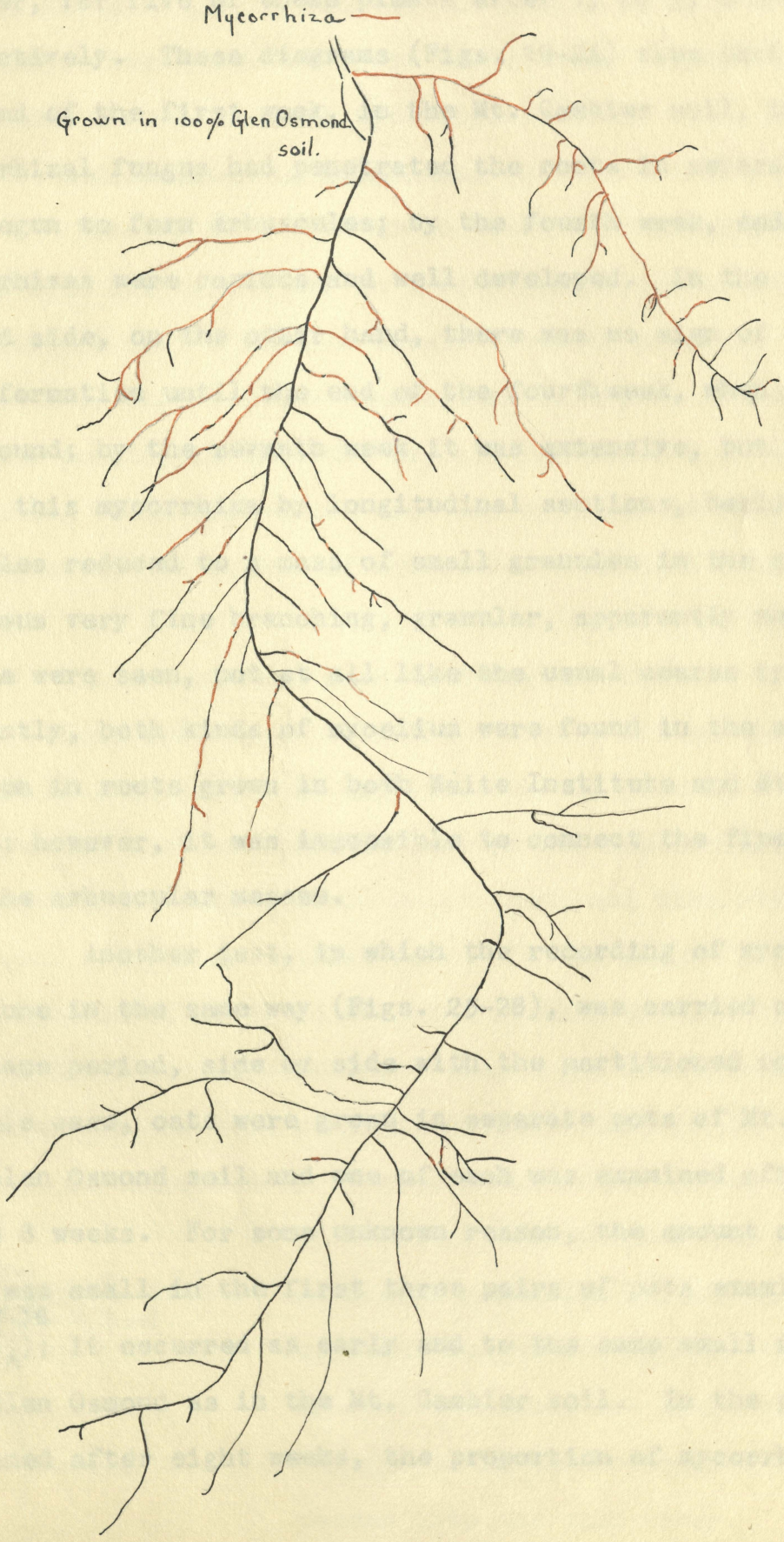
Part of the seminal root system.

Fig 27

7-11-32

Part of root system of oats 8 weeks old.

Nat. size.



For counterpart in Mt. Gambier soil, v. map of roots seven weeks in partitioned pots.

Fig. 28

minated until the three seminal roots were about one inch long and then they were fitted on the top of the partition with some roots on each side; one half of the pot was then filled with Mt. Gambier soil and the other with Glen Osmond soil, so that the one plant had its roots divided between the two soils. A diagram of the root system was made, as described earlier, for five of these plants after 1, 2, 3, 4 and 7 weeks respectively. These diagrams (Figs. 19-24) show that even at the end of the first week, in the Mt. Gambier soil, the mycorrhizal fungus had penetrated the roots in several places and begun to form arbuscules; by the fourth week, endotrophic mycorrhizas were copious and well developed. In the Glen Osmond side, on the other hand, there was no sign of mycorrhiza formation until the end of the fourth week, when a trace was found; by the seventh week it was extensive, but on examining this mycorrhiza by longitudinal sections, besides arbuscules reduced to a mass of small granules in the cells, only numerous very fine branching, granular, apparently non-septate hyphae were seen, not at all like the usual coarse type. Subsequently, both kinds of mycelium were found in the same section in roots grown in both Waite Institute and Mt. Gambier soils; however, it was impossible to connect the fine hyphae and the arbuscular masses.

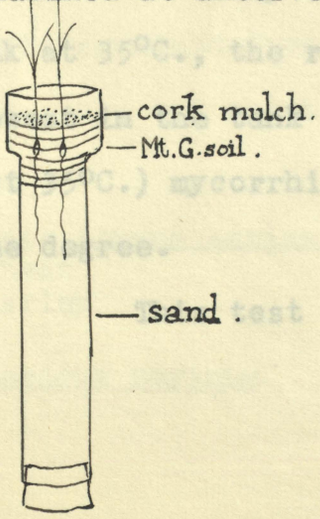
Another test, in which the recording of mycorrhiza was done in the same way (Figs. 25-28), was carried out during the same period, side by side with the partitioned pots. But in this case, oats were grown in separate pots of Mt. Gambier and Glen Osmond soil and one of each was examined after 2, 3, 6 and 8 weeks. For some unknown reason, the amount of mycorrhiza was small in the first three pairs of pots examined (v. Figs. 25-26); it occurred as early and to the same small degree in the Glen Osmond as in the Mt. Gambier soil. In the plants examined after eight weeks, the proportion of mycorrhiza in

both pots was quite large and comparable with that in plants of the same age from the partitioned pots. In spite of this variation, the general experience was that the time of mycorrhiza formation is very early in the life of the oat seedling in both Mt. Gambier and Corny Point soils and much later in Glen Osmond soil; the mycorrhiza persists for the rest of the life of the plant, which flowers and fruits normally. Adult oat plants from Waite Institute fields were examined (Fig. 32) and showed a great deal of mycorrhiza.

d. Localization of infections.

Reasons have been given above for thinking that the fungus does not spread far in a longitudinal direction inside the root and that a heavily invaded root is produced by the more or less simultaneous penetration of hyphae at numerous discrete points; so that the imposing investment of arbuscule-containing cells around the vascular cylinder is resolved into a close mosaic of groups of arbuscules each with a different origin.

An attempt was made to determine whether the fungus spreads along for any appreciable distance inside the root tissues by arranging that oat roots should grow in a glass cylinder for a short distance through Mt. Gambier soil, where the mycorrhizal fungus would enter them, and then abruptly pass into a layer of sterile sand. If the fungus did grow far along the roots, it would be found in them even when they had entered the layer of sterilized sand. The soils were kept at approximately the same constant moisture content and the plants examined at intervals up to nine weeks after sowing the seed.



