MEMOIRS OF THE COLLEGE OF SCIENCE, KYOTO IMPERIAL UNIVERSITY, SERIES B.
VOL. II, NO. 1, ARTICLE 3, 1926.

On the renewed Growth of the mycorrhizal Root.

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With 1 Plate and 4 Text-figures.

Received October 23, 1925.

While I was examining a considerable number of microtomic sections of fir mycorrhiza Form B[1] with the microscope, I found, by chance, a vigorous root in which the completed fungous mantle had been split at its terminal end. At that time I could not determine whether the fissure had been formed by a renewed growth of the root or by some another cause.

Touching on the growth of the completed mycorrhizal root, McDOUGALL[2], in 1914, stated: "It is evident that the root does not grow in length after a complete fungous mantle has been formed." It is sure that a thick mantle affects some pressure upon the root even if it still possesses the tendency for further growth. But it is yet problematical, whether the roots remain unaltered in that condition, or are induced to renew their growth in the following season, and whether they grow by pushing aside the fungous mantle, or hand in hand with the latter.

(1) Masui, K., A Study of the Mycorrhiza of *Abies firma*, with special Reference to its mycorrhizal Fungus. Mem. Coll. of Science, Kyoto Imp. Univ. Series B, Vol. II, No. 1, Article 2, 1926.

(2) McDougall, On the mycorrhizas of forest trees. Am. J. Bot., Vol. 1, P. 48–74, 1914.

I collected therefore numerous typically developed mycorrhizas Form B on 6 Sept. '24, for the investigation. But, as the fungous mantle is thin and almost transparent, mere examination was not suitable. Fortunately the mycorrhiza contains a great deal of tannic substances in the root tissue, and gives a dark brown coloring with chromic acid, while the fungous mantle remains unaltered and gives a distinct contrast in color. The reagent was useful moreover to fix the material adequately. For this purpose the following chromo-acetic solution was used:

Chromic acid	1 gm.
Glacial acetic acid	1 cc.
Distilled water	100 cc.

A quantity of fresh material was dipped in this reagent for 24 hours, and washed for a while until the yellowish color disappeared in the water. This method proved to be very serviceable for the investigation, and enable me to pick out easily the specimens in question even with a pocket lens. Through this procedure I was able to find that the mantleclad root-apex of the completed mycorrhiza in *Abies firma* (Form A and Form B), *Abies Mayriana*, *Alnus japonica* and *Pinus densiflora* can renew its growth breaking through the mantle.(1)

In all these case the mantle, which has completely covered the rootlet, is first split in various directions. Many of them are broken longitudinally as shown in Fig. 1;5, Pl. VI and Text fig. 1;1, 4, 6, 8 & 13, but sometimes it splits transversely (Text fig. 1;5, 7 & 9) or irregularly. (Text fig. 1;2, 3, 10, 11, 12 & 14) When the fissure is formed, the tip peeps out from it, and makes further growth in length free from the mantle.

Filaments, arisen from the margin of the fissure, follow then to the root along the epidermal wall, and thus the secondarily elongated portion of the root is, sooner or later, covered with them completely.

(1) This method may be applied to the ectotrophic mycorrhizas of many other plants if they are not too small.

Text fig. 1;8 shows a fissure of the old mantle at its middle portion and a newly developed mycelium has already covered half the area of the new surface. An interesting specimen is shown in Text fig. 1;2. As a split was made at the lateral portion of the apex of mantle, the root tip peeped out obliquely. The plastic growth of the root restricted by the mantle is therefore clearly seen.

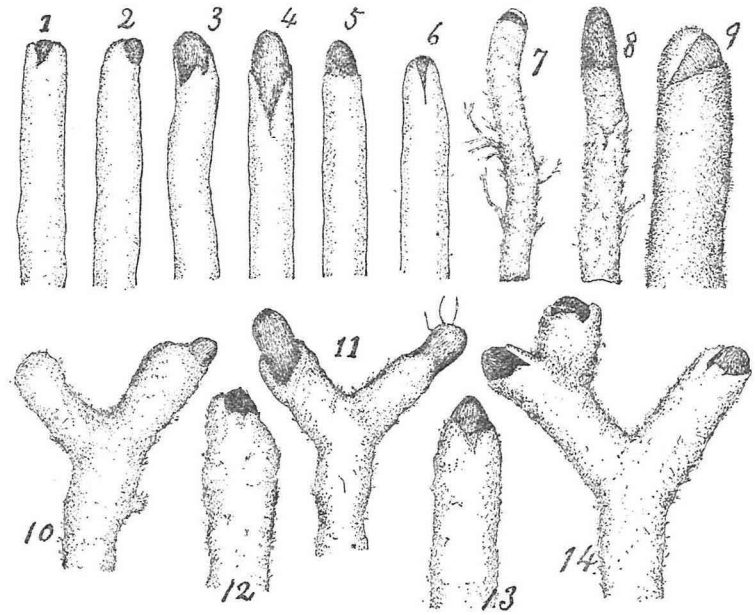

Fig. 1. Completed mycorrhizas which have renewed their growth. 1–6, Form B of *Abies firma* ×7; 7–8, Form A of *Abies firma* ×7; 9, *Abies Mayriana* ×16; 10–14, *Pinus densiflora* ×26.

