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DR. K. CEJP:

Notes on the biology of some Hepatics.

One speaks generally of the mycorrhize in hepatics, as they were first observed by KNY in *Marchantia* and *Lunularia* and by BÖTTCHER in 1879. Then, in 1897, JANSE described mycorrhize in the trop. genus *Zoopsis*. According to him the mycorrhize is as an endophyt-fungus a facultative aërobiont which penetrates the texture and assimilates nitrogen from the air. The plant gives it protection and carbohydrates.

The mycorrhize of hepatics was studied in detail by NĚMEC, especially in foliose-hepatics, with special regard to *Calypogeia trichomanis*. He found (1), that mycorrhize is widely spread among them, being only absent in *Lophocolea bidentata* and in one specimen of *Lepidozia reptans* growing on loam soil; in shady places rich in humus it had mycorrhize. Then, in places where there were still other kinds of hepatics, *Jungermannia bicrenata* had mycorrhize, where as the other kinds were without. In the rhizoid this hepatic had fungus-filaments, thin and scanty septate, and besides also thick filaments numerously septate, further hyphes with roundish cells, finally breaking down. Specimens grown in cultures are more exposed to the fungus than wild growing ones, and here the fungus appears as a distinct parasite as attacked cells generated.

Now I have to refer to the observations mady by NĚMEC in *Calypogeia*. Within the rhizoids of this hepatic, which come into contact with the substrat, hyphes were observed, carrying lateral short branches. Often they were seen to have pierced the membrane in several places, and sticking out. These mycels were but little articulated, with small nuclei within the cells, several in each cell. At their end the rhizoids are conically inflated, and in this part the hyphes are usually tangled in the depth, with one end often sticking out and continuing to grow. Where there are two parallel hyphes, they are often connected by a transverse one. At the base of the rhizoid, i. e. at its origin, the hyphes shorten and form a kind of pseudoparenchymatic network pressed close to the membrane of the neighbouring cells. This network has one or more layers, sometimes filling even the whole upper part of the cell, from which the rhizoid developed. These fungus-cells send into the neighbouring cells of the plant special digitate haustories of equal length, straight, often a little conical at the end, with a hyaline membrane. It is interesting that in the cells into which the shoots, the nucleus approaches them. Besides these endophyts there are many hepatics entangled and enveloped in the mycelium of the fungi which in many places develop even receptacles. In the genus *Calypogeia* NĚMEC folloved the thin Peziza *Mollisia Jungermanniae*, a tiny greenist blue fungus which lives on all living parts, in such places are living cells of normal organization. It has not yet been fully proved whether the endophyt mycelium and *Mollisia* are one and the same kind, though in cultures this seems to be so and that the hyphes present in the mycorrhize are bet transformed by circumstances. Cultures

grown in wet surroundings are positively heliotropical. Under vertical light they grow straight up. By experiments NĚMEC wished to ascertain whether the plant may exist without hyphes. Plants with hyphes were cut off and laid on sand. They grew rhizoids in which most were mycorrhize. Further plants were cut off, the hyphes removed under the microscope, and the plants again laid on sand. Of twenty specimens eight had mycorrhize, twelve had none. Plants with hyphes forming a network grew better than not infected plants which were more liable to droop. Finally, when all the spores of the receptacle of *Mollisia* had carefully been removed, cultures were grown and inoculated into sand, in which cut-off plants without mycorrhize were grown. After a short time almost all specimens grew beautifully and had hyphes in their rhizoids. Further NĚMEC studied how the hyphes intruded into the plants. For these studies he used *Calypogeia* from places rich in humus and sand. Here very complicated conditions were followed. The fungus enters the plant through the rhizoids, then in the upper part the pseudoparenchymatic network is formed from which digitate haustories grow into the neighbouring cells. This stage continues for a long time. In older plants or parts the haustories are usually conical. In dying parts they grow again and continue as new parts of the hyphes. When a part of the plant dies, a differentiation sets in. In some cases the thin-walled hyphes degenerate, the thick-walled hyphes continue to grow, pierce the dead part and again infect the plant (2).

