STUDIA FORESTALIA SUECICA

Nr 83 1970

Mycorrhiza and tree nutrition in poor forest soils

Mykorrhiza och näringsupptagning hos skogsträd i mager skogsjord

by

ERIK BJÖRKMAN
Department of Forest Botany

SKOGSHÖGSKOLAN
ROYAL COLLEGE OF FORESTRY
STOCKHOLM

ABSTRACT

When fertilizing forest trees with nitrogen a great quantity is bound in organic humus compounds and cannot be utilized by the trees. Extensive reserves of bound nitrogen also exist in comparatively poor forest soils. However, it has been found that it is possible to release these reserves by inoculation with certain soil fungi of the litter-decomposing type. It does not seem improbable that such measures can represent a biological alternative to chemical fertilization. In the present paper it is shown how *Boletus subtomentosus*, that normally is a mycorrhiza forming fungus, can stimulate the development of pine plants. A physiological strain of this fungus is used which has the ability to decompose litter. The typical mycorrhiza fungus *Boletus bovinus* also stimulated the plant growth to a certain extent. However, the stimulating effect ceased when strong fertilization was used or when the light intensity was reduced. Constant illumination resulted in the plants developing well and with good mycorrhiza formation. When the period of illumination was reduced to 16 hours a day the plants developed less vigorously and the mycorrhiza formation was reduced.

Ms. received 9 June 1970

ESSELTE TRYCK, STHLM 70

010546

1. Introduction

Even though the chemical fertilization of forest soil in Sweden is still percentually modest (approximately 1 % of the forest area, cf. Holmen, 1969), it is regionally of great importance. Much experience has been gained about the strong development of the annual ring after the fertilization of forest stands. It is another question, however, as to whether the one-sided use of plant nutrients can entail disadvantages in the long term for the natural nutrient balance and gradually bring about a fall in production capacity. Does the change in the nutrient balance, which fertilization can be assumed to cause, have any negative consequences? Cannot the one-sided use of nutrients in the long term upset the natural nutrient balance and gradually have a deleterious effect on production?

To answer these questions one must differentiate between those cases where nutrients are used once only and those where repeated fertilization takes place. In the first instance, where it is chiefly a question of fertilizing older stands 5 to 10 years before the final cut, it is hardly likely that any long-term damage will occur if the dose is suitable. In the latter instance, however, as is the case when fertilizing younger stands—a measure which is becoming ever more common—the one-sided use chiefly of nitrogen can be feared to result in dislocations not least in the composition of forest microflora and microfauna, which to a great extent affects the growth of trees. Intensified research has recently been started in this field, for which reason more extensive material is still not available. However, several observations indicate that the repeated one-sided use of nitrogen can even result in a deficiency of some other nutrient, e.g. magnesium or phosphorous.

Available material has shown that the fertilization of pine or spruce with nitrogen results in a more or less strong growth reaction in the tree. This growth increase in the annual ring often declines in pine after 3 to 4 years and in spruce after 5 to 6 years, after which in certain cases a poorer growth than in corresponding non-fertilized trees can be noted. To maintain the improved growth after fertilization, further fertilization is therefore necessary. Such a measure has more than an economic aspect, it undoubtedly entails biological consequences, not least on the soil's microorganisms.

Another recent experience in the field of forest fertilization is that the nitrogen introduced to the soil is not as hitherto thought leached in the soil, but is instead to a great extent bound in the complex compounds of the humus (Björkman, Lundeberg & Nömmik, 1967). Later experiments also establish that the tree utilizes only 10—15 % of the added nitrogen (Nömmik & Popovic, not published). As this is a very low degree of utilization it seems that efforts should be concentrated on discovering whether a more effective way of utilizing the nutrients can be found, which in turn should lead to an improvement in the growth of the tree.

As forest trees ingest a considerable part of their nourishment via certain soil fungi, which live in symbiosis with the roots and form mycorrhiza with them, this nutrient uptaking mechanism should be studied more closely if it is intended to improve the ability of the tree to ingest nutrition. It cannot be precluded for instance that different species of mycorrhiza fungi can be more or less efficient nutrient assimilators, and that the introducing of particularly active species can be possible if special measures are taken, e.g. the temporary sterilization of soil, to enable such beneficial fungi to establish themselves free from the competition of other soil organisms.

There is much proof from tropical areas that mycorrhiza fungi can have a very favourable effect on the growth of conifer plants. By introducing soil infected with such fungi, so that the formation of mycorrhiza occurs, such plants have grown many times bigger than corresponding plants without mycorrhiza (Cromer, 1935; Hatch, 1937; Rosendahl, 1942; Björkman, 1964; Hacskaylo & Vozzo, 1967; Mikola, 1969 a.o.). It has also been possible to demonstrate that it is chiefly phosphorous that the mycorrhiza fungi can make more available; this is of particular importance in the tropics, where there is often a deficiency of phosphorous. A real lack of phosphorous seldom occurs in forest soils in the Scandinavian countries, the common growth limiting nutrient factor there being mineralised and readily accessible nitrogen.

It has consequently been considered of importance to try to find mycorrhiza fungi which, either alone or in combination with other fungi, chiefly the so-called litter decomposers, can release and make available to trees and plants the relatively rich reserves of nitrogen which occur in the majority of forest soils. This problem is particularly important in such soils where the humus layer is thin, or where because of certain silvicultural precautions, such as burning or extensive clear-felling, a so-called heath degeneration of the microflora and the microfauna can have taken place.

In the Scandinavian countries, for example, where most of the land has been forested for a long period of time, a fungus flora has had time to establish itself practically everywhere; this flora also includes species which form mycorrhiza with the forest trees. It is therefore unlikely that there—as distinct from the tropics, which for the most part have never sustained conifers—it will be possible to achieve any considerable improvement in tree growth by introducing mycorrhiza fungi. The experiments which have been made by adding mycorrhiza fungi to young spruce plants in the period of stagnated development following planting have not been very positive. Even less positive effect has been obtained by adding pure cultures of mycorrhiza fungi because of the difficulty these fungi have in establishing themselves in the new habitat in fierce competition with other microorganisms. An alternative that has been tried and found to be practicable under certain conditions is at the nursery stage—using soil which has been temporarily sterilised—to infect the plants with certain species of mycorrhiza fungi. When mycorrhiza formation has occurred and the plants have reached a suitable age, they are planted on their final sites. In this way Moser (1956) has found in Austria that plants of *Pinus cembra* raised in a lowland nursery and infected with *Boletus plorans* after planting out on a site at an altitude of about 2500 m, where trees had previously not grown, developed more strongly than corresponding plants without mycorrhiza or with mycorrhiza formed by other species of fungi. Similar positive results were achieved in Puerto Rico when introducing *Pinus* species by adding fertile soil from Maryland pine stands or by adding pure cultures of certain mycorrhiza fungi (cf. Björkman, 1964; Vozzo, 1968; Hacskaylo, 1970).

In an attempt to ascertain the possibility of using certain species of fungi in a similar way under Swedish conditions, a number of nursery and laboratory experiments were carried out in 1968 and 1969 at the Department of Forest Botany, and a series of field experiments was also conducted. The following is an account of the former.