In order to understand the structural difference more precisely, I made microtomic sections. Fig. 6 and 7 Pl. VI show longitudinal sections of the mycorrhizas of *Alnus japonica*, which have made a renewed growth. The mechanical restriction of the mantle upon the growing root is here quite concievable if one compare the root of the former, which has grown obliquely through the laterally formed fissure, with that of the latter, which has grown straight forward because it was restricted equally at the sides. Text fig. 2 shows a root of *Abies firma* which has made further growth of 0.6 mm. from the broken

margin of the mantle. The mantle should have been broken by the growing root, at least in two portions at the tip of the mycorrhiza. The margin of the mantle at the right side of the root in the section was found to be 45.2 μ in thickness. From the margin of the obliquely torn mantle arose a rather thin fungous mantle, 6.5–16.1 μ in thickness, which has already grown 0.27 mm along the surface of the new portion of the root. At the left side of the section the same feature may be seen, though the mantle is yet quite thin. At the tip of the root it bears a piece of mycelial mass, which undoubtedly shows, that it was once a part of the mantle and torn off by the growth of the root. In the case of the mycorrhiza of *Alnus japonica* I could find a similar specimen. Cell division goes on profusely in the meristematic portion, indicating rapid growth of root.

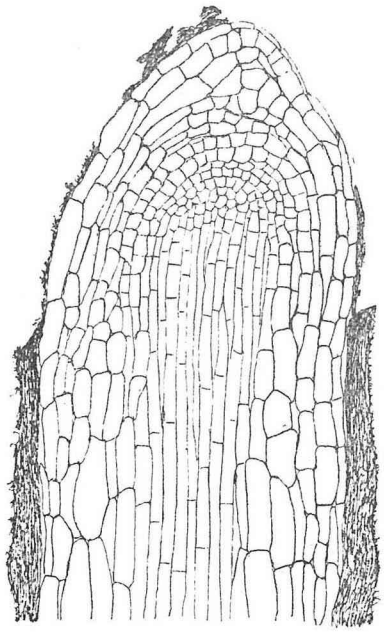

Fig. 2. A median section of a mycorrhiza of *Abies firma* (Form B), after it has made renewed growth. × 100.

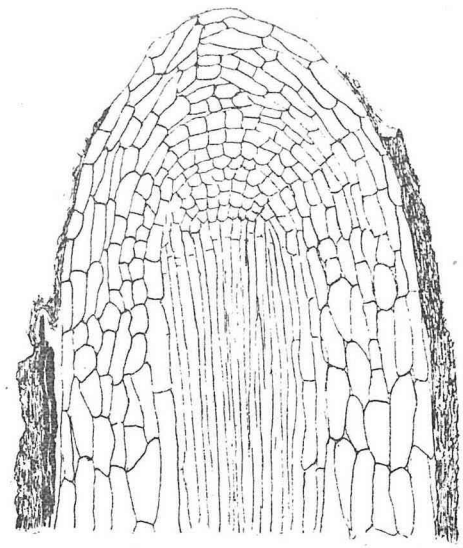

Fig. 3. A median section of a mycorrhiza of *Abies firma* (Form B), which has made renewed growth. × 100.

Another section of the same specimen is shown in Text fig. 3. A new mycelial network arisen from the margin is clearly seen. At

the left side the torn margin is more or less separated from the outermost portion of the cortical tissue, and the mycelium, starting from the inner layer of the mantle, has advanced fast adhering to the surface of the newly elongated root.

Moreover it is clearly shown in every section that the development of the HARTIG's network in the cortical tissue is still very scanty at the split portion of the mantle. The development of such a network is to retard, to some extent, the tearing of the fungous mantle by the growth of the root.