I studied *Calypogeia trichomanis* from a place of two different substrats, near Strašice, specimens from a soil very rich in humus and specimens from the neighbouring stumps. The specimens from the humus had well developed mycorrhize, where as those from the stumps had none. In the first case the hyphes of the rhizoids run upwards until they formed the well known pseudoparenchymatic network, but they did not form any haustories with the exception of some few cases. Specimens without mycorrhize grew very well in cultures, just as those with mycorrhize. Most specimens have on their surface heaps of mycelium-filaments which cannot be well removed and these can easily infect cultures of the plants. This is in keeping with GOLENKIN's opinion (3), who has worked much at the problem of mycorrhize in hepatics, that in some kinds mycorrhize is but facultative, a plant must not always have the fungus to live, perhaps only when it grows on a substrat under certain circumstances.

Further I followed that in conical rhizoids of *Calypogeia* the hyphes form a huge coil at the base and besides there are long, sparsely articulated, longitudinal hyphes stretching into the neighbouring cells, where they attach and fill the whole cell as a dense network of twisted hyphes. Similar nests in cells were followed by GARJEANNE who, just as NĚMEC, describes the formation of pseudoparenchymatic network staining brown-red with iodine and this indicates glycogen. GARJEANNE (10) followed also germinating fungus spores in the hyphes stretching into the rhizoid. The leaves of hepatics have numerous algae, often in colonies, and here the formation of the lichen was followed, how the fungus hyphes sticking out of the rhizoids or of the outer cells of the axis and leaves break through and wind round the algae-colonies. PEKLO studying the problem of mycorrhize in all Muscineae, adds that haustories does not always appear in other kinds. In younger networks the plant is fairly resistant against the intrusion of hyphes or haustories into the cells, but hyphes will intrude into the very centre of dying networks, and here continue to grow so that gradually they appear here as a parasite. According to NĚMEC (1) the haustories are the

organs by means of which the hyphes break into the neighbouring cells of the host from which they take their food. But he also admits that the plant stimulates the fungus to secure a better metabolisme in such places. It is sure that there is no ordinary intrusion of the hyphes into the cells, as the branches are of equal length so that they neither continue to grow nor otherwise hurt the plant. The movement of the nucleus towards the haustories is explained as produced by traumatic irritation due to the intrusion of digitate shoots into the cells. Later on GARJEANNE (Flora 1911) was able to isolate hyphes from folious hepatics and to cultivate *Mucor rhizophilus*. He made many experiments by infecting with this fungus and followed that infection always takes place at a certain time of the year and that it also depends on the nourrishment of the hepatic! — Quite recently an interesting case was described by MAGROU, Ann. Sc. Nat. 1925 in *Pellia epiphylla*. Numerous thallus of this hepatic, infected at the time of the maturation of sporogon, showed complete desintegrition, where as in younger thallus the hyphes in the neighbourhood of the archegons were seen to degenerate, so that the sporophyte part was not infected at all. Spores of *Pellia* cultivated on infected soil, was infected soon after its blossoming and died. But in some cases there were still certain meristematic zones from which new plants developed. Without hyphes the spores throve only on a substrat of ph = 4·85.