2. Experiment 1. Fertilized and non-fertilized pine plants with mycorrhiza fungi added (1968)

2.1. Materials and methods

In a so-called Möller greenhouse (with a glass roof and walls of netting), pine plants were set in 250 ml plastic pots containing low-nutritional peat to which had been added a similar volume of perlite. This peat—from Hällmyren in northern Sweden (cf. Björkman, 1942, pp. 61—64)—was taken from a depth of about 1 m, where the occurrence of microorganisms was negligible but where mycorrhiza fungi were however present. Each pot contained 5 plants. In general 10—15 pots were used in each test combination with the exception of the series where shading was used; here, for reasons of accommodation, somewhat fewer pots were used. The experiment started with the sowing on 25 May.

Approximately 5 weeks after sowing, pure barley cultures of two fungi which normally promote the formation of mycorrhiza were added. These were *Boletus bovinus* and *Boletus subtomentosus* ("C 30" according to Lundeberg, 1970). The former is known to promote mycorrhiza in several *Pinus* species (Masui, 1927; Hatch & Hatch, 1933; Eglite, 1954; cf. Melin, 1925), the latter in *Pinus mugo* (Modess, 1941) and *Pinus silvestris* (Lundeberg, 1970). However, Lundeberg has demonstrated that *Boletus subtomentosus* occurs in a number of races, of which some do not form mycorrhiza. Thus C 30, the strain used in the present experiment, lacked this ability under the conditions in which *Boletus bovinus* formed mycorrhiza. Because of the initially poor occurrence of microorganisms in the substrate the fungi introduced into it were able to develop. This is not normally possible in unsterilized soil.

After a further 2 weeks various kinds of nutritional substances in water solution were added twice weekly (10 ml each time) during the period 13 June to 9 September 1968. The solutions had the following composition:

O	= distilled water					
N	= NH_4-tartrate	1.0	g	per	litre	water
P	= H_3PO_4	1.4	g	,,	,,	,,
NP	= NH_4-tartrate	1.0	g	,,	,,	,,
	+ H_3PO_4	1.4	g	,,	,,	,,
NPK	= NH_4-tartrate	1.0	g	,,	,,	,,
	+ H_3PO_4	1.4	g	,,	,,	,,
	+ KCl	1.0	g	,,	,,	,,

The total amount per pot during the test period was therefore 240 mg NH_4-tartrate, 336 mg H_3PO_4 and 240 mg KCl respectively. Distilled water was given to the plants as required.

In a special test series using the same fertilization an admixture of 4.3 g $CaCO_3$ per litre (= totalling 1.032 mg per pot) was used to test the effect of calcium on the ingestion of nitrogen, phosphorous and potassium.

In addition to the above test series, where light conditions were optimal—hereafter called full daylight (1/1 light), although the light intensity was somewhat reduced by the greenhouse roof—a further two test series using one-half (1/2 light) and one-quarter (1/4 light) of the light in the main series were arranged. The shading was arranged with the help of lattice boxes of wood of the same construction as those described by Björkman (1942, p. 68).

When the test was terminated 9 September the height of the plants was measured, as also the length of the needles and roots. The roots were examined with regard to the frequency of mycorrhiza, which was expressed in relation to the number of mycorrhizal root points as a percentage of the total number of root points. Samples of needles and roots were prepared for chemical analysis. In this connection great care was taken with the samples intended for carbohydrate analysis in that the samples were taken simultaneously and tested immediately. This precaution was necessary because the content of soluble carbohydrates varies during different times of the day. For determining the "reducing substance" the method described by Meyer (1962 pp. 10—11) was employed, in which 0.1 *N* HCl is used for the hydrolysis. In addition to those on "reducing substance", total nitrogen and phosphorous analyses were made on needles. At the end of the experiment also the soil in the pots was analysed with regard to pH, total nitrogen, total phosphorous (P_{HCl}), total potassium (K_{HCl}) and soluble phosphorous, potassium and calcium (P-Al, K-Al and Ca-Al respectively). All these analyses were carried out by the National Agricultural Chemistry Laboratory employing the standard methods in use at that laboratory.

2.2. Results

The measurement data of the plants in the various test series have been collocated in Table 1. From this table and Fig. 1 it can be seen that the plants in the unshaded test series (1/1 light) grew much bigger if they were inoculated with *Boletus subtomentosus* (C 30). In the

Table 1. Development of fertilized and unfertilized 1 year-old pine plants raised in pots with low-nutrient peat in full daylight (1/1) and shaded (1/2 and 1/4) summer 1968.

Fertilization	Fungus added	Length, mm			Dry weight, mg		Short roots transformed to mycorrhiza, % of total
		Shoots	Needles	Roots	Shoots	Roots	
Full light (1/1)							
— (control)	—	45	21	70	52.1 ± 2.5	44	6
—	*Boletus bovinus*	42	24	86	58.3 ± 2.1	45	16
—	*B. subtomentosus*	65	34	94	156.4 ± 3.1	67	46
N	—	46	26	69	96.6 ± 4.2	48	0
P	—	40	20	96	50.3 ± 2.4	39	10
P	*Boletus bovinus*	44	24	102	61.3 ± 1.8	46	18
P	*B. subtomentosus*	56	32	98	117.8 ± 4.3	82	61
N + P	—	60	35	76	204.4 ± 6.2	74	0
N + P + K	—	76	34	58	292.7 ± 5.7	91	0
N + P + K	*Boletus bovinus*	75	35	64	281.3 ± 6.1	88	0
N + P + K	*B. subtomentosus*	75	36	71	306.1 ± 7.5	96	0
Ca	—	43	21	72	51.0 ± 2.2	43	0
Ca	*Boletus bovinus*	44	24	70	53.2 ± 2.3	45	0
Ca	*B. subtomentosus*	44	24	81	54.1 ± 2.1	39	0
Ca + N	—	43	21	70	49.7 ± 1.9	37	0
Ca + N + P	—	47	22	67	53.4 ± 2.4	35	0
Half light (1/2)							
— (control)	—	46	21	52	42.3 ± 1.2	27	0
—	*Boletus bovinus*	38	23	64	50.5 ± 2.4	31	0
—	*B. subtomentosus*	59	29	87	112.3 ± 5.3	32	0
N	—	48	28	47	83.6 ± 4.2	24	0
P	—	39	20	81	37.1 ± 2.0	29	0
P	*Boletus bovinus*	44	25	75	65.5 ± 4.3	25	0
P	*B. subtomentosus*	58	29	76	93.4 ± 3.9	24	0
N + P	—	54	30	60	101.2 ± 5.6	22	0
Quarter light (1/4)							
— (control)	—	41	18	30	19.6 ± 0.8	3	0
—	*Boletus bovinus*	40	18	33	20.4 ± 1.3	3	0
—	*B. subtomentosus*	39	16	36	18.5 ± 1.0	2.5	0
N	—	40	17	28	19.5 ± 0.9	2.5	0
P	—	40	17	36	18.2 ± 2.1	3	0
P	*Boletus bovinus*	39	18	37	22.4 ± 1.7	3	0
P	*B. subtomentosus*	40	18	32	23.6 ± 1.3	2	0
N + P	—	40	18	26	23.1 ± 1.8	2	0

unfertilized series the plants infected with *Boletus subtomentosus* were even considerably bigger than when only the N-additive was used.