In *Abies firma* such a new growth of the completed mycorrhizas is to be seen chiefly among the vigorous ones, while in the case of the mycorrhiza of *Abies Mayriana, Alnus japonica* and *Pinus densiflora*, there is almost no difference in thickness between those which have made it and those which have not. Of course the frequency may vary according to the season. The number which I counted in six kinds of mycorrhizas is as follows:

Mycorrhizas of	Number of mycorrhizas of which mantle had been split.	Total number of mycorrhizas examined.	Rate of splitting of the mantle.	Date.
Abies firma, Form A.	3	?		Sept. '24.
„ „ Form B.	16	144	ca 11 %	Sept. '24.
A bies Mayriana.	30	199	„ 15 %	Oct. '24.
Alnus japonica.	17	181	„ 9.4 %	June '25.
Pinus densiflora.	8	206	„ 3.7 %	Nov. '24.

To put it briefly: (1), the completed ectotrophic mycorrhiza of *Abies firma* (*Form A* and *Form B*), *Abies Mayriana, Alnus japonica* and *Pinus densiflora* is able to make renewed growth; (2), When the root grows the mantle is first split in various directions; (3), but the new root-tip is covered again, sooner or later, with a fungous mantle which arises from the margin of the fissure.

FRANK ('85)[1] seems to have believed in the continued growth of a mycorrhizal root by the gradual extention of the completed mantle and also in the renewal of growth after its mantle has perished. Concerning the former case he made the following statements:

"Es muss also angenommen werden, dass auch der Pilzmantel eines Wachstums, einer Erweiterung fähig ist, um mit der Verlängerung der von ihm eingeschlossenen Wurzel Schritt zu halten. In der That hat auch er dort, wo der Vegetationspunkt und die Region der Längenzunahme des Mykorhizakernes liegt, seine Zuwachsregion. Stets sind nämlich die Hyphen, welche den um die Wurzelspitze herumgehenden Theil des Pilzmantels zusammensetzen, viel dünner als diejenigen in dem weiter rückwarts liegenden, nicht mehr wachsenden Stück, nämlich 0.0008–0.0024 mm. breit und bis etwa 0.005 mm. lang, und gehen nach rückwarts ganz allmählich in die grösseren Zellen über. Der Pilzmantel vergrössert sich also dadurch, dass an der Spitze der Mykorhiza immer neue Fäden sich zwischen die vorhandenen einschieben und dass dann die Zellen des so gebildeten Pseudoparenchymas sich bis auf ihre definitive Grösse erweitern. Beide Teile der Mykorhiza halten in disein Wachsthum gleichen Schritt, so dass der Pilzmantel auch der wachsenden Wurzelspitze immer dicht anliegt."

In the case of the mycorrhiza of *Abies firma*, *Abies Mayriana*, and *Pinus densiflora*, on the other hand, the hyphae, which constitute the young portion of mantle, are almost the same in thickness as those behind, so that gradual elongation of the mantle is not implied. Only in the case of the mycorrhiza of *Alnus japonica*, is the apical portion of the mantle composed of much smaller hyphal cells and more or less looser in texture than the after part. In this case the gradual growth of the mycorrhizal root, according to FRANK's opinion, may be assumed to a certain extent. But the mere difference in the thickness of the mantle does not necessarily imply inequality of the mechanical tension. It might be introduced likewise by the nutritive conditions of the hyphae. FRANK's first case needs therefore still further investigation, even if it is not improbable.

As to the second case in FRANK's statement, it is not rare to meet with clear examples. As the completed mycorrhiza become old

(1) FRANK, B., Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. Ber. d. D. B. Ges., Bd. 3, S. 395–408, '85.

the cortical layers of the root die away, and accordingly the mycelial mantle also comes to the same fate. The meristematic portion of the root, however, maintains its growing capacity, and after the mantle has lost its power of resistance the root begins to extend further. In the case of the mycorrhiza of *Abies firma* this mode of new growth is also observable.

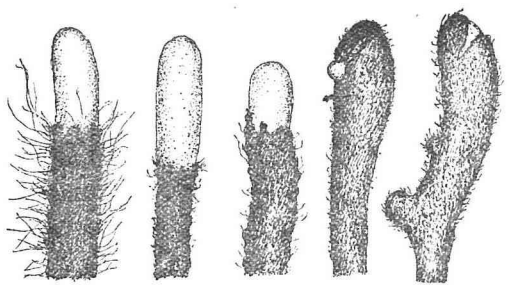

Fig. 4. Old fir-mycorrhizas which have made renewed growth. ×7.

On 3 October, '24, I collected numerous mycorrhizas at Kurama, about seven miles north from Kyoto. At that time the roots of two year-old tree were exclusively investigated. The roots bore numerous side-branchlets which were transformed into mycorrhizas. Among the mycorrhizas I could distinguish the following three stages of development: young white ones, middle-aged brown ones and dark old ones. Among the last, which had been formed perhaps in previous year, some roots were proved to have maintained the capacity for further growth, while the mantles had already perished.