Of eight plants, all but the very first examined, possessed as much mycorrhiza as usual in the Mt. Gambier soil right against the sand, but there was never any fungus in the roots once they had passed into the sand layer.

To have used also sterile Mt. Gambier soil in place of the sand might have yielded data for useful comparison of the relative preferences of the fungus for sand and this soil.

It would also be interesting to have cytological observations on the extent of spread of single infections such as might be obtained with small amounts of "inoculum" in a sterile soil.

6. Mycorrhiza and soil temperature.

In order to study the effect of certain environmental factors, a soil temperature test was conducted with Algerian oats. Six Wisconsin constant temperature units were used, each tank holding eight pots, four of Mt. Gambier soil and four of Corny Point soil. The temperatures at which the tanks were kept were 35, 30, 25, 20, 15 and 10°C. (the lowest tank, called 10°C. for convenience, was at the temperature of cold water during July and August and varied from 9°C.-13.5°C.). The soil moisture was kept constant at approximately 60% saturation capacity. One pot of each of the two soils from every tank was examined by the sampling method after 2, 3, 4 and 7 weeks.

Considering the plants in Mt. Gambier soil first: by the fourth week the percentage of mycorrhizal roots seemed to be near the same maximum in all the tanks, except in the one at 35°C. where there was nothing or only a trace throughout the experiment. Formation of mycorrhiza in the tank at 10°C. came on gradually, but in the other tanks a large number of roots was attacked at once and the proportion did not increase after the first fortnight.

In Corny Point soil, mycorrhiza did not appear in the tank at 35°C., the rate of increase seemed slower, and, as before, slowest in the tank at 10°C., but at all the temperatures (except 35°C.) mycorrhiza was ultimately formed to approximately the same degree.

This test showed clearly that the fungus has a wide

temperature range from at least 9°C. to somewhat over 30°C. It did not invade the oats at 35°C., but whether this was due to the death of fungus, or to an alteration in the nature of the roots at this temperature which did not permit penetration, was not determined. The oats themselves made very poor growth at 35°C. It would have been interesting to transfer some of the 35° pots to a cooler tank to determine whether the fungus was still alive and active. The formation of mycorrhiza proceeds slowly to the maximum at the lower temperatures, but reaches it very quickly at temperatures between 15 and 30°C. The first signs of manganese deficiency occurred in the tanks at 15, 20 and 25°C. four weeks after sowing the seed; by this time mycorrhiza was well established. F.R. Jones (1924) found a similar wide temperature range in the mycorrhizal fungus of legumes, although variation existed among different hosts.

Concurrently with the temperature tanks, oats were grown in pots in the glasshouse in Glen Osmond, Mt. Gambier and Corny Point soils. After six weeks, no mycorrhizal fungus was found in the roots of the plants grown in Glen Osmond soil; a week later there was a trace. Mt. Gambier and Corny Pt. soils under the same conditions had produced copious mycorrhiza.

7. Records of endotrophic mycorrhiza in various plants.

Though, for the experimental work, Algerian oats were always used, a few general observations were made on occurrence of mycorrhiza in other plants, mostly in the Glen Osmond district. The following list enumerates the plants examined, the majority during the winter months; negative results are given, but do not rest upon sufficient evidence to be important. Wheat plants examined from Waite Institute fields had no mycorrhiza (Fig. 30), but wheat from some other localities had.

Mycorrhiza present

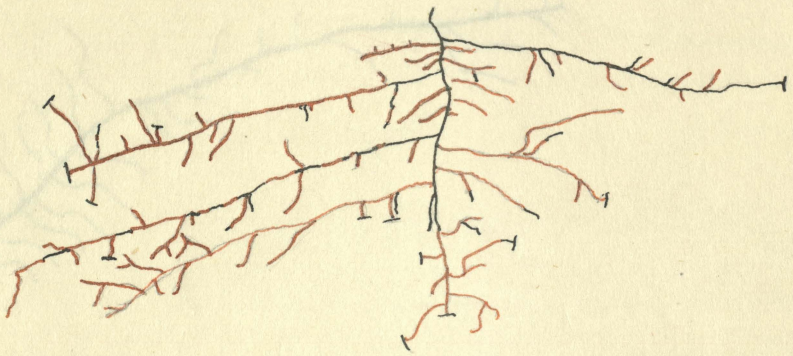
Oats (Avena sativa)	Glen Osmond, Streaky Bay and Roseworthy.
Wheat	Cooke's Plains, Streaky Bay.
Barley	" " " " and Glen Osmond. (Fig. 31)
Hordeum murinum	Glen Osmond.

Nat. size.

Trifolium arvense in flower, root system.

22·10·32

Waite Institute.



Several confirmatory sections made.

Fig. 29

Fig. 30

Wheat from the field, Glen Osmond. Root system three months old. 29·9·32
No mycorrhiza.

7.10.32.

Roots of Glen Osmond field plants

Nat. size.

Barley.

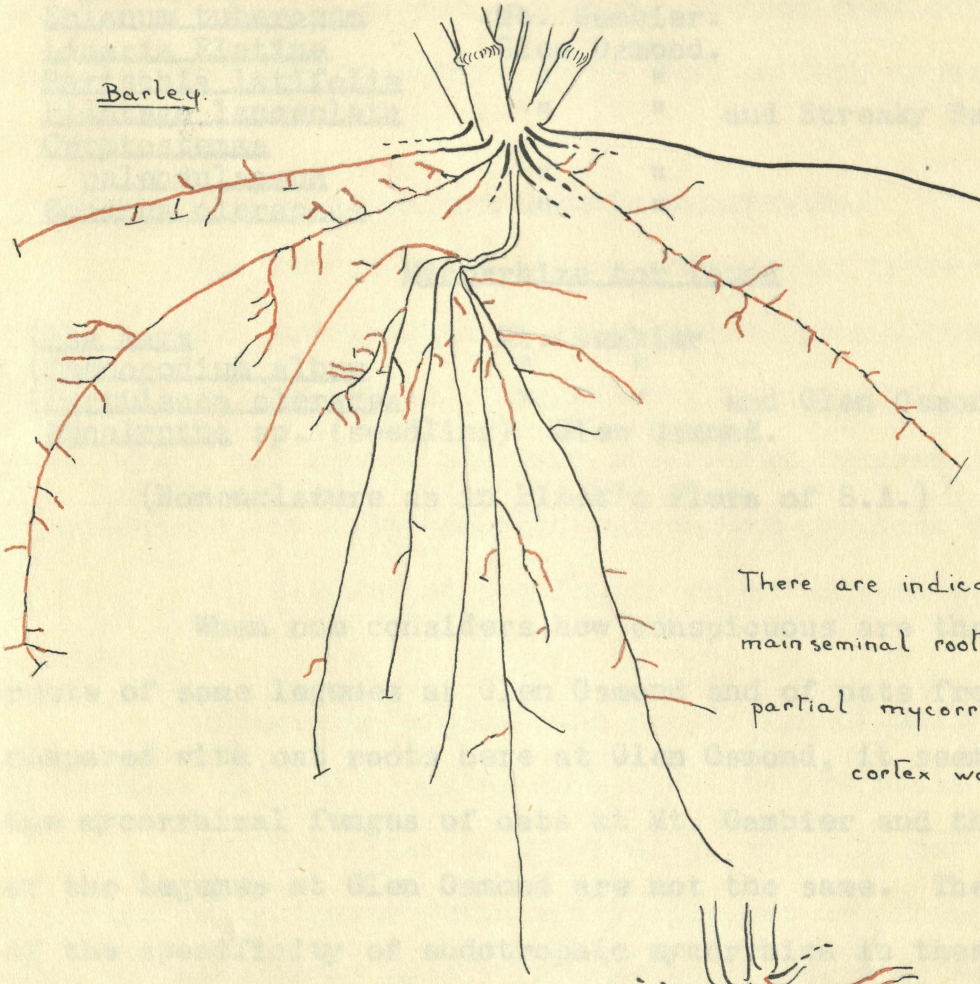


Fig.31

There are indications that the main seminal roots were at least partial mycorrhizas before the cortex was exfoliated.