If phosphorous was added, the plants grew approximately as big as in unfertilized pots without added fungus, no doubt because the nitrogen supply constituted the restricting factor in the subsoil.

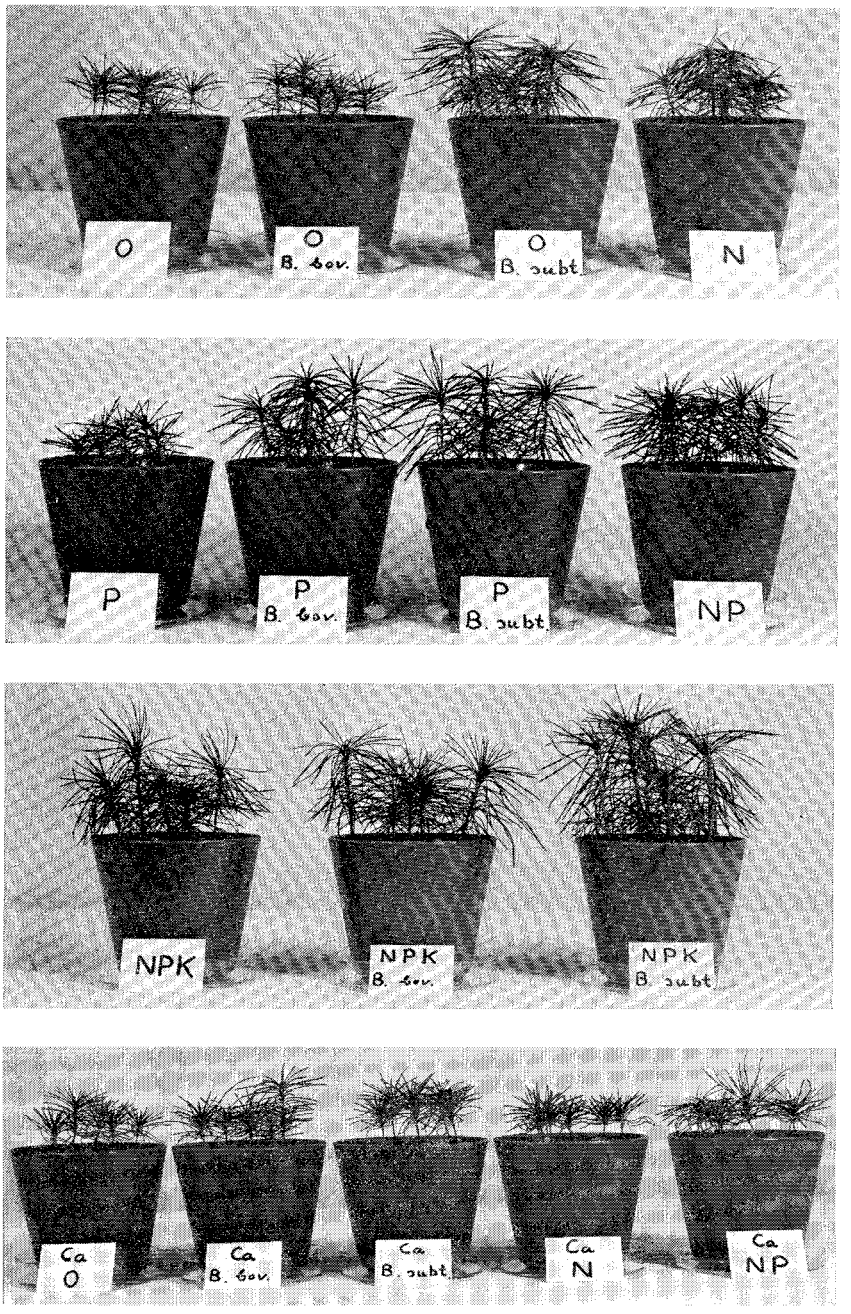

Fig. 1. One-year-old pine plants raised in full daylight in low-nutrient peat fertilized with N, P, K and Ca and to which the soil fungi *Boletus bovinus* and *Boletus subtomentosus* had been added.

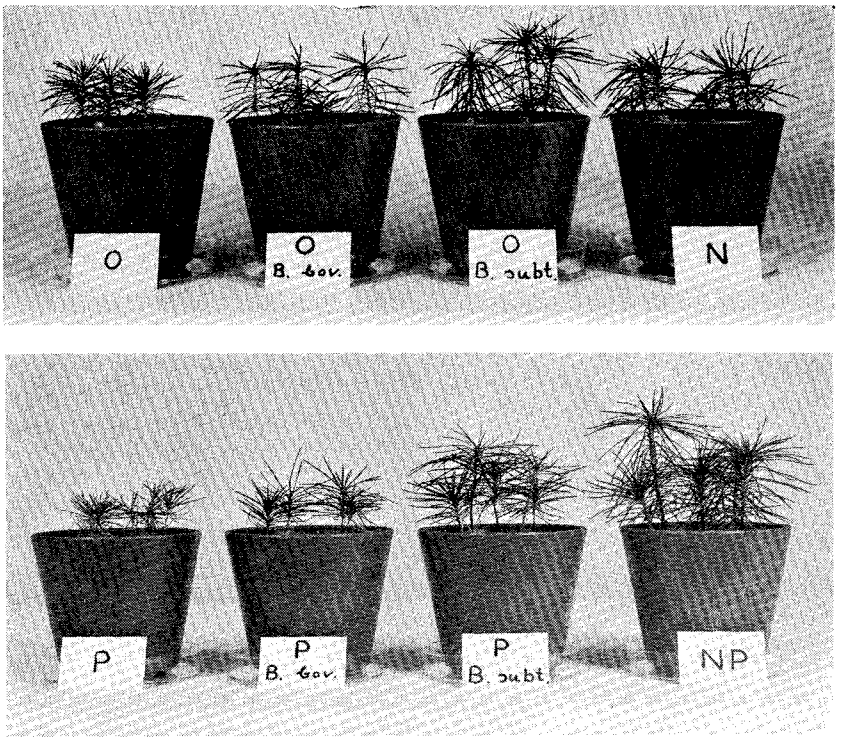

Fig. 2. One-year-old pine plants raised in 1/2 light in low-nutrient peat fertilized with N and P and to which the soil fungi *Boletus bovinus* and *Boletus subtomentosus* had been added.

If finally the full fertilizer was introduced in the form of NPK, all differences were levelled out, and the plants with added fungus and the control plants attained the same size.

In the calcium series the same tendencies could be seen as in the main series, although they were by no means as clear. Moreover, all the plants developed considerably less than the plants without calcium, an observation that has also been made in other experiments. The reason for this is not entirely clear and will not be discussed here.

