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METHODS OF INTRODUCING MYCORRHIZA-FORMING FUNGI
INTO SOIL OF USSR FOREST BELTS

V. Ya. Chastukhin

In the reforestation of steppes, the problem of mycotrophic nutrition of trees has assumed great significance. The importance of fungi in the life of trees has been demonstrated, but not enough is known for practical purposes about the organisms forming mycorrhizae.

Woodland soils are very rich in fungus flora. Many groups of fungi exist, but only a few, chiefly pileate fungi, are able to form mycorrhizae.

Most synthetic mycorrhizae have been produced with these organisms. It has been experimentally proved that mycorrhizae can be produced by many varieties of *Amanita muscaria*, *cortinarvius*, *Russula*, agarics, "maslyaniki", and representatives of the *Boletus* genus, such as the white mushroom (*Boletus edulis*), the *Boletus scaber* (edible mushroom), the "maslyaniki", etc.

It is highly probable that the lisichki, mokrukhi, and ezheviki (hedgehog mushrooms) also belong in this category. Various forms of truffles (tuberaceae) which are classified as ascomycetes also produce mycorrhizae.

In regard to the problem of specifying the mycorrhiza-forming fungus for individual varieties of trees, several groups can be isolated.

Some of the groups are highly specific, for example, the *Boletus elegans*, the specific variety for larch. Somewhat less specific are certain types of *Boletus*, for example, *Boletus luteus*, which forms mycorrhizae on a number of conifers (pine, spruce, etc). According to Melin (10), the least specific variety is *Amanita muscaria*, which forms mycorrhizae on various conifers and also on larches (for instance, birches).

In addition to true mycorrhiza-forming fungi, there are pseudomycorrhizae which have a harmful effect on trees.

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Hence, in artificial infection of future woodland soils in reforestation of the steppes, the first question must be what fungus from what site is most desirable for infecting woodlands. From this viewpoint, the study of mycorrhiza is especially significant.

We shall cite only general data from our observations on the fungus flora of oak and pine groves and from those made on the steppe rock shelf by the Institute of Agriculture imeni Dokuchayev.

The oak groves of the wooded steppe zone possess an unusually rich fungus flora, much of it mycorrhiza-producing. Scores of varieties have been recorded in the autumn in small experimental areas. Among them are such varieties as *Boletus subtomentosus*, *Amanita phalloides*, many varieties of *Cortinarius*, and *Russula*. In addition, the oak groves of the wooded steppe zones are strongly infected with the undesirable *Agaricus melleus*.

Compared with natural oak groves, the groves in the planted steppe forest belts investigated by us possessed a much poorer fungus flora both in quantity and quality. The presence of this limited number of varieties may depend on both the conditions of contamination and the ecological situation.

The predominant varieties of mycorrhizal fungi in forest belts were the *Inocybe* and the *Russula*.

Pine groves have an unusual abundance and variety of mycorrhiza. Here we could trace the gradual changes in these varieties according to the age of the planted trees.

The pioneer in young plantings of 3 to 4 years' growth was the mycorrhiza *Boletus luteus*; next came the *Lactarius rujus* or *Amanita muscaria* which developed luxuriantly, and, last of all, the *Boletus edulis*, which developed when the planting had matured.

In the basic belts inspected, *Boletus granulatus* had developed.

It is obvious that a detailed knowledge of fungus flora as affected by the conditions of natural forests and existing forest belts is needed in accurate experiments on the selection of the most favorable varieties introduced to solve problems of acclimatization, specificity of varieties of mycorrhiza, etc.

In addition to the importance of the mycorrhiza in growing trees, its usefulness as an alimentary product is very significant, since many of the mycorrhizal fungi are valuable as food--the white mushroom, the *Boletus scaper* and the "maslyaniki."

There are three methods of introducing mycorrhizal fungi into wooded belts: (1) infection of seeds and seedlings in planting by forest humus containing mycelia of mycorrhizal fungi, (2) infection by pure cultures, and (3) infection by fruit bodies and spore emulsions.