Oat

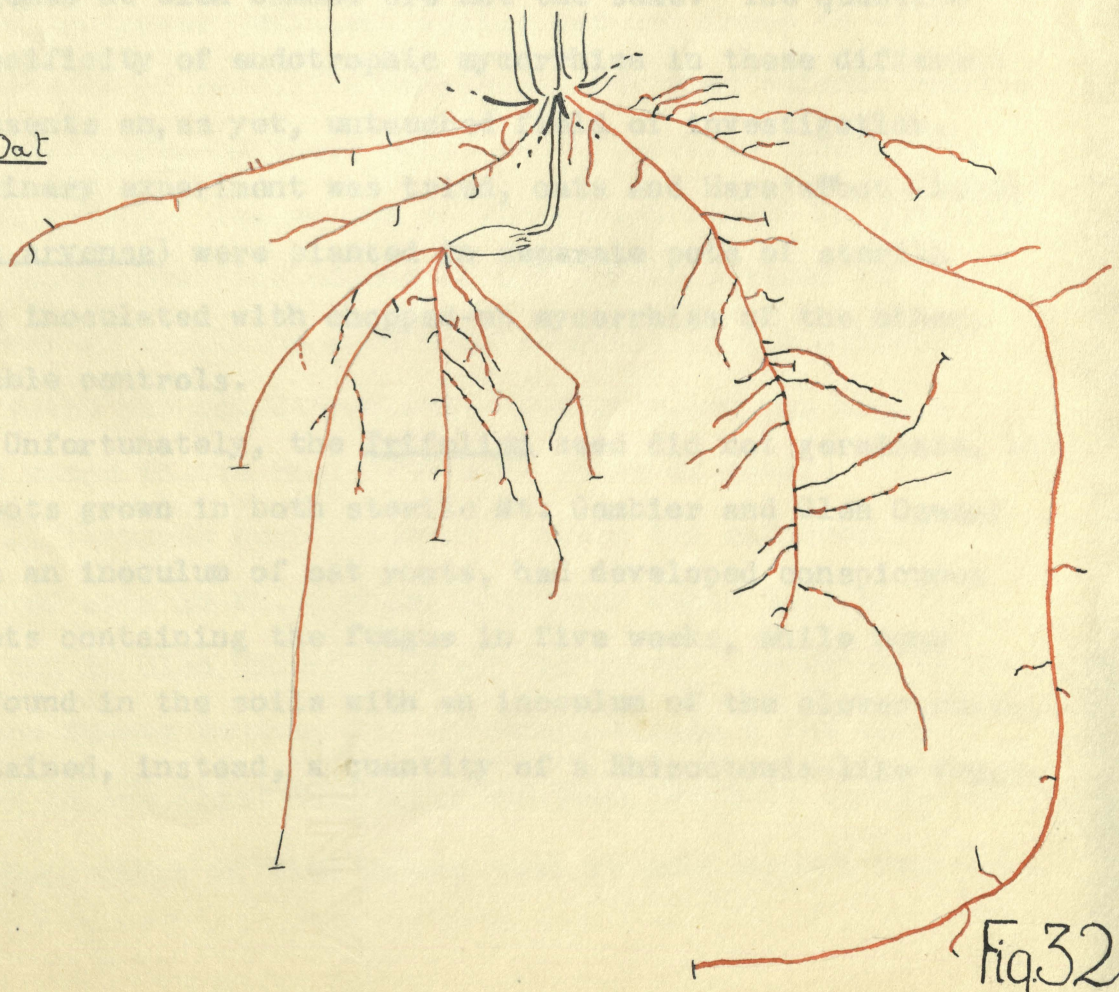


Fig.32

Poa annua	Glen Osmond.
Bromus sp.	Streaky Bay.
Stipa nitida	Koonamore. (A trace)
Lolium sp.	Streaky Bay.
Coronopus didymus	Mt. Gambier soil.
Medicago denticulata	Glen Osmond.
Trifolium arvense	" " (Fig.29)
Melilotus indica	Streaky Bay.
Vicia sativa	Glen Osmond--especially marked.
Pisum sativum	" "
French Bean	Mt. Gambier.
Oxalis corniculata	Glen Osmond.
Euphorbia peplus	" "
Solanum tuberosum	Mt. Gambier.
Linaria Elatine	Glen Osmond.
Bartschia latifolia	" "
Plantago lanceolata	" " and Streaky Bay.
Cryptostemma calendulaceum	" "
Sonchus oleraceus	" "

Mycorrhiza not found

Summer	Zea mays	Mt. Gambier
	Chenopodium album	" "
	Portulacca oleracea	" " and Glen Osmond.
	Eucalyptus sp. (seedling)	Glen Osmond.

(Nomenclature as in Black's Flora of S.A.)

When one considers how conspicuous are the mycorrhizal roots of some legumes at Glen Osmond and of oats from Mt. Gambier, compared with oat roots here at Glen Osmond, it seems likely that the mycorrhizal fungus of oats at Mt. Gambier and that of some of the legumes at Glen Osmond are not the same. The question of the specificity of endotrophic mycorrhiza in these different plants presents an, as yet, untouched field of investigation. One preliminary experiment was tried, oats and Hare's Foot Clover (Trifolium arvense) were planted in separate pots of sterile soil, each inoculated with chopped-up mycorrhiza of the other, with suitable controls.

Unfortunately, the Trifolium seed did not germinate, but the oat roots grown in both sterile Mt. Gambier and Glen Osmond soils with an inoculum of oat roots, had developed conspicuous yellow roots containing the fungus in five weeks, while none could be found in the soils with an inoculum of the clover roots; these contained, instead, a quantity of a Rhizoctonia-like fungus

which filled many of the cortical cells with a pseudoparenchyma of hyphae. There is no doubt that this Rhizoctonia was introduced with the clover roots, but these were certainly well supplied with mycelium bearing arbuscules and vesicles, whatever other fungi there might have been.

This experiment supports the opinion that the endotrophic mycorrhiza of oats is distinct from that of clover, but much further work will have to be done on this aspect of the problem.

8. Summary of work on endotrophic mycorrhiza.

The fungus which causes endotrophic mycorrhiza of oats, usually accompanied by a yellowish-green discoloration of the roots, is described. The hyphae, which usually enter through root hairs, are coarse and bear arbuscules, sporangioles and vesicles and are found only in the mature cortical tissue.

The culture of the fungus on artificial media was not attempted.

Efforts to express the proportion of endotrophic mycorrhiza in a root system on a quantitative basis are outlined.

The formation of mycorrhiza, which is particularly abundant on certain manganese deficient soils, was not found to depend upon the absence of available manganese, for when soluble manganese salts were added to these soils the mycorrhiza remained as abundant as before. The amount of mycorrhiza formed in the roots seemed to depend upon the amount of the fungus present in the soil, but why it should be much more abundant in the manganese deficient soils was not determined. The mycelium has only a restricted range in the root from each point of entry.