In the 1/2 light series the plants were less well-developed than those in the 1/1 light series but just as tall (Fig. 2). The tendency towards better developed plants in the unfertilized and phosphorous fertilized test series after infection with *Boletus subtomentosus* was consequently the same. In the 1/4 light series, however, no difference at all could be noted between the plants after the various forms of treatment (Fig. 3). The lack of light was the predominant restricting factor affecting the growth of the plants.

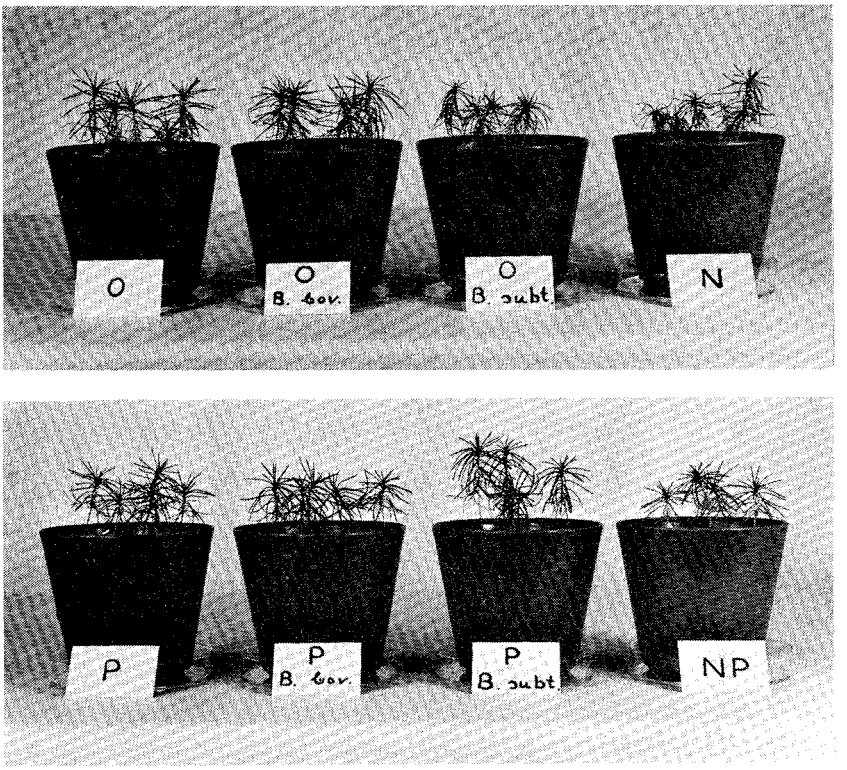

Fig. 3. One-year-old pine plants raised in 1/4 light in low-nutrient peat fertilized with N and P and to which the soil fungi *Boletus bovinus* and *Boletus subtomentosus* had been added.

The mycorrhiza analyses of the plants showed that mycorrhiza formation in the unfertilized test series in full light occurred in 16 % of the plants after infection with *Boletus bovinus* and in 46 % when *Boletus subtomentosus* was added. In the phosphorous series the corresponding frequencies were 18 % and 61 %. In the shaded tests using 1/2 and 1/4 light no mycorrhiza formation occurred. Neither was this the case when the nitrogen fertilizer was used. In these test series the dose was so strong that both the short root and long root points were frequently swollen and club-like (cf. e.g. Björkman, 1940).

The fungus which constituted the mycorrhiza could not be determined. There is much to suggest, however, that the two added fungi both helped to stimulate the "natural" mycorrhiza formation of the fungi already present in the substrate, rather than that they themselves formed mycorrhiza (cf. Experiment 2).

From Table 2, in which the results of chemical analyses of the

Table 2. Chemical analyses of the substrate (low-nutrient peat) after cultivation for one summer of pine plants fertilized in various ways and treated with different fungi. During the experiment the pots were stored in varying degrees of illumination. 1968.

Fertilization	Fungus added	pH	P-Al	K-Al	Ca-Al	P_{HCl}	K_{HCl}	N_{tot}
Full light (1/1)								
— (control)	—	4.2	9.4	24.5	210	22	105	0.38
—	*Boletus bovinus*	4.0	2.4	27.0	200	12	60	0.30
—	*B. subtomentosus*	4.1	4.0	31.0	210	14	60	0.40
N	—	3.9	1.8	23.0	220	8	50	0.61
P	—	3.7	242	20.0	238	230	50	0.37
P	*Boletus bovinus*	3.5	278	29.5	200	266	50	0.39
P	*B. subtomentosus*	3.6	198	21.5	190	190	40	0.41
N + P	—	3.7	151	15.0	160	184	40	0.47
N + P + K	—	4.3	190	280	320	230	355	0.34
N + P + K	*Boletus bovinus*	3.9	150	315	220	172	345	0.50
N + P + K	*B. subtomentosus*	3.8	172	360	240	188	350	0.54
Ca	—	7.3	5.7	11.5	6080	14	40	0.30
Ca	*Boletus bovinus*	7.3	8.2	14.0	6420	16	45	0.34
Ca	*B. subtomentosus*	7.2	10.0	15.0	6320	16	45	0.35
Ca + N	—	7.0	3.6	10.0	7600	10	40	0.43
Ca + N + P	—	6.8	220	10.5	6240	190	45	0.43
Half light (1/2)								
— (control)	—	4.1	3.6	21.5	240	14	65	0.36
—	*Boletus bovinus*	4.1	3.8	24.0	196	12	60	0.32
—	*B. subtomentosus*	4.2	3.6	21.0	210	12	55	0.32
N	—	4.2	2.4	19.0	200	8	50	0.45
P	—	3.5	198	22.0	150	216	55	0.32
P	*Boletus bovinus*	3.6	218	29.0	230	200	50	0.35
P	*B. subtomentosus*	3.7	227	34.0	180	198	65	0.33
N + P	—	3.6	165	22.0	190	168	50	0.49

substrate performed after the termination of the experiment are collated, it can be seen that the pH-value was approximately the same in all test combinations, namely 3.5—4.2, except when calcium was added, in which case it rose to 6.8—7.3. No changes resulted from the addition of other plant nutrients. For the rest the analyses quite naturally showed considerably higher P-contents after the addition of H_3PO_4 and much higher K-contents and Ca-contents after the addition of KCl and $CaCO_3$ respectively. On the other hand it was not possible to see any strong increase in the N-content in the substrate after the addition of NH_4-tartrate. Infection with the two fungi had hardly affected the nourishment constellation at the end of the experiment in comparison with that of non-infected substrate.

The chemical analyses of the needles (Table 3), admittedly in-

Table 3. Total nitrogen and phosphorous in needles of 1-year-old pine plants raised in low-nutrient peat and fertilized in various ways and treated with different fungi. During the experiment the pots were stored in varying degrees of illumination. 1968.