Far better known is the mycorrhize in frondose-hepatics, especially in the genus *Fegatella*. GOLENKIN followed mycorrhize in *Fegatella conica, Marchantia paleacea. Preissia commutata*, less in *Plagiochasma elongatum, Targionia hypophylla*. In rhizoids he found hyphes even in the thallus. In *Fegatella* these biological problems were also studied by BOLLETER, CAVERS, BEAUVERIE and NICOLAS. BEAUVERIE (5) followed hepatics in the vegetative stage and considers the fungus as a representative of the genus *Fusarium* after the culture he had been able to grow, though unfortunalety without obtaining seeds. Like GOLENKIN he describes infected specimens as resistant in comparison to the weak specimens without hyphes. According to him infection depends on the presence of humus the more humus, the greater the mycorrhize. Hepatics on soil without humus never reach such dimensions and have no hyphe-filaments in their body. With regard to respiration he followed that respiration increases assimilation, chlorophyle being but little active as its function is disturbed by fungus. From this BEAUVERIE draws the conclusion that the fungus helps the hepatic to draw part of the carbon and nitrogene for its nourishment from the humus, so that the hepatic becomes partly a saprophyte by means of the fungus. BOLLETER (6), in his morphologic-physiological monography of *Fegatella*, describes the presence of hyphes in thallus-cells and says that these cells stained red contain fungi. Here the hyphes have smooth walls, ramifications, and are filled with a granular plasme with very scanty membranes. They do not stretch into the network crowded with chlorophyle. BOLLETER finds a relation between the red stained membranes and the presence of hyphes, and the premature development of the sexual-organs. He followed that plants from a substrat rich in humus have heaps of mycorrhizes, where as there are none in plants from dry roks (and calcareous tuff). With regard to the relation between fungus and hepatic, he is of the opinion that perhaps the plant does not benefit at all by the fungus, but that the fungus is not injurious either, at the utmost absorbing some substances from the network. Recently CHAUDURI and RAJANAN (15) followed a fungus-infection in *Marchantia nepalensis* and according to them the infected cells are stained red and the infected thallus are remarkable especially by their resistance. The

interior hyphes, too, develop numerous chlamydospores. Therefore experiments were made with hyphes, hyphes and hepatics being cultivated separately, so as to prove that hepatics cannot exist without fungi and that this is the case of genuin symbiosis. But they only showed in cultures on highly maltose soils, that the fungus absorbs carbohydrates from its host, without ascertaining what it gives in return.

CAVERS (8) followed the development of spores in *Fegatella* and arrived at interesting conclusions. In sterilized soil they grew but little, giving small elongated thallus, whereas in not sterilized soil under unfavourable conditions fairly hupe thallus developed, infected by the fungus. Then these experiments were repeated by NICOLAS with the same results. PEKLO (4) however states that in *Fegatella*, mycorrhize which is no constant feature, was followed in plants from sandstom roks, therefore in specimens poor in water and light, directly clinging to the rocks and not on a humus-substrat. Then also in specimens from roots and bark of forest-trees.

NICOLAS (7) followed the thallus in various places and altitudes. Sterile thallus gathered in the region of Toulouse had all mycorrhize, were but little starch less elongated and thicker. Other circumstances, as altitude, humidity of the air, light, may have similar influence on the development of the sexual-organs as the presence of hyphes. Recently I followed hyphes in the body of *Marchantia polymorpha*, looking especially for the reasons of the presence of fungi within the cells of hepatics. For this purpose I studied specimens from various substrats and places, and I may say that there is no general rule with regard to infection by fungus. *Marchantia* is a hygrophile hepatic growing mostly on damp rocks, in notches, a. s. o., less on humus-soil. Though it is true that specimens from humus are richer in mycorrhize, most of the specimens from other substrats likewise harbour hyphes. Fewest or nearly no hyphes have specimens from damp, clayly soils. How mycorrhize depends on different conditions, is best indicated by such cases where in some places frondose-hepatics have no hyphes at all, where as some foliose hepatics growing in the same places and swingling with the others, have rhizoids full of hyphes.

With regard to the individual thallus, the infected thallus pieces carry far more sexual-organs than specimens without hyphes. There would be therefore a certain relation between the presence of hyphes and the sexuality of the thallus. This problem was already discussed by BOLLETER and NICOLAS in *Fegatella*. BOLLETER even describes a covering of *Fegatella* from the botanical garden in Zurich, sterile for eight years because it had no trace of any hyphes in the tissue. — Then also the sexual-organs mature earlier in thallus infected by fungi than in not infected one. NICOLAS (13) followed in many sterile thallus from various places the absence of fungi, whereas thallus with sexual-organs always had mycorrhize.

