D. N. Sen:

Root Ecology of *Tilia europaea* L. — I. On the Morphology of Mycorrhizal Roots

**Introduction**

As early as 1885, Frank postulated the theory that mycorrhizal fungi are symbionts closely related to tree growth. Much controversy since then has surrounded the problem of ectotrophic and endotrophic mycorrhizae. The major work in this field has been done by Melin and his collaborators on coniferous trees. They are of the opinion that most of the mycorrhizal fungi lack the ability to produce cellulase and therefore are unable to utilize cellulose as a source of carbohydrates. According to Frank (1885) the hyphae of the lower symbiont act as nutrient absorbing organs. Melin (1925) assumes after Stahl’s “Mineral Theory” (1900), that in addition to inorganic nutrients organic nitrogen compounds may also be made use of by mycorrhizal fungi.

It has been proved by many researches in the recent past that the mycorrhizal symbiosis is of decisive importance from the silviculture point of view, but the real mechanism of this symbiosis is still not clear and well understood. It has been assumed that roots and fungal hyphae exude certain substances, which are important for the initiation of this symbiotic relationship, which as a result bring about morphological variations. Conversion of Pine roots into mycorrhizae is always accompanied by mono- or dichotomously branched swellings of the root tips and an absence of root hairs on the swollen part.

A great deal of this study has been made on the coniferous trees by several workers, which have certain advantages. Little is known from the morphological point of view about the deciduous forest trees. One finds in this connection the works of Clowes (1951), Truszowska (1953), Wilusz (1953), Vlasov (1955), Jeník (1957), and Sobotka (1958). Besides these, there are classical works on coniferous trees by Melin (1927), Bjoörkman (1941), and a summary by Harley (1959). Special mention of the works done on *Quercus* species by Vlasov (op. c), and Jeník (op. c) on the classification of mycorrhizal roots on the morphological basis can be made here. Sobotka (1958) has studied the influence of organic substances in soil on the frequency and anatomy of nutritional roots (mycorrhizal) of *Tilia cordata* in nursery. He concluded that there was no influence on the roots of treated and controlled sets. He has also classified the mycorrhizal roots morphologically.

**Method**

The present study of morphological deviations mainly depend on the records from the seedlings of two years old grown in different soil types: A classified scheme of the soil types has been given in Table 1. The comparison has also been made with the adult trees growing under natural forest conditions in Central and Eastern Bohemia. Roots have been examined under microscope from different soil layers, but such differentiation could not be made in the roots from the experimental pots. In the present contribution no consideration on the anatomical features of the different roots have been made; further results on the root ecology of *Tilia europaea* will be communicated later.

**Observations**

In Lime (*Tilia europaea* L.) mycorrhizal roots dominantly being of ectotrophic type are more suitable for the morphological recognition. According to Clowes (1951, p. 1), “*Fagus* root systems differ conspicuously from that of *Pinus*, which shows inherent morphological differences between long and short roots”. A distinct difference can be ascertained in the long and short
roots of *Tilia europaea* by close observation. The difference might be due to mycorrhizal roots which have been found developing as small branches on the long roots. As a matter of fact it is difficult to miss the mycorrhizal roots of ectotrophic type if they are closely and properly examined under a lens. As far as the study of the author goes there is not much difference morphologically between the roots of *Fagus* and *Tilia*. Whether the character of the short roots is inherited or is the result of fungal presence is difficult to say, because some short roots, though lacking an outer mantle do show morphologically similar features to those which have an ectotrophic mantle. It is for certain that the presence of external fungal mantle on the normal roots must bring about some changes in the external morphology as described in the following pages, and also seen in other examples worked out by different authors. It may also be possible that only endotrophic type of mycorrhizae may not bring about any distinct morphological variations from the other short roots of limited growth as seen in case of *Fraxinus excelsior* (Jeník and Kubíková, 1961). The occurrence of morphological variations have been attempted in a semi-diagrammatic sketch, observing this state of affairs for mycorrhizal roots only, (Fig. 1).

The diagram, though it looks a little odd in the beginning, tries to explain the degree of morphological change in lateral roots of limited growth through mycorrhizal infection. The Pine mycorrhizal roots show a sharp distinction from non-mycorrhizal ones forming very often remarkable dichotomous branching; in Lime the short mycorrhizal roots differ by several forms from the uninfected ones, but the distinction is not so sharp as that in Pine; whereas in the case of Ash there is no external morphological distinction between mycorrhizal and non-mycorrhizal short roots. The last mentioned genus shows an endotrophic mycorrhiza, which is usually connected with the special anatomical make up of the root (presence of exodermis! — from an unpublished account on Ash by Jeník and Kubíková).