Fertilization	Fungus added	Percentage of air-dry sample N_{tot}	Percentage of air-dry sample P_{tot}
Full light (1/1)			
— (control)	—	1.21	0.20
—	*Boletus bovinus*	1.68	—
—	*B. subtomentosus*	2.02	0.59
N	—	3.04	0.14
P	—	1.96	0.38
P	*Boletus bovinus*	1.69	—
P	*B. subtomentosus*	2.07	—
N + P	—	2.89	—
N + P + K	—	2.69	0.31
N + P + K	*Boletus bovinus*	2.83	—
N + P + K	*B. subtomentosus*	2.82	—
Ca	—	1.62	0.09
Ca	*Boletus bovinus*	1.82	—
Ca	*B. subtomentosus*	2.47	—
Ca + N	—	2.25	—
Ca + N + P	—	2.77	—
Half light (1/2)			
— (control)	—	1.32	0.09
—	*Boleltus bovinus*	2.45	—
—	*B. subtomentosus*	2.34	0.17
N	—	2.94	—
P	—	1.51	—
P	*Boletus bovinus*	2.23	—
P	*B. subtomentosus*	2.64	—
N + P	—	2.67	—

complete because of the lack of material, showed that at the end of the experiment the needles had a total average nitrogen content of 1.21 % in the case of the plants in the unfertilized control pots in full light, while in the case of the plants fertilized with nitrogen the values represented 3.04 %. On adding *Boletus bovinus* to the unfertilized test series the needles had reached a content of 1.68 %, while plants infected with *Boletus subtomentosus* had an average content of 2.02 %. In the pots fertilized with NPK the addition of fungus had not led to any appreciable further ingestion of nitrogen. Phosphorous had been ingested from the substrate in considerable quantities after inoculation with *Boletus subtomentosus,* as an analysis from the unfertilized test

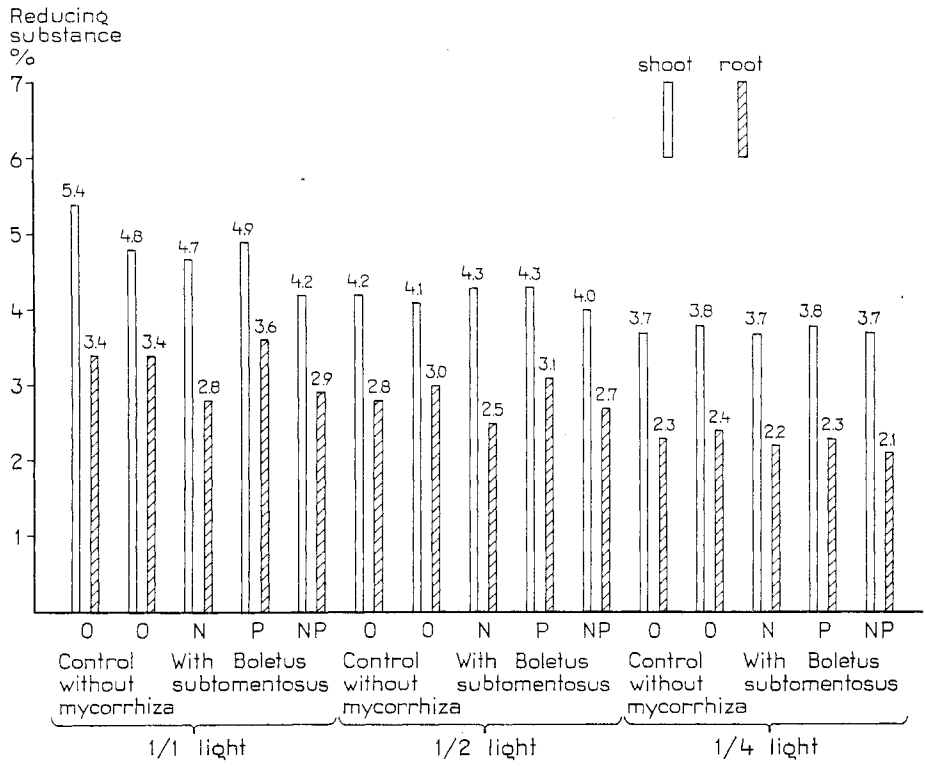

Fig. 4. "Reducing substance" in shoots and roots of fertilized and unfertilized 1-year-old pine plants raised in low-nutrient peat and infected with *Boletus subtomentosus* ("C 30"). Experiment conducted partly in full daylight ("1/1 light"), and partly shaded to half and quarter light respectively ("1/2 light", "1/4 light"). Analysis values the average of at least 4 double samples.

series in full light and from the same series in half light showed (0.20 % P without fungus and 0.59 % with fungus in full light, and 0.09 % and 0.17 % in half light respectively).

The carbohydrate analyses of the test material showed (Fig. 4) that the "reducing substance" in the needles was approximately the same at the end of the experiment for the plants in the various test combinations. However, the contents were uniformly lower in the case of the most shaded plants. In the same way a noticeably lower content could be established in the roots of the shaded plants. Another tendency was seen in the lower contents in the plants that had been fertilized with nitrogen, both in the full light and half light series. In the 1/4 light series, however, the values were the same. A corresponding inclination towards higher values was noticed after P-fertilization. It is

uncertain whether the addition of *Boletus subtomentosus* affected the sugar content of the roots.

The established incidence of mycorrhiza in the different test series (cf. Table 1) is of interest against the background of these analyses. Thus mycorrhiza occurred only in plants under full illumination, and particularly abundantly if fertilization with phosphorous had taken place. Only a slight tendency towards a higher content of "reducing substance" in the roots of plants to which mycorrhiza fungi had been added could be determined. The experiments did not support the theory propounded by Meyer (1962) that the mycorrhiza fungi *caused* the higher sugar content in the roots of mycorrhiza carrying plants as established by him.

3. Experiment 2. Fertilized and unfertilized pine plants in pots with added mycorrhiza fungi (1969)

In order to further verify the conclusions, the 1968 experiment was repeated in the same way the following year. The experiment started as late as 16 June 1969 but was terminated at the same time as the previous year's experiment, i.e. mid-September. No calcium series was included in the 1969 experiment. The addition of nutrients commenced 15 July, i.e. a month later than in the previous year's experiment.

Because of the short test period, the differences between the various phases of the experiment were not so great as in the 1968 experiment. This time, however, a distinct difference could also be established between the development of plants with and without added test soil fungi. As in the previous year, it was seen that *Boletus subtomentosus* had a stimulating effect on the growth of the plants.

In principle, analyses of the "reducing substance" in needles and roots gave the same result as in the previous year's experiment. The lower content in the roots of the shaded plants was particularly noticeable. Other differences that had been established in the 1968 experiment were not so clear, probably because of the shorter duration of the experiment and the limited time for mycorrhiza formation.

These circumstances show the great degree of care that must be taken when drawing conclusions in this particular branch of research. Comparisons between experiments started early in the spring using natural forest soil and those started late in the summer using an artificial substrate, as was done by Handley & Sanders (1962), can easily lead to misleading conclusions.

4. Experiment 3. Mycorrhiza-inoculated fertilized and unfertilized pine plants in unsterilized raw humus raised in cultivation boxes during varying periods of illumination (1969)

4.1. Materials and methods

In the winter of 1969 an experiment was arranged using pine plants raised in pots in low nutrient raw humus and perlite, which had been sterilized by heating to + 60°C for 60 hours. Unsterilized low nutrient raw humus was also used.

Five plants were raised in each pot. About one month after germination a peat culture of *Boletus bovinus* or *Boletus subtomentosus* was added to the pots in the sterilized series (cf. Moser, 1956).