These methods have not yet been well studied, and, in practice, only the method of infecting seeds and seedlings by forest humus has been applied. The two other methods require further research before application, but may prove very useful.

Contamination by Forest Humus or Mycorrhiza

The essence of this method is that forest humus, containing mycelia of mycorrhiza-forming fungi, is introduced while planting seeds or seedlings in the soil. As the tree develops, infection of the roots must take place. This method has been successfully employed by various research workers--N. A. Yurre (6), Baraney (1), Rayner (11), and others.

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Baraney introduced onto oak seedlings mycorrhiza taken from well grown oak roots, and transplanted with its soil. Analysis of the roots showed that the percentage of mycorrhizal seedlings in infected plots was considerably greater than in control specimens. The difference was especially noticeable in dark brown soils and less conspicuous in chernozem.

Rayner's experiments consisted of contaminating conifer seedlings by introducing forest humus into holes at the time of planting.

Rayner considers it essential to introduce the forest humus in the first 6 months of the year and to protect it from excessive drying. No further description is needed, as the method is well known and is already in use in reforesting the steppes.

The advantages of this method are its simplicity and availability. Its drawbacks are the necessity of transporting large amounts of soil to treeless regions and the impossibility of determining the composition of the fungus flora in the soil. It is, consequently, impossible to control the mycelia in the soil and to guard against the danger of introducing harmful, parasitic varieties. To some extent this danger can be eliminated through preliminary mycological investigations of the area from which the humus is obtained.

Contamination by Pure Cultures of Mycorrhizal Fungi

To put this method in practice two problems must be solved: (1) isolation of mycorrhizal fungi in pure cultures, and (2) working out a system of mass reproduction of them for technical purposes.

Unlike the easily isolated mold varieties, it is hard to obtain pure cultures from mycorrhiza, chiefly because it is symbiotic. But, in spite of difficulties, it is not a hopeless task since cultures have already been obtained from many mycorrhizae: *Boletus elegans*, *Boletus luteus*, *Boletus variegatus*, *Tricholoma flavobrunneum*, and certain others.

Rayner proved that it was possible to use pure mycorrhizal cultures to inoculate trees by infecting pine seedlings with a culture of *Boletus bovinus*.

The best method of obtaining pure cultures is to plant pieces of fungus fruit bodies in artificial nutrient media. Moreover, it must be borne in mind that young fruit bodies, preferably from the part where the pileus joins the stalk, must be used for planting. Since the development of mycelia is very slow, all the rules of asepsis must be observed. Any foreign infection will ruin the culture. The fruit bodies should be selected in dry weather since moist fungi (after a rain) are usually extremely susceptible to contamination by bacteria.

The most successful nutrient agent is composed of must and synthetic media with an admixture of activators containing growth substances. The latter, as proved by recent investigations, are very important.

For the next step--obtaining mass cultures of mycelioid fungi--two methods are employed at present: surface and immersion growths. The former method is carried out by cultivating mycelioid films in cultivation chambers on flat dishes filled with a nutritional fluid. (A description of the surface culture method can be found in Chastukhin's book, "Mass Cultures of Microscopic Fungi" (9).) This method is used for commercial production of citric acid and pectase with the aid of the mold fungus *Aspergillus niger*.

The subsurface method used to cultivate fungus mycelia in tanks with the aid of air blowing is the same as that employed in manufacturing penicillin.

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When mass culture technique is perfected, it will greatly facilitate solution of the problems related to introducing mycorrhiza to wooded belts.

Contamination by Fruit Bodies and Spores

Beside the two above-mentioned methods, a third procedure using spores from fruit bodies is possible.

The fruit bodies of pileate fungi have a hymenial layer on the underside of the pileus (Agaricaceae) or the tubes in the case of the Boletaceae.

In studying the fruit bodies of pileate fungi, Falk and Buller (Fal'k and Byuller) discovered the complicated structure which ensures the dissemination of spores with the aid of the wind (7).