Marchantia has two kinds of rhizoids: one of them smooth, more numerous, the other with strobiliform thickenings on the inner side and less numerous. This second kind never had any hyphes. The hyphes run through the rhizoids either individually; often several hyphes are parallel, stretch into the thallus tissue, and sometimes the end of the hyphes may even reach into the carpophores. PEKLO mentions a case where the fungus even spread into the walls of the antheridium hallows. The hyphes never entangle in a coil which would fill the whole cell, as it is the case in some foliose hepatics, they do not even form a pseudoparenchymatic network to suck from the neighbouring cells. The filaments only run through the tissue. In comparing the filaments with each other

we follow, that there are often considerable morphological differences. In the rhizoids of *Marchantia* I followed thin slender filaments with scanty membranes besides thicker hyphes with membranes closer together, and with shorter cells even with detaching conidies. Other filaments ramify. For these reasons the observations of various authors differ very much, and probably we have quite a number of different kinds of fungi living in different kinds of hepatics, and the mycelies of which spread once in humus, once also in other soils. It would be well if — as already stipulated by KAVINA (11) — the individual filaments could be isolated and grown in separate cultures until they develop receptacles, so that they might be more easily determined and the influence of each kind followed separately. Hitherto a representative of the genus *Pythium* was ascertained beyond doubt, followed by NICOLAS (9) in *Marchantia polymorpha.* Besides other mycorrhize-filaments he followed straight hyphes with scanty membranes, having in the interior of the cells globular thickenings of the dimensions 8—10 × 10—15 μ, either terminally isolated or rosary-shaped. According to these features they would belong to the genus *Cladochytrium*, but he also followed at the end of some filaments globes with thick walls, 12—13 μ in diameter, some carrying besides a pedunculated antheridium. Apparently in the first case sporocystes, and in the second oospheres were followed, and therefore they would represent the genus *Pythium.* Besides KAVINA mentions as fungi usually followed in hepatics: *Pythium de Baryanum,* especially in *Riccia* and *Pellia,* then the genera *Botrytis* and *Cladosporium, Helotium* and *Leptospora musciola*, living chiefly in some foliose hepatics. Apparently, howerer, these genera have nothing in common with the fungi of mycorrhize, as here we seem to have to do with true parasitism, though otherwise hepatics are sufficiently protected against parasitism, their cell-membrane being rich in sphagnol as stated by CZAPEK. In *Fossombronia Wondraczeki* there lives a fungus which breakes into the sporogons and disturbs the spores. In *Fegatella* NICOLAS describes two kinds of spores, larges ones, of several, cells easily germinating, without any traces of fungus infection; and smaller ones, of but one cell, badly germinating, and with fungus hyphes. Apparently here, too, there is a parasitic fungus which has nothing in common with the fungi of mycorrhize. — Similar well known parasitic fungi are *Saprolegnia Schachtii* in *Pellia epiphylla, Pythium cystosiphon* in some *Riccia,* a. o.

Now with regard to the relation between the mycorrhize filaments and the plant, some authors speak of parasitism on account of the formation of haustories of the probable sucking of nourishing substances from the body of the hepatic, or because the hepatic does not need the assistance of the fungus-filaments in absorbing certain substances from the humus, as it produces itself carbohydrates. Others however speak only of symbiosis. And really we do not know whether the fungus profits by the symbiosis, whether it is only a case of hospitality or whether it really takes in every case some nourishing substances from the body of the host. In the case of the hepatic of course, seems to profit in some respects by the presence of the fungus, (increase of the thallus to a certain degree, absorption of certain substances from the humus, relation to the sexual-organs, a. s. o.) In our opinion it is a case of an incidental symbiosis as well as of an incidental parasitism, that in hepatics many kinds of fungi live behaving differently under certain circumstances. In any case, mycorrhize in hepatics is no stable, uniform feature occuring always and everywhere as in some higher plants, and here in certain cases it assumes parasitic character. In some

cases we may speak of harmless parasitism, where the fungus does not imperil the host at all, though it does not profit by its presence; or there is a moderate parasitism, where the fungus takes certain substances from the plant without giving anything in return. That howerer in some cases the plant stands in close relation to the fungus, is proved by various cases of thallus without filaments and with hyphes in their interior, as well by various cultures.

Therefore the term mycorrhize does not apply to all cases of the relation between fungus and hepatic, it ought to be defined more accurately or at least stated as special case of mycorrhize in hepatics.

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