Fertilization of the pots was carried out as in Experiment 1 with NH_4-tartrate (N) and H_3PO_4 (P) at 1.0 g and 1.4 g per l respectively. Fertilization took place twice a week and the dose per pot was 10 ml each time. During the experiment each pot received a total of 300 mg NH_4-tartrate and 420 mg H_3PO_4.

The experiment was a pure laboratory experiment and the pots were kept in small climate chambers ("Thallotron" of ASSAB's construction) with a volume of approximately 0.25 m^3. Illumination was by means of a ramp of strip lights—Sylvania Electric F48T12/GRO/VHO —with a light intensity at plant level of about 6.000 lux. The temperature was kept at approximately 18°C during the illuminated period and was slightly less at night. Three different types of illumination were used in three different chambers, namely constant illumination, i.e. 24 hours a day, and 16 and 8 hours' illumination a day. The length of the experiment represented a normal summer period but was extended to exceed that of Experiment 1 by about 3 weeks.

At the end of the experiment a record was made of the length of shoots, needles and roots, as also of the average dry weight of needles and roots from a minimum of 30 plants in each and the same experiment combination. In addition, orientating analyses were made of the content of "reducing substance" in needles and roots.

4.2. Results

Table 4 and Fig. 5 and 6 show that the plants reacted very strongly to fertilization in the 24-hour series but negligibly in the 16-hour series.

Tabell 4. Development of fertilized and non-fertilized 1-year-old pine plants raised in pots partly vith sterilized, low-nutrient raw humus partly with unsterilized raw humus in constant light (6000 lux, 24 hours) and in 16 hours and 8 hours illumination per day. Two different fungi added to the sterile substrate. 1969.

Light and fertilization	Fungus added	Length, mm			Dry weight, mg		Short roots transformed to mycorrhiza, % of total
		Shoots	Needles	Roots	Shoots	Roots	
24 hours light							
Non-fertilized soil							
Sterile	—	41	37	108	176.2 +2.1	156	—
	Boletus bovinus	54	44	128	162.5 ±1.9	129	0
	B. subtomentosus	58	51	138	228.1 ±4.0	159	0
Unsterilized		50	39	120	206.1 ±2.4	142	36
Fertilized soil							
Sterile	—	65	56	49	535.2 ±8.3	177	—
	Boletus bovinus	76	102	76	548.6 ±6.6	124	0
	B. subtomentosus	82	92	64	514.5 ±6.5	117	0
Unsterilized	—	62	107	54	316.7 ±4.8	108	0
16 hours light							
Non-fertilized soil							
Sterile	—	32	26	68	49.4 ±2.6	59	—
	Boletus bovinus	37	28	59	67.5 ±2.1	70	0
	B. subtomentosus	36	27	72	61.4 ±2.8	53	0
Unsterilized	—	51	39	101	81.5 ±3.0	87	21
Fertilized soil							
Sterile	—	46	32	78	87.2 ±4.2	82	—
	Boletus bovinus	50	39	72	88.5 ±4.1	61	0
	B. subtomentosus	42	40	64	94.6 ±2.8	49	0
Unsterilized	—	46	41	70	115.1 ±5.1	98	0
8 hours light							
Non-fertilized soil							
Sterile	—	30	6	5	12.0 ±1.0	4	—
	Boletus bovinus	32	10	13	9.0 ±0.5	5	0
	B. subtomentosus	32	10	15	11.3 ±0.9	4	0
Unsterilized	—	31	7	14	8.7 ±0.2	16	0
Fertilized soil							
Sterile	—	32	7	6	10.4 ±0.6	4	—
	Boletus bovinus	32	8	15	12.0 ±0.9	7	0
	B. subtomentosus	31	9	10	18.3 ±0.8	6	0
Unsterilized	—	32	8	21	42.8 ±3.1	14	0

In the 8-hour series the plants grew equally, irrespective of fertilization. The low degree of illumination was the growth limiting factor in this series.

In the sterilized raw humus there was no mycorrhiza after artificial inoculation with *Boletus bovinus* and *Boletus subtomentosus*. On the other hand, the plants in the unsterilized and unfertilized humus dis-

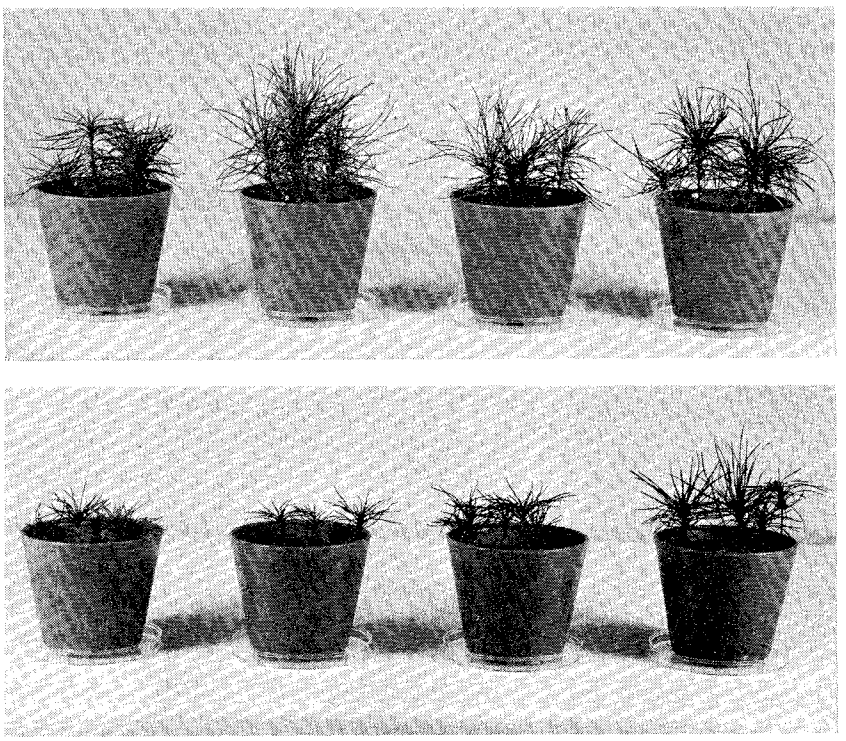

Fig. 5. One-year-old pine plants raised in climate chamber in sterilized, unfertilized low-nutrient raw humus, to which was added two different soil fungi, and in unsterilized raw humus (pot on extreme right) in 24-hour illumination (6000 lux) a day (upper row), partly in 16-hour illumination a day (lower row). From left to right: 1) plants in raw humus without added fungus, 2) plants with added pure culture of *Boletus subtomentosus*, 3) ditto of *Boletus bovinus* and 4) plants in unsterilized, fertile raw humus.

played abundant mycorrhiza formation in the 24-hour series, which showed that the illumination in the climate chamber was sufficient for the constitution of mycorrhiza.

The roots in the 16-hour series were usually quite different in appearance from those in the 24-hour series. Thus the roots in the former plants were considerably "thinner", and also had a different morphological inner structure, the reasons for which will be dealt with later.