When the cells of the meristematic portion of the root are impelled to further growth, the dead mantle, which had completely covered the root, is torn off easily as shown in Text fig. 4;4 & 5. From the split peeps out the root, or a primordium of rootlet as shown in figure 4. The newly developed portion of the root is of white color or translucent, and at first it seems to be free from the mycelium, but sooner or later, it may be infected again and transformed into a complete mycorrhiza.

In short, the growth of mycorrhizal roots may be stated as follows: When the root is weak, it can not grow more after the completion of the mantle. This is perhaps the most general case. If, however, the root still possesses the capacity for further growth, there

arises a struggle between the root and the mantle. There then probably follows a mechanical tension and plastic growth of the mantle by the pressure from within. But sooner or later this comes to a standstill according to the season and to the strength of the root. But after a pause some vigorous roots are impelled to new growth, split the mantle and peep out—the renewed growth through the living mantle. But the mantle is then completed again. When the cortex of the root dies, the mantle also perishes. But some roots may still have growing power, and then follows a renewed growth through the decayed mantle.

I must express my deep sense of gratitude for many helpful suggestions to Prof. K. KORIBA, under whose direction this work has been carried on.

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Explanation of plate.

Pl. VI, Fig. 1–7. Young mycorrhizas in which renewed growth of the roots has taken place, splitting their completed mantles.

Fig. 1. Mycorrhiza of *Abies firma* Form B. ×9.

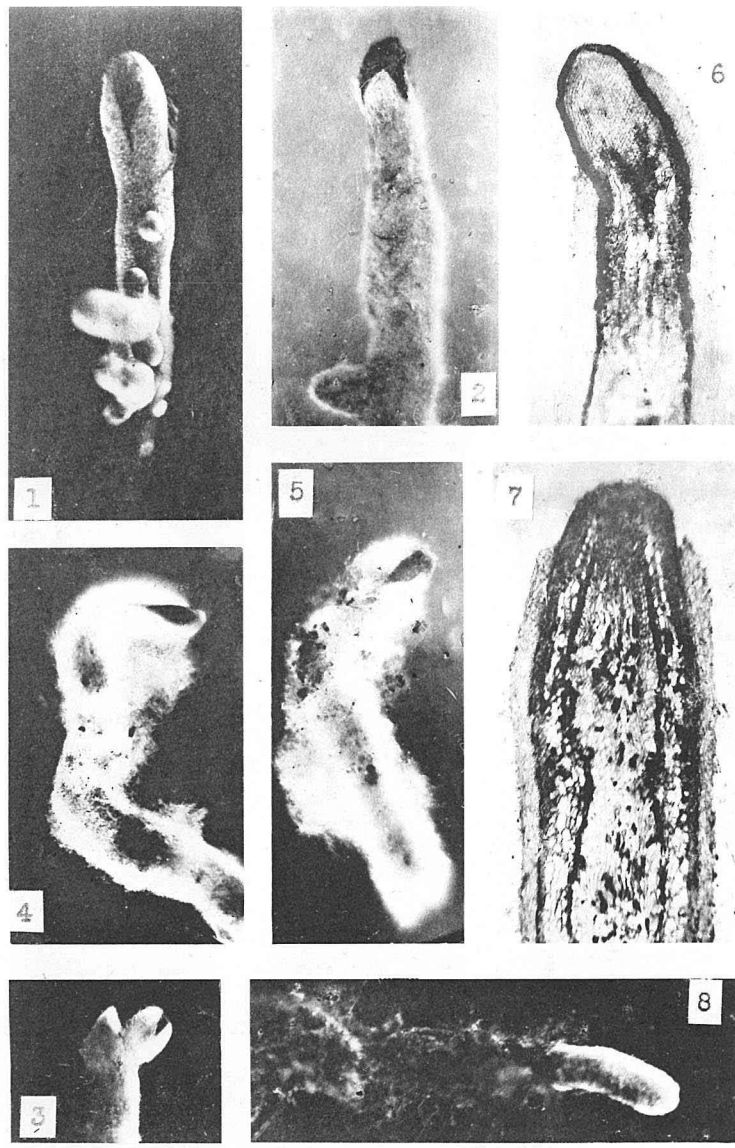
Fig. 2–3. „ „ *Abies Mayriana.* ×14.

Fig. 4–5. „ „ *Alnus japonica.* ×14.

Fig. 6. Longitudinal section of mycorrhiza of *Alnus japonica.* ×53.

Fig. 7. „ „ „ „ „ „ „ ×72.

Fig. 8. An old mycorrhiza of *Abies firma* in which renewed growth of young root has taken place. ×9.



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