On the basis of these studies, we developed a theory of disseminating spores by streams of air. But experiments did not always support this theory. A large number of experiments on pileate fungi (7) showed, in a count of the number of spores removed by air streams or rain as compared with the number remaining on the fungi, that the majority remained on the fruit bodies. These experiments also proved that the possibility of spores' being carried by rain into the ground was very limited since; because of the filtering power of soil, the spores were retained by it at depths not exceeding 0.5 to 1 centimeter.

Meantime, we observed that most fruit bodies were quickly contaminated by the larvae of flies or other insects. A whole series of fungi act as bait for insects, especially flies.

Further investigations showed that spores are very resistant, and do not lose their ability to propagate in passing through the intestines of flies and larvae.

Larvae, crawling out of fungi and taking to the earth to enter the chrysalis stage, carry masses of spores in the intestine and expel them into the soil on becoming chrysalises. It has been shown that the larvae of Fungivarideae can reach a depth of several centimeters in the ground, while other groups, e.g., Pegonia and Mydea, can even go for some distance in a horizontal direction.

The list of fungi whose spores were found by the authors in insects includes: (1) *Boletus versipellis*, (2) *Boletus scaber*, (3) *Boletus edulis*, (4) *Boletus luteus*, (5) *Pholiota squarrosa*, (6) *Pholiota mutabilis*, (7) *Hypholoma sublateritium*, (8) *Hypholoma fasciculare*, (9) *Paxillus involutus*, (10) *Lepiota procera*, (11) *Flammula* sp., (12) *Stropharia squamosa*, and (13) *Galera tenera*.

Thus, diptera, whether in the imago or larval stage, play an important part in disseminating fungus spores. The larvae are particularly important, since they deposit the spores in the immediate vicinity of the tree.

Large quantities of spores have also been discovered in the intestine of earthworms which had come in contact with decomposing fungi.

In practice, this knowledge can be utilized to infect trees in the steppes by insects carrying fruit bodies. So far, however, experiments along this line have been rather amateurish. Fungi have been planted in wooded zones under trees, but the results are not conclusive, since fungi might also have been carried in another way.

The most interesting experiments are those performed by Samutsevich. He planted little pieces of fungi fruit bodies near trees in furrows to facilitate penetration of spores to the roots.

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Reforestation of the steppe opens the way for extensive experiments along these lines. If trees can be contaminated by mycorrhiza with the aid of fruit bodies infected with larvae, the problem will be solved. The natural habitat of the insect must, of course, be considered; fruit bodies must be protected from drying out, etc.

One advantage of this method is the possibility it offers of infection with definite varieties, not mixtures of fungi. The drawback is the necessity for rapid delivery from the woods to the steppe region.

Another modification of this method might be the employment of either spore powder or spore emulsion for direct infection of seedlings and seed.

Emulsions can be obtained either from the dry spores fallen from the fungus or in the form of an emulsion from fruit bodies. Accurate tests have demonstrated that the spores of *Boletus circinans*, the marsh and grey *Boletus*, and *Boletus luteus* retain ability to germinate for a whole year, while the spores of *Boletus granulatus* and *Boletus bovinus* die in six months.

Spore emulsions can be applied to seeds or seedlings during planting. Watering the ground with spore emulsions is not very effective as the spores remain in the top soil.

Because of the small number of experiments with emulsions, it would be premature to make a definite evaluation of this method. Further experiments are necessary.

Up to the present only the method of introducing forest humus has been checked experimentally. The other methods must be carefully worked out. It is also important to conduct experiments on various ecological conditions and various types of soil.

Experiments on the infection of tree roots with the aid of larvae and spore emulsions need no complicated apparatus, but the technique of mass production of mycorrhiza can only be worked out in special laboratories.

Considering the importance of introducing mycorrhiza in connection with reforestation of the steppes, we believe that immediate attention should be given to these problems.

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