Differences were observed between the endotrophic mycorrhiza of oats in Mt. Gambier and Corny Point soil on the one hand, and in Glen Osmond soil on the other. Invasion of the roots by the fungus begins, for instance, within a few days of germination in the first two soils and soon produces conspicuous discoloration, while in Glen Osmond soil it only occurs much later

and is not so striking in appearance.

Mycorrhiza of oats was formed in two types of soil kept near 60% saturation capacity at soil temperatures fluctuating between 9 and 13.5°C. and intermediate temperatures up to 30°C., but not at 35°C.

Endotrophic mycorrhiza is recorded in twenty other species, and the possible occurrence of different strains of the fungus in various plants is discussed.

II. MYCORRHIZA OF PINUS RADIATA.

1. Introduction.

The second part of the work deals with quite a different type of mycorrhiza, the ectendotrophic mycorrhiza of conifers described by Melin.

It was for long suspected, and has now been definitely proved in a number of cases, that the fructifications of the Hymenomycetes found under many trees are produced by the same mycelium that forms mycorrhiza with the roots. The one tree may form mycorrhiza with several different fungi and the same fungus often has different hosts.

It is still a vexed question whether one of the symbionts obtains all the benefit and the other suffers only disadvantages, or whether it is one of mutual gain. Melin is led more and more to the latter view, though Masui (1927), on the results of a biochemical analysis of the food substances present in ordinary roots and in mycorrhizas, concludes that there are many obligate mycorrhizal fungi which are definite parasites; even the facultative ones rob the root of amino-acids and sugars and ultimately cause death of the mycorrhiza.

In agreement with the view of the beneficial nature of the mycorrhizal fungus, the failure of seedlings in nurseries established on new land has repeatedly been attributed to the absence of a mycorrhizal fungus. Kelley (A.P. Jour. Forestry 28: 34. 1930) records the failure of Mont Alto nursery stock in Pennsylvania and attributes it to this cause, so does Shedley in W.A. (loc. cit. 1926 and Austr. Jour. of Forestry 10: No. 5, p.130, 1927).

Pinus radiata Don. (P. insignis Douglas, P. montereyensis Hart.) is the Californian Monterey pine, so extensively planted for softwood forests in Australia and New Zealand. The roots so far examined have all had dichotomously forked structures

31·10·32.

Mycorrhiza of volunteer Pinus sp., Glen Osmond.

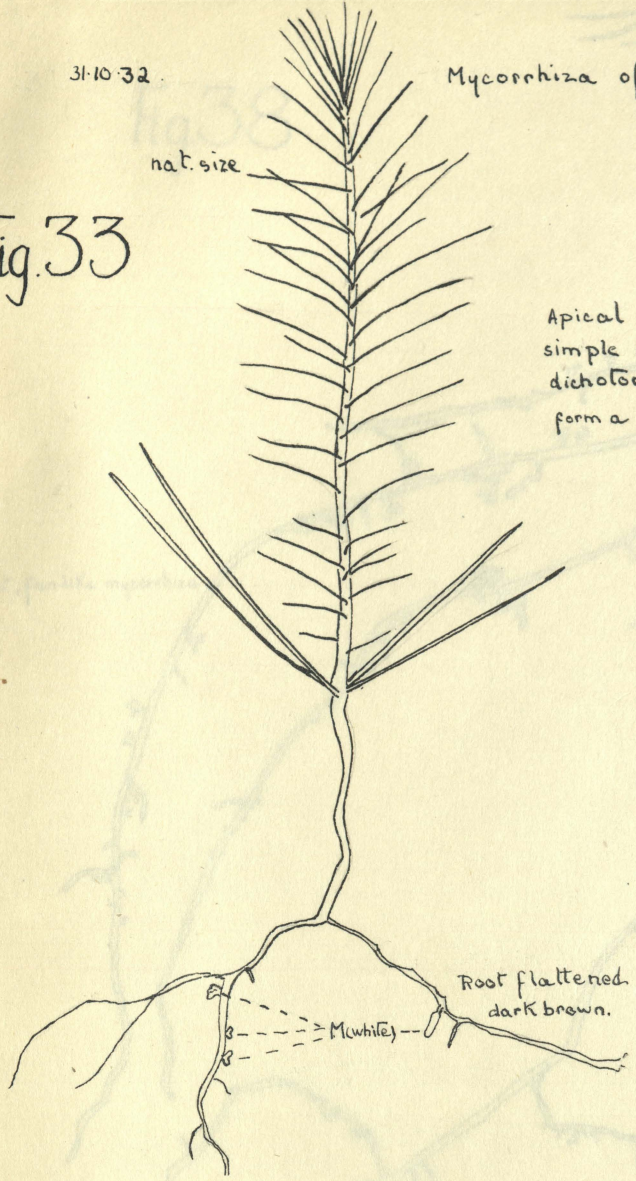
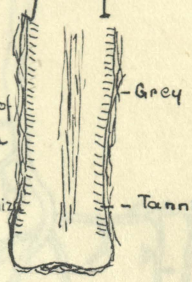
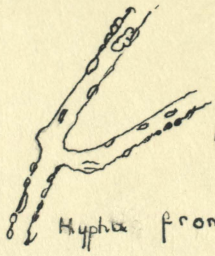


Fig. 33



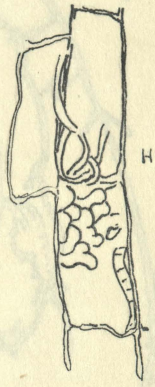
Apical meristem of simple mycorrhiza dichotomising to form a Gabelmykorhiza

Fig. 34



Hyphae from mantle.

Fig. 35



Hyphae in tannin-containing cell, from l.s.