**Enumeration of Naturally Occurring Mycorrhizae**

A number of morphological deviations have been noted in Lime, which have been ennumerated in classified form as per system followed by Vlasov (1955) and Jeník (1957) for morphological forms rather than type of infection.

Slankis (1958) states that there is a close relationship between the degree of deviations in root morphology and the concentration of auxin applied in case of Pine in his experiments. It may be inferred that these different morphological deviations in mycorrhizal roots depend upon the concentration of auxin given out and the specific form of mycorrhizal fungus. In the case of *Tilia* the swelling of short roots leading to different shapes and forms, depending on increasing intensity of infection, cause absence of root hairs on these swollen roots, the presence of distinct fungus mantle of different forms in most cases, and also branching at times. No ectotrophic mycorrhizae were observed in long roots, nor the presence of hairs on mycorrhizal roots, contrary to what has been observed by Clowes (1951) in *Fagus sylvatica*.

It is interesting to note that Truskowska (1953) in her survey reported *Tilia parvifolia* in *Fraxinetum-Alnetum* association to be endotrophic. Henry (1933) also recorded *Tilia americana* to be endophytic in his woodland types. The forms of mycorrhizae in the same plant and the soil under similar conditions are so variable that it becomes difficult to put them in a proper
scheme of classification. However an attempt has been made here to enumerate the morphologically different forms of mycorrhizae occurring in nature. Not much stress has been given in this classification on the laboratory experiments, considering that the ecological deviations should be worked out mainly under natural conditions.

Fig. 1. Mycorrhizal root series in case of Pine (a), Lime (b), and Ash (c), (Semi-diagramatic) (2 ×).

I - Swollen, nodule-shaped:

Swollen nodular ends have been observed as a normal feature with most of the short roots ultimately becoming mycorrhizal of one type or the other. Mother roots on which these roots are borne do not become mycorrhizal. Root hairs were very distinct on the mother root, but absent from the base of the swollen part. It is also possible that these may remain non-mycorrhizal. Seedlings grown in soil type LW showed this kind of variation more (Fig. 2, Plate XXII E).

II - Club-shaped:

Club-shaped mycorrhizal roots are quite common and characteristic, which have swollen tips with tapering long or short stalks like that in a club. Under this kind might occur five forms which are a little different from the other in their fungal pattern.

(a) Setaceous: This form has a swollen apex with fine hyphae projecting in the form of setae of equal length all around. It was noted in the seedlings grown in soil types LD, LW, CD and CW, (Fig. 3, Plate XX A and B).
Fig. 2. Different forms of swollen or nodule-shaped mycorrhizae with distinct root hairs on mother root (50 ×).

Fig. 3. Club-shaped: Setaceous mycorrhizal roots with distinct fungal setae at the swollen tips and root hairs on mother roots (50 ×).

Fig. 4. Club-shaped: Glabrous mycorrhizal roots with root hairs on mother roots (50 ×).
(b) **Glabrous**: The roots here are yellow to light brown in colour and have a straight club-shaped structure with slightly smaller stalks than the setaceous forms. Apparently the roots do not show any marked fungal presence. There might be some similarity between this and the previous form. The mother roots bearing mycorrhizal roots show abundant root hairs. Seedlings grown in soil type SW show this form in abundance, (Fig. 4).

(c) **Hairy**: This form has stiff and long projecting hyphae all over the mycorrhizal roots. It was recorded from the seedlings grown in soil type HD. In natural forest it was observed in clayey-loam layer also, (Fig. 5, Plate XXI C and D).

(d) **Loosely wefted**: The mycorrhizal roots may be in bunches or in pairs. They are of light brown colour and covered in a loose weft of hyphae all around. It was recorded from soil type SD, (Fig. 6, Plate XXII F).

(e) ** Branched**: A very similar form to that of spindle-shaped glabrous is the branched type. It has root hairs on the stalk of the mycorrhizal roots and is also branched quite irregularly. It is dark brown in colour and the fungal-mantle may be indistinct, (Fig. 7, Plate XXIII G) or distinct, (Plate XXIII H). It was noted in seedlings grown in soil type LW.

**III - Spindle-shaped:**

Spindle-shaped and club-shaped have similar variations. The mycorrhizal roots in this case lack the stalk bearing the swollen part, but the whole root is nearly pointed at both ends like a spindle. The following forms have been noted.

(a) **Setaceous**: It bears the small fine setae of equal length all over the surface of the mycorrhizal roots. The colour ranges from light to dark brown. This form was also observed from the seedlings grown in soil types LW and HW, (Fig. 8).