The orientating analyses of "reducing substance" showed clearly lower contents in the roots of the 8-hour series, but in other respects the values were fairly similar.

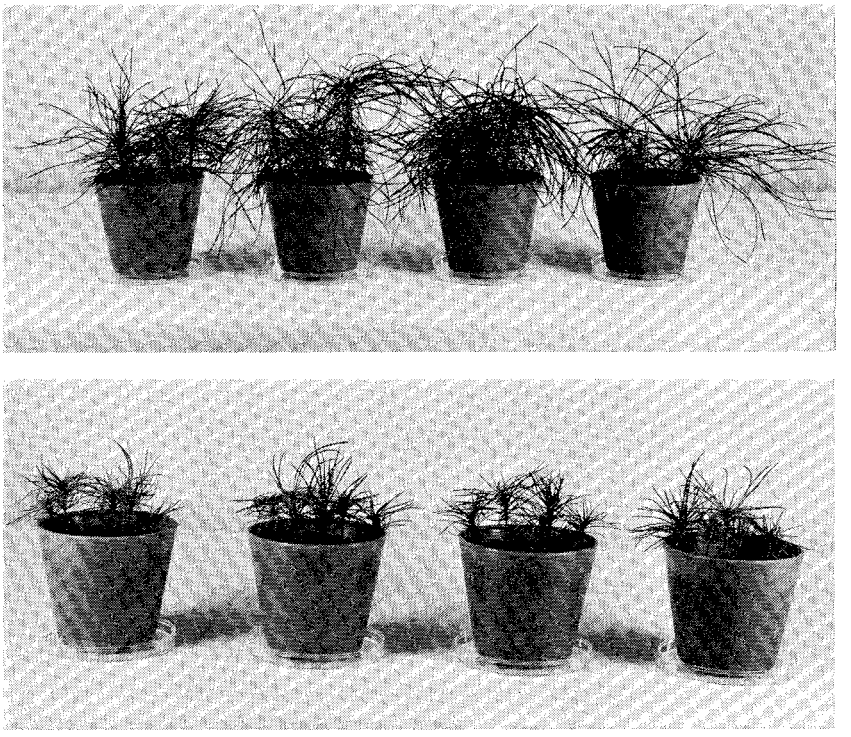

Fig. 6. Same conditions as in Fig. 5 but with fertilized plants.

5 Summary and discussion

Judging from the above experiments it is possible to influence the development of pine plants by the addition of suitable soil fungi. However, the way in which these fungi contribute towards the better development of the plants is not yet fully clear. It is known from earlier experiments that typical mycorrhiza fungi do not produce laccase or proteinase. On the other hand the so-called litter decomposing fungi or white rot fungi have an enzyme system which makes it possible to break down the complex humus compounds of the soil (cf. Lindeberg, 1948) thereby releasing nitrogen which can be ingested by the trees. It has also been demonstrated recently (Lundeberg, 1970) that one of the fungi tested in these experiments, *Boletus subtomentosus,* can occur in several physiological races with varying ability to break down complex humus compounds. The most likely course in natural conditions would seem to be an interaction between soil organisms with an ability of litter-decomposition and mycorrhiza fungi, which normally lack this ability, but which are able to assume symbiosis with tree roots and enable them effectively to conduct the ingestion of soluble soil nutrients.

The fact that the pine plants used in the above experiments grew stronger in sterilized soil to which pure cultures of *Boletus subtomentosus* had been added showed that this fungus was in some way able to bring about the formation of soluble nitrogen in forest soil. It is of great interest to note that this ability did not manifest itself when the plants were shaded and when illumination constituted the growth limiting factor (cf. Björkman, 1942). Thus the mycorrhiza formation which occurred during full illumination ceased entirely in 1/4 light.

Mycorrhiza formation failed completely to occur also in the heavily fertilized pots (cf. Björkman, 1942), which is probably partially due to the energy problem, even though the effect can in this respect be assumed to be of a more complex nature. The transformation of energy-rich assimilates into substances which are more or less toxic to the fungi, e.g. of phenol type, can in this respect play a part, as also morphologic changes in the roots caused by changes in the nutritional balance of the soil or other influences (cf. Slankis, 1958; Foster & Marks, 1967; von Hofsten, 1969). Thus when calcium was added all mycorrhiza formation was completely suppressed and the plants grew to about the same size.

In the third series of experiments (Experiment 3) it was possible to show that the plants grew much stronger if they were raised in constant light (24 hours a day) than if the light was limited to 16 or 8 hours a day. In the latter case the plants, and more particularly their roots, were very poorly developed and there was no sign of mycorrhiza formation. In the 16-hour illumination series mycorrhiza occurred only in non-fertilized plants which had been raised in unsterilized soil. Table 4 shows that such non-fertilized plants were consistently bigger in shorter periods of daylight than corresponding plants in sterilized soil. Whether this has to do with the fact that a formation of mycorrhiza was possible in this soil but not in the sterilized soil to which only one particular fungus had been added—which is not with certainty the most "effective" nourishment assimilator in this substrate—cannot be ascertained with the help of the above experiments, but it does not seem entirely improbable.

However, as it has been seen that fully-developed mycorrhiza does not necessarily always occur when a clearly stimulating effect of a certain strain of a normally mycorrhiza forming fungus or of a litter-decomposing fungus (cf. Lundeberg, 1970) has taken place. It therefore seems probable that such an inoculation can result in good plant development already without real mycorrhiza formation (cf. Levisohn, 1956). This characteristic has also been observed in earlier experiments and been attributed to a certain antagonistic effect on parasitical root fungi (Marx, 1966; Sasek, 1967; Hyppel, 1968). In the present inoculation experiment with sterilized substrate, where the actual structure of the soil itself can be assumed to have undergone rather insignificant changes, it has, however, been possible to establish a directly stimulating influence on the growth of the plants (cf. Wilde, 1968).

Even though generalisations should be avoided, and the results of nursery and laboratory tests not be indiscriminately transferred to natural conditions, it should be possible to assume that certain fungi can have a favourable influence also in natural conditions where there is a certain lack of nitrogen and where light conditions are good. It therefore seems justifiable to proceed along this biological path of utilizing nitrogen which is bound in the soil. If a suitable method or partial sterilization and the introduction of certain mycorrhiza fungi or other soil fungi can be devised for practical application, this would constitute a biological alternative to chemical fertilization, as rich stores of nitrogen which are inaccessible to trees are also present in so-called low-nutrient forest soil.

Orientating field tests along these lines are at present in progress.

ACKNOWLEDGEMENT

The investigation has been supported by grants from the Swedish Natural Science Research Council and is included in the Swedish International Biological Programme.

REFERENCES

BJÖRKMAN, E. 1940. Mycorrhiza in pine and spruce seedlings grown under varied radiation intensities in rich soils with and without nitrate added. Medd. Stat. skogsförs.-anst., 32, 23—74.

— 1942. Über die Bedingungen der Mykorrhizabildung bei Kiefer und Fichte. (On the conditions for the formation of mycorrhiza in pine and spruce). Symb. Bot. Upsalienses., 6: 2.