Fig. 36

T.s. of a dichotomizing mycorrhiza

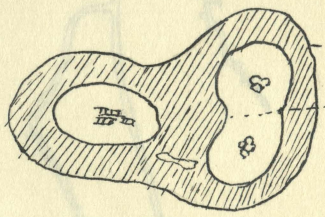
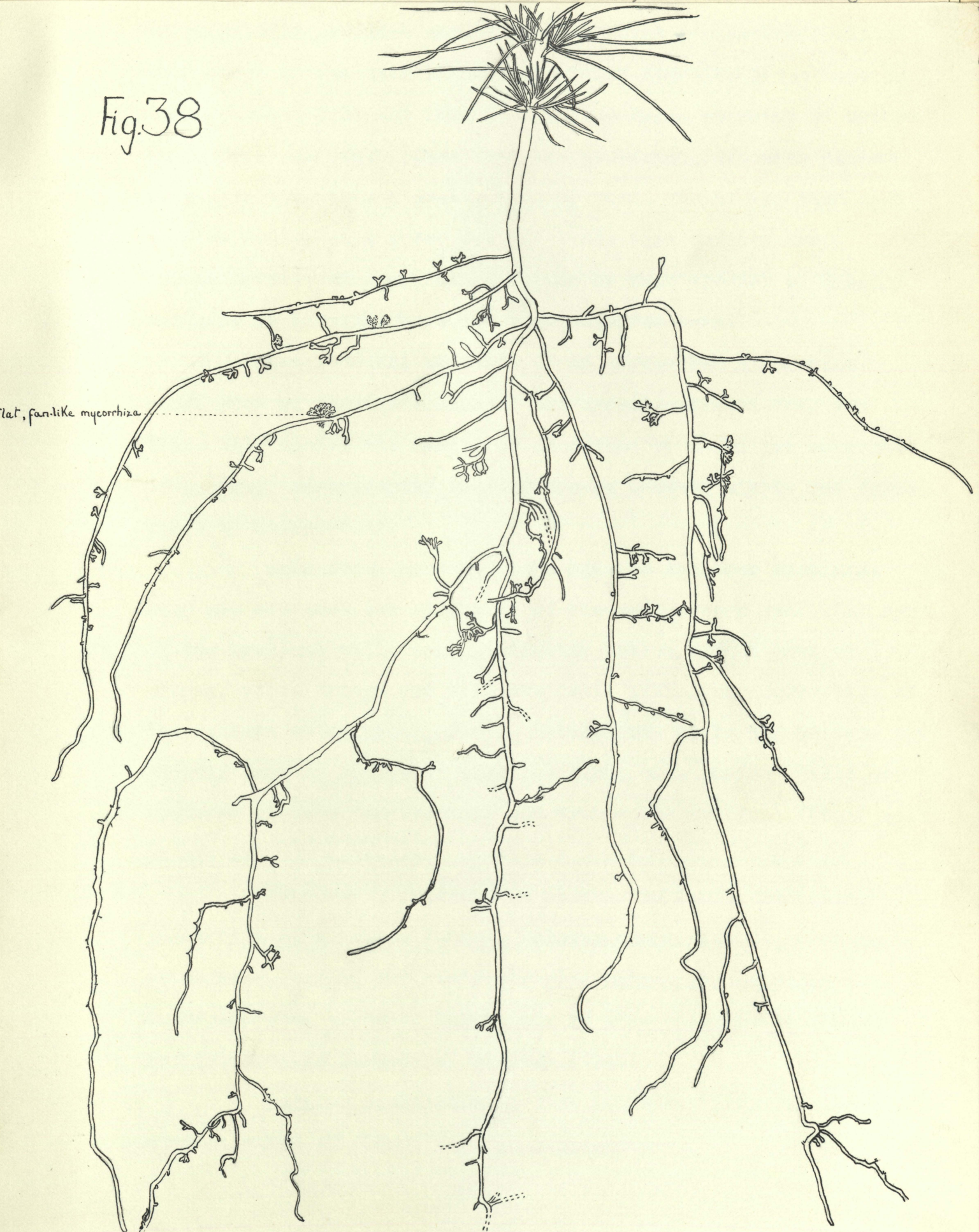


Fig. 37

Fig. 38



Part of root system of Pinus radiata, a seven months pot plant with mycorrhiza. Leafy shoot 3½" high. Nat. size.

8·11·32.

not more than 4-5 mm. long. At first, these mycorrhizas are a dirty white colour, but become brown. Judging by the number of thin, almost black dead ones found, they must be comparatively short-lived, as recorded by other workers.

Some unbranched ones are found, but those forking once or repeatedly are more usual; sometimes the successive forkings take place in the same plane and a flat, fan-like mycorrhiza is formed, whose ribs are soon lost in the thick covering of hyphae (Fig. 38). At other times they are irregular, and when coated with mycelium, form a small, nodular mass. Melin has seen such nodules as big as a green pea and finds that nodules are characteristic of mycorrhizas formed by many species of Boletus, but none so striking have yet been observed here.

Longitudinal sections of an unbranched mycorrhiza often show an equal division of the apical meristem into two (Fig. 34), inside the mantle as described by Masui for mycorrhiza of Quercus, so evidently the difference between simple and forked kinds is unimportant.

Only fresh hand-sections have so far been examined. They did not show the structure of the mantle very well, but many of the cortical cells were distinctly filled with a mass of hyphae, which branch and fill the cells with lines suggestive of the pattern of a finger print. Between the cells may be seen the "Hartig Net" or "Réseau," like the links of a chain. These are features of both longitudinal and transverse sections (Figs. 41, 42 and 43).

Wherever P. radiata is planted in South Australia, quantities of a yellow fungus, Boletus granulatus L., make their appearance, and in certain districts, Rhizopogon luteolus Fr., a Gasteromycete, as well; Rhizopogon is also recorded in Western Australian pine forests by Shedley (1926).

Boletus granulatus is just as common here in stands of other species of Pinus, e.g. P. halepensis.

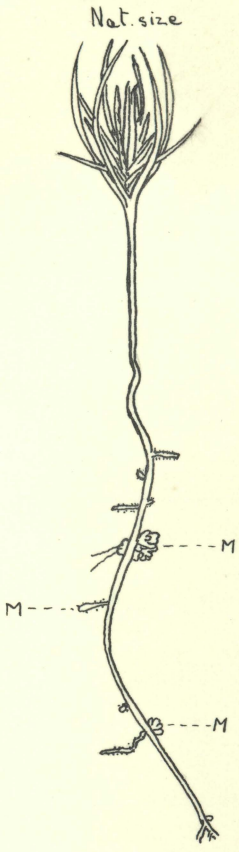


Fig. 39

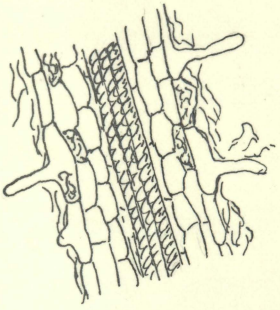


Fig. 40

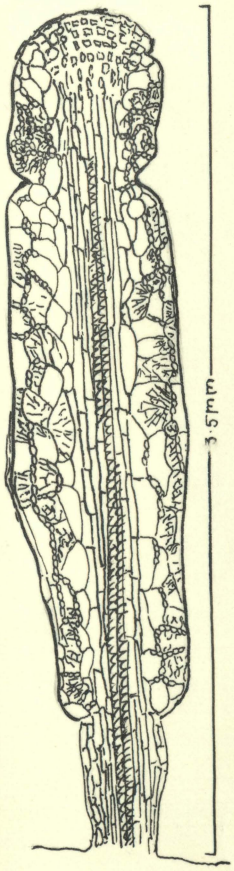


Fig. 41

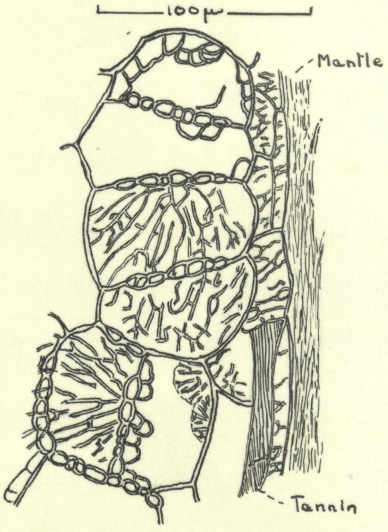


Fig. 42

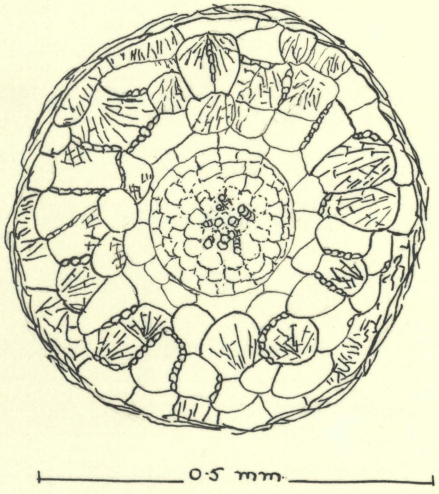


Fig. 43

In the present case, it is desired to establish beyond doubt that Boletus granulatus and Rhizopogon luteolus form mycorrhiza with Pinus radiata. The fact that some P. radiata nurseries on new land proved unsuccessful in Western Australia until soil was brought from old-established nurseries, will make any results of special interest (Kessell, 1926).