(b) **Glabrous**: This form is very similar to the other two described above and below in its colour, but it does not possess any distinct hyphae externally in any form. It is possible that on sectioning it might reveal some sort of mycorrhiza. It was noted in the seedlings grown in soil type LW also with other forms, (Fig. 9).

(c) **Mantled**: The root in this case is dark brown and has a covering of fungus mantle with hair like stiff hyphae coming out into the sand to which were attached a number of particles of sand, not easy to separate. The mother root bore root hairs. It was recorded from the seedlings grown in soil type SW under room temperature, (Fig. 10).

(d) **Hyphal Woven**: In natural forest it was recorded from brown loam with a thick black cover of hyphae all over the mycorrhizal roots. At one spot where the roots were found growing inside dead old rotten wood, the stiff projecting hyphae were found traversing through the whole length of the mycorrhizal roots, (Plate XXIV I).

**IV - Non-swollen or slightly swollen:**

Some mycorrhizal roots do not present any characteristic shape, and they are either non-swollen or slightly swollen at their tips. They are almost of uniform thickness throughout. Two distinct forms have been noted under this type.

(a) **Glabrous**: This form of mycorrhizal roots was recorded from about eight months and also two years old seedlings in soil types LW and CD. A progressive degree of glabrous
mycorrhizal roots could be very nicely observed. In younger roots the initiations of short roots in form of dots, (Plate XXIV J), and in older the short roots of uniform diameter throughout with slightly swollen tips can be seen, (Plate XXV K and L).

(b) M a n t l e d : Some mycorrhizal roots which branch racemously were found completely covered with a weft of fine fungal mantle of white colour all over. These roots were almost of uniform thickness and do not show a distinct swollen part. It was also observed from the seedlings grown in soil type LW, (Fig. 11).

V- Coralloid racemosed:

This type of mycorrhizal roots recorded from natural forests was found growing mostly in humus layer. The roots were racemously branched on the mother roots forming a group with rough surface. It was of dark brown colour and showed very fine hyphae in the form of setae when seen under the microscope, (Fig. 13).

From the above classification one gets an idea of deviations in the morphology of mycorrhizal roots, but this is not fixed or strict for the different soil types in which they have been mentioned to be occurring. A number of forms can possibly be seen occurring on one and the same mother root of the seedling, which bears them all in a series as is clear from the Figure 12, of a root recorded from a seedling about two years old grown in soil type LW under room temperature. A classified scheme of different mycorrhizal roots occurring in different soil types as described above is given in Table 1.

D i s c u s s i o n

Fig. 13. Coralloid racemosed type of mycorrhizal roots (2 x).

From the survey of the above account it can be seen that a variety of mycorrhizal roots exists in Tilia.

The above mentioned variety of mycorrhizal roots could be explained by the presence of different species of fungi and the different amount of auxins produced by them.

As shown by SLANKIS (1958) for Pine, it may be expected that the auxins exuded by the mycorrhizal fungi would not be strictly confined to mycorrhizal roots but would be partially translocated into the whole system of the root. Taking into consideration this presumption for Tilia, the presence of non-mycorrhizal but morphologically similar short roots could be explained here. But with this assumption one is confronted with another problem. Why this fungal produced auxins should effect only the short end-roots to bring a deviation from the normal morphology, so that it looks similar to mycorrhizal roots, and at the same time why should it not effect the long roots? No
mycorrhiza has been noted for long roots in the case of Lime. The same question might hold good for *Quercus* species also, where Jeník (1957) states that mycorrhizal roots are the changed forms of end-roots of limited growth, and no mycorrhiza was noted on long thick roots, though the presence of ectotrophic mycorrhizae have been reported by him for this tree. The explanation of this phenomenon is supposed to be in a different physiological activity of the long root which even covers the influence of the auxins. Therefore the primary differentiation into long and short roots dominates for the occurrence of mycorrhizae also, and so the influence of the auxins comes in the second place.