— 1964. Report and recommendations from IUFRO's Mycorrhiza Working Group (Section 24) meeting in Puerto Rico July 2—7, 1964. Publ. Inst. Skogsbot., Royal Coll. of Forestry, 137.

BJÖRKMAN, E., LUNDEBERG, G. & NÖMMIK, H. 1967. Distribution and balance of N^{15} labelled fertilizer nitrogen applied to young pine trees (*Pinus silvestris* L.). Stud. For. Suec., 48.

CROMER, D. A. N. 1935. The significance of the mycorrhiza of *Pinus radiata*. Austr. Comm. Forestry Bur. Bul., 16.

EGLITE, A. K. 1954. Daži izméginájumi par mikorizas sintezi. Latv. P.S.R. Zinatnu Akad. Vést., 8. 91—96.

HACSKAYLO, E. 1970. Biological amendments to improve forest soil. J. Forestry, 68, 332—334.

HACSKAYLO, E. & VOZZO, J. A. 1967. Inoculation of *Pinus caribaea* with pure cultures of mycorrhizal fungi in Puerto Rico. XIV IUFRO Congress Paper 5, 139—148.

HANDLEY, W. R. C. & SANDERS, C. J. 1962. The concentration of easily soluble reducing substances in roots and the formation of ectotrophic mycorrhizal associations—a re-examination of Björkman's hypothesis. Plant and Soil, 16, 42—61.

HATCH, A. B. 1937. The physical basis of mycotrophy in plants. Black Rock Forest Bul., 6.

HATCH, A. B. & HATCH, C. 1933. Some hymenomycetes forming mycorrhizae with *Pinus strobus* L. Jour. Arnold Arboretum, 14, 324—334.

HOFSTEN, A. VON 1969. The ultrastructure of mycorrhiza I. Ectotrophic and endotrophic mycorrhiza of *Pinus silvestris*. Sv. Bot. Tidskr., 63, 455—463.

HOLMEN, H. 1969. Skogsgödsling i Sverige. Medd. Kgl. Skogs- o. Lantbr. Akad. arb.gr. f. skogl. växtnäringsforskning, 1.

HYPPEL, A. 1968. Effect of *Fomes annosus* on seedlings of *Picea abies* in the presence of *Boletus bovinus*. Stud. For. Suec., 66.

LEVISOHN, I. 1956. Growth stimulation of forest-tree seedlings by the activity of free-living mycorrhizal mycelia. Forestry, 29, 53—59.

LINDEBERG, G. 1948. On the occurrence of polyphenol oxydases in soil-inhabiting Basidiomycetes. Physiol. Plant., 1, 196—205.

LUNDEBERG, G. 1970. Utilisation of various nitrogen sources, in particular bound soil nitrogen, by mycorrhizal fungi. Stud. For. Suec., 79.

MARX, D. H. 1967. Ectotrophic mycorrhizae as biological deterrents to pathogenic root infections by *Phytophthora cinnamomi*. XIV IUFRO Congress Paper 5, 172—181.

MASUI, K. 1927. A study of the ectotrophic mycorrhizas of woody pants. Col. Sci. Kyoto Imp. Univ. Mem. Ser. B., 3, 149—179.

MELIN, E. 1925. Untersuchungen über die Bedeutung der Baummykorrhiza. Fischer, Jena.

MEYER, H. 1962. Die Buchen- und Fichtenmykorrhiza in verschiedenen Bodentypen, ihre Beeinflussung durch Mineraldünger sowie für die Mykorrhizabildung wichtige Faktoren. Mitteilungen der Bundesforschungsanstalt für Forst- und Holzwirtschaft, 54. Reinbek.

MIKOLA, V. 1969. Comparative observations on the nursery technique in different parts of the world. Acta For. Fennica, 98, 1—24.

MODESS, O. 1941. Zur Kenntnis der Mykorrhizabilaner von Kiefer und Fichte. Symb. Bot. Upsaliens., 5: 1.

MOSER, M. 1956. Die Bedeutung der Mykorrhiza für Aufforstungen in Hochlagen. Forstwiss. Cbl., 75, 8—18.

ROSENDAHL, R. O. 1942. The effect of mycorrhizal and non-mycorrhizal fungi on the availability of difficulty-soluble potash and phosphate minerals. Soil Sci. Soc. Amer. Proc., 7, 477—479.

SASEK, V. 1967. The protective effect of mycorrhizal fungi on the host plant. XIV IUFRO Congress Paper 5, 182—190.

SLANKIS, V. 1958. The role of auxin and other exudates in mycorrhizal symbiosis of forest trees. In: The Physiology of Forest Trees (Ed. K. V. Thimann), Cap. 21. Ronald Press, New York.

WILDE, S. A. 1968. Mycorrhizae: Their role in tree nutrition and timber production. Research Bull., Coll. Agricult. Life Sci., Univ. of Wisconsin, 1—27.

VOZZO, J. A. 1968. Inoculation of pine with mycorrhizal fungi in Puerto Rico. Ph. D. Diss., Geo. Wash. Univ., Washington. D. C.

Sammanfattning

Mykorrhiza och näringsupptagning hos skogsträd i mager skogsjord

Det är känt att barrträdsplantor som uppdrages i näringsfattig skogsjord kan stimuleras i sin tillväxt genom tillförsel av lämpliga marksvampar, mykorrhizasvampar eller förnasvampar. De förra förfogar i princip ej över laccas eller proteinas, som gör det möjligt att nedbryta komplexa humusföreningar i marken. Gränsen är emellertid oskarp mellan mykorrhizasvampar och förnasvampar. En i den föreliggande undersökningen prövad stam av *Boletus subtomentosus,* som ofta utgör en typisk mykorrhizabildare, har visat sig kunna nedbryta komplexa organiska humusföreningar och frigöra för träden upptagbart kväve samt stimulera mykorrhizabildningen genom andra svampar.

Vid en beskuggning av plantorna till hälften av fullt ljus förefanns ännu ett stimulerande inflytande genom den nämnda svampen, men vid beskuggning till 1/4 av fullt ljus blev alla plantorna lika. En utjämning skedde även vid tillsats av NPK och $CaCO_3$, då svampens inflytande eliminerades och ingen mykorrhiza bildades. En tillsats av fosfor medförde en viss ökning av mykorrhizabildningen.

Vid 24 timmars belysningstid blev plantorna i ett annat försök betydligt kraftigare än vid kortare belysningstid. I krukor med osteriliserad och ogödslad råhumus förekom mykorrhizabildning vid 24 och 16 timmars ljus men ej vid kortare belysningstid.

Det konstaterade gynnsamma inflytande som förnanedbrytande svampar kan utöva genom att frigöra bundet kväve och förmedla upptagningen av sålunda för skogsträd tillgänglig näring, som kan försiggå genom typiska mykorrhizasvampar, borde i själva verket kunna utnyttjas som ett alternativ till kemisk gödsling. De stora förråd av för träden i allmänhet oåtkomligt kväve, som även finnes i näringsfattiga skogsmarker, skulle sålunda kunna utnyttjas på biologisk väg i praktisk skala. Försök härmed pågår.