The pure culture methods of Melin have been used, but there are advantages in growing the seedlings under more nearly normal conditions, so the experiment was performed of growing pines in six-inch pots in soil which had first been heat-sterilized to kill any naturally occurring mycelium or spores in it. Ordinary seed from a seedsman's stock was planted in the pots at the beginning of April and the latter were fully exposed and watered with tap-water. Six weeks later, the pots where seeds had germinated were taken and divided into three groups of seven pots each. The first group was used as a control. In the second, the pines were carefully turned out and the soil mixed with mycelium taken from the bases of Boletus fruit bodies in a P. radiata plantation, before the pines were replanted. The third group of pots had broken up pilei of Boletus mixed with the soil in the same way.

The seedlings grew well, especially at the beginning of spring, and after seven months they were carefully washed out and examined. All the pots, including the uninoculated controls, contained characteristic short, forked mycorrhiza on the long roots, so the soil is evidently infected rather easily by spores reaching it from the air, the water or clinging to the seed coat. Precautions can be taken against this by keeping the pots in a closed house, watering with distilled water and sterilizing the seed, but the other method is so much simpler that it was worth the trial.

Though, in this case, one cannot draw conclusions from the dozen or so plants in each group, there was no doubt that the

plants with the most copious mycorrhiza came from the group inoculated with broken-up fruit bodies(Fig. 38); a few plants with a fair amount of it were found in the other pots, but for the most part they had only a small number of isolated mycorrhizas. It is very likely that the large number of spores introduced with the fruit bodies was the direct cause of the prolific mycorrhiza formation.

Two months ago, seedlings germinated from seed sterilized with 1:1000 $HgCl_2$ were planted in pots of sterilised soil and inoculated with an artificially obtained culture of Boletus granulatus growing on sand containing 2% of cornmeal. These are not yet ready for examination.

2. The fungi in pure culture.

The mycelium of the fungi was successfully obtained in culture by carefully breaking open a sound, young fruit body and cutting out a piece of tissue under sterile conditions from the freshly exposed surface (Duggar, 1905) and placing it on agar plates or slants. The agar most frequently used was a nutrient agar of the following composition:-

1000 ml. distilled water
20 grm. glucose
0.1 grm. $MgSO_4$
0.5 grm. NH_4Cl
1.0 grm. KH_2PO_4
20 grm. Agar

Later, the mycelium was transferred to a sterilized mixture of 98% sand at 50% saturation capacity and 2% cornmeal in plugged flasks.

a. Boletus granulatus.

B. granulatus L. (Polyporaceae) has been obtained in pure culture before by Melin, who synthesized mycorrhiza between it and Pinus silvestris L. (1923), and P. montana Mill. (1924).

The media used in the present case were:-

(1) potato-dextrose agar,
(2) the mineral nutrient agar given above,
(3) ~~prime~~ prune agar.

The first did not appear to be as suitable as the second,

24·5·32. Aerial hyphae from Boletus granulatus culture on potato dextrose agar, eight days old.

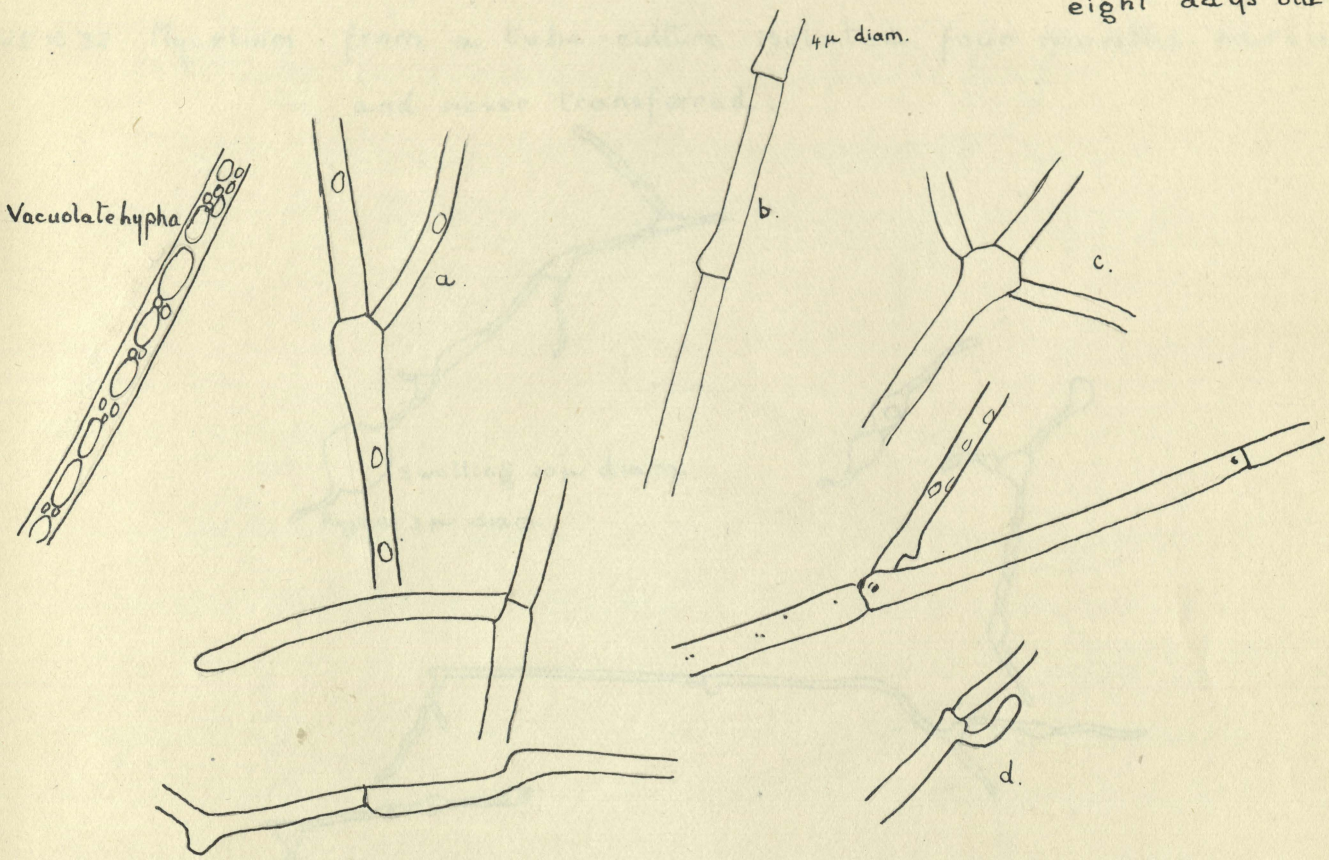


Fig. 44

25·10·32. Mycelium from a tube-culture isolated four months earlier and never transferred.