**Table 1.**

Morphology of the Mycorrhizal roots according to Soil types

<table>
<thead>
<tr>
<th>Kind of Soils</th>
<th>Symbol</th>
<th>Mycorrhizal Forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>SD</td>
<td>Club-shaped: Loosely wefted.</td>
</tr>
<tr>
<td>Wet</td>
<td>SW</td>
<td>Club-shaped: Glabrous; Spindle-shaped: Mantled.</td>
</tr>
<tr>
<td>Clay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>CD</td>
<td>Club-shaped: Setaceous: Non-swollen or Slighty swollen: Glabrous.</td>
</tr>
<tr>
<td>Wet</td>
<td>CW</td>
<td>Club-shaped: Setaceous.</td>
</tr>
<tr>
<td>Loam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>LD</td>
<td>Club-shaped: Setaceous.</td>
</tr>
<tr>
<td>Wet</td>
<td>LW</td>
<td>Swollen or nodule shaped: Club-shaped: Setaceous, Branched; Spindle-shaped: Setaceous, Glabrous; Non-swollen or Slighty swollen: Glabrous, Mantled.</td>
</tr>
<tr>
<td>Humus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>HD</td>
<td>Club-shaped: Hairy.</td>
</tr>
<tr>
<td>Wet</td>
<td>HW</td>
<td>Spindle-shaped: Setaceous.</td>
</tr>
<tr>
<td>Forest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humus Layer</td>
<td></td>
<td>Coraloid Racemosed.</td>
</tr>
<tr>
<td>Clayey Loam</td>
<td></td>
<td>Club-shaped: Hairy.</td>
</tr>
</tbody>
</table>
Clowes (1951) makes no notice of this problem at all when he states that both long and short roots of *Fagus silvatica* may become mycorrhizal, and ectotrophic being more frequent. He also states that *Fagus* root system differs conspicuously from that of *Pinus*, which shows inherent morphological differences between long and short roots, and further that the occurrence of short roots is a character of the plant (*Fagus*) and not the result of mycorrhizal infection. Robertson (1954) also states in case of Pine that the cortex of long root is also mycorrhizal, having a well developed Hartig net but no pronounced mantle. And further he states also that the infection of the long root does not lead to any morphological change, which is obvious to the unpractised eye. But he gives no reason for the later case. No reason for this difference in short and long roots has been given by any of the above mentioned authors, except for Slankis (1958) who considers it due to the large difference in volume between the mycorrhizal and the long roots.

From the observations made by the author for short morphologically similar non-mycorhizal roots which in most cases have a great development of tanin in the rhizodermis and the cortical cells, so that the cells are filled with it, and turns red-brown, comes out a probability that it acts as a barrier for fungal infection. But the development of tanin in cortical cells take place where the fungus infection has taken place and a mantle is formed, so that the cortex can be marked sometimes into two regions, the outer cells with tanin and the inner ones without it. At the same time long thick roots have been observed without any tanin and are not infected. It appears that it is not a decisive factor but its importance will be discussed later. Slankis (1958) shows for Pine that auxin exuded by fungus determines broadly the sequence and frequency of short and long roots and thereby the pattern of the root system.

Keeping all this in view it becomes quite difficult to put the morphogenetic deviations of Mycorrhizae in *Tilia* in any of the systems of older classifications. Melin's (1923, 1927) classifications do not hold good considering the deviations observed in Lime. An attempt has been made by Clowes (1951) to classify the types of mycorrhizal infection of Beach upon external morphology for a deciduous tree. Types have been separated by mode of branching, diameter and colour etc., but he feels that in general the kinds very much grade into one another. Vlasov (1955) has stressed first the colour and the shape of the mycorrhizae in his classification for Oak. Jenik (1957) mostly classifies the different types of ectotrophic mycorrhizae of Oak on morphological forms and the arrangement of fungal mantle on them. Sobojka (1958) has classified the short lateral roots (mycorrhizae) of *Tilia cordata* into five classes, mostly depending on the colours, measurements and their adherence to the soil. It appears that he missed several forms in his study which is based only on seedlings.

The author is unable to fit the variety of forms observed in Lime in any of the classifications mentioned above. The classification of Sobojka (op. c.) appears to be arbitrary and from his few photographs it is difficult to make a distinction into a great variety of forms occurring in Lime. Among all the classifications mentioned above, the author agrees most to the ones put forth by Vlasov (1955) and Jenik (1957) which appear to be more helpful for enumerating different forms by shape and fungal pattern on mycorrhizae. The colour changes here from yellow to brown black, the dimension of the
similar roots on a mother branch is so different and variable that it is again
difficult to make a correct assessment in general. The anatomical differences
are not discussed in this paper. A classification has been proposed on the
basis of records made from the seedlings grown in culture pots under natural
conditions with a comparison to naturally growing trees in the forest.

Summary

1. The roots of seedlings and adult trees of Lime (Tilia europaea L.) have been studied.
It has been observed that great morphological deviations occur in mycorrhizal roots. A scheme
for the classification of such distinct mycorrhizae has been worked out.