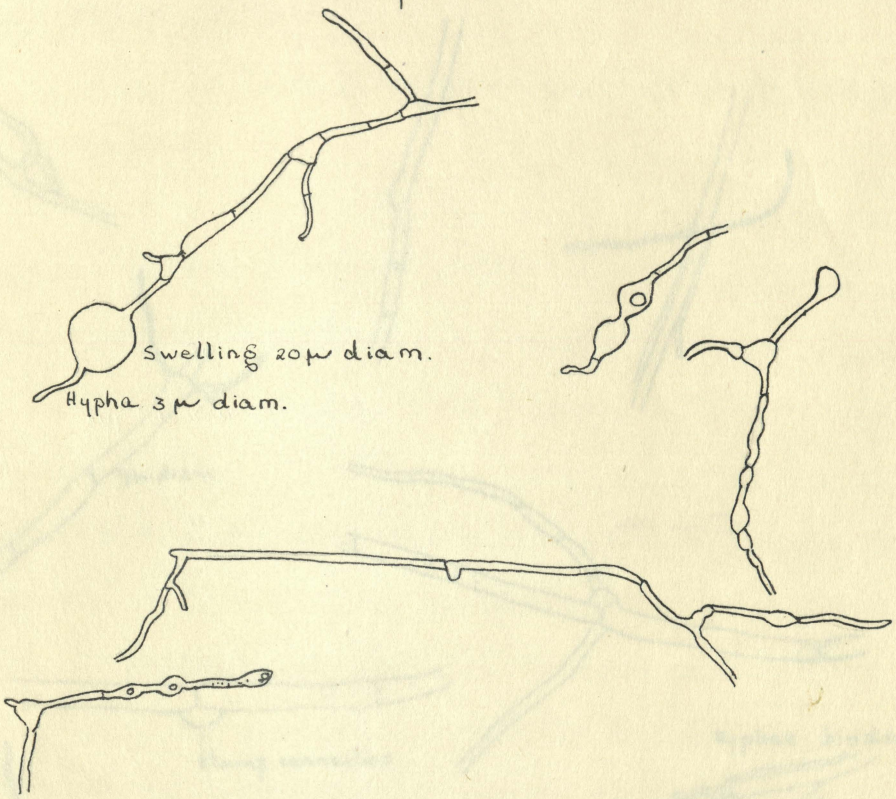


Fig. 45

20.7.32. Rhizopogon luteolus mycelium from culture on nutrient agar, 26 days old.

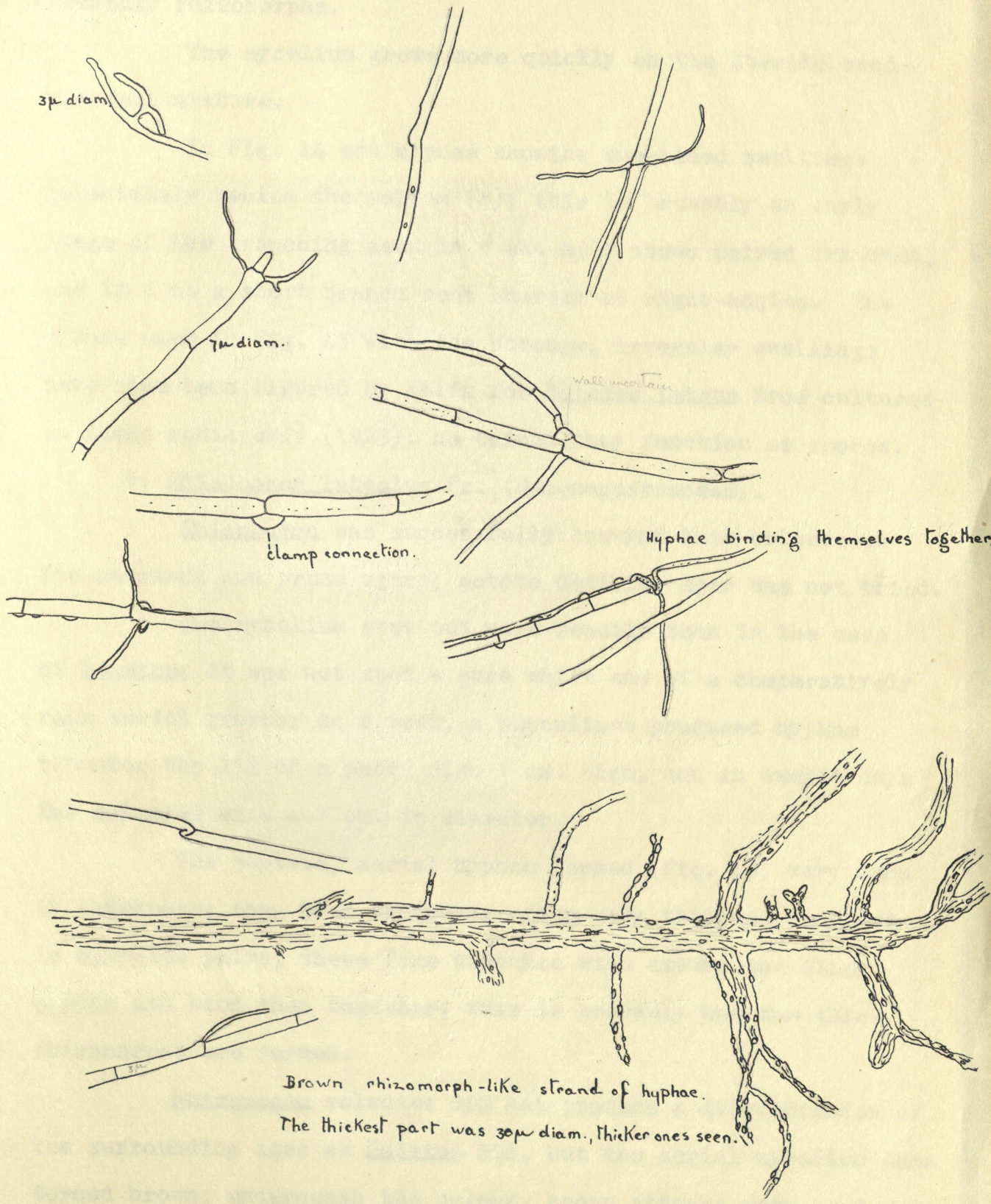


Fig. 46

which was used most.

After four to eight days at 25°C., the pieces of tissue were covered with a short, snow-white, furry mycelium which slowly grew down onto the agar, preserving its short, dense character. A yellow colouring matter diffused out into the agar and thick, brown, radial strands could later be seen in the submerged part of the colony through the bottom of the dish - probably rhizomorphs.

The mycelium grows more quickly on the sterile sand-cornmeal mixture.

In Fig. 44 are hyphae showing one-sided swellings immediately behind the septum (b); this is probably an early stage of the branching seen in c and d; c shows paired branches, and in d is a short branch bent sharply at right-angles. The hyphae seen in Fig. 45 with the strange, irregular swellings have also been figured by Melin for Boletus luteus from cultures on fluid media only (1923); he thinks they function as spores.

b. Rhizopogon luteolus Fr. (Hymenogastraceae).

Rhizopogon was successfully brought into culture on the nutrient and prune agars; potato dextrose agar was not tried.

The mycelium grew out more readily than in the case of Boletus; it was not such a pure white and of a comparatively rank aerial growth; in a week, a subculture produced hyphae touching the lid of a petri dish 1 cm. high, and in twelve days the colonies were 4-7 cm. in diameter.

The septate, aerial hyphae formed (Fig. 46) vary much in thickness; they bear branches, often very thin, and produced in opposite pairs, these fine branches wind around the other hyphae and bind them together; this is probably how the thick rhizomorphs are formed.

Rhizopogon colonies did not produce a discoloration of the surrounding agar as Boletus did, but the aerial mycelium soon turned brown; underneath the colony, brown streaks were produced.

Fig. 4.7

In cornmeal sand culture, these distinctions are lost and both mycelia are pure white and of much the same density.

3. The pines in pure culture.

Sterile seedlings were raised in the following way. The seeds (from Hackett's) were first soaked in water to become thoroughly wetted, or for preference alternately soaked and dried to assist germination, then treated with 1:1,000 $HgCl_2$ for 1½-5 minutes, after which they were thoroughly washed in at least three changes of sterile water and placed on agar-poured plates (about ten seeds per 9 cm. diameter petri dish) and put to incubate at 25°C.; only the seeds that germinated without producing any bacterial or fungal contamination in the agar were used. The germination of P. radiata seeds under these conditions was much slower than in sand, for example.

The sterile seedlings were planted by twos in a sterile sand-culture, or a culture with granulated cork and sand designed partly for drainage, in 300 c.c. Erlenmeyer flasks stopped with a cotton plug and a brown paper cap (Fig. 47).

The sand was river sand which had been treated with hydrochloric acid to make it neutral in reaction and to destroy the small quantities of humus which would otherwise produce toxic products on heating, then washed well with tap water and dried. 150 grm. dry sand made up to 75% saturation capacity with a nutrient solution was put into each flask, and formed a layer about 1 inch deep. The nutrient solution used consisted of:-

distilled water	1,000 ml.	$MgSO_4,7H_2O$	0.3 grm.
KH_2PO_4	1 grm.	$FeCl_3$	0.01 grm.
$CaCl_2$	0.1 grm.	NH_4Cl	0.5 grm.
NaCl	0.1 grm.	glucose	0.5 grm.

(after Meyer, 1903, with the addition of NH_4Cl and glucose).

The flasks were then plugged and autoclaved at one atmosphere for thirty minutes. The sterile pine seedlings germinated on agar were carefully planted in these flasks as they were ready.

The seedlings, under these conditions, looked green and healthy enough, though growth was slow and the foliage softer and finer than usual.

The percentage of granulated cork (80% by volume) in the flasks with the cork-sand mixtures made too open a substrate which soon dried out.

4. Synthesis of mycorrhiza.

Besides the largely uncontrolled method described earlier, of using sterilized soil mixed with pilei and mycelium, in which to grow the pines, pure culture syntheses are also being attempted. Of a series of Pinus radiata seedlings in Erlenmeyer flasks, some were kept as controls, and the rest were inoculated with Boletus granulatus, either when the seedling was planted in the flask and had only a radicle showing, or else later, when it was well established. The inoculum, a small piece of either agar or sand-cornmeal culture, was dropped into the flask close to the stem of the pine seedling. The flask was then plugged again and left till examined some months later. The plants were not watered and did not appear to need it; this, of course, would have to be done carefully with sterile water and increases the risk of contamination.

Ten of these flasks were examined 2½-4 months later; there were very few lateral roots and no perceptible growth of the mycelium over the surface of the sand; no mycorrhiza had formed, so it was considered advisable to leave the rest of the flasks for a longer period.

In the meantime, more tests of the same kind will be prepared.

5. Summary of work on mycorrhiza of Pinus radiata.

The mycorrhiza of P. radiata from Kuitpo Forest in South Australia, where Boletus granulatus is the dominant basidiomycete on the forest floor, are of the dichotomizing, ectendotrophic type, with the usual intercellular Hartig Net and intracellular cortical hyphae.

Two basidiomycete fungi, Boletus granulatus and Rhizopogon luteolus, suspected of forming mycorrhiza with P. radiata

have been isolated and grown in pure culture from their respective fruit bodies.

Attempts have been made to synthesize mycorrhiza from pure cultures of Boletus granulatus and seedlings of Pinus radiata under sterile conditions.

No positive results have yet been obtained, but insufficient time has elapsed since the most satisfactory technique has been established. Good mycorrhiza formation can apparently not be expected in much less than eight months, and the present series of cultures will be carried on for at least that period.

DESCRIPTION OF FIGURES

I. Endotrophic Mycorrhiza.

Fig. 1. "Map" of root system of oat plant from Mt. Gambier soil.

2. " " " " " " " Corny Point "

3. Hypha of mycorrhizal fungus in root hair of oat plant from Mt. Gambier soil.

4. Two hyphae in one hair.

5. Almost empty vesicle in an oat root containing only a few oil globules.

6. Longitudinal section of an oat root containing mycorrhizal fungus; the cortex on only one side of the vascular cylinder is shown. Three hyphae enter from a root hair and branch through the cortex; some of the hyphae contain oil globules. There is a richly protoplasmic vesicle in the fourth layer of the cortex and another full of oil globules among the bushy arbuscules of the two innermost layers of the cortex.

7. Fungal hypha entering the root hair of an oat plant from a germinating spore (?)

8. Hypha entering hair of oat root.

9. Young protoplasmic hypha in root hair of oat.

10. A cell from an oat root containing an arbuscule; both intercellular and intracellular hyphae are seen.

11. Mycelium found outside oat roots containing mycorrhizal fungus and closely resembling the latter; a typical vesicle is present.

12. A mycorrhizal hypha and a finer septate one are in the same cell; both fungi were widespread in this oat root.

13. Hyphae entering the cortex of an oat root (in longitudinal section) and forming arbuscules in the two innermost layers; the arbuscules are in the granular state.

14. Sporangiole-containing cell from the inner cortex of an oat root.

15. Sporangiole from a much discoloured oat root; the globules are greenish and do not give the fat reaction with Sudan III.

16. Longitudinal section of an oat root with sporangioles around the vascular cylinder.

17. One side of the cortex in the longitudinal section of an oat root. The hyphae are ramifying through the cells and the granular arbuscules form a solid row just outside the endodermis. N = nucleus.

18. Microphotographs of two roots mounted whole in water; the one is a normal root, the other full of mycorrhizal fungus and very discoloured. The thick opaque jacket of arbuscules makes the vascular cylinder appear abnormally thick and uneven at the edges.

Fig. 19, 20, 21, 22, 23 and 24. Root maps of oat plants grown in partitioned pots after 1, 2, 3, 4, 7 and 2 weeks respectively.

25, 26, 27 and 28. Root maps of oat plants grown in Mt. Gambier and Glen Osmond soils as a parallel series, after 2, 3, 5 and 8 weeks.

29. Root map of Trifolium arvense (Hare's foot clover) from the field.

30. Root map of mature wheat plant from the field, Glen Osmond.

31. " " " " barley " " " " " " "

32. " " " " oat " " " " " " "

II. Mycorrhiza of Pinus.

33. Volunteer Pinus sp. seedling with a few mycorrhiza. M = mycorrhiza.

34. A simple mycorrhiza bifurcating to form a "Gabelmykorrhiza."

35. Hypha from mantle of 34.

36. Cell from cortex of 34 containing fungal hyphae.

37. Transverse section of a dichotomizing mycorrhiza with the branches embedded in a mass of hyphae.

38. Root system of Pinus radiata with mycorrhiza.

39. Volunteer seedling of Pinus sp. showing at what an early stage mycorrhiza may be formed. There are coralloid branched mycorrhiza on the right of the main root (M) and a simple one on the left (M) where the mantle is just beginning to form as seen in the next figure.

40. Longitudinal section of young mycorrhiza with the hyphae of the mantle just beginning to extend down from the root tip among the hairs. Some of the cortical cells contain hyphae.

41. Longitudinal section of a simple mycorrhiza of P. radiata (same material as for Fig. 38). There is a thin mantle of fungal hyphae. The chain-like Hartig Net may be seen between the cells of the cortex, some of which are filled with a branching mass of hyphae. The constriction near the tip of the mycorrhiza may be due to the sudden flush of growth in spring after the winter rest.

42. The Hartig Net and intracellular hyphae of Fig. 41 are seen in greater detail.

43. Transverse section of a mycorrhiza of P. radiata.

44. Mycelium of Boletus granulatus in culture; for discussion, see text.

45. Mycelium of Boletus granulatus; see text.

46. Mycelium of Rhizopogon luteolus in culture; see text.

47. Photo showing method of growing pine seedlings under sterile conditions in flasks, for the synthesis of mycorrhiza.

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