2. The scheme for the classification does not present much similarity with other proposed
upto this time, not even the one proposed by Sobotka (1958) for Tilia cordata L., but agrees
to a certain extent with that of Vlasov (1955) and Jenik (1957) given for Quercus species.

3. In Pine there is a sharp distinction between mycorrhizal and non-mycorrhizal roots;
in Lime this difference is not so distinct though they differ from the uninfected ones by several
forms, whereas the endotrophic mycorrhizae in Ash show no external morphological distinction.
These trees, mentioned as examples, appear to present a mycorrhizal series.

4. It has been observed that there is a more variation of forms in seedlings than in adult
trees. Sobotka (op. c.) whose study is mainly based on seedlings of Tilia cordata L., does not
mention such a variety of forms described here in the text. The greater variability of mycorrhizae
has come out by the use of a larger number of soil types and by the study of adult trees.

5. It is presumed after other works that non-mycorrhizal roots with similar morphology as
that of the mycorrhizae, are the result of the flow of certain substances produced by the fungus
in the root system. The presence of tannin in short roots probably does not play a decisive role
in this connection, but its importance will be discussed later.

Acknowledgements

This work is being carried out under the guidance of Doc. Ing. J. Jenik, to whom I am very
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sincere thanks to Prof. Dr. B. Fott, Chief of the Botanical Institute, for his interest in the work
and providing facilities for publication; to Dr. J. Kubiková for help in laboratory work. I am
also grateful to the governments of Czechoslovakia and India, for the award of this research scho-
larship, which enabled me to pursue this study.

D. N. Sen:

Ekologická studie kořenů lípy (Tilia europaea L.). —
I. Morfologie mykorrhizních kořenů

Autor studoval kořeny semenáčků a vzrostlých stromů lípy (Tilia europaea L.). U mykorr-
 rhiz této dřeviny byla pozorována značná morfologická proměnlivost a na základě srovnávacího
studia v různých půdách bylo vypracováno schema pro jejich třídění.

Navržené klasifikační schema není v žádném vztahu k dřívější použitým klasifikacím, ani
k třídění, které pro mykorrhizy lípy navrhl Sobotka (1958). Třídění navazuje do určité míry
na schema vypracované Vlasovem (Vlasov 1955) a Jeníkem (Jeník 1957) pro dub.

U borovic je morfologicky odlíšení kořenů mykorrhiziních od kořenů nemykorrhiziních velmi
ostře; u lípy je tato diferenciaci řádově nižší a konečně u jasanu, který má mykorrhizu endo-
trofní, nevzniká žádné vnější rozdělení. Tyto dřeviny, jmenované jako příklad, představují
tedy s hlediska stupně své morfologické diferenciacie vůči kořenům neinfikovaným určitou mykorr-
 rhizní serii.

Autor pozoroval větší variabilitu tvarů mykorrhiz na mladých semenáčcích nežli na vzrost-
lých stromech. Sobotka (op. c.), opírající se převážně o studium semenáčků lípy malolisté,
(Tilia cordata L.), se nezmiňuje o takové proměnlivosti forem, která je v této práci popsána.
Rozdíly ve výsledcích vznikly zajisté použitím většího množství různých druhů půd a studiem
mykorrhiz u vzrostlých stromů.
Podle teoretických výsledků fysiologico-anatomické práce Slankisovy (Slanks 1958) se dá předpokládat, že morfologická podobnost některých nemykorrhizních kořenů s mykorrhizami je výsledkem přesunu určitých morfogenetických substancí, produkovaných mykorrhizní houbou, do celého kořenového systému. Přítomnost tánínu nemá pravděpodobně v této souvislosti rozhodující úlohu, jak se o tom autor ještě podrobněji zmíni při anatomickém popisu kořenů lipy.

References


Explanations of Plates

Plate XX — A and B. Club-shaped: Setaceous mycorrhizal roots (120 x).

Plate XXI — C and D. Club-shaped: Hairy mycorrhizal roots (50 x).

Plate XXII — E and F. Swollen or nodule-shaped mycorrhizal roots, with distinct root hairs on mother roots (120 x). Loosely-wefted mycorrhizal roots (150 x).

Plate XXIII — G and H. Club-shaped: Branched mycorrhizal roots with indistinct fungal mantle in G, distinct in H (70 x).

Plate XXIV — I and J. Spindle-shaped: Hyphal woven mycorrhizal roots (120 x). Non-swollen or slightly swollen: Glabrous type with root initiations (120 x).

Plate XXV — K and L. Non-swollen or slightly swollen: Glabrous mycorrhizal root of uniform diameter with a little swollen tip in K (120 x).

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